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SSPhS
Swiss Society
of Pharmaceutical
Sciences

www.sgphw.ch

SAPhS
Swiss Academy
of Pharmaceutical
Sciences

10/12

SGPhW Schweizerische Gesellschaft der Pharmazeutischen Wissenschaften
SSPhS Swiss Society of Pharmaceutical Sciences
SSSPh Société Suisse des Sciences Pharmaceutiques

Die Gesellschaft fördert die Schweizer Pharmazie in ihren wissenschaftlichen Aspekten und insbesondere den wissenschaftlichen Nachwuchs. Sie erfüllt Ihre Aufgaben vornehmlich durch:

- Unterstützung der Bestrebungen aller nationalen und regionalen pharmazeutisch-wissenschaftlichen Organisationen.
- Pflege nationaler und internationaler wissenschaftlicher Kontakte durch die Zusammenarbeit mit wissenschaftlichen Gesellschaften.
- Vertretung der pharmazeutischen Wissenschaften in Fachkreisen, den Behörden und der Öffentlichkeit.
- Kommunikation pharmazierelevanter Erkenntnisse und Informationen aus Wissenschaft, Forschung und Industrie.
- Organisation von Veranstaltungen zum Zwecke der Fortbildung, des wissenschaftlichen Austausches und des Networkings.
- Auszeichnung von Personen, die sich um den Fortschritt der pharmazeutischen Wissenschaften verdient gemacht haben.

The Society fosters the scientific aspects of pharmacy, especially young pharmaceutical scientists, in Switzerland by:

- Support of activities of national and regional organisations related to pharmaceutical sciences.
- Contacts to national and international scientific societies.
- Representation of pharmaceutical sciences to professional organisations, authorities and the general public.
- Publication of relevant findings and informations obtained in science, research and industry.
- Organisation of events dedicated to education, scientific exchange and networking.
- Awarding individuals who have significantly contributed to the advancement of pharmaceutical sciences.

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5th SWISS PHARMA SCIENCE DAY 2012

The 5th SWISS PHARMA SCIENCE DAY – "MISSION ACCOMPLISHED" (again)!

Prof. Dr. Rudolf Brenneisen, University of Bern, President Swiss Academy of Pharmaceutical Sciences (SAPhS)
Prof. Dr. Gerrit Borchard, University of Geneva, University of Lausanne, School of Pharmaceutical Sciences (EPGL), President Swiss Society of Pharmaceutical Sciences (SSPhS)

At its inception five years ago, the Swiss Pharma Science Day was intended as a gathering of Swiss pharmaceutical scientists working in academia and industry, with a special focus on the support of young scientists. This idea has been met with enthusiasm by pharmaceutical scientists and the support by the pharmaceutical industry and regulatory authorities. The organizers were thus highly motivated to make this year's event again a success for the participants, and pharmaceutical sciences in Switzerland as such. Here is our report on the 5th SWISS PHARMA SCIENCE DAY of August 29, 2012 held at the University of Bern.

SPhSD Pre-Seminar on Editorial Policies in Publishing Scientific Papers

On August 28, 2012, preceeding the 5th SWISS PHARMA SCIENCE DAY, the Swiss Society of Pharmaceutical Sciences (SSPhS) together with the publisher Elsevier organized a seminar on editorial policies in publishing scientific papers. As speaker, Prof. Gerrit Borchard, president of SSPhS, gave advice on "How to write a world-class paper" to the audience in a fully packed Langhans Auditorium.

Addresses of welcome

Prof. Dr. Gerrit Borchard, President, SSPhS
Prof. Dr. Rudolf Brenneisen, President, SAPhS
Prof. Dr. Christian Leumann, Vice-Rector Research, University of Bern

The next day, the 5th edition of the Swiss Pharma Science Day (SPhSD) was opened by Prof. Borchard, who thanked the University of Bern for its hospitality and readiness to host again the SPhSD. This was followed by a welcoming speech of the University of Bern's Vice-Rector Research, Prof. Christian Leumann, a chemist by training with an interest in the synthesis and characterization of base-modified DNA-analogues for applications in nanotechnology. Prof. Leumann pointed out that research at the University of Bern follows the paradigm that "excellent research requires excellent infrastructure", the latter reflected by the presence of not less than 5 NCCRs (National Centers of Competence in Research) at Bern. The increase in student numbers – the number of 15,000 was reached in 2011 for the first time – does not necessarily meet with increasing budgets, thus acquisition of "soft money" is required. Prof. Leumann was happy to mention that acquisition of exterior funding rose by 97% and represents about 60% of total budget today. Rounding up his speech, Prof. Leumann deemed the creation of a new drug an intellectual challenge with unparalleled societal impact.

Prof. Gerrit Borchard, President SSPhS and organizer of SPhSD

Prof. Christian Leumann, Vice-Rector Research, University of Bern

Prof. Rudolf Brenneisen, President SAPhS, organizer of SPhSD

The morning session was chaired by Prof. Brenneisen, President of the Swiss Academy of Pharmaceutical Sciences (SAPhS). The scientific program was opened by the President of Novartis Switzerland, Pascal Brenneisen (no relation to the chairman!). In his lecture, he analyzed the position of the pharmaceutical industry in Switzerland, and the current and future challenges to be overcome. Nominal figures mentioned by Mr. Brenneisen are indeed impressive: The pharmaceutical industry in Switzerland is directly and indirectly assuring about 144,000 jobs, contributes about 6% to the Swiss GDP and 29% of total Swiss exports. According to Mr. Brenneisen, in spite of high labor costs, high salaries and the lack of tax incentives to promote R&D, the pharmaceutical industry remains the most sustainable industry, with five pharmaceutical companies among the top ten investors in research and development in Switzerland. In his words, the pharmaceutical companies are faced today with the crisis in the global economy with exchange rate fluctuations, the pressure to innovate due to patent expiry meeting with risk aversion on the side of regulatory authorities, and the lack of sufficient recognition of the benefits of drug development especially in an aging population. To face these challenges, Mr. Brenneisen suggested to improve the regulatory process (more efficient and faster approval) adjusted to EMEA and FDA standards, and the strengthening of clinical research by the creation of a national ethical committee as a central institution.

Lecture 1: Keynote Speech

Pascal Brenneisen, Country President Switzerland, Novartis International AG, Basel:
„The Pharmaceutical Industry in Switzerland: Trends and challenges"

Abstract:
On a global scale, Switzerland is considered to be one of the most competitive nations. The pharmaceutical industry has contributed significantly to this success:

- In 2008, the Chemical and Pharmaceutical sectors combined accounted for 44% of Switzerland's total R&D investments of CHF 12 billion.
- Novartis spends more than CHF 3 billion per year on R&D in Switzerland alone. The Knowledge Campus in Basel remains the biggest research center of Novartis worldwide.
- In 2010, the pharmaceutical industry contributed 5.7% to the Swiss GDP and secured, both directly and indirectly, more than 135,000 jobs.
- In 2011, Pharma accounted for CHF 62 billion or 30% of total Swiss exports.

The world economy is slowing down. Yet, regardless of the prospect of growing national and international challenges, too little is done by the public sector to mitigate the consequences of the Euro crisis and the overvalued Swiss franc and to strengthen the R&D investment capabilities of the pharmaceutical industry in Switzerland. Research productivity will not compensate for revenue losses resulting from a record level of upcoming patent expiries.
Scientifically acknowledged benefits of drugs to the national healthcare system remain insufficiently recognized and valued. Costs for medicines will continue to be contained by authorities and payors.
Social and demographic developments will place a heavy burden on the Swiss national health system. The medical needs of an aging population will require more economic resources. Yet, even though medicines accounted for just 9.7% of total health sector expenditure in Switzerland in 2011, the pharmaceutical industry continues to be falsely labeled by politicians and hence seen by the public as the sole factor responsible for the rising costs of the healthcare sector. Future aggressive price fixing for new drugs by the authorities as well as price cuts for new indications remain a constant threat. Continuing on this path could well lead to the decline of the pharmaceutical industry of Switzerland.
The industry recognizes that the healthcare sector as a whole does need wide-ranging political and structural reforms. It would, however, be unwise to have the industry bear the brunt of such an endeavor.

Instead, while focusing on the overall sustainability of the Swiss healthcare system, all stakeholders should urgently get together to come up with a comprehensive master plan to increase the country's attractiveness to the industry.

Pascal Brenneisen, Novartis Pharma Switzerland, keynote speaker

Lecture 2: Pharmaceutical Biology

Prof. Dr. em. Kurt Hostettmann, Champex-Lac: "Medicinal plants: What is new?"

The following lecture on medicinal plants was given by Prof. Kurt Hostettmann, whose recent retirement from the University of Geneva obviously did not have an impact on his scientific activities. Focusing on polyphenols as scavengers of oxygen radicals, Prof. Hostettmann discussed several natural sources of polyphenols whose consumption would have positive effects on diseases such as Alzheimer's. Citing a poem by Johann Wolfgang von Goethe, he pointed out the positive effects of Ginkgo in Alzheimer's and age-related cognitive problems. Similar improvement of cognitive function was seen in an animal experiment with mice consuming blueberry juice, in the words of Prof. Hostettmann: "Blueberry juice for a better memory". Rosemary, yet another source of polyphenols, is also considered to boost the brain and improve memory function, thus potentially able to mitigate the degenerative process in Alzheimer's and dementia. Prof. Hostettmann showed that this had already been known in Shakespeare's time, citing from the great author's Hamlet, where Ophelia says (Act 4, Scene V): *"There's rosemary, that's for remembrance; pray, love, remember: and there is pansies. That's for thoughts."*. Pansies *(Viola tricolor),* the name derived from the French word "penser", of course, are also said to have pharmacological properties for treatment of skin diseases. Rounding up his talk, Prof. Hostettmann pointed out the beneficial effects of coffee, best consumed green and unroasted, on the avoidance of prostate cancer, recently shown in an epidemiological study.

Abstract:
There is presently a tremendous increase of interest in plants containing large amounts of polyphenols of various chemical structures (flavonoids, tannins, anthocyanidines, etc.). Research in fruit polyphenols is attracting more and more scientists due to their strong antioxidant and radical scavenging properties. It is quite well-known that eating large amounts of fruits can prevent different types of cancers and cardiovascular diseases. More recently, research has focused on the intake of fruits rich in polyphenols in relation to cognitive decline in aging persons. Several *in vivo* studies using animal models and also clinical trials have shown that eating regularly fruits such as apples, blueberries and strawberries can contribute to slow down the progression of Alzheimer's diseases. A study was conducted with blueberry juice. Mice receiving the juice had a better sense of orientation in a maze than control mice. Dietary supplementation of blueberries resulted in a neurocognitive benefit in aging patients suffering from early memory troubles. Thus, the slogan blueberry juice for a better memory is becoming popular in the USA. The blue pigments of blueberry are responsible for the memory boosting effect. Very recently, a clinical study showed that persons inhaling essential oil of rosemary experienced an increase of the cognitive functions which was associated with the plasmatic concentration of 1,8-cineole, the main constituent of the essential oil. These findings are interesting as in Ancient Greece rosemary needles were rubbed on the head of kids to make them more intelligent! Even William Shakespeare is mentioning in Hamlet „There's rosemary, that's for remembrance". Esophageal cancer is the third most common gastro-intestinal cancer and strawberry appears to be efficient for its prevention and even its treatment. *In vivo* studies showed that freeze-dried strawberries inhibited tumor development in the esophagus of rats. Based on these results, clinical trials were undertaken with 36 patients with esophageal precancerous lesions. They consumed 60 g of freeze-dried strawberries per day during a period of 6 months. Biopsy before and after strawberry consumption showed that 29 out of 36 participants experienced a decrease of the precancerous lesions. Coffee lowers risk of prostate cancer according to an epidemiological study carried out with around 48,000 men during a period of 16 years. A clear association between coffee consumption and prostate cancer could be established. Those who drank 6 cups of coffee or more per day were 60% less likely to develop a lethal form of the disease. It did not matter whether the coffee was normal or decaffeinated. This indicated clearly that polyphenols are responsible for the protective effect and not caffeine! Finally, there is now a registered drug available to fight stress and burnout, namely *Rhodiola rosea* L. (Crassulaceae). The extract of the roots is decreasing the salivary concentration of cortisol, called also the stress hormone.

Prof. Kurt Hostettmann, Champex-Lac

Lecture 3: Molecular Biology

Prof. Dr. Dario Neri, Institute of Pharmaceutical Sciences, ETH Zurich: "Vascular targeting – from the bench to the clinic"

The last presentation of the morning session was given by Prof. Dario Neri of ETH Zurich, focusing on the translation of the concept of vascular targeting in cancer therapy from basic research to clinical application. While cytokine/antibody fusion proteins (immunocytokines) are able to deliver cytokines to tumors, the association of cytotoxic drugs to antibody formats (full antibodies, single chain or

Fab fragments) requires strategies developed by Prof. Neri's group. The target of this research is the (neo)vasculature in tumors, with its importance for growing tumors, the expression of surface marker targets not expressed in healthy tissue, and its accessibility from the systemic circulation. In addition to antibody binders, small molecules are also considered to fulfill the role of Paul Ehrlich's "magic bullets". Such molecules, binding specifically to antigens expressed by cancer cells or endothelial cells of the tumor neovasculature, may be identified by DNA-encoded chemical libraries.

Abstract:
Antibodies can be used to deliver bioactive molecules (drugs, cytokines, photosensitizers, radionuclides, etc.) to the tumor environment, thus sparing normal tissues. The targeting of modified sub-endothelial extracellular matrix components using armed antibodies is particularly attractive, because of:

(i) the abundance and stability of some of these antigens (e. g., splice isoforms of fibronectin and tenascin-C);
(ii) the dependence of cancer on new blood vessels
(iii) the accessibility of these structures from the blood-stream
(iv) the fact that some of these antigens are very abundant in many different cancer types, while being virtually undetectable in most normal adult tissues [1-3].

Vascular targeting approaches can be used beyond oncology, for the treatment of other serious conditions which are characterized by the over-exuberant proliferation of new blood vessels. While cytokines can be conveniently delivered at site of disease by the construction of fusion proteins with antibody vehicles ("immunocytokines"), the targeted delivery of cytotoxic drugs requires more sophisticated chemical strategies. We have recently explored the development of linkerless strategies for the coupling of potent cytotoxic drugs to tumor-targeting antibodies [4-5].
Advanced preclinical and clinical data on armed antibodies will be presented in this lecture. In addition, we have recently started to explore whether small organic ligands, specific to tumor-associated antigens, can be used for pharmacodelivery applications *in vivo*. Selective ligands can be conveniently isolated from large DNA-encoded chemical libraries [for a recent review, see ref. 6].

References:

[1] D. Neri, R. Bicknell. Nature Rev Cancer 2005; 5: 436-446.
[2] D. Neri, C. Supuran. Nature Rev Drug Discov 2011; 10: 767-777.
[3] D. Neri, N. Pasche. Drug Discov Today 2012; 17: 583-590.
[4] G. Bernardes et al. Angew Chemie Int Ed Engl 2012; 51: 941-944.
[5] G. Casi et al. J Am Chem Soc 2012; 134: 5887-5892.
[6] L. Mannocci et al. Chem Commun 2011; 47: 12747-12753.

Prof. Dario Neri, ETH Zurich

Poster session

After the morning session and the excellent Italian lunch buffet, the over 160 participants had the opportunity to view and discuss the 64 posters (all abstracts can be found at the end of this article) presented by young scientists. As in previous years, awards were given to three outstanding poster presentations, and one poster was given a special award due to its originality. These awards were sponsored by Debiopharm, AKB Foundation and the Pharmaceutical Society of Zurich (first, second and third poster award, respectively), as well as by Vifor Pharma (special award). In addition, the jury of distinguished scientists was tasked with finding candidates for two more awards, i. e. the best poster in Pharmaceutical Biology (sponsored by Zeller AG) and in Pharmaceutical Technology (sponsored by TTC Glatt AG).

Serving the Italian lunch buffet

Prof. Ulrich Honegger, former Vice-President SSPhS and Fellow SSPhS, and Dr. Irmgard Schmitt-Koopmann, board of AKB

Dr. Christian Lanz, organizer of SPhSD, and Karin Fürer, PhD student, enjoying (healthy!) coffee break

Prof. Matthias Hamburger, Fellow SSPhS, and Prof. Kurt Hostettmann, together with Mrs. Hostettmann

Prof. Jörg Huwyler, University of Basel, and Benoîte Kaeser, board member of SSPhS

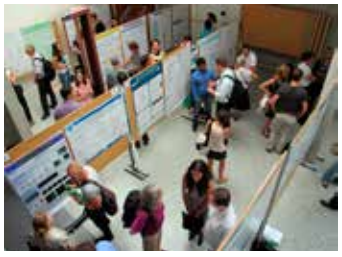
Prof. Gerrit Borchard, President SSPhS, Prof. Stefan Mühlebach, Vice-President and Fellow SSPhS, Dr. Manuela Langos, Swissmedic

Dominique Jordan, President pharmaSuisse, and Prof. Hans Leuenberger, former President SSPhS

Heinz Schmitter and Prof. Bruno Gander, ETHZ, together with Vroni Jakob, PharmGZ and Prof. Rudolf Brenneisen, President SAPhS

Professor Ursula von Mandach, University Hospital Zurich, and Miss Dixa Thakrar (poster no. 5)

Two participants (left Klaus Eichler, TTC Glatt), critically looking at posters



Poster session

Dr. Felix Wüst, publisher of SWISS PHARMA and Fellow SSPhS.

Lecture 4: Pharmacoepidemiology

Prof. Dr. Christoph Meier, University Hospital Basel: "Contraception and risk of venous thromboembolism – a drug safety issue of high public interest"

In opening the afternoon session, chaired by Prof. Borchard, Prof. Christoph Meier (Basel Pharmacoepidemiology Unit, University Hospital Basel), commented on the drug safety risk of thromboembolism by the use of oral contraceptives. While generally burdened with rare and mild side effects, oral contraceptives may in some cases lead to serious adverse events such as venous thromboembolisms. In contrast to the long existing paradigm, these adverse effects may be caused by progestogen derivative by a much greater extent than the estrogen component of the "pill". In addition, as recent studies have shown, side effects may differ between various products and depend on the progestogen derivative contained. A 2-fold increased risk of venous thromboembolisms is considered when comparing third to second generation oral contraceptives. Prof. Meier therefore suggested performing a randomized clinical trial to investigate this safety risk, which, however, would be of considerable size due to show a significant outcome.

Abstract:
Millions of women worldwide use oral contraceptives, the most reliable method for birth control. Most oral contraceptives contain a combination of an estrogen (mostly ethinylestradiol) and a progestogen derivative. Oral contraceptives are often well tolerated, causing only minor adverse effects in the beginning of a treatment such as gastrointestinal intolerance, breast tenderness, or sleep problems. In rather rare instances, more serious adverse reactions can occur, such as for example dangerous and potentially even fatal venous thromboembolisms. While for a long time the estrogen derivative was considered to be the main reason for the increased risk of venous thromboembolism, recent research suggested that the progestogen derivative may actually be an even more relevant contributor to venous thromboembolism. Large epidemiological studies suggest that the risk of venous thromboembolism differs between various pill products, depending on which progestogen derivate the pill contains. This led to a substantial controversy in this field, because massive financial interests are at stake. To the current day, the available literature provides substantial evidence that third generation oral contraceptives and the newer drospirenone containing pill exert an approximately 2-fold increased risk of venous thromboembolism as compared to the older contraceptives of the second generation. In this presentation the currently available evidence supporting the notion that newer pill products are associated with a higher risk of venous thromboembolism than older preparations is discussed. These findings are put in the context of potential beneficial effects of the newer pills and of the magnitude of the problem in general from a public health perspective.

Prof. Dr. Christoph Meier, University Hospital Basel

Lecture 5: Ethics in Science

Dr. med. Peter Kleist, GlaxoSmithKline AG, Münchenbuchsee: „Bias and ethics in publishing"

Following up, Dr. Peter Kleist, Medical Director at GSK, stated as an opener that "not everything is economy in the pharmaceutical industry, it is also important *how* the money is earned". He specifically pointed out that about 50% of results from (failed) clinical trials are not published, leading to the selection of positive data and hiding non-significant results, resulting in the so-called "publication bias". This, of course, is not beneficial for the clinical development of new drugs. However, even published studies may be subjected to a bias, e. g., in data interpretation (change of trial protocol) or if data are selectively reported (outcome reporting bias). In addition, editors of scientific journals are more interested to publish "positive results" and manuscripts from high reputation institutions (Halo effect). Last not least, reports authored by "eminences" are less often challenged during the review process (Matthew effect). To cope with this situation, Dr. Kleist demanded that all clinical trial data are submitted for publication, and then reviewed by open peer review and commentary followed by open access publishing.

Abstract:
A publication bias can lead to an imbalanced database for the benefit/risk assessment of a medical intervention and in consequence may harm patients. Furthermore it can exert inappropriate influence of cost/benefit analyses and finally contribute to a waste of limited resources of our health system. Although these facts have been discussed for almost 20 years and are widely accepted today, 50% of all performed clinical studies are still not going to be published (Non Publication Bias), predominantly those studies with a statistically non-significant result. But also published studies can corrupt the basis for a correct evaluation if study outcomes are selectively reported (Outcome Reporting Bias). According to systematic analyses, more than 50% of published clinical studies appear to have at least one primary efficacy or safety outcome that was changed in comparison to the trial protocol. In addition, the proportion of results being published does not represent the real word of all clinical research; publication is frequently driven by individual interests, impact and attraction of the readers (the "Wow Factor") – rather than by research quality.
Publication bias is not a problem that can be limited to an individual group of people – on the contrary, all major stakeholders of the science system, i. e. academic researchers, the pharmaceutical industry and medical journals contribute to it. They all are affected by a conflict of interests which explains why efforts of self-regulation are of modest success. The overlap of the secondary interests of various stakeholders makes it even more difficult to stimulate an ethical behavior with isolated approaches. For example, a high number of reprints of an article does not only distort the perception of the value of an intervention but also increases popularity of the author and the journal's impact factor.
In the last years the main focus has been on study registration as a measure to enhance transparency in research. The need for public registration of a clinical study, particularly if this is mandated by law, potentially increases the pressure for a later complete and unbalanced publication of study results. However, recent analyses of the impact of study registration have demonstrated that correct reporting has not significantly improved yet. A stricter control of quality and a stricter sanction of non-compliance seem to be necessary. But just fostering public study registration will not be sufficient; it becomes more and more evident that wrong incentives of the science system need to be overruled by more radical changes of the whole system. Open access publishing, open peer review and commentary, and departure from summing up impact factors as major means for the assessment of academic performance are further steps towards the right direction.

