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# 6th SWISS PHARMA SCIENCE DAY 2013

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)

***"Being the link between Industry and University!" – This is the aim of the Swiss Pharma Science Day (SPhSD). This mission was fulfilled for the 6th time this year, 2013. The highlight of the day was the lecture of Prof. Ernst, winner of the Nobel Prize for chemistry in 1991. The SPhSD was also a great opportunity for the professionals to meet colleagues from other fields of Pharmaceutical Sciences, and for the motivated students to present their work at the university on a poster.***

Nowadays, it is easily possible for academics and students to access e-sources of publications. Every written publication gives undoubtedly detailed information, but nothing can replace the human contact! Thus, the SPhSD offers the chance to scientists, active in Pharmaceutical Sciences, to meet each other in a stimulating environment for discussion and exchange of thoughts and experiences. The topic of the lectures was chosen in order to show different aspects of current scientific, political and historical issues.

Dr. Christine Moll, Dr. Benoîte Kaeser, and Prof. Ursula von Mandach, board members of the SSPhS, at the registration desk

## Addresses of Welcome

**Prof. Dr. Gerrit Borchard, President SSPhS**
**Prof. Dr. Rudolf Brenneisen, President SAPhS**
**Prof. Dr. Peter Eggli, Dean Faculty of Medicine**

The SPhSD was opened by Prof. Gerrit Borchard, president SSPhS, who thanked the University of Bern for its hospitality and willingness to once again host this event. His words were followed by a welcoming speech of the Dean of the Faculty of Medicine, Prof. Peter Eggli. The morning session was chaired by Prof. Rudolf Brenneisen, president SAPhS. It started with a political speech from the state Secretary for Education, Research and Innovation, Dr. Mauro Dell'Ambrogio, followed by a presentation from Prof. Dr. Brigitte Kopp, University of Vienna, who presented her research in Traditional Chinese Medicine (TCM). After this, Prof. em. Dr. Peter Gehr, University of Bern, gave an insight in the "Opportunities and Risks of Pharmaceutical Nanoparticles". After lunch, sozializing, networking and the poster session, the afternoon session was chaired by Prof. Borchard, starting with „Systems Pharmacology – Towards Multitarget Therapeutic Interventions" presented by Prof. Meindert Danhof, University of Leiden. Then, Uwe E. Jocham, CSL Behring Bern, discussed the future of immunoglobulins in the pharmaceutical industry. The last lecture was given by Prof. em. Richard R. Ernst, ETH Zurich, winner of the Nobel prize of chemistry in 1991 and SSPhS Reichstein medal 2000 – a most impressive and charming personality. What a fascinating retrospect in NMR and MRI, highly essential tools in chemistry and medicine!

Prof. Rudolf Brenneisen, President SAPhS, organizer of SPhSD

Prof. Peter Eggli, Dean Faculty of Medicine, and Dr. Umut Soydaner, member of the organizing team

## Lecture 1: Keynote Speech

**Dr. Mauro Dell'Ambrogio, State Secretary for Education, Research and Innovation, Bern:**
**„Federal Promotion of Research and Innovation 2013–2016: Guidelines, Objectives and Instruments"**

Dr. Mauro Dell'Ambrogio, State Secretary for Education, Research and Innovation

*(The full text of the Keynote Speech is published on the pages 9 through 11 of this issue of SWISS PHARMA 10/13).*

## Lecture 2: Pharmaceutical Biology

**Prof. Dr. Brigitte Kopp, University of Vienna:**
**„Assessment of Quality and Safety of TCM Drugs as Challenge for the Future"**

Traditional Chinese Medicine (TCM) is an ancient Chinese practise that employs different methods for the treatment of medical conditions including the use of medicinal plants. Chinese Traditional Herbal Drugs (CTHD) have become increasingly popular in the Western world during the last decades. This also generated observations regarding frequent plant material falsification and mix-up. In many cases, CTHD also undergo paozhi-processing, a technique that implies different treatments like roasting or curing prior to formulation into herbal medicines. Processing of plant material is new to Western herbal medicine and leads to changes of the chemical composition. This can affect the therapeutic properties of the plant and can also serve for detoxification purposes. For European patients, the safety and quality of CTHD is of major importance. Therefore, monographs of the most prominent plants used in TCM have been included in the European Pharmacopoeia and efforts continue to focus on quality standards for CTHD.