Dr. Peter Kleist, GlaxoSmithKline AG

Lecture 6: Analytics

Carina Lämmle, Biberach University of Applied Sciences, Biberach (D): "Belladonna puzzle – Application of mass spectrometry"

The Swiss Pharma Science Day is dedicated to the benefit of especially young researchers. However, the organizers did not expect to have as presenter a docent being younger than a PhD student. Carina Lämmle, a tutor of mass spectrometry at the University of Applied Sciences in Biberach (Germany), was accompanied by her grandparents due to her age of merely 17 years. Ms. Lämmle presented in a very professional way in what was her first presentation in English the solving of the "Belladonna puzzle", an analysis of a yet not described atropine derivative in belladonna fruits by means of mass spectrometry. Involving the fascinated audience in her presentation, she soon put all skeptics seen her very young age to shame. A true accomplishment of a talented young scientist.

Abstract:
In search for new chemical compounds for medical use and more efficient just as more accurate methods in pharmaceutical research, there is a high interest in the investigation and characterization of ingredients in a variety of fruit and plants. For these reasons alkaloids and especially anthocyanins of *Atropa belladonna* L., a deadly nightshade, have been analyzed. Some alkaloids are already well established in therapy due to anticholinergic and central nervous system activities. Besides, previous studies on anthocyanins showed beneficial effects against the initiation and development of vascular diseases. Several analytical procedures including XAD, countercurrent chromatography (CCC), hydrolysis, high performence thin layer chromatography (HPTLC) and liquid chromatography-mass spectrometry (LC-MS^n) were used for purification and structure elucidation.
There is an amount of predictive value in a mass spectrum which may lead to a preliminary idea of molecules. Furthermore, chromatographic or physicochemical properties help to gain more information on molecules. Nevertheless, it is not possible to elucidate every detail of the sterical structure of alkaloids and anthocyanins using these methods.
However, a presumably so far not yet described atropine derivative could be detected in addition to interesting, novel anthocyanins. The main component responsible for the color of the belladonna fruits is with a high probability a petunidin-based molecule consisting of petunidin, coumaric acid, rutinose and hexose or caffeic acid. In addition, malvidin-based molecules and a delphinidin-based one

could be found as well. All in all, the process of getting those chemical structures of compounds through mass spectrometry – comparable to a puzzle – is the main part of this lecture.

References:
[1] Q. Du, G. Jerz, P. Winterhalter. Isolation of two anthocyanin sambubiosides from bilberry (Vaccinium myrtillus) by high-speed counter-current chromatography. J Chromatogr A 2004; 1045: 59-63.
[2] S. Kim, M. Joo, S. Yoo. Structural identification and antioxidant properties of major anthocyanin extracted from Omija (Schizandra chinensis) fruit. J Food Sci 2009; 74: C134-140.
[3] Y. Ito, W.D. Conway (Eds). High-Speed Countercurrent Chromatography, Chemical Analysis Series, Vol. 132, Wiley Interscience, New York, 1996, pp. 255–263.

Carina Lämmle, Biberach University of Applied Sciences

Recognitions and Awards

Fellows 2012

After a short coffee break it was time for the recognitions and awards. Two outstanding scientists, Prof. Jean-Luc Veuthey and Dominique Jordan were honored with the Fellowship of the Swiss Society of Pharmaceutical Sciences (SSPhS), and appointed members of the Swiss Academy of Pharmaceutical Sciences (SAPhS).

Prof. Dr. Jean-Luc Veuthey, Vice-Rector of the University of Geneva and Professor in Pharmaceutical Analytical Chemistry, was nominated as Fellow by the Swiss Society of Pharmaceutical Sciences (SSPhS) and Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) "For his merits in pharmaceutical research, specifically in analytical chemistry, in teaching and education, as well as in representing the Pharmaceutical Sciences nationally and internationally".

Dominique Jordan, President of pharmaSuisse, has been elected as Fellow of SSPhS and Member of the SAPhS "For his merits and efforts in the training and formation of pharmacists, such as approval of FPH specialization, his socio-economic and political contributions to the Swiss public health system and his support to foster the SSPhS and SAPhS".

The Swiss Society of Pharmaceutical Sciences, and its Scientific Council, i.e. the Swiss Academy of Pharmaceutical Sciences, are very proud to count Prof. Jean-Luc Veuthey and Dominique Jordan among their ranks.

Prof. Dr. Jean-Luc Veuthey (middle), receiving the SSPhS Fellowship 2012

Dominique Jordan (left), receiving the SSPhS Fellowship 2012

Poster award winners

1st Prize, sponsored by Debiopharm:
Mi Liu, ETH Zurich
P-24: "Comb-Polymer Monolayers Engineered to Display Molecular Sieving Properties for the "Smart" PEGylation of Proteins"

Colleague of the winner Mi Liu (not present), receiving the first poster prize from Dr. Vuaridel, Debiopharm

2nd Prize, sponsored by the Foundation of the Association of Bernese Pharmacists (AKB):
Sakthikumar Ragupathy, University of Geneva
P-34: „Decrease in Human Bronchial Epithelial Cell Monolayer Permeability Triggered by TLR2 Ligation is Mediated by Atypical Protein Kinase C Zeta"

Sakthikumar Ragupathy, winner of the second poster prize (AKB Foundation)

3rd Prize, sponsored by the Pharmazeutische Gesellschaft Zürich (PharmGZ):
Claudia Dührkop-Sisewitsch, University of Bern
P-20: "C1 Esterase Inhibitor Treatment in Skeletal Muscle Ischemia / Reperfusion Injury"

Claudia Dührkop-Sisewitsch, winner of the third poster prize (PharmGZ)

Prize for best poster in Pharmaceutical Technology, sponsored by TTC Glatt Group:
Zdravka Misic, University of Applied Sciences NW Switzerland and University of Basel
P-30: "Novel Thermoplastic Capsules for Robust Encapsulation of Hydrophilic Lipid-Based Formulations"

Zdravka Misic, winner of the TTC Glatt poster prize

Prize for best poster in Pharmaceutical Biology, sponsored by Zeller: Olivier Potterat, University of Basel
P-10: "Library-based Discovery and Characterization of Daphnane Diterpenes as Potent and Selective HIV Inhibitors in *Daphne gnidium*"

PD Dr. Olivier Potterat, winner of the Zeller poster prize

Special prize, sponsored by Vifor Pharma:
Isabelle Arnet, University of Basel
P-46: "Erroneous Prescription of Half Tablets in a Swiss University Hospital"

Dr. Isabelle Arnet, winner of the Vifor Pharma poster prize

Thanking and invitation to the 6th Swiss Pharma Science Day on Wednesday August 28, 2013

The 5th Swiss Pharma Science Day ended with drinks and snacks at the beautiful setting of the House of the University of Bern. The organizers would like to thank all speakers for their excellent presentations, and the Faculty of Medicine of the University of Bern as the host of this event. The Verlag Dr. Felix Wüst AG Küsnacht, AKB Foundation, Debiopharm, Elsevier B.V., TTC Glatt, the Pharmaceutical Society of Zurich (PharmGZ), Vifor Pharma, Mundipharma Medical, Zeller, Galexis and pharmaSuisse are recognized for their continued financial support.
The organizers are looking forward to welcome young pharmaceutical scientists to the 6th edition of the SPhSD on August 28, 2013, again in Bern.

Prof. Gerrit Borchard, Geneva, Prof. Rudolf Brenneisen, Bern

SWISS PHARMA SCIENCE DAY 2012

Poster Abstracts

P-1

Design of Biorelevant Test Setups to Predict *In Vivo* Drug Features in Orodispersible Dosage Forms

M. Guhmann[1,2], F. Gerber[2], N. Pöllinger[2], S. Klein[1], W. Weitschies[1]
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Introduction: Progress in pharmaceutical formulation design has given rise to novel oral drug delivery systems, like orodispersible formulations, offering advantages like administration without water, ease of swallowing, rapid onset of action and convenience of dosing. Rapid disintegration of the dosage form in the mouth may lead to drug dissolution and affect the drug product bioperformance [1]. Pharmaceutical scientists need to gain more knowledge on orodispersible dosage forms performance to better predict *in vitro* the *in vivo* drug behaviors [2].
Aims: Biorelevant test setups that mimic physiological conditions experienced by oral formulations during disintegration in the mouth and arrival in the stomach were designed and evaluated *in vitro*.
Methods: With a focus on composition, volume, pH and residence time, biorelevant models simulate mouth and gastrointestinal contents. The developed biorelevant schedule consists consecutively of Simulated Salivary Fluid (SSF) (pH 7.4, 5 mL, 3 min), FaSSGF (pH 1.6, 50 mL, 30 min) and FaSSIF (pH 6.8, 250 mL, 60 min). Diclofenac was chosen as drug model using the acid, sodium and potassium forms. Dissolution studies were performed applying biorelevant test setups and compared to USP method (apparatus 2, 50 rpm, 120 min, 750 mL 0.1N HCl pH 1.1 and 60 min 1000 mL phosphate buffer pH 6.8). Using HPLC and optical morphogranulometry, chemical and physical state characterizations were investigated to elucidate peculiar drug behaviors.
Results: Using USP method, similar low drug releases were observed for the three drug forms in the acidic phase that slowly reached 68%, 83% and 100% for diclofenac free acid, sodium and potassium respectively in the basic phase. In contrast, using biorelevant models, after partial (about 18%) and almost complete release in SSF for diclofenac free acid and salts respectively, the precipitation that occurred in FaSSGF for the 3 drug forms was characterized by a transitional state only for the salts. All drug releases were fast and complete in FaSSIF. Applying these models, drug cyclization and formation of aggregates were subsequently observed for both salts in FaSSGF. The important dissolution of the salts in SSF leads to increased precipitation in FaSSGF that alters their chemical and physical states. Owing to low dissolution in SSF, this is not observed for the free acid. Based on these observations, it is suggested that *in vivo* behaviors of diclofenac may strongly be determined by pH modifications along the mouth and the gastrointestinal tract after oral administration.
Conclusions: Investigating diclofenac as a drug model, the present study indicates that characterizing drug behaviors under biorelevant conditions considering mouth and stomach conditions is a key tool for the understanding and the development of orodispersible dosage forms.

Keywords: Orodispersible forms, design, biorelevant models.

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P-2

Taste Sensing System for the Selection of Drug Candidates in Early Development Stage and Rational Development of Taste Masked Orodispersible Dosage Forms

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Introduction: To achieve patient compliance, the organoleptic aspects of drug substances play a key role in the development of oral pharmaceutical preparations. For novel solid oral drug delivery systems like orodispersible dosage forms, for which the exposition of unpleasant tasting active pharmaceutical ingredients is highly critical, drug taste masking techniques and palatability testing become of increasing importance [1].
Aims: The ability of an electronic tongue to guide the selection of drug candidates and rationalize the development of taste masked orodispersible formulations was evaluated.
Methods: Diclofenac was used as drug model investigating the free acid, the sodium and the potassium salt forms. Calibrations curves were established with different concentration ranges. Measurements were performed using the electronic taste sensing system TS-5000Z (Insent Inc., Japan) qualified based on ICH guideline Q2 [2]. The system was equipped with 7 lipid membrane sensors representing bitterness (1, 2 and 3), sourness, saltiness, umami, and astringency and corresponding aftertaste qualities. Sensor responses (mV) of 3 consecutive measurements were analyzed by univariate data evaluation. Using the most suitable drug form considering taste modalities, diclofenac taste masked Orally Disintegrating Tablets (ODTs) were developed and further evaluated by the electronic tongue versus placebos by Principal Component Analysis (PCA).
Results: High contrasts between the 3 diclofenac drugs were disclosed by the outputs of the electronic tongue and clearly discriminate the free acid from its salt counterparts. Diclofenac salts induced bitter (taste and aftertaste) and astringent (taste) responses with different detection intensities; saltiness stimuli was additionally recorded for the potassium salt. In contrast, only a bitter aftertaste from sensor 3 was detected for the free acid form. Based on these results, diclofenac acid was selected for manufacturing taste masked ODTs. Taste masking strategies used granules produced by coating of single drug crystals or wet granulation, further compressed into ODTs. The PCA map showed that intermediated prod-

ucts (granules) and finished products (ODTs) could be distinguished by the electronic tongue. All formulations differed from the pure diclofenac acid. Representing PC-1 (77% of the information), the taste reduction was 33% and 54% for drug coated granules and matrix granules, respectively, 67% and 71% for their corresponding ODTs.
Conclusions: Investigating diclofenac as a drug model, the electronic tongue proved to be a valuable tool for the selection of drug candidates in the early pharmaceutical development phase and the rational development of taste masked orodispersible dosage forms.

Keywords: Electronic tongue, selection, rational development.

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P-3

A New HPLC-Method to Determine Sennoside A and Sennoside B in Sennae Fructus and Sennae Folium

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Introduction: The current monographs in the Ph. Eur. 7.3 for Senna pods and Senna leaves describe a photometric assay based on the Bornträger reaction to determine hydroxyanthracene glycosides, calculated as sennoside B. The method is time-consuming, unspecific for sennosides and the precision is not adequate for a modern assay.
Aims: The photometric method shall therefore be replaced by a modern HPLC method. According to the literature [1] about 70% of the total anthrachinone content is due to sennoside A and sennoside B. These substances are therefore suitable for the standardisation of Senna products. The *Pharmacopoeia* of the People's Republic of China *(PPRC)* and the Ph. Jap. already describe HPLC methods to determine sennoside A and sennoside B in the respective monographs. The Ph. Jap. uses ion-pair chromatography with tetraheptylammoniumbromide. The unmodified procedure has a runtime of 70 min.
Methods: The adapted and validated method [2,3] uses solid-phase extraction (SPE) which allows a selective sample preparation by using an anion exchange phase. We used a conventional RP C_{18} column Tosh TSKgel ODS-80TS (4.6 mm x 150 mm), 5 µm, as stationary phase and acetonitrile-water-phosphoric acid 200:800:1 v/v/v as mobile phase. The flow rate was 1.2 mL/min, the column temperature 40 °C, the detection wavelength 380 nm, and the injection volume 20 µL. The runtime is 10 min, the chromatogram shows 2 peaks due to sennoside A/B and 2 additional smaller compounds. One of them is rhein-8-O-glucoside.
Results: The procedure has been successfully validated according to ICH guidelines. We analyzed 6 batches of Senna. The pods *(Senna angustifolia)* showed a total content of sennoside A and B of 1.74 to 2.76% and the content of leaves was clearly lower with 1.07 to 1.19%, respectively.
Conclusion: The suggested method is considered to be suitable to determine sennoside A and sennoside B in Sennae folium and Sennae fructus. The consideration is based on the performed validation and on the results for the analyzed samples. Shorter run time and better resolution are clear advantages of the suggested method, compared to other methods.

Keywords: HPLC, Senna, sennoside A, sennoside B.

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P-4

Capillary Electrophoresis Combined with Time-of-Flight Mass Spectrometry for the Analysis of Carbohydrate-Deficient Transferrin

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Introduction: Transferrin (Tf) is the most important endogenous iron transporting protein containing 2 metal binding sites and 2 N-linked carbohydrate chains with a total of up to 8 negatively charged sialic acid terminals. The major glycoform of Tf contains 4 sialic acid residues (tetrasialo-Tf) but minor isoforms with 2 to 8 sialic acid residues can be identified in normal human serum. Carbohydrate-deficient transferrin (CDT) is the most specific marker for chronic alcohol intake and encompasses isoforms without (asialo-Tf) and with 2 (disialo-Tf) sialic acid residues. The disialo-Tf fraction is increased up to 10 times in sera of alcohol abusers, while asialo-Tf is highly specific to alcoholic patient since it is not observed in normal sera.
Capillary electrophoresis (CE) is currently recognized as a high efficiency separation technique for proteins analysis. Nevertheless, one major drawback is the potential protein adsorption onto the negatively charged surface of the capillary. Proteins adsorption can be minimized by coating the capillary. For routine analysis of CDT, a commercial CE method providing a bilayer capillary coating is used for the relative quantitation of asialo- and disialo-Tf. However, this method suffers from a lack of sensitivity and selectivity due to the UV detection at 195 nm. CE can be hyphenated with mass spectrometry (MS) to increase the sensitivity and to readily assess the presence of specific asialo-Tf, but routine CE conditions for CDT determination are not MS compatible (bleeding of coating material, ionization suppression).
Aims: This study consists in developing CE and coating conditions as well as ionization and MS parameters to ensure Tf analysis and CDT determination by CE-MS.
Methods: Various acetate and formate-based background electrolytes (BGE) at different concentrations and pH were tested on Tf standard at 1.5 mg/mL. Bilayer and neutral polymeric coatings were investigated in CE-UV configuration with optimization of polymers concentration and coating procedure. Electrospray (ESI) and time-of-flight MS (TOF/MS) operating conditions, *i. e.* sheath liquid composition, nebulizing gas flow rate, drying gas flow rate and temperature) were investigated to maximize ionization.
Results: Best results for glycoforms separation in CE-UV in terms of resolution, efficiency, and coating stability were obtained with a polybrene-dextran sulfate coating at 10% each. BGE consisted of 20 mM ammonium acetate at pH 8.5. Sheath liquid was composed of H_2O-isopropanol-formic acid 50:50:5 (v/v/v). Nebulizing gas was set at 4 psi, and drying gas at 4 L/min and 350 °C. Under these conditions, most abundant glycoforms were detected in CE-TOF/MS but unambiguous determination of CDT could be hardly achieved due to poor Tf ionization and loss of efficiency.

Conclusions: MS-compatible coating and BGE conditions were successfully developed in CE-UV to allow Tf glycoforms separation. Further investigations in CE-TOF/MS will be performed to enhance Tf ionization and glycoforms resolution.

Keywords: CE-MS, carbohydrate-deficient transferrin, capillary coating, intact protein analysis.

P-5

Blood Pressure and the Menstrual Cycle in Teenage Females

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Introduction: Studies have suggested that exogenous doses of the ovarian steroids, estrogen and progesterone, in oral contraceptive pills and hormone replacement therapy, affect blood pressure (BP). However, the effects on blood pressure, of naturally occurring ovarian steroids, which change during the normal menstrual cycle, remain elusive. A few studies have suggested that there is an association between BP and the menstrual cycle. However, all of these studies were conducted in elder females and yielded conflicting results.
Aims: This prospective primary data collection study investigates whether BP changes with the phases of the normal menstrual cycle in teenage females.
Methods: Systolic blood pressure (SBP) and diastolic blood pressure (DBP) readings were taken from 22 postmenarchal teenage females in grade 11, attending the International School Basel. Measurements were taken each day (Monday to Friday) for eight weeks. The onset of menstruation, for each subject, was noted during these eight weeks. BP readings taken on and after the onset of the first menstruation contributed to the primary analysis. Readings taken before the first menstruation were used to investigate consistency in any trends observed in the primary analysis. BP was compared between the different phases of the menstrual cycle using analysis of variance, which adjusted for between-subject variation. The study also included five teenage males for control purposes.
Results: The primary analysis revealed that crude SBP was higher in the luteal phase than follicular (114.14 vs. 111.61 mm Hg). The adjusted mean difference, however, was 0.61 (95% CI: -1.29 to 3.51; P = 0.53), revealing no significant difference. There was no difference in DBP between the phases -0.07 (-1.62 to 1.48; P = 0.93). In the previous cycle, however, SBP and DBP were higher during the follicular phase than the luteal. This finding may be due to greater stress response, as these measurements were taken earlier on in the experiment. Stress response is likely to have a greater impact on the follicular phase, as it occurred closer to the start of the experiment. This stress response was also observed in the control males.
Conclusions: Blood pressure does not appear to vary with the phases of the menstrual cycle in teenage girls and hence, naturally changing levels of endogenous ovarian steroids have no effect on BP or do not vary enough to produce an effect in teenage females. This finding has potentially important clinical implications. Namely, physicians cannot attribute BP variations in a teenage female to her menstrual cycle and therefore may warrant further diagnosis. Another finding of the study is that researchers investigating BP should consider a long familiarisation stage, between the subjects and the experimental environment, to minimise stress response in the results.

Keywords: Blood pressure, menstrual cycle, follicular phase, luteal phase.

P-6

Systematic Method Development in HILIC: Which Parameters are the Most Relevant for Tuning Selectivity and Retention?

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Introduction: Hydrophilic interaction liquid chromatography (HILIC) is an interesting alternative to reversed phase liquid chromatography (RPLC). Indeed, HILIC is appropriate to retain polar compounds but also to analyze ionisable analytes of various polarity, with a very different elution order, compared to RPLC. Moreover, HILIC conditions also provide a higher sensitivity in ESI-MS and a lower backpressure than in RPLC due to higher volatility and weaker viscosity of highly organic mobile phase. However, the choice of suitable experimental conditions in HILIC is often challenging for the analyst because of the important diversity of stationary phase, mobile phase pH, ionic strength and organic modifier that could significantly affect the retention, selectivity, peak width and peak shape.
Aims: The goal of this work was to determinate which parameters are the most relevant for tuning selectivity and retention.
Methods: In this study, 5 different stationary phases packed with fully porous sub-2µm particles (i. e. hybrid silica, silica, diol, amide, zwitterionic) were used with 4 mobile phase pH varying from 3 to 6, two ionic strengths (10 and 50 mM) and 3 organic modifiers (ACN, MeOH/ACN 20:80, IPA/ACN 20:80; v/v). These conditions were applied to a set of ~ 80 pharmaceutical compounds covering a wide range of physico-chemical properties. Principal component analysis (PCA) was performed and highlights the most different experimental conditions in terms of selectivity and retention.
Results: The most relevant parameters for tuning selectivity in HILIC are stationary phase and pH. In case of method development, organic modifier and ionic strength could be considered as secondary parameters.
Conclusions: Amide, bare silica, hybrid silica and zwitterionic phases sould be initially tested at pH 3 and 6 with pure ACN and one ionic strength in case of method development.

Keywords: HILIC, method development, selectivity.

P-7

How Many Plants Have Been Studied Chemically and Pharmacologically? – A Case Study of the Swiss Flora

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Introduction: Estimations of how many of the worlds ~250,000 higher plant species have been studied chemically or for bioactivity are contradictory, and range from 0.05% to > 15%. This survey provides a definition of what could be considered "studied" and provides a systematic literature analysis – exemplified by the plants native to Switzerland.
Methods: We retrieved 454,535 literature references for the 2,677 native plants using SciFinder Scholar™, which were screened, analysed and classified in subcategories.
Results: In summary, 55% of plant species native to Switzerland have been investigated phytochemically. Forty-two % have had compounds isolated from them, 19% have been studied for fatty acid composition, 15% have had essential oils composition analysed, and 9% have been studied for phyto-sterols. With regard to bioactivity 28% of all species have been investigated, 24% have

been studied using *in vitro* methods, 17% have been studied *in vivo*, 16% have been tested for antimicrobial effects, and 4% have been investigated clinically. Furthermore, we analysed the data with respect to habitats and growth forms.
Conclusions: This case study shows that a substantially larger proportion of Swiss plants have been studied than has been postulated for the global flora. We demonstrate that random sampling of every 10th or 20th species in the list is sufficient for a prediction of the outcome of the comprehensive study, with a deviation of a few percent. A systematic study of a representative section of the global flora, using this approach, would give a sound estimation of how many plants have been studied phytochemically, and for bioactivities.

Keywords: Flora, phytochemistry, bioactivity, biodiversity.

P-8

Renaissance Remedies: Treatments for Tuberculosis in 16th and 17th Century German Herbals

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Introduction: A third of all humans are currently infected with *Mycobacterium tuberculosis* bacteria, which kill 1.7 Mio. people a year [1]. Before antibiotics were developed tubercolosis was treated with herbal remedies. In this study we systematically searched for plants used to treat tuberculosis in 9 German herbals from the 16th and 17th centuries, including those by Hieronymus Bock, Adam Lonitzer, Pietro Andrea Mattioli, Theodor Zwinger, Jakob Theodor, Otto Brunfels, and Leonhart Fuchs.
Methods: We documented the plants, identified them botanically, and did an extensive search of the scientific data banks Medline and SciFinder scholar to find recent results concerning the phytochemistry and possible antimycobacterial activities.
Results: This survey is the first systematic study of herbal tuberculosis treatments in Renaissance Europe. We report 208 plant species, which to a large part have not attracted much scientific attention, as 87% of them have never been studied for antimycobacterial effects before. Of the 29 that had been studied, 12 had shown activity.
Conlusions: Further research on the antimycobacterial activity of these plants could help our understanding of plant use in the past and may lead to the discovery of new active compounds.

Keywords: History, plants, Renaissance herbals, Tuberculosis, Mycobacterium.

Reference:
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P-9

Comprehensive Metabolite Profile of *Phyteuma Orbiculare* L., a Wild Alpine Food Plant

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Introduction: Plants which have been traditionally eaten by the alpine population may provide new opportunities to develop mountain agriculture. In this context we have investigated the chemical composition of *Phyteuma orbiculare* L. (Campanulaceae), a herb whose leaf rosettes and flowers have been eaten as salad by rural populations in the Canton of Valais (Switzerland).
Aims: Aims of this study were to analyze the phytochemical profile of *P. orbiculare* and to quantify compounds relevant for nutrition in the flowers as well as in leaves. Finally, the study was extended to include the taxonomically closely related species *P. spicatum, P. hemisphaericum,* and *P. ovatum.*
Methods: Extracts of different polarities were subjected to a comprehensive metabolite profiling using a dereplication platform combining HPLC-PDA-MS and offline microprobe NMR analyses. Quantitative data on fatty acids, minerals, and carotenes were obtained according to standard procedures described in the literature. Antioxidant properties were assessed by an ORAC *(Oxygen Radical Absorbance Capacity)* test.
Results: Fatty acids, triterpenoids, and phenolic glycosides were identified online or after targeted isolation. The compounds include a new dimeric phenylpropanoid glucoside and two new triterpene saponins with unprecedented skeletons [1]. The leaves of *P. orbiculare* contained large amounts of ß-carotene (3.7 ± 0.3 mg/100 g fresh weight (f.wt.)), potassium (689.3 ± 161.8 mg/100 g f.wt.), magnesium (105.3 ± 20.7 mg/100 g f.wt.), and calcium (596 ± 70.1 mg/100 g f.wt.). The leaves contained about 2.5 times more omega-3 (308.9 ± 43.5 mg/100 g f.wt.) than omega-6 (125.1 ± 19.1 mg/100 g f.wt.) fatty acids, which represents a nutritionally favorable ratio. Interestingly, the ratio was reversed in the flowers (10:7). ORAC values of 26768 ± 113 and 19933 ± 1722 µmol of Trolox equivalents·100 g^{-1} f.wt. were determined for the leaves and flowers, respectively, and are significantly higher than data reported for green leafy vegetables (460 to 8330). The total phenolic contents of 6850 ± 460 and 8240 ± 580 mg GAE·100 g^{-1} f.wt. in leaves and flowers, respectively, correlated with the ORAC values. The HPLC-PDA-MS profiles showed a similar composition for *P. orbiculare, P. spicatum,* and *P. ovatum,* while free fatty acids and saponins were not detected in *P. hemisphaericum*. The data support the parallel use of these three species as food plants.
Conclusions: Different types of secondary metabolites including triterpenoid glycosides with unique structural features and a new dimeric phenylpropanoid glucoside were identified. No compounds with reported toxicity, nor substance classes with known toxicological risks were detected. Based on their chemical composition combined with pleasant gustatory properties, rampion species may be considered for future cultivation as food plants.

Keywords: Food, *Phyteuma orbiculare,* alpine plants.

Reference:
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P-10

Library-Based Discovery and Characterization of Daphnane Diterpenes as Potent and Selective HIV Inhibitors in *Daphne Gnidium*

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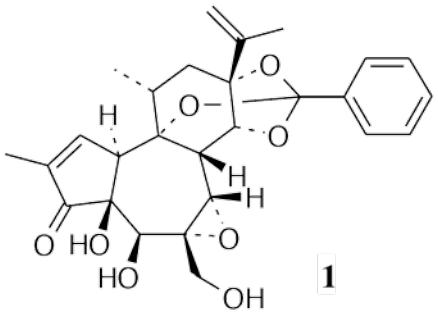
Introduction: Despite the existence of an extended armamentarium of effective synthetic drugs to treat HIV there is a continuing need

for new potent and affordable drugs. Given the successful history of natural product based drug discovery, a library of close to one thousand plant and fungal extracts was screened for antiretroviral activity.
Aims: The present study set out to identify new natural HIV inhibitors.
Methods: A library of close to one thousand plant and fungal extracts was screened for antiretroviral activity. To identify new inhibitors, the active extracts were submitted to a process integrating physico-chemical data with biological information, referred to a HPLC-based activity profiling.
Results: A dichloromethane extract of the aerial parts of *Daphne gnidium* (Thymelaeaceae) exhibited strong antiretroviral activity and absence of cytotoxicity. With the aid of HPLC-based activity profiling, the antiviral activity could be tracked to 4 daphnane derivatives, namely, daphnetoxin (**1**), gnidicin, gniditrin, and excoecariatoxin. Detailed anti-HIV profiling revealed that the pure compounds were active against multidrug resistant viruses irrespective of their cellular tropism. They inhibited equally well the replication of both CXCR4- and CCR5-tropic HIV-1, but differed in their potency, with daphnetoxin and gnidicin being the most potent with EC_{50}s in nanomolar range, and excoecariatoxin the least active compound against both viruses. Importantly, none of the purified compounds displayed cytotoxic activity at the doses assayed, thus yielding a high selectivity index in each case. Mode of action studies that narrowed the site of activity to viral entry events suggested a direct interference of daphnetoxin (**1**) with the expression of the 2 main HIV co-receptors CCR5 and CXCR4 at the cell surface.
Conclusions: Daphnetoxin was identified as a potent inhibitor of HIV replication, and its mode of action could be partly elucidated. From a more general perspective, this study demonstrates the potential of HPLC-based activity profiling as an efficient strategy for the identification of antiviral compounds and their targeted isolation from complex plant extracts.



1

Keywords: HIV, *Daphne gnidium,* daphnetoxin.

P-11

Metabolomic Studies on *Isatis Tinctoria* – Comparison of Different Origins, Harvesting Dates, and the Effect of Repeated Harvesting

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Introduction: *Isatis tinctoria* (Brassicaceae) is an ancient dye and medicinal plant with potent anti-inflammatory and anti-allergic properties [1].
Aims: We investigated metabolic differences by NMR spectroscopy of plants grown on experimental plots at the Agricultural Field Station of Thuringia in Dornburg, Germany, under identical conditions. Comparisons were carried out for plants of different geographic origins, different harvesting dates, and between single and repeatedly harvested plants. For the study, 6 origins were compared, which had been harvested at 6 time points. In addition, the effect of repeated harvesting was investigated.
Methods: Leaf samples were shock-frozen with liquid N_2 immediately after harvest, freeze-dried, and cryomilled prior to extraction. Extracts were prepared by pressurized liquid extraction (PLE) with EtOAc and MeOH. EtOAc extracts were dissolved in $CDCl_3/CD_3OD$ (7:3) with TMS as internal standard for NMR measurements, and spectra analyzed by multivariate analysis.
Results: The score plots produced by Principal Component Analysis (PCA) revealed differences in the metabolic profile between the origins and harvesting dates. Partial Least Square Discriminant Analysis (PLS-DA) plots exhibited differences between the single and repeatedly harvested plants. Its loading plots showed mainly unsaturated fatty acids to be responsible for these differences.
Conclusions: The results demonstrated the metabolic differences of the origins and harvesting dates. Furthermore, repeated harvesting has an influence on the metabolic profile.

Keywords: *Isatis tinctoria,* metabolomics, NMR, multivariate analysis.

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P-12

Antiprotozoal Isoflavan Quinones from *Abrus Precatorius ssp. Africanus*

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Introduction: A library of 207 extracts from selected South African plants was screened *in vitro* against a panel of protozoan parasites, *Plasmodium falciparum, Trypanosoma brucei rhodesiense,* and *Leishmania donovani.* A CH_2Cl_2/MeOH (1:1) extract of *Abrus precatorius* L. *ssp. africanus* Verdc. (Fabaceae) strongly inhibited *P. falciparum* (97.8%), *T.b.rhodesiense* (100%), and *L. donovani* (75.5%) when tested at a concentration of 4.8 mg/mL.
Aims: To identify the compounds responsible for the antiprotozoal activity of CH_2Cl_2/MeOH (1:1) extract of *A. precatorius.*
Methods: The active constituents were tracked by HPLC-based activity profiling [1] and isolated by preparative and semi-preparative RP-HPLC. NMR spectroscopy (^1H, ^{13}C, COSY, HMBC, HSQC, NOE difference) was used to elucidate the structures and establish the relative configuration. The absolute configuration was determined by comparison of electronic circular dichroism (ECD) spectra with calculated ECD data. Isolated compounds were evaluated for their *in vitro* activity and cytotoxicity by using established protocols [2].
Results: Five active compounds were obtained and identified as 2 isoflavan hydroquinones and 3 isoflavan quinones, among them 2 new natural products. Hydroquinones derivatives exhibited low

activity in both antiprotozoal and cytotoxicity tests. In contrast, quinone-type isoflavonoids showed strong activity against *T.b. rhodesiense* (IC_{50}s of 0.3 and 0.2 µM) and low cytotoxicity in the L-6 cell line (IC_{50}s of 22.1 and 10.1 µM).
Conclusions: (3*R*)-Abruquinone B and (3*R*)-abruquinone I showed strong activity against *T.b. rhodesiense* and good selectivity (selectivity indices, as calculated from cytotoxicity data, of 78.3 and 61.3, respectively), which qualifies the compounds as good candidates for assessment of *in vivo* activity in a murine model.

Keywords: *Abrus precatorius,* antiprotozoal, isoflavan quinone, ECD.

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P-13

Insulin Ionization Profile in CE-ESI-MS

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Introduction: The on-line combination of capillary electrophoresis (CE) with mass spectrometry (MS) is an attractive option for the analysis of intact proteins that are multi-charged with electrospray ionization (ESI). Nowadays, two ESI interfaces exist to achieve this coupling and they differ according to their operating flow rate. The sheath liquid interface works at the µL/min flow rate (electrospray regime) and is pneumatically assisted by addition of a nebulizing gas and a sheath liquid (make-up liquid). In this configuration, only the composition of the sheath liquid should drive the ionization. The sheathless nanospray interface operates in the nL/min flow rate (nanospray regime) without any pneumatic assistance and the composition of the background electrolyte (BGE) is the key parameter of ionization. Furthermore, each ESI needle (or tip) is manufactured with diverse type of materials (*i. e.* platinum or stainless steel for sheath liquid interface and silica for sheathless interface) presenting its own electrochemical reactions. All these features lead to distinct droplets formation which influences the ionization of intact proteins so that their charge state distribution (CSD) is modified.
Aims: In this study, both types of CE-MS interfaces (*i. e.* sheath liquid and sheathless) were evaluated and compared regarding the CSD of insulin selected as a model protein.
Methods: For each interface, insulin was analyzed with a 30 mM ammonium formate buffer at pH 2.5 and 9.0 with 10% acetonitrile. Different ESI needles were investigated in the sheath liquid configuration, whereas the influence of pH and organic solvent proportion in the BGE were studied with the sheathless nanospray interface. Repeatability and sensitivity of each set-up were evaluated as well.
Results: In sheath liquid configuration, the two different needles (stainless steel and platinum) exhibited the same sensitivity and the same behavior toward insulin. The sheathless nanospray interface led to significant modification of the CSD and higher charge states were detected as well as lower limits of detection compared to the sheath liquid interface.
Conclusion: The specific properties of each interface as well as pH of the BGE presented a clear influence on the CSD of insulin.

Keywords: Capillary electrophoresis, mass spectrometry, ionization profile, insulin, CE-MS.

P-14

The Capillary Isoelectric Focusing: A Novel Approach for the Fast Determination of pK_a Values of Small Compounds

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Introduction: Inappropriate ADME behaviour (absorption, distribution, metabolism and excretion) leads to the rejection of new chemical entities (NCEs) during drug development. Thus, the determination of physicochemical properties such as lipophilicity or solubility is of crucial importance in the early steps of drug discovery. Ionization constants are also widely determined since the ionization states govern other physicochemical and pharmacokinetic properties. Nowadays, pK_a values are determined by powerful methods such as potentiometry, spectrophotometry or capillary zone electrophoresis [1].
Capillary isoelectric focusing (cIEF) is an electrophoretic technique allowing to separate polyelectrolytes according to their isoelectric point (pI), *i. e.* the pH where an amphoteric compound is under its neutral form, using a pH gradient created within a capillary. cIEF is used to separate proteins in proteomic applications, analysis of complex proteins mixtures and microheterogeneity determination [2].
Aims: The aim of this study was to evaluate the potential of cIEF for the determination of pK_a values of small compounds in one injection from the determination of the pH range where compounds are under their neutral form.
Methods: Simple and monofunctional compounds were used to explore the performance of this approach. The time corresponding to the appearance of the neutral form was measured and compared to pK_a values from the literature.
Results: Linear correlations (r^2 = 0.997, slope = -3.67, intercept = 55.00 and r^2 = 0,998, slope = -2.80, intercept = 43.94 for five acidic and five basic compounds, respectively) were obtained. These relations can be used as calibration curves for the pK_a determination of unknown compounds.
Conclusions: Capillary isoelectric focusing was successfully used for the rapid determination of pK_a values of simple and monofunctional compounds and suggests interesting perspectives for early drug discovery.

Keywords: ADME, pK_a, cIEF.

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P-15

Terpene Trilactone Bilobalide and PC-12 Neuronal Cells: A Structure-Activity Relationship Study

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Introduction: *Ginkgo biloba* extracts have been postulated to provide improvement of memory and beneficial effects to patients suffering from Alzheimer's disease [1]. Bilobalide (BB), isolated from *G. biloba,* is a unique terpene trilactone with a cage-like skeleton consisting of four five-membered rings. Unlike other trilactone

ginkgolides [2] a structure-activity relationship (SAR) study of BB has not been accomplished, yet.
Aims: In order to elucidate the role of the lactone moiety of BB by means of a SAR, BB was isolated from *G. biloba* leaves. Derivatives, including non-lactone BB, were then prepared via organic reactions, and bioassays were conducted on PC-12 neuronal cells.
Methods: The isolation of BB from *G. biloba* leaves was achieved by refluxing in AcOEt followed by separation using silica gel chromatography. The reduction of the isolated BB lactones was then performed using DIBAL-H, followed by treatment with Et_3SiH and $BF_3{\cdot}Et_2O$ to afford a BB-diether, which does not contain any lactone moiety. Several other derivatives of BB were also prepared utilizing organic reactions [3]. The prepared BB derivatives were then tested for biological activity on PC-12 cell lines.
Results: Natural BB showed proliferating cell activity, neurite outgrowth effects, and neuroprotecting effects against Aß(1-40). Some of the derivatives containing lactones showed similar biological activity. However, the BB-diether did not exhibit neurite outgrowth effects.
Conclusions: The results of the SAR study strongly suggest that the lactone moieties of BB play an important role in exerting neurite outgrowth effects for PC-12 cells.

Keywords: Terpene trilactone, bilobalide, PC-12, structure-activity relationship.

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P-16

Is Raman Spectroscopy a Potential Pat Tool for Drug Quantification in Self-Emulsifying Drug Delivery Systems?

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Introduction: Self-emulsifying drug delivery systems (SEDDS) are complex mixtures in which drug quantification is challenging especially in the context of real-time process analytics.
Aims: We aimed at evaluating Raman spectroscopy as a process analytical technology (PAT) for drug quantification in complex lipid mixtures.
Methods: The model drugs fenofibrate, indomethacin, and probucol were quantitatively assayed in two different SEDDS. The drug content was first determined in bulk formulations using a single-fiber immersion probe. The formulations were then manually filled into hard-gelatin capsules and quantitatively assayed using a multi-fiber P^hAT probe. Raman spectra were recorded in the backscattering mode using a Raman RXN1 analyzer (Kaiser Optical Systems Inc.). HPLC was used as reference method. We developed multivariate calibration models and calculated the limit of quantification (LOQ), the relative standard error of prediction (RSEP), as well as the mean recovery to validate the models.
Results: Each formulation revealed at least one spectral region where the Raman signal was highly specific for the drug. Drug quantification in the bulk formulation provided RSEP between 1.5 and 3.8% and LOQ in the range of 0.04–0.45% w/w. Interestingly, LOQ values obtained from drug quantification in the capsules were in the range of those obtained from measurements in the bulk solution (0.01–0.41% w/w). However, the RSEP of quantification in capsules evidenced that the analytical performance was here depending on the specific composition of the lipid mixtures. Mean recovery values were in the range of 99.1–102.0% and 99.2–102.7% for drug quantification in the bulk formulation and in capsules, respectively. All values were sufficiently low to fulfill the general acceptance criteria of performance parameters in analytical validation.
Conclusions: Our results encouraged the use of Raman spectroscopy as an accurate method for API quantification in complex semi-solid formulations such as SEDDS. Good analytical performance was demonstrated for both, bulk formulations as well as liquid-filled capsules.

Keywords: Self-emulsifying drug delivery systems, Raman spectroscopy, drug quantification, process analytical technology.

P-17

Electrophoretically Mediated Microanalysis for Characterization of the Enantioselective CYP3A4 Catalyzed N-Demethylation of Ketamine

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Introduction: Enantioselective capillary electrophoresis (CE) is a well established and attractive methodology. Due to high resolution, short analysis time and low consumption of chemicals and solvents, it is often applied to assess the stereoselective metabolism of a drug and to characterize enzymatic metabolic pathways *in vitro* [1]. Execution of an enzymatic reaction performed in a capillary with subsequent electrophoretic analysis of the formed products is referred to as electrophoretically mediated microanalysis (EMMA).
Aims: The aim of this work is to develop an EMMA method to investigate the stereoselectivity of the CYP3A4 mediated N-demethylation of ketamine.
Methods: Ketamine was incubated in a 50 µm ID bare fused-silica capillary together with human CYP3A4 Supersomes using a 100 mM phosphate buffer (pH 7.4) at 37 °C. A plug containing racemic ketamine and the NADPH regenerating system including all required co-factors for the enzymatic reaction was injected, followed by a plug of the metabolizing enzyme CYP3A4 (500 nM). These two plugs were bracketed by plugs of incubation buffer to ensure proper conditions for the enzymatic reaction. The rest of the capillary was filled with a pH 2.5 running buffer comprising 50 mM Tris, phosphoric acid and 2% w/v of highly sulfated γ-cyclodextrin. Mixing of reaction plugs was enhanced via application of -10 kV for 10 s. After an incubation time of 8 min at 37 °C without power application, the capillary was cooled to 25 °C within 3 min followed by application of -10 kV for the separation and detection of the formed enantiomers of norketamine.
Results: Norketamine formation rates were fitted to the Michaelis-Menten model and the elucidated values for V_{max} and K_m were found to be comparable to those obtained from the off-line assay of a previous study [2]. The data obtained revealed that CYP3A4 N-demethylation of ketamine occurs in a stereoselective manner.
Conclusions: An on-line method was developed to investigate the *in vitro* N-demethylation of ketamine via CYP3A4, with the incubation performed in-capillary with subsequent electrophoretic separation and detection of the ketamine enantiomers. After additional improvements, the in-capillary method should be widely applicable to assess enzymatic activity in a fast, low-cost and automated way.

Keywords: Electrophoretically mediated microanalysis (EMMA), capillary electrophoresis, ketamine, CYP3A4, stereoselective metabolism.

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P-18

Gram-Scale Purification of Bioactive Natural Products – A Challenge Exemplified by the hERG Channel Inhibitor Dehydroevodiamine

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Introduction: Many studies in the field of natural product drug discovery report promising *in vitro* activities of pure compounds. However, follow-up studies investigating pharmacokinetic profiles and *in vivo* effects of natural products are rarely performed. A major limiting factor for preclinical and clinical studies is that these compounds are usually not commercially available. Preparative isolation of gram amounts of a compound within a short time and with high purity (> 98%) remains a challenge in natural product research.
Aims: In the course of a project evaluating the risk potential of natural products for cardiotoxicity, a plant extract library was screened using an *in vitro* hERG assay. Dehydroevodiamine (DHE) and hortiamine, two alkaloids isolated from the TCM herb *Evodia rutaecarpa,* were identified as potent hERG blockers. The aim of this study was to develop a method for large-scale purification of DHE for assessment of its arrhythmogenic potential in animal models.
Methods: Enrichment of an alkaloidal fraction was achieved by filtration of the crude extract over a cation-exchange resin (Lewatit® MonoPlus SP 112). The alkaloid-enriched fraction was further purified by isocratic preparative RP-HPLC.
Results: Alkaloids were selectively extracted by ion-exchange chromatography. Optimization of the HPLC conditions with respect to sample loading, resolution, and separation time led to the implementation of a medium-throughput purification protocol. Purity of DHE was 98% (HPLC, NMR).
Conclusions: An efficient and rapid method for the gram-scale purification of DHE from the extract was developed. Using this approach can promote the commercialization of DHE as a standard reference compound.