Prof. Brigitte Kopp, University of Vienna

## Lecture 3: Nanotechnology

**Prof. em. Dr. Peter Gehr, University of Bern:**
**„Opportunities and Risks of Pharmaceutical Nanoparticles"**

Prof. Peter Gehr, University of Bern

Nanoparticles (particulate matter ≤100 nm) are the building blocks of one of the key technologies of the 21st century. While the size of these structures is growing smaller, their importance is growing bigger in both technological and economic terms. Nanoparticles are in the process of revolutionizing technological applications from industry through to pharmacy and medicine. Despite their enormous potential, the generation, use and disposal of nanoparticles can be a risk to humans and the environment.
Areas have to be identified and promoted where research is needed in order to better understand the major opportunities and possible risks posed by products including pharmaceutical products based on engineered nanoparticles.
The research projects of the National Research Program 64 "Opportunities and Risks of Nanomaterials" of the Swiss National Science Foundation will help to solve problems and answer questions related to such particles. The research carried out under this program will provide a scientific basis for recommendations and appropriate measures with regard to the generation, use and disposal of engineered nanoparticles, including pharmaceutical nanoparticles. The insights gained from these studies and their applications will benefit the society at large and help protect the consumer and the environment.
Nanoparticles mainly enter our organism by inhalation. The intact skin is a good barrier against nanoparticles. Most of the nanoparticles entering via oral way will pass through the gastrointestinal tract; only few may enter the tissue, mainly in the small intestines. Nanoparticles may, however, also be intravenously injected.

In the lungs, deposited particles may penetrate through the air-blood tissue barrier and enter the vascular circulation. These particles as well as those directly injected into a vessel will translocate into all organs, but in different amounts. It is not clear yet, by which mechanisms they enter and leave the blood stream and what their effect is in the different organs. These are questions under extensive investigation worldwide.
Pharmaceutical aerosols are planned to be used for therapeutic reasons. Depending on the purpose any type of nanoparticle can be engineered. Such nanoparticles can contain a magnetic core to be guided to a certain location. They can contain another metal core which can be warmed from outside to kill diseased cells and tissue. They can also be coated with certain receptors to dock at specific target locations.
This talk will present the lung as the main portal of entry for nanoparticles. It will discuss the fate of inhaled nanoparticles including possible mechanisms of entering cells and tissue, and it will finally present some pharmaceutical aerosols.

## Poster session

During the lunch break, the participants of the SPhSD had the opportunity to enjoy discussions between each other about many different subjects, but most importantly they were able to see the unique 72 posters being presented. The authors of the posters were available for comments and questions related to their research.

Participants, enjoying lunch break

Prof. Kurt Hersberger, University of Basel, lively discussing with Dr. Stephan Buchmann, President GSIA

Helene Kettiger, poster author, presenting her data to Dr. Alfred Bretscher, Honorary Senator University of Bern

Dr. Isabelle Arnet, University of Basel, checking poster list

Prof. Georg Imanidis, University of Applied Sciences Basel-Muttenz and board member SSPhS, Prof. Hans Leuenberger, former President SSPhS, and Helene Kettiger, University of Basel

Prof. Muriel Cuendet, University of Geneva, and Prof. Gerrit Borchard, University of Geneva and President SSPhS

Prof. Matthias Hamburger, Fellow SSPhS, and Prof. Henriette Meyer zu Schwabedissen, both University of Basel

Participants, enjoying coffee break

## Lecture 4: Pharmacology

**Prof. Dr. Meindert Danhof, University of Leiden: "Systems Pharmacology – Towards Multitarget Therapeutic Interventions"**