Keywords: Dehydroevodiamine, large-scale purification, ion-exchange chromatography, preparative RP-HPLC, preclinical testing.

P-19

Abietane Diterpenoids From *Salvia Sahendica* – Antiprotozoal Activity and Determination of Their Absolute Configurations

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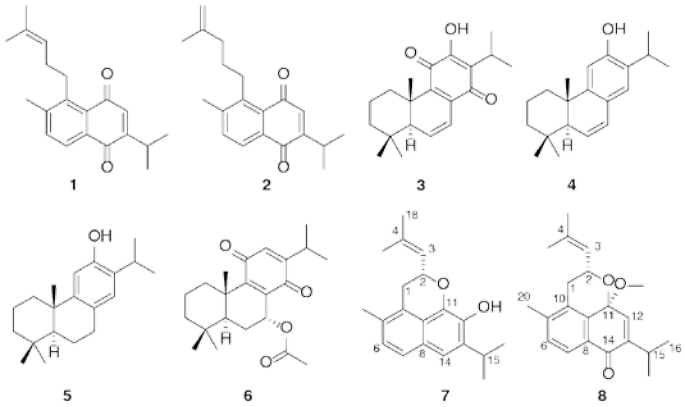
Introduction: *Salvia* is the largest genus of Lamiaceae, with over 900 species found throughout the world. It is represented in the Iranian flora by 58 species, of which 17 are endemic. *Salvia* species are particularly rich in diterpenoids which are responsible for various biological activities of these plants. The endemic plant, *Salvia sahendica,* is used in Iranian folk medicine for antibacterial and antifungal purposes as well as treatment of dyspepsia. In a screening of Iranian plants for antiprotozoal activity, a *n*-hexane extract of roots of *S. sahendica* potently inhibited the growth of *Plasmodium falciparum* K1 strain.
Aims: Isolation of antiprotozoal compounds from from *S. sahendica.*
Methods: HPLC-based activity profiling of an active extract of *S. sahendica* was used to determine active constituents which were isolated by RP-HPLC in a semi-preparative scale. Structure elucidation was achieved by analysis of spectroscopic data including 1D and 2D NMR and HRESI-MS. Seven known and one new abietane-type diterpenoids were identified (1-8). The absolute configuration of sahandol (7) and sahandone (8) were assigned by comparison of experimental electronic circular dichroism (ECD) spectra with calculated ECD data, using time dependent density functional theory (TDDFT) and methanol as solvent.
Results and conclusion: *In vitro* biological activity against *P. falciparum* and *Trypanosoma brucei rhodesiense* STIB 900 strain and cytotoxicity in rat myoblast (L6) cells were determined. The IC_{50} values of the compounds ranged from 0.9 to over 8.8 µM against *P. falciparum,* and from 1.8 to over 32.3 µM against *T. brucei rhodesiense.* The cytotoxic IC_{50} values ranged from 0.5-15.5 µM. Selectivity indices for *P. falciparum* were 0.1–18.2 and 0.1–1.2 for *T. brucei rhodesiense,* respectively.

Keywords: *Salvia sahendica,* Lamiaceae, diterpenes, *Plasmodium falciparum, Trypanosoma brucei rhodesiense.*



P-20

C1 Esterase Inhibitor Treatment in Skeletal Muscle Ischemia / Reperfusion Injury

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Introduction: Acute limb ischemia is one of the most common peripheral vascular emergencies and associated with extensive morbidity and mortality. Ischemia/reperfusion injury (IRI) of extremities has been analyzed before, but until now an effective treatment of this critical situation is missing. During the period of ischemia the endothelium is altered and contributes to IRI. It could be shown that endothelial proteins are changed, expose neo-epitopes and

bind natural antibodies, resulting in the activation of a complex inflammatory cascade. It is well known that C1 esterase inhibitor (C1-INH) has an important physiological role in regulating the complement, the kallikrein-kinin as well as the coagulation system. The use of C1-INH may therefore be effective to attenuate IRI, where these systems play a crucial role.
Aims: We hypothesize that the concentration of naturally circulating plasma C1-INH is not sufficient to prevent skeletal muscle IRI. Therefore, we want to investigate the effect of treatment by C1-INH (50 U/kg, Berinert® P, isolated from human plasma) on reduction of IRI in a rat hind limb IRI-model.
Methods: Three groups of male Wistar rats (non-treated, vehicle control- and C1-INH-treated) were subjected to 3 h of hind limb ischemia and 2 h of reperfusion. For induction of ischemia the femoral artery was ligated and a tourniquet placed to block collateral circulation, under maintenance of the venous return. Either C1-INH (50 U/kg) or vehicle was systemically injected via the tail vein 5 min before ischemia, mimicking pre-treatment for elective surgery. Muscles were removed for histological and edema assessment as well as analysis of deposition of antibodies and complement components.
Results: Activation of the kallikrein-kinin system was assessed by analysis of the wet/dry weight ratios. These ratios were significantly reduced by C1-INH (106 ± 2.7%) as compared with the non-treated (127 ± 10.6%) and control-treated rats (120 ± 7.9%). Histological preparations showed maintenance of muscle integrity when rats were treated with C1-INH. Furthermore, we detected significantly reduced amounts of deposited IgG in the reperfused muscle, whereas no differences could be found for IgM, C1q and C3b/c.
Conclusions: C1-INH treatment (50 U/kg) is able to significantly reduce I/R injury by decreasing edema formation and IgG deposition as well as preserving tissue integrity and maintaining muscle viability. However, C1-INH treatment does not affect C1q or C3b/c deposition in this model and it is therefore unclear whether the beneficial effect of this treatment is due to complement inhibition. We are currently more precisely analyzing effects of C1-INH treatment on the kallikrein- kinin- and coagulation cascades. All in all, C1-INH might be a promising approach for reduction of IRI in critical limb ischemia.

Keywords: C1 esterase inhibitor, ischemia/reperfusion injury, protection, hind limb.

P-21

Searching for Antitrypanosomal and Antiplasmodial Natural Products From Plants and Fungi

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Introduction: For the last six years we have cooperated in screening plant and fungal extracts against protozoan parasites and identifying their active compounds.
Aim: With our work we contribute to the discovery of new leads for the development of drugs to treat these neglected tropical diseases, and to gain a better understanding of antiprotozoal natural products.
Methods: Promising extracts were analyzed by HPLC-based activity profiling to identify active constituents which were subsequently isolated and tested against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* [1].
Results: So far libraries containing more than 2500 plant and fungal extracts have been screened. Some of these extracts were traditionally used in South African, Renaissance European, or Iranian folk medicine to treat malaria. The compounds with highest *in vitro* activity against *P. falciparum* were the alkaloids carpaine (IC_{50} 0.8 µM) from *Carica papaya,* berberine (IC_{50} 0.1 µM) from *Coptis chinensis,* and the triterpenoid perovskone B (IC_{50} 0.18 µM) from *Salvia hydrangea* [2]. *In vivo* tests with carpaine in the *P. berghei* model, however, showed no decrease in parasitaemia. Selected compounds with a sesquiterpene lactone or a miltirone-type diterpene scaffold showed high *in vitro* activity against *T. b. rhodesiense* (IC_{50}s 0.3–0.8 µM). Cynaropicrin (IC_{50} 0.3 µM) was tested *in vivo* and reduced the parasitaemia in the acute sleeping sickness mouse model [3].
Conclusions: Our lead discovery project against protozoan parasites resulted so far in 55 natural products with *in vitro* activity. Of these, 8 were new compounds. Most compounds showed moderate activity in the 1–10 µM range, with little selectivity. Two compounds were tested *in vivo,* and one of them was able to reduce parasitaemia. Cynaropicrin is the first plant derived natural product with *in vivo* activity against African sleeping sickness.

Keywords: *P. falciparum, T.b.rhodesiense,* extract library, drug discovery, leads.

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P-22

Anti-inflammatory Compounds Isolated From *Diospyros Bipindensis*

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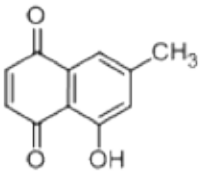
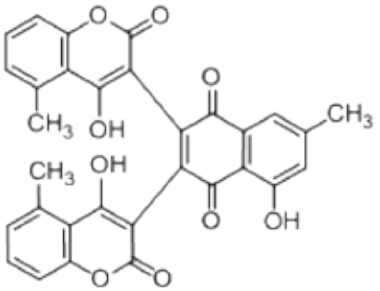
Introduction: *Diospyros bipindensis* (Gürke) stem barks are used in Cameroon by pygmies Baka for the treatment of pulmonary diseases. A common process in all of these diseases is inflammation. Thus, we used the inhibition of the pro-inflammatory mediator nuclear factor-kappa B (NF-κB) transcriptional activity as a target.
Aims: To identify the compounds responsible for the anti-inflammatory activity.
Methods: Bioassay-guided fractionation was performed on the active dichloromethane extract. A C_{18} reversed solid phase column was used to separate the extract in 5 fractions. Active fractions 3 and 4 were further purified by semi-prep HPLC to afford 4-hydroxy-5-methylcoumarin, plumbagin, canaliculatin, ismailin and betulinic acid as the main constituents. The anti-inflammatory activity was measured in 293-HEK cells stably transfected with a luciferase reporter construct regulated by the NF-κB response element.
Results: Plumbagin and ismailin inhibited NF-κB with an IC_{50} of 0.9 and 29.5 µM, respectively. Together with minor compounds, they could contribute to the anti-inflammatory activity of the extract.



Plumbagin



Ismailin

Conclusions: These results may support the traditional use of *Diospyros bipindensis* for the treatment of pulmonary diseases.

Keywords: *Diospyros bipindensis,* plumbagin, ismailin, NF-κB inhibition.

P-23

Bryophyllum Pinnatum, a Well Regarded Phytomedicine in Obstetrics and Gynecology – New Data on its Chemical Composition

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Introduction: *Bryophyllum pinnatum* (syn. *Kalanchoe pinnata,* Crassulaceae) is a succulent perennial plant native to Madagascar. *B. pinnatum* leaf extract has been used in anthroposophical medicine as a sedative and as a tocolytic agent to treat premature labor [1, 2]. We recently showed that *B. pinnatum* leaf press juice also inhibits porcine detrusor contractility *in vitro* [3]. To explore the potential of *B. pinnatum* as a treatment for patients suffering from overactive bladder syndrome, a pilot study in humans was recently performed. A positive trend compared to placebo could be shown [4]. For clinical applications as well as for safety and quality control, in-depth knowledge of the chemical composition of *B. pinnatum* preparations was needed.
Aims: The aim of the investigation was to characterize the constituents of a methanolic extract of *B. pinnatum* and to determine whether potentially toxic bufadienolides were present.
Methods: The leaves of *B. pinnatum* were lyophilized and extracted with methanol. The extract was submitted to TLC and HPLC-UV/ESI-MS analyses, and subsequently fractionated by a combination of Sephadex LH-20 CC, Diaion HP-20 CC, and preparative and semi-preparative HPLC on RP-18. For comparison, bufadienolides were isolated from the dichloromethane fraction of another species, *B. daigremontianum.* Purified compounds were identified by ESI- and APCI-MS as well as 1D and 2D NMR spectroscopy.
Results: Preliminary HPLC-UV/ESI-MS analyses revealed that flavonoid glycosides were the main UV-absorbing constituents of *B. pinnatum.* A total of 9 flavonoids including kaempferol, quercetin, myricetin, acacetin and diosmetin glycosides, and syringic acid ß-D-glucopyranosyl ester and 4'-O-ß-D-glucosyl-cis-p-coumaric acid were unambiguously identified. Four bufadienolides, which were isolated from the related species *B. daigremontianum,* namely bersaldegenin-1-acetate, bryophyllin A, bersaldegenin-3-acetate, and bersaldegenin-1,3,5-orthoacetate, were detected as trace compounds in *B. pinnatum.*
Conclusions: Flavonoid glycosides are the characteristic constituents in the methanolic extract of *B. pinnatum* leaves. It appears that potentially toxic bufadienolides are only present in trace amounts in this extract. As a next step, a quantitative determination of the bufadienolides and the main flavonoids will be undertaken. Our findings are consistent with clinical observations showing that *B. pinnatum* preparations are well tolerated.

Keywords: *Bryophyllum pinnatum,* HPLC-UV/MS profiling, flavonoids, bufadienolides.

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P-24

Comb-Polymer Monolayers Engineered to Display Molecular Sieving Properties for the "Smart" PEGylation of Proteins

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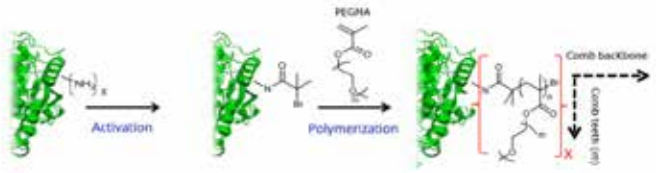
Introduction: As biotherapeutics proteins and peptides are attracting more and more attentions of scientists by virtue of their high biological activity and specificity. However, the delivery of therapeutic proteins and peptides in their unmodified form has several drawbacks, including poor stability, low solubility, short *in vivo* circulation, and immunogenicity. Conjugating proteins and peptides with polymers is an effective strategy to overcome some of these limitations [1].
Aims: In this study we examined a "polymer engineering" approach for tuning the bioactivity of protein–polymer conjugates by manipulation of architecture and the conformation of polymer chains growing from the surface of an enzyme.
Methods: We have prepared >100 well-defined conjugates of α-chymotrypsin (αCT) with PEG-based comb-shaped polymers and tested the bioactivities of these conjugates toward different sizes of substrates, including protein substrate, peptide substrate and amino acid substrate [2,3]. The grafting density, backbone length, and side-chain length of the polymer can be adjusted to control the properties of the polymer layer on the surface of αCT.
Results: Polymer architecture and conformation were found to be important parameters in bioconjugate design. A change of polymer conformation from *star* to *rigid worm* to *flexible worm* greatly influenced the diffusion properties of the polymer layer. In the *rigid worm* regime, a 100% active (vs. native) conjugate was prepared and the latter was completely insensitive to the addition of glycoprotein inhibitors.



Conclusion: This "polymer engineering" approach is applicable to a large class of protein drugs and provides a general modification methodology for improving their pharmacological profiles.

Keywords: Protein-polymer conjugates, PEG, comb-shaped polymer, ATRP.

References:
[1] M.A Gauthier, H.-A. Klok. Polym Chem 2010; 30: 134-1520.
[2] B.S. Lele, H. Murata et al. Biomacromolecules 2005; 6: 3380-3387.
[3] W. Gao, W. Liu et al. Proc Natl Acad Sci 2009; 106: 1523-15236.

P-25

MLP-Tools – A Comprehensive Software Package to Apply the Molecular Lipophilicity Potential in PyMOL

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Introduction: The Molecular Lipophilicity Potential (MLP) is a molecular interaction field that describes the lipophilic properties of chemical identities based on experimental 1-octanol/water partition coefficients (log P_{oct}) of small chemical compounds [1]. It has found broad application in computational chemistry and ligand-based drug design and it is nowadays routinely used for 3D-QSAR [2]. Further developments in describing the protein cavity lipophilicity have recently shown how MLP can also be applied successfully in molecular docking [3]. PyMOL is a popular open source software package to render molecule structures [4].
Aims: Building a MLP-based computational toolbox as a plugin for PyMOL.
Methods: Implementation of the MLP and a comprehensive collection of programs in the programming language Python, combined with a user-friendly graphical user interface (GUI) as a plugin for PyMOL.
Results: Conception of a new MLP-based computational tool as a plugin for PyMOL able to characterize the spatial lipophilic distribution around a chemical compound (log P_{oct}) and to calculate its *virtual* log P. In addition, the novel tool will give access to polar and apolar properties of protein binding sites allowing the quantification of ligand-protein interactions ($Score_{MLP}$).
Conclusions: The MLP-Tools program for PyMOL provides a free and easy-to-use GUI, helpful to understand lipophilic properties and interactions in computational drug design.

Keywords: Lipophilicity, MLP, Pymol, Python.

References:
[1] P. Gaillard, P.-A. Carrupt, B. Testa, A. Boudon. J Comput Aided Mol Design 1994; 8: 83-96.
[2] G. Ottaviani, S. Martel, P.-A. Carrupt. J Med Chem 2007; 50: 742-748.
[3] A. Nurisso, J. Bravo, P.-A. Carrupt, A. Daina. J Chem Inf Model 2012; 52: 1319-1327.
[4] The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.

P-26

Synthesis of a Novel Amphiphilic Chelating Molecule for Magnetic Resonance Imaging

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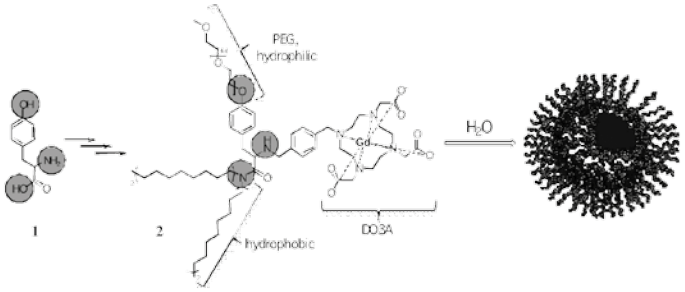
Introduction: Magnetic Resonance Imaging (MRI) is a powerful non-invasive method for clinical diagnostic procedures, frequently carried out after injection of formulations of contrast agents containing a paramagnetic gadolinium complex. Conjugating poly(ethylene glycol) chains (PEG) at their external surface would provide contrast agent with prolonged circulation time and tissue accumulation ability via the Enhanced Permeation Retention (EPR) effect.
Aims: The aim of the study was the chemical synthesis of a novel amphiphilic molecule as part of a micellar MRI contrast agent.
Methods: The synthesis relies on the use of a (L)-tyrosine-OH (**1**) as starting material. This amino acid displays three functional sites that were selectively grafted. The carboxylic acid, the amino and the hydroxyl moieties were substituted by a hydrophobic NH-$(C18)_2$ chain, a DO3A coordinating metal centre separated by a benzyl spacer, and a hydrophilic methoxy-poly(ethyleneglycol)$_{2000}$ (M-PEG_{2000}), respectively, to give the ligand (**2**) as presented in the figure.
Results: The synthetic optimized conditions were designed in order to do a "one-pot" synthesis using the same solvent of reaction. A specificity of the molecule obtained is the various signals resulting from the NMR spectra in different solvents. It was clear from the peak surfaces in solvent bearing different polarities that the ratio of the hydrophobic chain versus the hydrophilic PEG tail corresponds to the state of a micelle-like behaviour due to the amphiphilic property of the molecule.



Conclusions: We reported here the synthesis of a novel amphiphilic chelate. Further work will be focused on the formulation of the micelles and the characterization of the complex as MRI contrast agent.

Keywords: MRI contrast agent, ligand, amphiphilic molecule, DO3A.

P-27

Impact of Chronic Kidney Disease on the Onset of Diabetes: It's Not Only Sugar After All!

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Introduction and aims: Among the several metabolic complications seen in chronic kidney disease (CKD), the well documented insulin resistance remains an obstacle highly important to consider, given its ability to increase the risk of morbidity and mortality in this disease. Such complication may lead to a progressive deterioration of glycemic control and possibly type II diabetes. We therefore believe that the insulin resistance seen in CKD could accelerate the onset of diabetes in patients already predisposed.
The NOD (non-obese diabetic) model consists of mice with insulin-dependent diabetes generally exhibiting clear signs of lymphocytic infiltration in the islets of Langerhans (insulitis), evidence that suggests destruction of cells of the endocrine pancreas. However, when subjected to an additional stress induced by CKD (3/4th nephrectomy), we believe that mice should develop diabetes at an earlier stage.
Methods: NOD mice were nephrectomized (3/4th nephrectomy) at 6–8 weeks of age. Nephrectomized and control mice were monitored daily for any signs indicating the occurrence of hyperglycemia.

When hyperglycemia was confirmed, the mice were sacrificed and their pancreas removed. Sections of 4 µm (one every 50 µm) were obtained from the pancreas and stained with hematoxylin-eosine (HE). In order to quantify lymphocytic infiltration, a rating between 0 and 4 was given on the first 20 pancreatic islets observed (0 being no infiltration and 4 complete insulitis). The percentage of islets belonging to each grade was calculated in order to procure an average result for each group (CKD vs CTL ± diabetes).

Results: We observed an earlier onset of diabetes in CKD-NOD mice compared to CTL-NOD mice. The average time of appearance for diabetes (time to affect 50% of the population) was 24 weeks of age for mice impaired with CKD as of 36 for the control group. Furthermore, the difference set for the incidence of diabetes between these two groups was much more significant in the female population (15 weeks of age vs 36; CKD vs CTL). Moreover, the analysis of histological sections of pancreas performed on these animals showed little to no sign of lymphocytic infiltration characteristic of type I diabetes in pancreas of CKD mice while control NOD mice had an important infiltration score as expected. On average, more than 60% of the islets of Langerhans of CKD mice presented either no sign of insulitis to slight lymphocyte infiltrates (grade 0 and 1) compared to over 75% of more pronounced insulitis (grade 2–4) in control mice ($P<0.05$).

Conclusions: These results suggest that CKD would not only accelerate the onset of diabetes in a predisposed population but could also promote the development of type II diabetes given the fact that lymphocytic infiltration of the Langerhans islets is usually more pronounced in type I diabetes.

Keywords: CKD, diabetes, NOD model, pancreas.

P-28

Potent and Selective *In Vitro* Antiplasmodial Alkaloids from *Carica Papaya* L.

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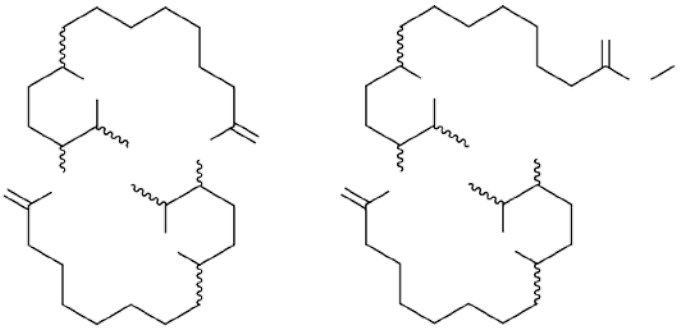
Introduction: Decoctions of papaya leaves are consumed for prevention and treatment of malaria in endemic areas of Eastern Indonesia. Extracts and alkaloid fractions reportedly show antiplasmodial activity.

Aims: To identify the active compounds from the leaves of *Carica papaya* L. (Caricaceae) responsible for the antimalarial properties.

Methods: HPLC-based activity profiling was used to locate the active compounds in the extract. Enrichment of the alkaloids was performed using a cationic ion exchange resin. Isolation of the active compounds was done by semi-preparative HPLC with MS detection. *In vitro* bioactivity of the compounds was determined against *Plasmodium falciparum* (K1 strain), and cytotoxicity with rat myoblast L-6 cells. The *Plasmodium berghei* acute mouse model was used for assessment of *in vivo* activity.

Results: The methanolic extract from leaves inhibited growth of *P. falciparum* by 51% when tested at a concentration of 4.8 µg/mL. HPLC-based activity profiling located the active compounds. Semi-preparative LC-MS separation of the enriched alkaloids fraction, obtained from ion exchange chromatography, led to isolation of 3 alkaloids. Compounds **1** and **2** were characterized as 2 stereoisomers of carpaine, a dimeric piperidine alkaloid previously reported from papaya leaves. Compound **3** was a methyl ester of a related piperidine derivative. Compounds **1-3** showed good *in vitro* antiplasmodial activity, with IC_{50}s of 1.02, 0.21, and 1.94 µM, respectively. Selectivity indices (SI) of 27, 98, and 24 were calculated on the basis of the IC_{50}s in rat myoblast L-6 cells. On the basis of its potent activity and high SI, alkaloid **2** was selected for *in vivo* testing in the *P. berghei* mouse model. However, no reduction of parasitemia was seen on day 4 after treatment with 5 mg/kg b.wt. i.p. daily from day 1 to 4.



1–2 **3**

Conclusions: HPLC-based activity profiling localized three antiplasmodial piperidine alkaloids in the methanolic extract of papaya leaves. Compounds **1** and **2** are stereoisomers of the known alkaloid carpaine; their stereochemistries remains to be established. Alkaloid **2** showed high *in vitro* activity against *P. falciparum* but was inactive in the *P. berghei* acute mouse model.

Keywords: *Carica papaya* L., antiplasmodial, alkaloid, HPLC-based activity profiling.

P-29

Establishment and Validation of a Reliable Human *In Vitro* BBB Model for Early Screening of Bioactive Natural Products

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Introduction: In drug discovery, well-based *in vitro* blood-brain barrier (BBB) models using Transwell® systems with tissue culture inserts are one of the major approaches to estimate brain permeability of new substances. In the human brain, endothelial cells are firmly connected through tight junction proteins and form a sealed barrier which protects the brain from xenobiotics circulating in the blood. Assessment of barrier integrity in BBB *in vitro* models is therefore of utmost importance when establishing high-quality models.

Aims: The aim of this study was to establish a reliable *in vitro* human BBB model from several immortalized brain endothelial cell lines for the screening of promising bioactive natural products.

Methods: Four different immortalized human brain endothelial cell lines (hCMEC/D3, BB19, hBMEC, and TY10) were screened for their ability to build a tight barrier, by measuring the TransEndothelial Electrical Resistance (TEER) using a CellZscope system [1] and a conventional Endohm-6 volt-ohm meter.

Results: Barrier tightness assessment with the CellZscope system and the Endohm-6 showed a good correlation of TEER values. Maximal TEER values were obtained with hBMEC and hCMEC/D3 monocultures on tissue culture inserts from Corning® and Greiner Bio-One®.

Conclusions: Compared to conventional TEER measurements, the CellZscope system is a time-saving and convenient tool to assess barrier tightness of cell layers *in vitro.* TEER values and confluency are recorded automatically every hour, and a continuous monitoring is hence possible. As the multi-well device (24 wells) is placed

directly into the incubator, disturbance of cell layers and contamination can be avoided. Besides TEER measurements, permeability assays using fluorescent integrity markers will provide valuable information on barrier tightness. The most suitable cell line, the best tissue culture insert, and the best culture conditions for our human BBB model will be selected after having obtained additional data from these studies.

Keywords: Blood-brain barrier, *in vitro,* TEER, integrity markers.

Reference:
[1] J. Wegener et al. BioTechniques 2004; 37: 590-597.

P-30

Novel Thermoplastic Capsules for Robust Encapsulation of Hydrophilic Lipid-Based Formulations

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Introduction: For decades gelatin has been used in a rotary die process as shell-forming material of soft capsules due to its unique physicochemical properties. Nevertheless, with respect to encapsulation of hydrophilic lipid-based formulations, gelatin has a considerable drawback. It is a large amount of water (up to 35%) that the gelatin shell contains immediately after production. There is the potential for this water to migrate from the capsule shell into the formulation, which will lead to a decrease in drug solubility and in turn, the potential for drug crystallization.
Aims: The present study introduces a novel capsule material that was obtained from extrusion as compared to the casting process of gelatin bands. We produced soft gelatin capsules (SGC) and the novel thermoplastic capsules (S-PVA-C) were of a starch-based polyvinyl alcohol material. Both technologies were used to encapsulate a hydrophilic lipid-based formulation of fenofibrate. We tested the hypothesis, whether the novel shell material can avoid pronounced water exchange and therefore avoid a potential drug precipitation in the fill mass.
Methods:
Drying kinetics-water activity measurements
During the drying process samples of SGC and S-PVA-C were taken at predetermined time points. Each capsule was opened, fill mass and capsule shell were separated, and analyzed for water activity (Novasina AG, Switzerland).
Temperature cycle test
SGC and S-PVA-C were left 14 days at 5 °C and then the next 14 days at 20 °C/40% rH. Both SGC and S-PVA-C were then opened with a sharp blade. The drug formulation was taken out and centrifuged. The supernatant was analyzed for drug content by HPLC.
Dispersion/precipitation test
The dispersion/precipitation test was performed in 1000 mL of 0.05 M SDS in water using USP dissolution apparatus 2 (Erweka, Germany). The paddles were rotated at 75 rpm and the medium was maintained at 37 ± 0.5 °C.
Results: Visual observation of drug formulation in SGC stored at 5 °C for 2 weeks (after centrifugation) revealed drug precipitation. The drug content in SGC after the temperature cycle test demonstrated reduced values (83 ± 1.5%). In case of S-PVA-C there was no visible drug precipitation at 5 °C after 2 weeks and also after the temperature cycle test (98.9 ± 6.2%). The comparatively high SD was mainly due to mass variability in the not optimized new filling process.We observed marked water migration from the soft gelatin shell to the hydrophilic fill mass during first hours of drying. This rapid water uptake caused drug precipitation in SGC, which was reflected by the results of a temperature cycle test. However, a different water migration mechanism (slight water exchange) was observed with S-PVA-C.
Conclusions: The hypothesis was confirmed that drug precipitation in the fill mass can be avoided by using the novel thermoplastic shell material. Thus, the novel capsule technology has a high potential for robust encapsulation of hydrophilic lipid-based formulations.

Keywords: Dissolution, oral drug delivery, material science, physical stability, precipitation.

P-31

High Resolution HPLC Biological Profiling for the Rapid Identification of Antifungal Compounds in Plants From French Polynesia

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Introduction: In early drug discovery, developing high-throughput and high-resolution methods has become a major challenge, especially to identify new potential lead compounds in crude plant extracts.
Aim: Micro-fractionation aims at enhancing the efficiency of lead finding from natural sources by miniaturising isolation, structure identification and biological testing working in the microgram range.
Methods: In order to rapidly identify antifungal compounds in crude plant extracts from French Polynesia, high resolution biological profiling was performed by semi-preparative HPLC micro-fractionation used jointly with a microdilution assay with the opportunistic yeast *Candida albicans.* The micro-fractions exhibiting antifungal activities were further analysed by Cap-NMR for dereplication purposes.
Results: The methanolic leaf extract of *Alphitonia zizyphoides,* a plant traditionally used to treat dermatomycoses [1], was the most active extract. Micro-fractionation followed by dereplication with microNMR showed the presence of betulinic acid, estimated to about 0.5% by NMR in the extract.
Conclusions: Coupling the performance of HPLC micro-fractionation with an at-line antifungal microdilution assay represents an efficient method to successfully localize bioactive peaks. For the methanolic leaf extract of *A. zizyphoides,* micro-fractionation followed by dereplication with microNMR has significantly speeded up the identification of the active compound, which could explain the traditional use of *A. zizyphoides.* The presented method is generic and easy-to-implement for the screening of crude plant extracts containing polar to medium polar substances.

Keywords: Micro-fractionation, *Candida albicans, Alphitonia zizyphoides,* betulinic acid, dereplication.

Reference:
[1] P. Pétard. Plantes utiles de Polynésie, Edition Haere po no Tahiti, 1986.

P-32

Stability Study of a Mutated Version of Recombinant Human Granulocyte Colony Stimulating Factor (rhG-CSF)

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Introduction: In the recent decades, proteins have become commonly used as biotech drugs and analytical tools. However, protein stability and extension of shelf-life remain major issues. We present here a study in which we have investigated the stability of a modified G-CSF under the stress of freezing. The protein was characterized by circular dichroism (CD), fluorescence spectrometry and differential laser light scattering (DLS) upon synthesis, subsequently frozen and characterized again upon thawing.
Aims: The objective of the study was to compare and evaluate the stability of a modified protein before and after freezing at –80 °C.
Methods: The mutated version of rhG-CSF was expressed in auto-induction medium in a widely used *E. coli* BL21(DE3)pLysS strain and a pET expression system. The resulting inclusion bodies were solubilized and protein was refolded and formulated as described by Boubeva et al. [1].
As one single method is not sufficient to evaluate the state of a protein, a set of different analytical methods was applied. Circular dichroism to determine the secondary structure, fluorescence spectroscopy to analyze changes in conformation and dynamic light scattering to determine aggregate size were employed. After characterization of the native protein, we froze aliquots at –80 °C. Aliquots were gently thawed on ice (+4 °C) for a monthly monitoring of stability during 3 months.
Results: Circular dichroism and fluorescence spectrometry using Nile red showed a good comparability between measures prior to freezing and thawed samples, however, fluorescence with ANS and intrinsic fluorescence indicated aggregation. Dynamic light scattering showed heterogeneity of samples, which indicates the presence of aggregates even directly after purification. However, the size was still five times lower compared to aggregated protein and during the first 3 months we did not observe a significant increase of the average aggregate size.
Conclusions: These results, although ambiguous, still show some promise that the protein is reasonably stable under the stress of freezing and thawing.

Keywords: Protein stability, protein analysis, aggregates.

Reference:
[1] R. Boubeva, C. Reichert, R. Handrick, C. Mueller, J. Hanneman, G. Borchard. Chimia 2012; 66: 281-285.

P-33

Metformin Does Not Alter the Risk of Lung Cancer: A Case-Control Analysis

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Introduction: Metformin use has been linked to a decreased cancer risk.
Aims: We explored the association between use of metformin or other antidiabetic drugs and the risk of lung cancer in particular.
Methods: We assessed the association between metformin and other antidiabetic drugs and lung cancer using a case-control analysis in the UK-based General Practice Research Database (GPRD). Cases were people with an incident diagnosis of lung cancer. Up to 6 controls per case were matched on age, gender, calendar time, general practice, and number of years of active history in the GPRD prior to the index date. The contribution of various potential confounders including tuberculosis, chronic obstructive pulmonary disease (COPD), diabetes mellitus, and co-morbid conditions to diabetes was evaluated in univariate models, and final results were adjusted for BMI and smoking.
Results: Long-term use (≥ 40 prescriptions) of metformin was not associated with an altered risk of lung cancer (adj. OR 1.19 (95% CI 0.96–1.48). Overall, use of sulfonylureas was duration-dependently linked to a marginally decreased risk of lung cancer (adj. OR 0.77 (95% CI 0.64–0.91). This risk decrease was observed in men (adj. OR 0.71, 95% CI 0.57–0.88) but not in women (adj. OR 0.90, 95% CI 0.66–1.21) and this risk decrease was not statistically significant in an analysis restricted to diabetic patients only (adj. OR. 0.88, 95% CI 0.72–1.07). Long-term use of insulin was associated with a slightly increased risk of lung cancer (adj. OR 1.33, 95% CI 1.04–1.71), however, no consistent trend across duration strata was observed.
Conclusions: Metformin did not decrease the risk of lung cancer.

Keywords: Epidemiology, cancer, antidiabetic drugs.

P-34

Decrease in Human Bronchial Epithelial Cell Monolayer Permeability Triggered by TLR2 Ligation is Mediated by Atypical Protein Kinase C Zeta

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Introduction: The airway epithelium acts as a crucial physical and immunological barrier against several inhaled antigens and allergens. Toll-like receptor (TLR) 2 has been shown to enhance the tight junction associated epithelial barrier function of human intestinal epithelial cells.
Aim: To investigate the regulation of TLR2 in maintaining epithelial barrier integrity in human bronchial epithelial cells (Calu-3).
Methods: Transepithelial electrical resistance (TEER) and paracellular flux experiments were used in parallel to RT-PCR, Western blotting and immunofluorescence experiments to study the tight junctional regulation by TLR2. Inhibitor assays were applied to elucidate the mechanism of tight junctional regulation by TLR2 ligands.
Results: Activation of TLR2 by Pam3CysSK4 and peptidoglycan showed a concentration-dependent increase in epithelial barrier function as characterized by TEER. Pretreatment with polyclonal anti-human TLR2-neutralizing antibody caused a significant reduction in TEER values. Treatment of Calu-3 cell monolayers with Pam3CSK4 resulted in a significant upregulation of claudin-1 and zonula occludens-1 (ZO)-1 tight junction proteins at the transcriptional level. This was reflected in a significant increase of the claudin-1 and ZO-1 protein expression level. Bisindolylmaleimide I, a PKC inhibitor at concentrations to inhibit classical and novel PKC isozymes did not inhibit the increase in epithelial barrier function. However, the concentrations at which it can inhibit atypical protein kinase C zeta, the increase in barrier function was significantly inhibited. These results are indicative that atypical PKC isoform zeta might mediate the increase in barrier function by TLR2 stimulation.
Conclusions: Stimulation of airway epithelial TLR2 enhances the tight junction associated barrier function via PKC zeta.

Keywords: Tight junctions, airway inflammation, protein kinase C, Calu-3 cell line.

P-35

Hormone Replacement Therapy and the Risk of Developing Gout

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Introduction: Estrogens have been reported to have a uricosuric effect which may explain to some degree the late onset of an increased gout risk for women. Hormone replacement therapy (HRT) increases the plasma volume and has been shown to slightly decrease the uric acid level.
Aims: We aimed at studying the association between use of HRT and the risk of developing an incident gout diagnosis.
Methods: We conducted a case-control study using the UK-based General Practice Research Database (GPRD). We identified female cases aged between 18 and 80 years with an incident gout diagnosis between 1995 and 2009 and matched them to one control women on age, general practice, calendar time, and years of history in the database. Conditional logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CIs) of developing gout in relation to previous use of HRT. We stratified by timing of use and by number of prescriptions, and additionally adjusted for potential confounders.
Results: The study encompassed 23,707 cases with a first-time gout diagnosis and the same number of matched controls. As compared to non-users, current users of 1–9, 10–19, or 20+ prescriptions of HRT showed tendency towards an increased OR of developing gout of 1.28 (95% CI 1.07–1.54), 1.09 (95% CI 0.91–1.31), and 1.32 (95% CI 1.14–1.52), respectively. Past use of HRT was not associated with an altered risk of developing gout. Further analysis regarding administration route (oral versus transdermal systems) or product composition (opposed versus unopposed) are currently under work and results will be presented at the conference.
Conclusions: This analysis suggests that patients who currently use HRT are at a slightly increased risk of developing gout, whereas past users are not. Use of HRT does not seem to materially alter the risk of a gout diagnosis in postmenopausal women.

Keywords: Gout, hormone replacement therapy.

P-36

NAD$^+$-Dependent Deacetylases as Promising Biotargets to Fight Against Chagas Disease and Leishmaniasis

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Introduction: *Trypanosoma cruzi* and *Leishmania spp.* are protozoan pathogens responsible for Chagas disease and Leishmaniasis, respectively. Current therapies rely only on a very small number of drugs, most of them inadequate because of their severe host toxicity or due to drug-resistance mechanisms. In order to find therapeutic alternatives, the identification of new biotargets is highly desired. SIR2, a NAD$^+$-dependent deacetylase belonging to the sirtuin family, is known to be essential for the life cycle of both parasites and, for this reason, widely used in anti-parasitic drug design [1]. Recent studies also highlighted the therapeutic potential of other NAD$^+$-dependant deacetylases found in both parasites [2].
Aims: The construction of robust 3D models of NAD$^+$-dependent deacetylases from parasites for future drug design studies.
Methods: Homology modeling, molecular docking, molecular dynamics simulations were used.
Results: The structures of NAD$^+$-dependent deacetylases from *T. cruzi, L. infantum* and *L. brasiliensis* were retrieved by homology modeling. The analysis of their active sites and the comparison with the human NAD$^+$-dependant deacetylase SIRT2 revealed differences between host and parasitic targets useful for selective drug design. Moreover, information from docking and molecular dynamics involving the general inhibitor nicotinamide was in line with the available experimental data.
Conclusions: The homology models of NAD$^+$-dependent deacetylases from parasites, supported by the the structural analysis presented in this work, represent reliable and attractive targets for the conception of novel selective antiparasitic compounds.

Keywords: NAD$^+$-dependent deacetylases, Chagas disease, Leishmaniasis, molecular modeling.

References:
[1] S. Kaur et al. Mol Diversity 2010; 14: 169.
[2] M.B.P. Soares et al. Acta Tropica 2012; 122: 224.

P-37

Set-Up of a Predictive *In Vitro* Sublingual Model as a Novel Platform for High Throughput Screening of Drugs and Vaccines

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Introduction: The sublingual route is increasingly considered a very attractive route of application for drugs, and especially for vaccine administration [1]. It is therefore surprising that the sublingual epithelium as a specific tissue of the oral mucosa remains poorly studied and characterized. Using a co-culture model combining human epithelial sublingual cells and dendritic cells, we developed and characterized a predictive tool for early stage drug screening prior to animal and human administration.
Aims: We first aimed to improve cell culture conditions and to confirm the *in vivo*-like feature of the *in vitro* co-culture model developed. Then, stimulation of dendritic cells after incubation with antigenic moieties was investigated. We anticipated obtaining substantial information on drug/tissue interaction mechanisms *in vivo* owing to the human origin of the cells.
Methods: An *in vitro* co-culture model based on human epithelial sublingual cells (HO-1-u-1 cell line [2]) and dendritic cells isolated from human blood was developed adapting a protocol previously described [3]. The co-culture was characterized using light and electron microscopy and compared to biopsies of human sublingual tissue. The release of inflammatory cytokines from both cell types

was studied on incubation with influenza virus and liposomes and compared to incubation with buffer only (negative control). Using an orientating qualitative multi-analyte ELISA, we first selected two cytokines, whose secretion appears to be specifically related to the presence of immune cells. Then, we performed a semi-quantitative ELISA on IL-6 and GM-CSF.
Results: Common histological features, such as the presence of microvilli and desmosomes, between *ex vivo* tissue and reconstituted *in vitro* epithelium were demonstrated. We observed *in vitro* a higher rate of cytokine release in the case of virus administration. Furthermore, in the reconstituted tissue, despite the intrinsic heterogeneity of dendritic cells due to their human origin, preliminary results show reproducible GM-CSF release data.
Conclusions: Histological characterization and stimulation of the underlying dendritic cells after incubation with antigenic moieties are encouraging. Indeed, they testify of the *in vivo*-like features of the *in vitro* reconstituted sublingual epithelium developed. Improvements in epithelial differentiation need to be performed and complementary information on specific markers remains to be addressed.
Keywords: Sublingual administration, HO-1-u-1 cell line, co-culture model, dendritic cells, vaccine.

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From Compound to Target: Chemical Proteomics and *In Silico* Screening Identify HSP90 and CDPK2 as Putative Targets in *Plasmodium Falciparum*

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Introduction: For several years now, *Plasmodium falciparum* is developing resistance to drugs in use. There is hence an urgent need for new treatments as well as new targets. By identifying the targets of novel or known active molecules with unknown mechanisms of action, it is possible to guide the development of new chemical entities towards their clinical application. Here we present our recent results on CP1, a new molecule in the development phase, and triclosan, a well-known antibacterial and fungicide.
Aims: This project aims to find the putative target(s) of CP1 using chemical proteomics and triclosan by means of ligand-based inverse virtual screening.
Methods: The parasite lysate was incubated with the affinity matrix and retained proteins were analyzed by LC-MS/MS. Yeasts complemented with plasmodial Hsp90 were grown in minimal media. Viability was calculated by comparison with untreated strains. Binding of CP1 derivatives to purified N-terminal PfHsp90 was evaluated with Differential Scanning Fluorimetry. For the identification of potential triclosan binders, molecules were evaluated *in silico* using the molecular docking program GOLD. Inhibition of PfCDPK2 and mechanism of action of triclosan were evaluated with a radiometric assay using myelin basic protein (MBP) as substrate.
Results: For CP1, chemical proteomics identified heat shock protein 90 (Hsp90) as a putative binder. Subsequent assays confirmed that the viability of yeast cells where the wild-type Hsp90 has been substituted with the plasmodial one was strongly reduced in presence of CP1. Moreover, CP2 was proven to bind the N-terminal domain of PfHsp90. R triclosan, the *in silico* inverse screening proposed the calcium-dependent protein kinase 2 (PfCDPK2) as its potential binding partner. Enzymatic assays confirmed inhibition of PfCDPK2 with an IC_{50} of 48 µM. Furthermore, the mechanism of action was determined to be non-competitive towards ATP.
Conclusion: This study shows that both chemical proteomics and *in silico* approaches are valuable tools for the identification of potential targets or binders of active molecules. The results obtained so far for PfHsp90 point definitely towards an interaction with the protein, although a direct proof of inhibition is still needed. On the other side, the confirmation of the inhibition of PfCDPK2 by triclosan opens new perspectives in the use of this molecule and derivatives thereof against *Plasmodium falciparum.* In both cases, such results represent a starting point towards the optimization of the molecules and the development of new therapeutics against malaria.