Prof. Meindert Danhof, University of Leiden

Systems therapeutics constitutes a novel therapeutic approach to the treatment of chronic progressive disorders. Systems therapeutics is characterized by: (i) individualized treatment modalities, (ii)

disease modifying rather than symptomatic drug effects, (iii) focus on preventive and pre-emptive treatments and (iv) the use of multi-target drugs or rational drug combinations. Due to their inherent complexity systems therapeutic approaches cannot be developed, nor be clinically applied, by trial and error. Model based approaches are essential to the success of systems therapeutics.
In recent years important progress has been made in the field of systems biology. The emphasis in this research has been on the identification of complex networks rather than linear pathways underlying biological processes. The interactions of drug molecules with multiples targets in the biological network are of considerable therapeutic interest as they may lead to enhanced therapeutic efficacy resulting from synergistic interactions.
In recent years progress has also been made in the field of in vivo quantitative pharmacology. Research in this field has focused primarily on the modeling of linear pathways of drug action. Quantitative pharmacology models have been proposed which contain specific expressions to characterize a) the target distribution, b) the target interaction/activation, c) transduction/homeostatic control mechanisms [1,2], and d) the disease processes and disease progression [3]. This modeling relies on novel bio-markers to quantify processes on the causal path between drug administration and response [4].
The combination of systems biology and quantitative pharmacology concepts opens a novel perspective on the development of systems therapeutics, based on multi-target drugs and/or rational drug combinations leading to synergism. The modeling of pharmacodynamic interactions will be essential for the development and the clinical application of these complex therapeutic interventions [5].

**References**:

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## Lecture 5: Biotechnology

**Uwe E. Jocham, Senior Vice President and General Manager CSL Behring AG, Bern:**
**„Immunoglobulins: Locally Successful in Global Niche of Biotech Industry"**

CSL – a global biopharmaceutical company headquartered in Melbourne, Australia, has over 10'500 employees working in more than 25 countries. With its subsidiary, CSL Behring, a global leader in the plasma protein therapeutics industry and headquartered in the U.S., the company researches, develops, manufactures and markets a range of live-saving and live-improving plasma-derived and recombinant products. With major manufacturing facilities in Australia, the U.S., Germany and Switzerland the company also operates one of the world's largest plasma collection networks, CSL Plasma.

CSL Behring AG is the "Center of Excellence" for immunoglobulins of the CSL Behring group. At its facility in Bern, Switzerland, CSL Behring AG employs more than 1'200 people. The company can look back on a long and successful history in the market for immunoglobulin products. The combination of quality, innovation and the latest technology has made CSL Behring AG a world leader in the field of immunotherapies. In its production facilities, therapeutically important proteins are isolated from more than five million litres of human plasma each year in accordance with the strictest safety and quality standards and manufactured into medicines. CSL Behring AG's products, immunoglobulins and albumin are distributed worldwide.
Over the years CSL Behring AG has continuously developed and improved its products and manufacturing processes by using the latest technologies. In 1999 CSL Behring AG was the first company in the sector to include an additional safety step to the manufacturing process for its immunoglobulins by introducing virus filtration (nanofiltration). The production of therapeutics from plasma is a complex process involving fractionation. Here, ethanol, filter aids and buffer substances are added to tanks with a capacity of several thousand litres to bring about precipitation of the specific active agents, which are trapped with filter presses and isolated. These pastes are the starting material for the subsequent process steps used to manufacture the products.
In 2008 CSL Behring launched its new generation immunoglobulin in the U.S., the first proline-stabilized 10% liquid intravenous immunoglobulin, a joint development between the CSL R&D teams of Broadmeadows (Australia) and Bern. Only 2 years later, in 2010, the successful launch was followed by the U.S. launch of the first and only FDA approved 20% immunoglobulin for subcutaneous administration. Both products are manufactured in the new state of the art facility at the Bern site. Since 2000 more than 300 million Swiss Francs have been invested in new facilities and capacity expansions at the Bern site. The company has grown from 560 employees in 2000 to more than 1'200 today. This makes CSL Behring AG the largest industrial employer in the city of Bern and an employer of choice.
CSL Behring AG maintains a close collaboration with universities, scientific and educational institutions, and as a member of several economic and scientific associations, e. g. Medical Cluster and others, is well positioned within the regional network.
With the support of numerous institutions, the company acknowledges the promotion of young academic talents. CSL Behring AG makes a valuable contribution to the local community by supporting local cultural institutions and events.