Keywords: PfHsp90, PfCDPK2, triclosan, chemical proteomics, inverse screening.

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Targeted Cutaneous Drug Delivery Using Polymeric Micelles

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Introduction: Ciclosporin A (CsA) is a potent orally administered immunosuppressant used for the treatment of autoimmune skin diseases including psoriasis. However, systemic administration of CsA exposes the patient to the risk of severe side effects. Hence, topical delivery of CsA, targeted directly to diseased skin, would offer significant advantages. However, CsA suffers from low cutaneous bioavailability and limited efficacy. Micelle formulations using a novel amphiphilic block copolymer (MPEG – dihexPLA), have recently been shown to incorporate several poorly water soluble drugs with high loading efficiencies and increase their skin bioavailability [1].
Aims: To quantify cutaneous delivery of CsA into porcine skin *in vitro* following topical application of a MPEG-dihexPLA micelle formulation and to visualize micelle distribution and penetration pathways by confocal laser scanning microscopy (CLSM) using Nile Red (NR) labelled MPEG-dihexPLA micelles (NR-MPEG-dihexPLA) containing fluorescent dyes (either DiO (3,3-dioctadecyloxacarbocyanine perchlorate) or fluorescein).
Methods: CsA-loaded micelles were prepared by drop-wise addition of the copolymer and CsA dissolved in acetone under sonication into ultrapure water. The organic solvent was removed by evaporation under reduced pressure. CsA content in the formulation was quantified by an in-house HPLC-UV method. Micelle size and morphology were characterized by dynamic light scattering and by transmission electron microscopy. Fluorescent micelles were prepared by adding NR-MPEG-dihexPLA to MPEG-dihexPLA copolymer. Fluorescent markers, either DiO or fluorescein, were loaded into the labelled micelles. The skin transport experiments were performed using dermatomed porcine ear skin and standard Franz diffusion cells (A = 1.9 ± 0.1 cm^2). CsA formulations were applied for 8 or 24 h. HPLC-UV was used to quantify CsA deposited in the skin. For the CLSM studies, skin samples were placed on a glass slide with the stratum corneum side up. The microscope fluorescence excitation wavelengths were set with a filter of 469–511 nm for DiO, 504–530 nm for fluorescein, and 607–674 nm for NR.

Excitation was performed using an Ar laser at 488 nm for all dyes. Skin samples in contact with saturated aqueous solutions of DiO and fluorescein for 24 h served as controls.
Results: A micelle formulation of 6.5 mg/mL CsA was prepared (325 mg CsA / g copolymer). The micelles were found to be spherical with an average hydrodynamic diameter of 82 nm. The amounts of CsA deposited in porcine skin after formulation application for 8 and 24 h were 6.3 ± 2.3 and 12.2 ± 4.0 µg/cm^2, respectively. The estimated concentration of CsA in the skin after 8 h was 84 µg/mL, which was 84-fold higher than the IC90 (1 µg/mL) in psoriatic patients with low sensitivity to CsA [2]. No CsA was detected in the receiver compartment. CLSM images using the NR-MPEG-dihexPLA micelles loaded with DiO showed that fluorescence of the labelled copolymer and the incorporated dye was co-localized in the uppermost skin layers. In contrast, for the fluorescein loaded micelles, although the labelled copolymer remained at the surface, the incorporated dye seemed to penetrate deeper into the skin. It can be inferred from these results that the physicochemical properties of the encapsulated molecule (in this case, the dye) may govern release from the micelle and subsequent skin penetration. The fluorescence of both dyes was higher from the micelles than from the saturated solution controls. The CLSM images of the regions surrounding hair follicles following formulation application suggested that the NR-MPEG-dihexPLA micelles might be preferentially deposited in and around these appendageal structures.
Conclusions: CsA was successfully delivered in supra-therapeutic amounts into the skin using nano-sized MPEG-dihexPLA micelles. CSLM images point to the localization of micelles in and around hair follicles suggesting that they may also facilitate delivery to these structures.

Keywords: Ciclosporin A, topical delivery, skin, dermatology, psoriasis, micelles, confocal microscopy.

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Development of Selective PET Tracers for $\alpha_5\beta_1$ Integrin Receptors

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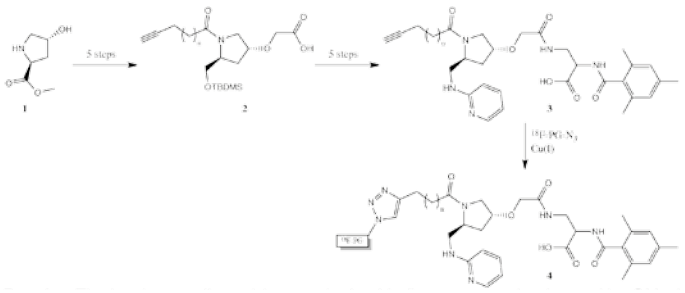
Introduction: Integrins are a large family of cell adhesion molecules, which are widely involved in tumor angiogenesis, growth and metastasis. Although some remarkable successes with $\alpha v\beta_3$ antagonists were recently obtained, it is presumed that inhibition of $\alpha_5\beta_1$ may be even more effective. $\alpha_5\beta_1$ is significantly up-regulated on endothelium during angiogenesis resulting in cell migration and survival, but not on quiescent endothelial cells [1].
Aims: Consequently, one needs molecular imaging probes to non-invasively quantify $\alpha_5\beta_1$ expression and to monitor $\alpha_5\beta_1$-mediated antiangiogenic therapy.
Methods: RGD-containing peptides have been traditionally used to target integrin receptors. Recently, several pyrrolidine derivatives were developed as potent and selective antagonists of $\alpha_5\beta_1$ [2]. Based on this pharmacophore, we have synthesized a series of PET peptidomimetics to image $\alpha_5\beta_1$ expression (Figure 1).

Figure 1. Synthesis of $\alpha_5\beta_1$ integrin selective PET ligands.



Results: The key intermediates (**2**) were obtained in five steps starting from 4-Hpr-OMe (**1**). Precursors (**3**) were obtained in five steps, which consisted mainly of coupling compounds (2) with *tert*-butyl-3-amino-3-oxo-2-(2,4,6-trimethylbenzamido)propanoate, and reductive amination with 2-amino-pyridine. Finally, Huisgen's cycloaddition of azido derivatives with the alkynyl pyrrolidines (**3**) yielded the final $\alpha_5\beta_1$ antagonists (**4**). The F-18 radiolabeled analogs were obtained by click chemistry addition of the corresponding ^{18}F-prosthetic groups to (**3**).
Conclusions: We have successfully synthesized novel selective peptidomimetic ligands towards $\alpha_5\beta_1$ integrin. The F-18 labeled analogs were prepared and are promising candidates for noninvasive imaging of $\alpha_5\beta_1$ expression in tumors.

Keywords: $\alpha_5\beta_1$ Integrin, antiangiogenic therapy, peptidomimetics, synthesis, noninvasive imaging.

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Nanocarriers for DNA Vaccination: Simultaneous TLR9 and NLR2 Stimulation Leads to Synergistic Enhancement of Pro-Inflammatory Cytokine Release from Murine Macrophages

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Introduction: DNA vaccination is an approach to induce protection against intracellular pathogens such as *Mycobacterium tuberculosis* (MTb) by establishing a cellular immune response. Targeting of innate immune receptors leads to the induction of Th1 cells that produce pro-inflammatory cytokines and are cytotoxic towards pathogen infected cells.
Aims: The aim was to produce cationic nanoparticles with a DNA vaccine encoding for MTb antigen 85A adsorbed at the outer surface. Being a Toll-like receptor-9 (TLR-9) ligand, plasmid DNA serves also as an adjuvant. Furthermore, a NLR2 ligand, muramyl dipeptide (MDP), was incorporated into the formulation to investigate the ability to elicit stronger immune responses when applied in combination with plasmid DNA. The nanocarrier systems were characterized and their ability to transfect murine RAW264.7 macrophages investigated. Toxicity profile and potential to induce pro-inflammatory cytokine release were examined *in vitro.*
Methods: The cationic chitosan derivative N-trimethyl chitosan (TMC) was synthesized and nanoparticles formed by complex coacervation with chondroitin sulfate. The plasmid was adsorbed to the surface by electrostatic interaction. Adsorption was visualized by scanning electron microscopy (SEM). MDP was added to the formulation and size and zeta potential of the nanoparticles were measured by means of dynamic light scattering and laser Doppler anemometry. The loading level of pDNA and MDP was determined

by fluorescence spectroscopy. RAW 264.7 murine macrophages were transfected and stimulated with pDNA and MDP loaded nanoparticles. Transfection was visualized by confocal microscopy and release of IL-12 and TNF-alpha determined. Finally, a cell proliferation assay (XTT) was performed with the same cells to investigate the toxicity of these nanocarrier systems.
Results: The cationic polymer TMC was successfully synthesized at a trimethylation degree of 20%. Nanoparticles (size below 300 nm) were formed and plasmid DNA adsorbed to the surface, slightly increasing the size of the nanoparticles. Indications of DNA adsorption are the decrease in surface charge, SEM image analysis and indirect quantification of adsorbed pDNA by fluorescence spectroscopy. A high adsorption and incorporation efficiency of pDNA and MDP was achieved and low toxicity of the loaded particles was determined (cell viability ca. 80%) after 24 h of incubation. Compared to the use of either receptor ligand alone, synergistic enhancement of cytokine release was induced by the combination of two innate immune receptor ligands in the same formulation.
Conclusions: This study demonstrated that NOD ligand containing nanoparticles can be prepared by a complex coacervation method and decorated with TLR-9 ligand plasmid DNA. Combination of both ligands in one carrier system very significantly increased pro-inflammatory cytokine release from murine macrophages in a synergistic fashion.

Keywords: DNA vaccine, chitosan nanoparticles, Toll-like receptor, NOD-like receptor, cell mediated immunity.

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Ex Novo Rhodanine Derivatives Targeting InhA

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Introduction: The 4-thiazolidinone moiety (also named as rhodanine) has been reported as privileged scaffold for compounds targeting the bacterial pyridine nucleotide NAD(P)H-dependent oxyreductases [1].
Aims: Identification of potential druggable targets involved in the type II FAS pathway, whose inhibition has been validated as therapeutic strategy against *Mycobacterium.*
Methods: Molecular docking simulations have been carried out by means of Surflex Dock (Sybyl-8.0) as structure-based approach aimed at identifying putative binding modes/affinities between selected *hit* compounds and a key target, the 2-trans-enoyl-acyl carrier protein reductase (InhA).
Results: According to the results from molecular docking, several possible interactions have been identified between the ex *novo* synthesized compounds and *M. tuberculosis* InhA. H-bond network interactions between (i) the sulfur atom of thiocarbonyl group, (ii) the carbonyl group at the position 4 of the rhodanine ring, (iii) the carbonyl group of -COOH from the tryptophan moiety as acceptors, and the hydroxyl group of tyrosine 158 in InhA and the hydroxyl group of NAD^+ as donors, respectively. in addition, those derivatives, bearing the naphthylidene residue, hydrophobically interact with the side chains of Phe 149, Met 199, Trp 222, Leu 218, Met 155, and Met 161.
Conclusions: The *ex novo* rhodanine derivatives have been identified as compounds targeting the InhA towards the inhibition of FAS II pathway.

Keywords: Rhodanine, thiazolidinone, *M. tuberculosis,* InhA, *in silico.*

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A New High-Throughput Screening Test Measuring Artificial Permeability Coupled With P-Glycoprotein Interaction

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Introduction: Inappropriate pharmacokinetic properties (PK) have been recognized as being one of the major factors leading to the withdrawal of new chemical entities (NCEs) from drug development process. Therefore, a large number of compounds have to be screened before matching one drug candidate disclosing good ADMET (absorption, distribution, metabolism, elimination, toxicity) properties during the early stage of drug discovery. In this context high-throughput methods thus becomes a real need to early assess compounds' PK and in particular their ability to penetrate biological membranes. It is well known that passive permeability of compounds through membranes is of prime importance in bioavailability. But it is also recognized that the efflux protein P-glycoprotein (Pgp, MDR1, ABCB1) affects the ADME of a large variety of compounds. This protein is well known to be responsible for some resistance in chemotherapy treatment, by overexpressing itself in cancer celles [1]. The Pgp is also naturally involved in the human body for biliary excretion, blood-brain barrier, and gastrointestinal track interference [1]. Most *in vitro* assays which are used to determine the interaction between NCEs and Pgp are made on cells such as Caco-2 or MDCKII.
Aims: The aim of this study was to develop a high-throughput assay able to predict simultaneously passive permeability through intestinal track and affinity of compounds for Pgp.
Methods: The assay is based on PAMPA (parallel artificial membrane permeability assay) technique developed to predict passive permeability through biological membranes, where a donor and an acceptor compartment are separated by a liquid artificial membrane. Depending on the nature of the artificial membrane, different biological barriers can be targeted [2]. In this study, hexadecane has been used as artificial membrane to mimic the passive diffusion through the intestinal track [2]. Purified membrane vesicles from Sf9 *(Spodoptera frugiperda)* expressing a high level of human recombinant Pgp was also added in the donor or in the acceptor compartment with the addition of compounds inhibiting all other ATPase pump present in the vesicles such as calcium or sodium/phosphate pump.
Results: Permeability obtained in presence and in absence of efflux protein was compared with each other. Compounds which interact with the Pgp have then a potential equilibrium with this protein which should lower there rate of permeability through the artificial membrane.
Conclusion: As the developed assay must be simple and easy to use the compounds' concentration was determined with an UV/Vis spectrophotometer. The obtained values were compared at first with well-known compounds with *in vivo* permeability and Pgp substrates data available. This assay makes it possible to get two informations which are nowadays essential in the conception of NCEs.

Keywords: ADME, permeability, PAMPA, glycoprotein P.

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Novel Biocompatible Hyaluronic Acid-Chitosan Hybrid Hydrogel for Osteoarthritis Therapy

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Introduction: One of the common therapies for osteoarthritis is the intra-articular injection of biodegradable, very high molecular weight hyaluronic acid (HA), requiring a high number of injections due to its limited retention time [1].
Aims: In order to reduce the frequency of injections, we followed a strategy to protect HA with another biopolymer forming a so-called hybrid hydrogel. Chitosan was chosen for its structural similarity with synovial glycosaminoglycans, its anti-inflammatory effect and ability to promote cartilage growth [2]. Since the two biopolymers show incompatibility and precipitation due to opposite charges, various excipients were evaluated to obtain homogeneous gels.
Methods: Viscosity was measured by rotational rheometry. Biocompatibility was assessed by subcutaneous injections in rats and histopathological evaluation at 1 week of the tissues in contact with the formulations [3].
Results: Excipients such as calcium chloride and sodium chloride were able to prevent hydrogel aggregation. Buffered formulations could be steam-sterilized, leading to stable, transparent gels with a viscosity comparable to that of a commercial product (Ostenil®). Following injection in rats, a good biocompatibility was observed for sodium chloride formulation whereas calcium chloride formulation led to calcium deposits.
Conclusions: Transparent homogeneous HA-Chitosan hydrogels could be formulated using appropriate anti-aggregant excipients. Selected formulations with sodium chloride show good biocompatibility in the rat subcutaneous model. Ongoing investigation will provide insight into the intra-articular fate of these hydrogels in a rabbit model.

Keywords: Osteoarthritis, hyaluronic acid, chitosan, hybrid hydrogel, biocompatibility.

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Three-Dimensional Localization of Sub-Micron Particles in the Lung Parenchyma by Multimodal Imaging

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Introduction: In order to judge the risks and chances of the deposition of sub-micron particles in the lung parenchyma a precise three-dimensional (3D) localization of the sites of deposition is very important – especially because an inhomogeneous deposition and local hot spots of very high particle concentrations are expected in the acinar tree as well as in individual alveoli.
Aims: The aim of this study is to combine different imaging modes – synchrotron radiation X-ray tomographic microscopy (SRXTM), light and electron microscopy – for the 3D localization of sub-micron particles.
Method: Unstained rats lung samples, containing 200-nm gold particles (applied by tracheal instillation), were embedded in paraffin, scanned at the beamline TOMCAT (Swiss Light Source at the PSI, Villigen) and three-dimensional visualizations were obtained. To verify the excepted gold deposition sites and to overcome the limitation of the resolution of SRXTM, electron microscopy (EM) or light microscopy (LM) were applied to the same samples. EM and LM images were registered in stack of SRXTM sections using an in-house written Matlab routine, based on "Scale-invariant feature transform".
Results: In the SRXTM data we observed small clusters of 200-nm gold particles. Because one voxel (370 nm side length) was larger than one gold particle, the number of gold particles contained in the observed clusters could not be counted precisely with this detection method. Using EM we were able to show that the smallest cluster contained one gold grain only. Also LM structural investigations were accomplished by using this matlab routine.
Conclusions: We conclude that the presented workflow is suitable for the investigation of deposition patterns of any kind of particles in the pulmonary gas-exchange area at the acinar and alveolar level.

Keywords: Lung, sub-micron particles, multimodal imaging, registration.

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Erroneous Prescription of Half Tablets in a Swiss University Hospital

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Introduction: Prescription of ½ tablets is a widespread practice, mainly for dose flexibility, ease of swallowing, and cost reduction. However, tablet splitting includes several disadvantages, like destruction of galenic formulation, stability problems, and unequal amount of active ingredient that may reduce effectiveness or re-

sult in a greater risk of toxicity. Prescribing ½ tablets may thus be considered as a prescribing error according to the most accepted definition of medication error.
Aims: The aim of this retrospective analysis was to assess the rate of prescribed ½ tablets in discharge prescriptions at the University Hospital in Basel (UHBS, 600 beds) and to evaluate its consequences for community pharmacists.
Methods: Discharge prescriptions with the term "½" were extracted from the electronic patients' data management system of the UHBS between January 1st and December 31st 2011. Presence of a score line and suitability for splitting were retrieved from the Swiss Online Compendium [1] and the internal pharmacy hospital list on splitting and crushing drugs [2]. Drugs were classified as (i) tablet with a score line, (ii) tablet with no score line, (iii) no information about score line, (iv) suitable for dose splitting, (v) not suitable for dose splitting, (vi) no information about splitting, and (vii) unnecessary splitting. Erroneous prescription was assigned for drugs that were not suitable for dose splitting.
Results: Of the 36,751 discharge prescriptions that were recorded in 2011 at the UHBS, 3,724 (10.1%) contained at least one prescription item with the term "½". The recipient patients were on average 72.9 ± 14.8 years old (median 76 years), 50.9% were women. After exclusion of 171 item (not a tablet, not available in Switzerland, major writing errors, missing strength, off the market) 4,517 item were analysed. For 49% of them, the prescribed splitting was unnecessary because of availability of adequate dosage strength. Most prescribed ½ tablets were scored (87%) and suitable for dose splitting (77.6%). Erroneous prescription rate of ½ tablets was 16.4% (inexistent score lines (12.6%); clear ban in the sources of drug information (3.8%) and concerned 2.8% of all prescriptions. When the lack of information on splitting suitability (5.6%) and on score lines (0.5%) was taken into account, erroneous prescription rate of ½ tablets reached 22.4%. More than half of all erroneous prescriptions (54.6%) could be assigned to 19 different products that were prescribed with an overall rate between 3.1 and 0.2%. Seroquel® (Quetiapin) at all strengths was the most often erroneously prescribed tablet to split (3.1%; unscored), followed by Sortis® (Atorvastatin) at all strengths (1.3%; unscored) and Seresta® (Oxazepam) 15 mg (1.2%; with decorative score line).
Conclusions: Prescribing of ½ tablets is a common issue, affecting 10.1% of all discharge prescriptions of the UHBS. However, every 6th prescription with ½ tablets is erroneous. Prescription of ½ tablets is a pharmaceutical care issue. Community pharmacists have to detect and to handle prescription errors since these might lead to drug related problems, which is amongst their core competences. In many cases, tablets of half the dosage are commercially available and pharmacists can offer a substitution. In all cases, time consuming and costly clarifications must be undertaken, ultimately the physician must be consulted, in order to modify the prescription or to dispense the prescribed ½ tablets as off-label use. If splitting is allowed, the patient's cognitive and physical capacities have to be clarified and appropriate aids have to be offered, e. g. a pill splitter.

Keywords: Medication error, prescribing error, splitting tablets, pharmaceutical care.

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Drug-Eluting Beads for Transarterial Chemoembolization: Sunitinib Loading, Release Properties and *In Vivo* Pharmacokinetics of DC Bead™ Microspheres

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Introduction: Microspheres combined with an anti-angiogenic drug are an attractive treatment approach for unresectable advanced carcinoma, particularly hepatocellular carcinoma, in the frame of transarterial chemoembolization. The two purposes of an injection of a drug-loaded vector into an artery consist on the one hand in vascular occlusion of the tumor-feeding vessels and on the other hand in prolonged *in situ* dwell time and therefore higher drug levels to damage the tumor tissue.
Aims: To study *in vitro* the loading and release of the multiple tyrosine kinase inhibitor sunitinib from differently sized commercially available embolization microspheres (DC Bead™) and the resulting pharmacokinetic profiles in rabbits.
Methods: 70–150 and 100–300 µm drug-eluting beads were loaded by immersion in a specially developed sunitinib solution. Drug was quantified by spectrophotometry at a wavelength of 430 nm. Drug release was measured over one-week periods in NaCl 0.9% at 37 °C, using the European Pharmacopeia/US Pharmacopeia flow-through apparatus IV. Release was normalized using an internal standard of 30% ethanol in NaCl 0.9%. As for the *in vivo* part, New-Zealand white rabbits received either 100–300 µm sized beads loaded with 6 mg of sunitinib by injection into the hepatic artery or 6 mg of sunitinib per os. Drug concentrations in plasma and liver tissue were assessed by LC-MS/MS.
Results: High, close to complete and homogeneous drug loading was obtained for both microsphere sizes, with a slightly faster loading for the smaller beads attributed to their higher surface area. Particle shrinking was observed with adsorption of sunitinib onto the hydrogel beads, which maintained their spherical shape throughout loading and release. Almost complete release was detected under physiological conditions, with similar and fast release profiles for both sizes of DC Bead™ particles. After embolization drug plasma levels remained low (<50 ng/mL) whereas in the liver tissue, very high concentrations at 6 h (14850 ng/mL) and 24 h (4226 ng/mL) were found.
Conclusions: The microspheres were efficiently loaded with the anti-angiogenic drug by an ionic exchange mechanism. Both sizes showed a fast release in saline. Sunitinib-eluting beads were well tolerated by rabbits without observation of unexpected effects. High liver drug concentration and low systemic levels indicated the potential of sunitinib beads for hepatocarcinoma treatment.

Keywords: Drug-eluting beads, sunitinib, chemoembolization, drug loading and release.

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Development of a New Artificial Membrane for the Study of Permeation Through the Blood-Brain Barrier Using PAMPA

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Introduction: The drug discovery process aiming at targeting the central nervous system suffers from one of the highest failure rate, due to the presence of the blood-brain barrier (BBB) and the highly represented transmembrane transporter proteins. High throughput screening techniques thus need to be developed in order to detect lead compounds disclosing good pharmacokinetic properties among a large set of drug candidates.
Aims: This work was devoted to the development of a new artificial membrane for PAMPA (parallel artificial membrane permeability assay), able to predict passive permeability through the BBB with a high throughput screening rate. PAMPA is an *in vitro* technique already used for the prediction of passive diffusion through biological membranes relying on a specific immobilized artificial membrane separating two aqueous compartments. Besides, a PAMPA-BBB technique has been developed in 2003 to predict permeability through the BBB, using porcine polar brain extract, allowing the classification of the tested compounds into passively transported (CNS+) or not passively transported (CNS-) categories [1]. The aim of this work was to generate a new artificial membrane for PAMPA, resulting in a continuous classification of passive permeation through the BBB, avoiding the drawbacks encountered when handling biological material and improving the throughput of the assay.
Method: The new artificial membrane was developed following correlations between the permeability generated with PAMPA, and the one predicted by a well-known cellular-based model: the bovine brain capillary endothelial cells model [2]. A design of experiments allowed the determination of the optimal composition of the artificial membrane. The permeabilities predicted with this new model were then compared to the ones generated with other *in vitro* and *in vivo* models used in the drug research process.
Results: The resulting correlation generated with the new PAMPA model (r^2 = 0.92; N = 13) indicates that our membrane is able to predict passive permeation through the BBB. Regarding the results, the passive permeation of a test set of compounds has been compared to *in vivo* log PS (r^2 = 0.81; N = 6) and to PAMPA-BBB (r^2 = 0.80; N = 13). Compounds known to be substrates of efflux transporters at the BBB have also been tested on our model. As expected, their positions on the different correlations indicate that the PAMPA model overestimates the permeation of those compounds, because of the ability of PAMPA to predict only passive transport.
Conclusions: The generated results indicate that the new artificial membrane is able to predict passive permeation through the BBB. Moreover, a possible active transport (either influx or efflux) can be highlighted when comparing our new PAMPA model with a cellular model expressing specific transporters.

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P-49

Localized and Combined Hyperthermia and Chemotherapy: A Novel Approach to Treat Bone Tumors

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Introduction: Bone metastases might be efficiently treated using intraosseous implants. In this view, we propose novel formulations that, once injected intratumorally, form a solid implant. Poly(methyl-methacrylate) (PMMA) cements are relevant formulations already used in vertebroplasty. They can be loaded with both an anticancer agent and superparamagnetic beads for combining chemotherapy and hyperthermia, the latter being an effective adjuvant in cancer therapy [1].
Aims: To develop acrylic cement formulations carrying superparamagnetic silica beads (SSB) embedding superparamagnetic iron oxide nanoparticles (SPIONs) and anticancer agents (doxorubicin, DOX). The implant can be heated applying an external magnetic field, sensitizing the surrounding tumoral tissues, while releasing the chemotherapeutic agent. By combining hyperthermia and chemotherapy, a synergistic effect may be reached improving the therapeutic effects of the implant.
Methods: Cement was prepared through an exothermic polymerization reaction by mixing PMMA and its monomer in presence of an initiator and an activator. SSB at 24% or 30% (w/w) and DOX at 2.5% (w/w) were loaded within the cement. Cement polymerization was followed in terms of temperature increase as a function of time and parameters such as setting time and maximum temperature (MT) achieved were determined. Heating capacity was assessed by measuring cement temperature increase under an external alternating magnetic field (6 mT and 140 kHz). *In vitro* DOX release was carried out in a saline media at 37 °C and the DOX was analyzed by spectrophotometry at 479 nm. *In vitro* toxicity of the implants was tested using XTT proliferation assay. Immortalized human prostate cancer cells, PC3, were incubated for 24 h before the cell viability was measured and compared with a control of non-treated cells. Young modulus was determined by compression of Ø 6 x 7 mm cylinders. Finally, the cements were injected within bovine vertebra and the radiopacity was measured using a micro CT-scan.
Results: PMMA cement was able to generate heat in the range of 43–44 °C and displayed sustained release over at least 10 days. The release profiles were not influenced by the heat generated during a 25 min-hyperthermia session at 6 mT and 140 kHz, allowing further studies on the synergetic effects of hyperthermia and chemotherapy. The heating power of the implants, so-called specific power loss (SPL), indicates the potential for hyperthermia-induced antitumoral effect. Cement for intraosseous injection might provide some mechanical support to the weakened bone as the Young compression moduli are in the range of cancellous bone. *In vitro* toxicity of eluted DOX on PC3 cells shows preserved drug cytotoxicity. The *ex vivo* injection showed that the SSB acts as radiopacifier for the cements. Finally, addition of SSB kept setting time within clinically acceptable values and maximal temperature below necrotic threshold, allowing a potential clinical use of these formulations.
Conclusions: Acrylic cement was successfully loaded with DOX and superparamagnetic nanoparticles, providing a sustained anticancer agent delivery and potential cytotoxic temperature. These data show within clinically acceptable parameters the feasibility of combining SPIONs for hyperthermia with local anticancer agent release.

Keywords: Hyperthermia, SPION, in situ forming implants, bone metastases, PC3.

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P-50

VMAT2 – Synthesis of Reserpine Analogs

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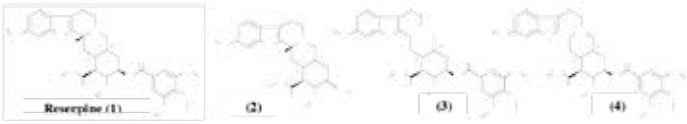
Introduction: The vesicular monoamine transporter type 2 (VMAT2) is a membrane protein that transports monoamines from the cytoplasm into synaptic vesicles. The discovery of small and brain-penetrable molecules that are effective and selective regulators of either dopamine or serotonin release without affecting the transport of other monoamines could be of value in the treatment of neurological diseases such as Parkinson, Alzheimer or Huntington. In fact, the VMAT2 inhibitor tetrabenazine is approved for the treatment of Chorea Huntington, although the compound does not only act on VMAT2 and is associated with significant side effects [1]. The plant product reserpine is the most potent VMAT2 inhibitor known [2].
Aims: This contribution will discuss the synthesis of a series of reserpine analogs and their evaluation as potential VMAT2 ligands.
Methods: A series of reserpine (**1**) analogs has been prepared based on natural reserpine as the starting material. Removal of the trimethoxybenzoyl moiety in (**1**) can be achieved in a single step and the resulting methyl reserpate was the key intermediate in the synthesis of analogs of type (**2**). Ring-opened products (**3**) and (**4**) were obtained by reserpine C-N and C-C bonds clevage, respectively.



Results: Amide and amine reserpine analogs show IC50 values for the dopamine uptake inhibition that is quite similar to that of reserpine. Dihydroreserpine (**3**) was 10-times less potent than reserpine.
Conclusions: Amide and amine reserpine analogs are the most potent compounds, both molecules suggest neither a loss of activity nor an improved efficacy compared to reserpine. Dihydroreserpine (**3**) appears to be significantly less potent.

Keywords: VMAT2, reserpine, dopamine, serotonin.

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P-51

Evidence for Bidirectional Endocannabinoid Transport Across Cell Membranes

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Introduction: Despite extensive research on the trafficking of anandamide (AEA) across cell membranes little is known about the membrane transport of other endocannabinoids such as 2-arachidonoylglycerol (2-AG). Previous studies have provided data both in favor and against a cell membrane carrier-mediated transport of endocannabinoids, using different methodological approaches.
Aims: To investigate whether endocannabinoids are transported across cell membranes or pass by passive diffusion.
Methods: Radioligand assays, TLC, GC-MS, pharmacological synergy.
Results: Since AEA and 2-AG undergo rapid and almost complete intracellular hydrolysis, we used a combination of radioligand assays and absolute quantification of cellular and extracellular endocannabinoid levels. In human U937 leukemia cells, 100 nM of AEA and 1 μM of 2-AG were taken up through a fast and saturable process reaching a plateau after 5 min. Using differential pharmacological blockage of endocannabinoid uptake, breakdown and interaction with intracellular binding proteins, we show that eicosanoid endocannabinoids harboring an arachidonoyl chain compete for a common membrane target that regulates their transport, while other N-acylethanolamines did not interfere with AEA and 2-AG uptake. By combining fatty acid amide hydrolase or monoacyl glycerol lipase inhibitors with hydrolase inactive concentrations of the AEA transport inhibitors UCM707 (1 µM) and OMDM-2 (5 µM) a functional synergism on cellular AEA and 2-AG uptake was observed. Intriguingly, structurally unrelated AEA uptake inhibitors also blocked the cellular release of AEA and 2-AG. We show, for the first time, that UCM707 and OMDM-2 inhibit the bidirectional movement of AEA and 2-AG across cell membranes.
Conclusions: Our findings suggest that a putative endocannabinoid cell membrane transporter (EMT) controls the cellular AEA and 2-AG trafficking and metabolism.

Keywords: Endocannabinoid transport, 2-AG, anandamide.

P-52

The Antinociceptive Triterpene ß-Amyrin Inhibits 2-Arachidonoylglycerol (2-AG) Hydrolysis Without Directly Targeting CB Receptors

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Introduction: Pharmacological activation of cannabinoid CB(1) and CB(2) receptors is a therapeutic strategy to treat chronic and inflammatory pain. It was recently reported that the natural product mixture α/ß-amyrin selectively binds to the cannabinoid CB(1) receptor with a subnanomolar K(i) value (133 pM). Orally administered α/ß-amyrin inhibited inflammatory and persistent neuropathic pain in mice both CB(1) and CB(2) receptor-dependently.
Aims: To investigate the effects of amyrins on the major targets of the endocannabinoid system different assays available in our groups were carried out.
Methods: We measured CB receptor binding interactions of α- and ß-amyrin in validated binding assays using hCB(1) and hCB(2) transfected CHO-K1 cells and further explored potential effects on endocannabinoid transport in U937 cells and breakdown in BV2 cell and pig brain homogenates, as well as purified enzymes.
Results: We were unable to detect significant binding of neither α- nor ß-amyrin to hCB receptors in our assays (K(i) >10 µM). Intriguingly, the triterpene ß-amyrin potently inhibited 2-arachidonoylglycerol (2-AG) hydrolysis in pig brain homogenates, but had no effect on anandamide degradation. Although ß-amyrin only weakly inhibited purified human monoacyl glycerol lipase (MAGL) it also inhibited α,ß-hydrolases (ABHDs) and more potently inhibited 2-AG breakdown than α-amyrin and the MAGL inhibitor pristimerin in BV2 cell and pig brain homogenates.
Conclusions: We propose that ß-amyrin exerts its analgesic and anti-inflammatory pharmacological effects via indirect cannabimimetic mechanisms involving the inhibition of degradation of the endocannabinoid 2-AG without interacting directly with CB receptors. Triterpenoids appear to offer a very broad and largely unexplored scaffold for inhibitors of 2-AG enzymatic degradation.

Keywords: Amyrin, 2-AG, CB(1) receptor, hydrolases, MAGL.

P-53

Development of Potent Selective Endocannabinoid Reuptake Inhibitors From a Plant Derived N-Alkylamide Scaffold

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Introduction: Plant fatty acid derivatives such as N-alkylamides are structurally related to endocannabinoids (EC). We found that some of the C12 N-alkylamides from purple coneflower (*Echinacea* spp.) weakly affect the reuptake of anandamide (AEA) into cells. ECs such as AEA are lipophilic signaling molecules which modulate several physiological processes such as pain and inflammation. A prolongation of the EC tone may therefore be pharmacologically favorable. We have synthesized a library of 140 compounds based on the natural dodeca-*2E,4E*-diene amide scaffold and screened them for AEA uptake inhibition and further targets within the EC system.
Aims: The lack of selectivity among present inhibitors of AEA reuptake requires the development of a novel class of EC uptake inhibitors. While to date the differentiation between degradation and uptake inhibitors of AEA is still difficult, we aim to provide new pharmacological tools to better understand EC transport. Since the identification of responsible protein(s) of EC uptake is still elusive, these novel compounds could help to target identification.
Methods: *In vitro* assays were performed using liquid scintillation counting. AEA uptake was measured in U937 and HMC-1 cells using [^{3}H]-AEA. Fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL) assays were performed with cellular and pig brain homogenates. CB receptor binding was determined using CHO-K1 hCB1 and hCB2 membrane preparations. Behavioral studies were performed in BALB/c mice using the hot plate test to evaluate analgesia.
Results: Conserving the aliphatic *2E4E*-dodecadiene chain of the natural isobutyl alkylamide while modifying its head group several novel and potent lipid AEA uptake inhibitors were obtained. Among the highly potent hit compounds WOBE437 showed an IC_{50} = 10 nM in AEA uptake inhibition while keeping high selectivity over the AEA degrading enzyme FAAH and CB receptors. Further, the competitive AEA reuptake inhibitor WOBE437 leads to analgesic effects *in vivo* confirming its indirect agonistic mechanism at CB1 receptors.
Conclusions: The natural dodeca-*2E,4E*-diene amide scaffold led to the successful development of a novel class of AEA reuptake inhibitors which exceed former arachidonic acid derivative reference inhibitors both in potency and selectivity. Overall, FAAH inactive AEA reuptake inhibitors will be crucial for the study of the EC transport system and may serve as tools for target identification. Additionally, these novel EC reuptake inhibitors are currently extensively tested in preclinical models and under investigation for the treatment of inflammatory skin disorders.

Keywords: Endocannabinoid system, anandamide reuptake inhibitor, WOBE437.

P-54

Development of a Decontamination Method for Degradation of Platinum Complexes Utilizing Vapor Phase Hydrogen Peroxide

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Introduction: Vapor phase hydrogen peroxide (VPHP) process is a modern decontamination method utilizing vapor phase hydrogen peroxide as an effective agent. So far, it has been used for bio-decontamination such US postal service buildings remediation after anthrax terrorist attacks in 2001, medical tools or areas sterilization or in food industry. Intensive research of its possible use for degradation of environmental polluting chemical contaminants (pesticides, industrial poisons, etc.) chemical weapons and especially biological active compounds like drugs, hormones and so on, is now performed. This work is aimed to study the decontamination process utilizing VPHP as an effective agent for the degradation of biologically active platinum complex compounds.
Aims: The aim of this work is to examine the influence of humidity and VPHP concentration on the kinetics of the degradation of a platinum complex model compound (potassium trichloroammineplatinate, TCAP).
Methods: The experiments were performed in the sophisticated laboratory experimental device called "peroxybox" especially developed for these purposes. It allows setting up various reaction conditions such as temperature, relative humidity and VPHP concentration. The samples of model substance deposited on microscope quartz slides were exposed to the set degradation conditions and then withdrawn in specific time intervals. The samples were evaluated using a new developed HPLC method.
Results: It is evident that VPHP is an applicable method for the degradation of platinum complexes. The relative humidity was proven to be a very important parameter for the degradation efficiency. The wet process is more efficient and about 1.3 times faster than the dry process. The minimal effective VPHP concentration was found to be 400 ppm for the dry process and 200 ppm for the wet process. Complete TCAP degradation was achieved in less than 1 and 2 h during the wet process and dry process, respectively, perfectly fulfilling the demands for practical usage.
Conclusion: Results show a good presumption for future studies and possible use of the VPHP method for the degradation of platinum cytostatics.

Keywords: VPHP, decontamination, TCAP, platinum cytostatics, hydrogen peroxide.

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P-55

Spray Drying of a Poorly Water Soluble Plant Extract From Organic Solution

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Introduction: Spray drying is one of the most frequently used methods of drying and in industry it is almost an exclusive technique for drying of thermolabile plant extracts. The most common way of increasing solubility of an active pharmaceutical ingredient in water is mostly based on increasing the specific surface area and/or preparation of specific formulations, for example an amorphous form, due to the higher thermodynamic chemical potential than its crystalline counterpart [1]. The co-spray drying of active substance with a matrix former has an advantage toward solubility as well, since the insertion of high-energy in the process is avoided [2].
Aims: The aim of this work is increasing of stability and aqueous solubility of a poorly soluble plant extract.
Methods: The plant extract was dissolved in an organic solvent and mixed with different types of excipients. The blend of active substance and matrix former was heated to reflux and spray-dried at this temperature. Different operational and geometrical parameters of the process were applied (e. g. 2-pump system, various inlet temperatures). Due to safety reasons, the spray-drying was performed in a closed loop system of Büchi Mini Spray Dryer B-290, equipped with absorption unit and Büchi Inert Loop B-295. Dissolution tests were performed with all powders, concentration levels of the active substance were measured using a HPLC/UV method, and the dissolution rates were compared with each other.
Results and conclusion: Amorphous powders of a plant extract with 5 different matrix formers could be successfully prepared yielding above 80 wt.%. The water content of the resulting samples was less than 1 wt.%. The produced particles had mostly spherical shape, with a mean size of about 5 µm. Narrow particle size distribution could be observed since the size of most particles (X_{90}) is less than 12 µm. Dissolution tests revealed differences in dissolution rates, varying with the matrix former. It was found, that more than 50% of the active substance was dissolved after 15 min.

Keywords: Spray-drying, plant extract, matrix former.

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P-56

Bilayered Electrospun Vascular Grafts for Improved Tissue Regeneration and Reduced Blood Leakage

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Introduction: Shelf-ready synthetic biodegradable vascular grafts are promising alternatives to autologous vascular material for small diameter vascular replacements in the clinic.
Aim: In order to improve tissue regeneration in the graft wall while preventing blood leakage upon implantation, bilayered vascular grafts with two distinct micro-architectures were prepared and evaluated.
Methods: The bilayered grafts were made by electrospinning polycaprolactone into a high porosity graft and adding a low porosity barrier layer either on the luminal or the adventitial side. Grafts were characterized *in vitro* for fiber size, pore size, total porosity, water and blood leakage, mechanical strength, burst pressure, and suture retention strength. The two types of grafts were then evaluated *in vivo* in the rat abdominal aorta replacement model for 3 and 12 weeks.
Results: The *in vitro* blood leakage through these barrier grafts was significantly reduced compared to the single layer high porosity graft. *In vivo,* the cell invasion at 3 and 12 weeks (6.4 ± 2.3% and 12.5 ± 0.7% for the outside barrier grafts vs. 23.5 ± 5.5% and 35.3 ± 5.3% for the inside barrier grafts) and neovascularization at 3 weeks (3.2 ± 1.1 vs. 14.7 ± 3.1 capillaries/field of view) was significantly reduced in outside barrier grafts, but there was no significant difference between the grafts for endothelialization rate or intimal hyperplasia.
Conclusions: Multi-layered grafts are therefore promising approaches for achieving optimal tissue regeneration in synthetic biodegradable vascular grafts, while avoiding the problem of blood leakage during surgery.

Keywords: Vascular grafts, polycaprolactone, electrospinning, tissue regeneration.

P-57

Risk of Developing Alzheimer's Disease in Association with Influenza Infections

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Introduction: Several epidemiological studies suggest a potential involvement of viral pathogens in the development of Alzheimer's disease (AD). While recent research focuses on herpes simplex virus type 1 (HSV-1), the role of influenza infection or vaccination is largely unknown.
Aims: To explore the association between influenza infection, exposure to influenza vaccines and the risk of developing AD in a large population-based study.
Methods: We conducted a case-control analysis using the UK-based General Practice Research Database (GPRD). We identified cases aged 65 years or more with an incident diagnosis of AD between 1998 and 2008, and we matched one control patient without dementia to each case on age, gender, general practice, calendar time, and years of history in the database. Conditional logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) of developing AD in relation to previous influenza infections, to a subgroup of influenza infections that occurred within one year after an influenza vaccination, or to another subgroup of influenza infections that were followed by bacterial superinfection (indicated by an antibiotic treatment within 30 days), stratified by number of infections and adjusted for various potential confounders.
Results: We identified 7,086 cases with an incident diagnosis of AD and the same number of matched controls. After adjusting for vari-

ous potential confounders, a history of influenza infection was not associated with an altered risk of developing AD. The OR for patients with 3 or more previous infections was 0.96 (95% CI 0.49 to 1.88). In the subgroups of those who developed an influenza infection despite a previous influenza vaccination or those who received antibiotic treatment, the OR of developing AD was not altered either.
Conclusions: In the current study population, a history of influenza infection was not associated with an altered risk of developing AD.

Keywords: Alzheimer's disease, influenza infection/vaccination.

P-58

Dual Activity of MicroRNA Precursors

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Introduction: A customary method of investigating the properties of microRNAs is to exogenously deliver commercially available microRNA "mimics" into cells and to observe their biochemical, biological and pharmacological effects. However, as our knowledge of microRNAs grows, it becomes increasingly clear that the chemical modifications introduced into mimics to facilitate synthesis, to maximize inhibitory activity of the "mature" strand, and to eliminate the "off-target" activity of a "passenger" strand in fact removes some of their most interesting and important biological properties.
Aims: Generate and evaluate a toolset to gain deeper insights into microRNA biogenesis and the biological effect of the "passenger strand" and its targets.
Methods: Here we describe the ready synthesis, purification and characterization of Drosha products using standard RNA reagents.
Results: We demonstrate how synthetic Drosha products fully recapitulate recently discovered properties of pre-microRNAs including their processing by dicer into simultaneously active 5p- and 3p-derived microRNAs. We use these synthetic pre-microRNAs to reveal that pre-microRNA-34a, an important tumor suppressor and candidate anti-cancer drug, is processed in cells to 5p- and 3p-active microRNAs, which target simultaneously important pathways in the cell.
Conclusions: Our results demonstrate that synthetic pre-microRNAs can be prepared by any laboratory equipped for RNA synthesis using standard reagents and that they are true microRNA mimics. We anticipate that the recent flurry of literature describing the properties of the hairpin loop and the roles of the passenger strand in the biogenesis and function of pre-microRNAs and this work will stimulate RNA researchers to switch to synthetic pre-microRNAs as a new generation of RNA reagents.