Uwe E. Jocham, CSL Behring, acknowledged by Prof. Gerrit Borchard

## Lecture 6: Special Lecture of the Chemistry Nobel Laureate 1991 and SSPhS Reichstein Medal Winner 2000

**Prof. em. Dr. Dr. h.c. mult. Richard R. Ernst, ETH Zurich: „NMR and MRI, History and Prospects"**

Prof. Richard Ernst, ETH Zurich, Nobel Prize Laureate and SSPhS Reichstein Medal Winner

The lecture sketches the exemplary importance nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) have reached in the natural sciences. Virtually all sciences can be addressed in a direct or more remote way by powerful exploratory tools that all relay on the interrogation of nuclear spins, functioning as sensors in objects and beings. Certainly, the pharmacological context is touched in many ways between "molecules and man". Magnetic resonance is still a young science since its inception 1946. It started as a physical phenomenon that soon has found fruitful applications in chemistry, biology, and in medicine. It became an enormously flexible tool, allowing the exploration of simple molecules to addressing clinical medical and even psychological questions. Magnetic resonance is still in an expansive phase of development and many further astounding applications can be perceived to become reality in the near future.

## Recognitions and Awards

The Swiss Academy of Pharmaceutical Sciences (SAPhS) consists of scientists who are distuinguished by their outstanding research and professional contributions in the field of Pharmaceutical Sciences. Usually, every year two scientists are awarded "Fellows of the SAPhS". In addition, at the end of the event six prizes are given to students, who have presented the best abstracts and posters.

## Fellows 2013

**Prof. Dr. Ursula von Mandach, University of Zurich Hospital**
**Prof. Dr. Bruno Gander, ETH Zurich**
**New Fellows of the Swiss Society of Pharmaceutical Sciences (SSPhS) and new Members of the Swiss Academy of Pharmaceutical Sciences (SAPhS)**

Prof. Dr. pharm. Ursula von Mandach, Department of Obstetrics and Gynecology, University of Zurich Hospital, has been designated a Fellow of the Swiss Society of Pharmaceutical Sciences (SSPhS) and a Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) "For her merits in establishing and promoting research and education in the field of Perinatal Pharmacology".

Prof. Ursula von Mandach, SSPhS Fellow 2013

Prof. Dr. sc. nat. Bruno Gander, Institute of Pharmaceutical Sciences, ETH Zurich, has been designated a Fellow of the Swiss Society of Pharmaceutical Sciences (SSPhS) and a Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) "For his merits in establishing and promoting research and education in the fields of advanced drug delivery and biomaterials".

Prof. Bruno Gander, SSPhS Fellow 2013

## Poster Award Winners

**1st Prize, sponsored by the Foundation of the Association of Bernese Pharmacists (AKB):**
**Stephanie Haller (coauthors: J. Reber, R. Schibli, C. Müller), ETH Zurich & PSI Villigen**
**P-19: „Comparative Study with Two Folic Acid Radioconjugates Showing Differences in Anti-Tumor Efficacy and Kidney Dose Burden"**

Stephanie Haller, First Poster Prize winner, awarded by Michele Bordoni, President AKB

**2nd Prize, sponsored by the Foundation of the Society of Industrial Pharmacists (GSIA):**
**Omar Sakr (coauthor: Gerrit Borchard), University of Geneva**
**P-65: "Double Chambered LbL Nanoparticles Coated with Viral Proteins as a Novel Intracellular Delivery System"**

Omar Sakr, Second Poster Prize winner

**3rd Prize, sponsored by the Pharmazeutische Gesellschaft Zürich (PharmGZ):**
**Lionel Sacconnay (coauthors: M. Angleviel, G.M. Randazzo, M. Marçal Ferreira Queiroz, E. Ferreira Queiroz, J. L. Wolfender, P.A. Carrupt, A. Nurisso), University of Geneva**
**P-5: "Antitrypanosomal Natural Compounds and Epigenetics: A Target-Fishing Approach Involving Sirtuins"**

Lionel Sacconnay, Third Poster Prize winner, awarded by Vroni Jakob-Alther, PharmGZ

**Special Prize, sponsored by Vifor Pharma Fribourg:**
**Stefan Gaugler (coauthors: T. Hettich, G. Schlotterbeck), University of Applied Sciences and Arts Northwestern Switzerland**
**P-44: "Biomarker Analysis in Dried Blood Spots"**

Dr. Sergio Mantelli, Vifor Pharma, awarding the Special Poster Prize to Stefan Gaugler

**Prize for best poster in Pharmaceutical Technology, sponsored by Technology Training Center Glatt, TTC:**
**Leonie Hattler (coauthors: R. Alles, J. Huwyler, M. Puchkov), University of Basel**
**P-39: "TIC – Tablet-In-Cup Drug Delivery Device"**

Ansgar Meermann, TTC Glatt, awarding the prize for the best poster in Pharmaceutical Technology to Leonie Hattler

**Prize for best poster in Pharmaceutical Biology, sponsored by Max Zeller & Söhne AG Romanshorn:**
**Simon Nicolussi (coauthors: J.M. Viveros-Paredes, M.S. Gachet, M. Rau, M.E. Flores-Soto, M. Blunder, J. Gertsch), University of Bern, University of Guadalajara, Mexico, and University of Uppsala, Sweden**
**P-56: "Guineensine is a Potent CNS-Active Inhibitor of Endocannabinoid Uptake and Cyclooxygenase-2 Showing Analgesic and Anti-inflammatory Effects"**

Simon Nicolussi, receiving the prize for the best poster in Pharmaceutical Biology from Dr. Catherine Zahner-Daniel, Max Zeller Söhne AG

## Acknowledgements and Invitation to the 7th SWISS PHARMA SCIENCE DAY in Bern on Wednesday, August 20, 2014

It is a tradition to finish the Swiss Pharma Science Day with an apéro in the beautiful House of the University of Bern, allowing to socialize and network facilitated by wine and beer.
The organizers would like to thank the following sponsors for supporting this event:
Novartis AG (platinum sponsor), AKB Foundation (gold sponsor, 1st poster prize and lecture Prof. Gehr), GSIA Foundation (2nd poster prize), PharmGZ (3rd poster prize), TTC Glatt Group (best poster in Pharm. Technology), Max Zeller Söhne AG (best poster in Pharm. Biology), Vifor Pharma (special poster prize), Mundipharma Medical Comp., CSL Behring AG, Bruker BioSpin AG (lecture Prof. R.R. Ernst), Galexis AG, Verlag Dr. F. Wüst AG, pharmaSuisse, University of Bern, Swiss Society of Pharmaceutical Sciences.
Last but not least, the thanks goes to all speakers for their excellent lectures.

Christophe Aeby (right), President asep and photographer, together with colleagues

Participants, relaxing after SPhSD 2013

Dr. Astrid Czock, pharmaSuisse

Proff. Gerrit Borchard and Stefan Mühlebach, President and Vice-President SSPhS

The SSPhS board

6th SWISS PHARMA SCIENCE DAY 2013 (www.sgphw.ch), Bern, 28th August 2013 (Keynote Lecture)

# Federal promotion of research and innovation for 2013–2016: guidelines, objectives and instruments

Speech[1] by Dr. Mauro Dell'Ambrogio, State Secretary for Education, Research and Innovation, Bern

Mr Chairman,
Ladies and Gentlemen,

It is an honour and a privilege for me to be speaking to you at the SWISS PHARMA SCIENCE DAY 2013. I am very glad to accept invitations such as this one since such symposiums or thematic events give me the opportunity to "visit the front" and gain important momentum for my work.
It is always a good idea to keep the entire system in mind when working within the Federal Administration and when preparing policies for Switzerland's education, research and innovation sector (ERI sector). The individual components and levels within this system may be likened to the gears and cranks of a complex machine, all interacting closely with one another: if we turn a tiny screw in one part of this machine, even with the best of intentions, we create undesired effects in an entirely different part – I shall come back to this briefly at the end of my presentation.
At the same time, we cannot gain a coherent overview of the entire system without a clear understanding of these individual components, their specific needs and performance levels. As a case in point, pharmaceutical sciences have long played a supporting role across the entire chain "Training => Fundamental research => Applied research and development => Marketable innovation" in Switzerland. Without the numerous small-, medium- and large-sized chemical and pharmaceutical companies and their considerable economic weight, Switzerland would not be the country it is today. To use a very common cliché, it would be like depriving us of milk, chocolate and the Matterhorn – entirely unthinkable!
Ladies and Gentlemen, pharmaceutical science – with its often interdisciplinary approaches and specialisations in such fields as biotechnology, pharmacology and molecular biology – has helped to make Switzerland a competitive location for research and production. And for this, I would like to thank you wholeheartedly. In fact, all of you deserve recognition for your individual contributions to teaching, science and research. I would also like to thank the Swiss Society of Pharmaceutical Sciences for its commitment.
In a country such as ours, where policymakers and government officials remain as far removed as possible from top-down management of higher education and research institutions, the bottom-up commitment of professors and researchers is very important. We need strong and independent personalities and we place our trust in their work with Swiss Academies of Arts and Sciences, in specialist associations, commissions and working groups. This is how highly specialised knowledge emerges from a very complex knowledge landscape. The outcome of this is *orientation knowledge* and *application knowledge* that are both shared and reviewed by peers.
*Application knowledge:* It is certainly not by chance that the chemical-pharmaceuticals industry is one of the main contributors of research expenditure in Switzerland or that the CEO of a leading international pharmaceuticals company will be speaking to you this afternoon. No, both are the expression of the partnership and traditionally close relationship between scientific research and market-based innovation in pharmaceuticals. It is also an expression of the fact that publically funded fundamental research in pharmaceuticals science at Swiss higher education institutions is fertile ground for subsequent innovative processes within the Swiss private sector.
This is a good thing, and not merely for *economic* reasons. A close and beneficial public-private partnership in this area is very much in the interests of *society as a whole*. Citizens expect the best quality of life, all the way into their oldest years. And this naturally includes high-quality healthcare. In order to satisfy these expectations, there is a need for smart, creative and promising responses to the very large number of unresolved issues in the areas of medicine and medical treatment. I am convinced that pharmaceutical sciences and the chemical-pharmaceuticals industry will continue to make significant contributions in the future.