Keywords: MicroRNA, precursor, synthetic hairpin, biogenesis.

P-59

Determination of Degradation and Saturation Solubility of an Unstable Phytopharmaceutical Compound

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Introduction: Micellar and liposomal vehicles can be used to improve stability and solubility of drugs. Direct measurement of the equilibrium solubility of an unstable compound is experimentally difficult especially if degradation takes place fast.
Aims: The aim of this work was to determine the degradation of the phytopharmaceutical compound nobilin and to develop a kinetic model to calculate the saturation solubility of this compound in different vehicles.
Methods: Aqueous media (aq-TM_{caco}), fasted state simulated intestinal fluid (FaSSIF-TM_{caco}), fed state simulated intestinal fluid (FeSSIF-TM_{caco}), and two liposomal formulations with the same lipid concentrations as FaSSIF-TM_{caco} and FeSSIF-TM_{caco} in aq-TM_{caco}, respectively, were used as vehicles in stability and solubility studies. The degradation was described by first order kinetics. The saturation solubility was calculated with a kinetic model which included the dissolution rate, the diffusion rate into and out of colloidal particles and the degradation rate in aq-TM_{caco}.
Results: The degradation constant of nobilin in solution with aq-TM_{caco} was approximately 0.4 h^{-1} and degradation occurred mainly by water addition. FaSSIF-TM_{caco} and FeSSIF-TM_{caco} reduced the degradation constants 1.3-fold and 8.6-fold, respectively, in comparison to aq-TM_{caco}. The two liposomal formulations with low and high lipid concentration decreased the degradation constant 8.7-fold and 44.6-fold, respectively. The degradation constants decreased with decreasing drug-to-lipid ratio. The calculated saturation solubility of nobilin in aq-TM_{caco}, FaSSIF-TM_{caco}, and FeSSIF-TM_{caco} was 106 µg/mL, 151.8 µg/mL, and 559.1 µg/mL.
Conclusions: The vehicles improved stability to an extent depending on the drug-to-lipid ratio. It was possible to calculate the saturation solubility with the kinetic model. The vehicles increased the saturation solubility. Both observations are probably because of the encapsulation of the compound in colloidal particles.

Keywords: Stability, solubility, kinetic model, lipid vehicle, phytopharmaceutical compound.

P-60

Stability Evaluation to Formulate a Nutraceutical with Polyunsaturated Fatty Acids (PUFAs) and Oxidation-Catalyzing Iron and Zinc

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Introduction: For "mental and cognitive health performance" EFSA (European Food Safety Authority) accepts RDA (recommended daily allowances) of Fe (15 mg/d) and Zn (10 mg/d). Ω3 polyunsaturated fatty acids (PUFAs) are essential for brain development and recommended upon deficiency, during pregnancy and early childhood. Equazen®, a marketed PUFA nutraceutical, demonstrated positive effects on working memory (learning skills) [1,2]. To check redox interactions of Fe, Zn and a vegetable antioxidant (AO) with PUFA a simple liquid formulation test was evaluated.
Methods: Tocopherol-stabilized PUFA triglycerides (DHA($C_{22:5}$): EPA($C_{20:5}$):GLA = 9:3:1, rel. conc.) was assayed for peroxides [PV] and p-anisidine [AV] for primary and secondary oxidation products (Ph.Eur.). The oil-mineral suspension was filled in brown snap cap vials. Different Fe(II)salts, a Fe(III)-hydroxy-polymaltose complex (Maltofer®), inorganic and organic Zn salts in RDA were tested at 40 °C and 75% rel. humidity over 4 weeks. Three samples per time point were centrifuged (10 min, 4000 rpm) and the supernatant analyzed. A plant-derived AO, rich in phenols and rosmarinic acid,

was checked for stabilizing. Saturated medium chain triglycerides (Miglyol®) served as blank.
Results: PV/AV [mean ± SD] at 40 °C/75% humidity; *exceeding Ph. Eur. limits.
(i) Blank: 0/0
(ii) Equazen® (t = 0): 1.0 ± 0.0/6.9 ± 0.9; (t = 4 weeks): 38.7 ± 0.2*/24.1 ± 2.5.
(iii) Equazen® + Fe sulfate or fumarate or Maltofer® (t = 4 weeks): 18.2*/143* or 25.6*/330* or 27.5*/150*.
(iv) Equazen® + Zn sulfate or Zn lactate (t = 4 weeks): 19.0*/140.0* or 11.0*/31.3*.
(v) Equazen® + Maltofer® + Zn lactate: without AO (t = 0): 1.2 ± 0.0/2.1 ± 0.3; (t = 4 weeks): 20.8 ± 0.1*/49.8 ± 0.2*; with AO (t = 0): 0.0 ± 0.0/0.6 ± 0.1; (t = 4 weeks): 16.3 ± 0.1*/14.1 ± 0.4.
Conclusions: (1) Ambient air and elevated temperatures oxidize liquid PUFA readily despite tocopherol content (Ph.Eur. limits for PV (<10) and/or AV (<30) exceeded within 4 weeks). (2) Transformation of peroxides into secondary products is not concentration-correlated, but catalyzed by Fe(III) < Fe(II); Zn lactate < Zn sulfate. The AO significantly increased the stability (PV and AV within the limits after 4 weeks stress test). (3) The proposed oxidation test in a liquid formulation is sensitive, reliable, non-expensive and assists a rational evaluation of PUFA formulations with potentially interacting pro-oxidatives.

Keywords: Polyunsaturated fatty acids (PUFAs), iron, zinc, tocopherol.

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P-61

Is Growth Inhibition of *Trypanosoma Brucei* Induced by 4-[5-(4-Phenoxyphenyl)-2H-pyrazol-3-yl]morpholine Due to an Alteration of the Adenylate Energy Charge?

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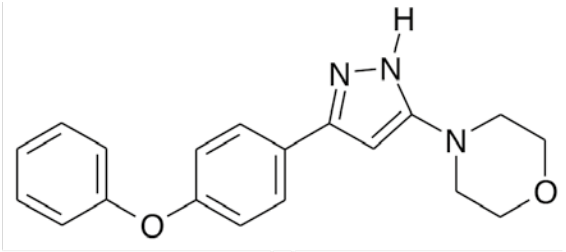
Introduction: *Trypanosoma brucei* is the causative agent of Human African Trypanosomiasis (HAT; sleeping sickness). The existing drugs against HAT have severe side effects, are expensive and need to be administrated over long periods and by injection. Therefore safe and effective new medicines are required to treat this disease. Our previous research reported 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine (Fig. 1: **1**) to exhibit antitrypanosomal activity with an IC_{50} of 1 μM and chemical proteomics identified *Trypanosoma brucei* adenosine kinase (TbAK) as the intracellular target. Compound 1 is a strong activator of TbAK, an important enzyme involved in the purine salvage pathway [1,2].
Aims: The aim of the present project is the elucidation of the mechanism of action of (1) leading to growth inhibition of *Trypansoma brucei.*
Methods: The intracellular purine levels of Trypanosomes in presence and absence of 1.5 μM of (**1**) were analyzed using an ion-pair HPLC/UV method.
Results: The results revealed that the adenylate energy charge [3] as well as ADP/ATP and AMP/ATP ratios were altered upon incubation of the cells with the compound. Furthermore, the ATP/AMP ratio varied as the square of the ADP/ATP ratio while the GTP/ATP balance remained unaffected.
Conclusions: Taken together, we find that (**1**) interferes with adenine nucleotide levels, with AMP being the key regulatory molecule of the adenylate energy charge in the parasite.

Figure 1. Chemical structure of 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]-morpholine.



(1)

Keywords: *Trypanosoma brucei,* adenosine kinase, purines, ion-pair HPLC/UV.

References:
[1] S. Kuettel et al. J Med Chem 2007; 50: 5833.
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P-62

Synthesis and Biochemical Characterization of Pyrimidine Derivatives as Potential PET Reporter Probes

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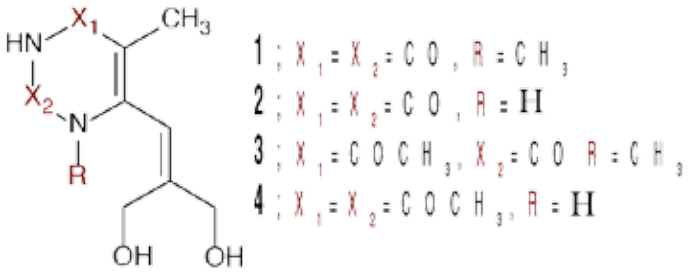
Introduction: Many purine and pyrimidine analogues are known to localize selectively in herpes simplex virus–infected cells due to unique monophosphorylation by virus-encoded thymidine kinase (TK). The monophosphates are converted to diphosphates, and then to the corresponding triphosphates by cellular enzymes [1]. The triphosphates prevent viral replication by inhibition of the viral DNA polymerase which has grater specificity. Radiolabeling of these HSV-1 TK substrates with a positron-emitting isotope allows noninvasive imaging of viral-kinase-enzyme activity by means of positron-emission tomography (PET) [2,3].
Aims: This project aims at synthesizing molecules with an unsaturated alkyl chain as a sugar mimicking moiety in the C-6 position and biochemically characterizing them in order to select the best candidate for developing PET tracers.
Results: *Chemistry:* 6-[(3-Benzyloxy-2-benzyloxymethyl-2-hydroxy) propyl]-1,5-dimethyl-2,4-dimetho-xypyrimidine was prepared as a starting material for the synthesis. Compounds **3** and **4** were prepared according to the 5-steps synthesis and compounds **1** and **3** were obtained in 2 steps starting from compound **3** and **4**. The identity of the synthesized compounds was confirmed by 1H and 13C NMR and MS. The purity of the compound is at least 98% as assessed by HPLC and TLC.
Biochemical Evaluation: The phosphorylation pattern of the newly synthesized compounds in presence of HSV-1 TK or hTK was monitored up to 90 min using a protocol based on HPLC-UV/DAD [4]. Three independent experiments were performed in triplicate. All four synthesized compounds were phospho-rylated in presence of HSV-1 TK but not in presence of hTK. Interestingly, compound **2** is diphospho-rylated by HSV-1 TK. This is the first time that a non natural substrate is diphosphorylated as the natural substrate thymidine.



Conclusions: The synthesis of 4 compounds was successful and their biochemical characterization revealed that they are all substrates of HSV-1 TK, thus promising lead structures for the design and synthesis of a new PET tracer.

Keywords: Pyrimidine analogs, hTK, positron-emission tomography (PET), HSV-1 TK.

References:

[1] Y. C. Cheng et al. J Biol Chem 1983; 258: 12460.
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P-63

In Vitro Silencing Activity of Amphiphilic Antisense Oligonucleotides

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Introduction and Aims: Antisense oligonucleotides (AONs) are a class of compounds with high therapeutic potential. One of the challenges facing this platform is the development of effective techniques to achieve cellular delivery. In the present study, a library of amphiphilic AON derivatives was synthesized and the *in vitro* silencing activity upon carrier-free transfection of the compounds was evaluated. It was found that sub-micromolar overnight incubation of carrier-free docosanoic acid (DSA)-conjugated antisense drug could downregulate up to 80% of the targeted Bcl-2 mRNA. Lastly, the impact of serum proteins addition, and more specifically, human serum albumin (HSA), on the transfection activity of the unformulated AONs was also examined, with the aim of identifying new strategies for improving the *in vivo* delivery of amphiphilic AONs.
Methods: Standard phosphoramidite solid-phase synthesis conditions were used for the synthesis of all chemically-modified and unmodified AON. Corresponding lipophilic moiety was conjugated via an amino-hexanol-linker to the 5′-end of chemically-modified AON sequences. Final products were purified by reverse phase HPLC. 800 nM of synthesized conjugates were incubated, in a carrier-free fashion, overnight in presence or absence of serum protein on PC-3 cells. Seventy-two hours post-transfection, the gene expression levels of Bcl-2 were assessed by qRT-PCR, as described elsewhere [1].
Results: Docosahexaenoic acid, cholesterol, and DSA were successfully attached to the AON. For all coupling reactions, good conversions were obtained (70–80%). In absence of serum, it was found that derivatives featuring a DSA (i.e., a saturated C22 fatty acid) moiety exhibited the highest knockdown efficiencies compared to all other lipophilic-grafted compounds (up to 80% mRNA suppression). The efficacy of the carrier-free amphiphilic AON was, however, decreased in a dose-dependent fashion when HSA was added in the transfection medium. Furthermore, it was observed that blocking the binding site of amphiphilic AONs to HSA with a short, free, fatty acid could re-establish the gene silencing of the non-formulated conjugates.
Conclusions: In the absence of serum protein, it was found that the most potent amphiphilic structure was the AON-DSA. However, introduction of serum or HSA in the transfection media decreased the antisense activity due to association with the (lipo)proteins. Interestingly, pre-incubation of HSA with free fatty acids completely restored the transfection activity of the amphiphilic AON. We believe that a strong association of hydrophobized AON to plasma proteins would in most cases impair the AON activity, and therefore strategies aiming at locally preventing such interactions could be tested in the future.

Keywords: Antisense oligonucleotide, fatty acid, albumin.

Reference:

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P-64

Homogeneous Antibody Conjugates: Site-Specific Modification by Transglutaminase

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Introduction: One of the remaining challenges of antibody conjugates is to overcome product heterogeneity: depending on the site of attachement and the physico-chemical characteristics of the ligand, the properties can vary between conjugates with different ligand-to-antibody ratios. Thus, methods which yield site-specific and stoichiometrically uniform antibody conjugates are crucial for the future development. Transglutaminases (TGase) catalyze the acyl transfer reaction between the γ-carboxyamide group of glutamine residues and various primary amines [1]. Hence, TGase is a powerful tool for protein conjugation as it allows the production of stoichiometric homogeneous conjugates with site-specific attachment of various ligands [2,3].
Aims: The aim of this study is to develop two novel strategies to produce homogeneous antibody conjugates using TGase. The technique should be applicable for a broad range of immunoglobulins without the need of prior molecular engineering of the antibody.
Methods: Here, we use two different approaches to generate novel antibody conjugates: (i) a ligand, containing a primary amine, is directly attached to a glutamine residue in the natural sequence of a deglycosylated antibody by microbial translglutaminase (MTGase) of *Streptomyces mobaraensis,* (ii) a Fc-binding domain (ZZ-domain) is labeled with a ligand of interest by MTGase. Mixing of coupled ZZ-domain with antibody yields a non-covalent conjugate with defined sites of attachment. Because the ZZ-domain lacks an intrinsic sequence which is is modified by MTGase, we designed different glutamine-tags suitable for enzymatic modification with MTGase and attached them to the ZZ-domain by using standard molecular biology techniques. ZZ-domains were expressed in *E.coli* HB101 and purified on an IgG-sepharose column. The coupling reaction is monitored by liquid chromatography coupled to a mass spectrometer with ESI source (LC-ESI-TOF).
Results: Enzymatic modification of deglycosylated anti-L1-antibody (chCE7degl) and aglycosylated variant N297Q (chCE7agl) could be accomplished in a quantitative yield. Defined ligand-to-antibody ratios enabled us to directly compare the influence of the ligand on pharmacokinetics (biodistribution study) and physico-chemical properties (isoelectric focusing) of the immunoconjugate. Expression of ZZ-domains yielded ~4 mg per L medium for each tagged

variant. Furthermore, we successfully labeled tagged ZZ-domain at a specific glutamine residue in the tag with various primary amine containing ligands including biotin, fluorescent dyes or chelators for radioisotopes. Using trypsin digestion to map peptides revealed the tag-glutamine as sole site of modification.

Conclusions: Using MTGase for enzymatic antibody conjugation proved to be a promising tool for the generation of homogeneous immunoconjugates. Moreover, tagged ZZ-domains can be functionalized with a variety of ligands using MTGase. They can therefore be used as versatile adaptor-molecules for non-covalent modification of antibodies.

Keywords: Microbial transglutaminase (MTGase), antibody conjugate, mass spectrometry, ZZ-domain.

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Swiss Society of Industrial Pharmacists

GSIA SSPI

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The Swiss Society of Industrial Pharmacists is an association of primarily pharmacists and other academic life-science professionals working in the Swiss pharmaceutical industry. To its members, the society is providing services and opportunities for networking and contacts within the pharmaceutical industry. Moreover, the society supports and rewards young academics, particularly in industrial pharmacy.

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Production Pharmaceutique Unité de Séparation des Gaz de l'Air

7–8/12 Des gaz liquéfiés extraits de l'air: la gamme VERISEQ® destinée à l'industrie pharmaceutique
Production de gaz liquéfiés dans l'unité de séparation des gaz de l'air de Muttenz – Les gaz VERISEQ® répondent aux exigences spécifiques de l'industrie pharmaceutique
Maja Studer, Dipl. Lm-Ing. ETH, Techniques & Services, Segments industriels, Chimie, Pharma, et Biotechnologie, PanGas, Muttenz

Spirig Pharma AG • Dermatologika

9/12 Spirig Pharma AG: Seit vielen Jahren unbestrittener Marktleader im Bereich Dermatologie im Pharmamarkt Schweiz
Nach der Ausgliederung der Generikasparte in die Spirig HealthCare AG und deren Verkauf an die STADA Arzneimittel AG setzt die Spirig Pharma AG ihre Wachstumsstrategie in der Dermatologie mit der Suche nach einer strategischen Partnerschaft mit einem multinationalen Player konsequent um
Gespräch mit Dr. Silvio Inderbitzin, Delegierter des Verwaltungsrates, und mit Dr. Beat Sägesser, CEO, Spirig Pharma AG, Egerkingen

Pharmaverpackung • Stickpacks • SVI

9/12 Stickpacks – Die neue Convenience-Verpackung für die Pharmaindustrie
Bericht über den vom Schweizerischen Verpackungsinstitut SVI am 28. August 2012 bei der Firma Invers-Lee AG, Burgdorf, durchgeführten SVI Pharma Roundtable zum Thema «Stickpack- die ideale Portionenverpackung»
Dr. Peter Schüpbach, Ivers-Lee AG, Burgdorf
Prof. Dr.-Ing. Eugen Herzau, Hochschule für Technik, Wirtschaft und Kultur Leipzig, Leipzig (D)
O. Becker, Merz Verpackungsmaschinen GmbH, Lich (D)
Dr. Georg Kokkinis, Dr. Kokkinis GmbH, Basel

Pharmaherstellung • Sterilproduktion • Parenteralia

9/12 Die neue «State of the art» Parenteralia Produktion von Roche in Kaiseraugst: Von der Projektidee zur Realisierung
Die Schweizerische Gesellschaft der Pharmazeutischen Wissenschaften (SGPhW) begrüsste Dr. Rainer Schmidt, Site Head Kaiseraugst, Drug Product Manufacturing, Pharma Technical Operations Biologics, der F. Hoffmann-La Roche Ltd. in Kaiseraugst als Gastreferent an ihrem PharmaLunch vom 28. September 2012
Gespräch mit Dr. Rainer Schmidt, F. Hoffmann-La Roche Ltd., Kaiseraugst

Pharmazeutische Mikrobiologie • CPM-Meeting

9/12 Curriculum für pharmazeutische Mikrobiologie (CPM)
Bericht vom 17. CPM-Meeting in Hoyerhagen/Niedersachsen (D)
Dr. Michael Rieth, Merck Serono, Darmstadt (D)

Swiss Pharma Science Day 2012

10/12 University of Bern, 29 August 2012
Swiss Societey of Pharmaceutical Sciences (SSPhS)
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SWISS PHARMA: «Mehr als 30 Jahre im Gespräch mit der Pharmazeutischen Industrie der Schweiz» – Live-Interviews der Jahre 1979 bis 2011

Felix Wüst

In unserem Verlag erschien im Gründungsjahr 1979 – neben vier weiteren Titeln – auch die erste Ausgabe der Zeitschrift SWISS PHARMA, Schweizerische Zeitschrift für die pharmazeutische Industrie (ISSN 0251-1673). Der Titel erscheint nunmehr im 34. Jahrgang (2012) und darf trotz Internet weiterhin grossem Interesse begegnen.

Von Anbeginn an haben wir in SWISS PHARMA Live-Interviews mit Spitzenpersönlichkeiten aus der Pharmaindustrie veröffentlicht. Niemand «durfte sich melden». Wir haben ausnahmslos sämtliche Gesprächspartner immer selber ausgewählt. Niemand wurde dafür je honoriert. Alle haben sich ausnahmslos spontan zu den Gesprächen bereit erklärt. Nie hatte es eine Absage gegeben. «Bedingung» für die Interviews war allerdings immer, dass die Gespräche unvorbereitet, eben «full live» stattzufinden hatten. Und so war es, und das war immer ein grossartiges Erlebnis.

Immer wieder erreichten uns Anfragen nach früher erschienenen Interviews, die wir aber leider nicht befriedigend beantworten konnten, war es doch ein Ding der Unmöglichkeit, von allen Heften seit 1979 auch nur 10 oder 20 Exemplare zu lagern. Nun haben wir sämtliche in SWISS PHARMA je erschienenen Interviews mit genauen bibliographischen Angaben aufgelistet (mit Angabe der Seitenzahlen), so dass ein Interessent bei der Zentralbibliothek Zürich bequem und für wenig Geld Fotokopien anfordern kann. Der Verlag stellt ein Verzeichnis aller SWISS PHARMA-Interviews gerne kostenlos in elektronischer Form zur Verfügung. Mit dieser Dokumentation wird auch mitgeteilt, wie man bei der Zentralbibliothek Zürich per E-Mail Fotokopien eines oder mehrerer Interviews anfordern kann. Das ist möglich, weil die Auflistung wie erwähnt jeweils die Seitenzahlen in den betreffenden Heften aufführt, so dass der Interessent exakt jene Druckseiten als Fotokopien anfordern kann, die er benötigt. Die Zentralbibliothek Zürich berechnet sehr vernünftige Preise für diese Fotokopien: Bis zu 20 A4-Seiten pauschal CHF 10.–; jede weitere A4-Seite zu CHF –.50 (50 Rappen). Die Kopien werden per Briefpost und mit Rechnung an den Besteller zugestellt.

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