***Speaker at the 6th SPhSD:***

***Dr. Mauro Dell'Ambrogio – State Secretary for Education, Research and Innovation***

Mauro Dell'Ambrogio, the holder of a Doctorate in Law from the University of Zurich, held a number of public offices in canton Ticino from 1979 to 1999 after passing his bar exam: Judge, Chief of the Cantonal Police, Secretary-General for Education and Culture, project manager for the creation of the University of Lugano (USI), and Secretary-General of the USI.
After four years heading up a group of private clinics, he became Director of the University of Applied Sciences of Southern Switzerland (SUPSI) in 2003. He has been mayor of Giubiasco, a member of the Ticino cantonal parliament and chairman of the Ticino electricity works.
From 2008 to 2012 he has been State Secretary for Education and Research.
In January 2013 he took up the post of State Secretary for Education, Research and Innovation.

1 Check against delivery.

What does the Confederation do to maintain and nurture this fertile ground? What does it do to ensure that a public-private partnership in your field is explicitly and implicitly possible? Well, if we consider the federal promotion of education, research and innovation for 2013–2016, I would say: quite a bit actually!
To begin with, the Confederation allocates a great deal of funding to the ERI policy area. Compared with the previous budget period, the total amount of funding has increased by around CHF 5 billion to CHF 26 billion. Of course, this is much less than what the Cantons have set aside for education, research and innovation (ERI). However, in keeping with the principle of federal *subsidiarity* set forth in the Federal Constitution, the Confederation is authorised, without limitation, to become actively involved in the ERI policy. And it has done precisely that over the past decade.
During this time, around 10% of all federal expenditure has been channelled to the ERI sector. Of the thirteen different policy areas, ERI has the fourth largest budget. This is a clear indication of how strategically important investment in "grey matter" has become.
The CHF 26 billion in federal funding devoted to ERI – more broadly to natural sciences and medicine and more narrowly to pharmaceuticals/chemistry – may be summed up in the following keywords:
– Higher education funding;
– Research funding;
– Support for young researchers;
– Support for international cooperation.

## Higher education funding

The Cantons are the main supporters and funders of Switzerland's ten cantonal universities. Nevertheless, the Confederation supports the Cantons in their efforts to ensure high-quality teaching and research.
First of all, the Confederation pays the Cantons a lump-sum amount for each student enrolled. This lump-sum amount is also calculated on the basis of the different costs associated with a given branch of study. Therefore, a cantonal university will receive around CHF 7600 from the Confederation for each pharmaceuticals student but would receive considerably less for a student enrolled in a humanities or social sciences discipline.
While this basic contribution by the Confederation (which in 2013–2014 stood at around CHF 2.6 billion) makes up a larger or smaller portion of a cantonal university's treasury, none of the cantonal universities can do without it. The University of Bern, for instance, received CHF 83 million last year, nearly one-third of the amount received from the Canton of Bern (CHF 277 million).
Secondly, the Confederation also provides cantonal universities with funding for construction and renovation as well as for the purchase and installation of scientific equipment and IT hardware and software. To give a current example, the Confederation will contribute CHF 70 million for the University of Basel's new Centre for Molecular Life Sciences, which cost a total CHF 310 million.
For the current budget period 2013–2016, a total of CHF 260 million in federal funding is available for university expenditure.

## Research funding

The Confederation has a constitutional mandate to encourage research, which it does through the provision of funding. In the current budget period, the Swiss National Science Foundation (SNSF) has over CHF 3.7 billion at its disposal for research grants. The SNSF has allocated around 40% of this amount to its Division III "Biology and Medicine" for the purpose of awarding grants for free fundamental research projects. I am convinced that many of you here today have already secured funding from the SNSF in competitive calls for project proposals.
Another source of funding for pharmaceuticals/chemistry are national research programmes (NRPs), which have become increasingly important for this field. In recent years, we have seen several NRPs such as NRP 49 "Antibiotic Resistance" or NRP 50 "Endocrine Disruptors: Relevance to Humans, Animals and Ecosystems". Both of these NRPs have led to pilot initiatives that have been recognised within the European context.
NRP projects tend to receive large amounts of federal funding, are intentionally limited to periods of four to five years and have rather loose structures. In contrast, national centres of competence in research (NCCRs) are entirely different. NCCRs are created for the purpose of making a long-term impact on the Swiss research landscape. They help to make already good-quality higher education institutions even more competitive through the creation of networks with national and international partners. At the same time, they support the development of Leading Houses for top-notch research. NCCRs also seek to help young researchers achieve peak levels of performance. Thanks to cooperation with economic partners, these efforts lead to patents, new processes and innovative products.
This federal funding instrument has demonstrably bolstered pharmaceuticals science, which has now started to gain momentum. Here, the NCCR "Molecular Oncology" or the new NCCR "TransCure" are two good examples alongside the NCCR "Chemical Biology", which has recently started work at the University of Geneva in cooperation with the Federal Institute of Technology Lausanne (EPFL). Also this year, the Federal Department of Economic Affairs, Education and Research (EAER) will launch the 4th series of NCCRs; I sincerely hope that your research fields will be a part of this endeavour.

## Support for young researchers

There is an old proverb that says "as you sow, so shall you reap", and this naturally applies quite specifically to the area of education and research. As you know, ladies and gentlemen, shortages in qualified workers specialised in mathematics, IT, natural sciences and engineering are a source of increasing concern in Switzerland. These shortages are particularly apparent in the areas of IT, engineering and construction. However, shortages have also been observed to a greater or lesser extent in natural sciences, namely in the field of chemistry and life sciences.
On the one hand, labour force shortages are highly dependent on economic cycles. At the same time, however, they are also determined by structural factors. Solid research studies have shown that young people already have a clearer idea of their occupation and career choices as early as age fifteen, i. e. in lower-secondary school. While the Confederation is not responsible for this part of the education system, it works to actively promote "hard sciences" at various levels. We therefore provide over CHF 5 million per year to the Swiss Academy of Sciences (SCNAT), which, among other things, seeks to encourage young people in lower- and upper-secondary education to pursue studies in science.
Upon completion of lower-secondary education, most young people choose the enrol in upper-secondary level vocational education and training (VET), which falls under the scope of the Federal Vocational and Professional Education and Training Act (VPETA). The funding allocated to the VET sector by both the Confederation and the cantons by virtue of this Federal Act will hopefully also encourage companies to create apprenticeship positions to train young people to become highly qualified in the fields of chemistry and pharmaceuticals technology. Those who obtain the Federal Vocational Baccalaureate may enrol at Swiss universities of applied sciences (part of the higher education sector), which open up even greater career prospects.
We should also not forget the extraordinarily broad range of funding possibilities that the Swiss National Science Foundation (SNSF) offers to individually help the very best minds in all scientific disciplines to succeed in their careers. This support includes exchange fellowships for PhD and postdoctoral students, SNSF-sponsored professorships

and specific instruments to advance careers in biology and medicine. The federal funding that the SNSF is able to spend each year (CHF 150 million) is quite important and is also intended to have an impact, ladies and gentlemen, in your field of expertise as well.

## Support for international cooperation

By their very nature, science and research are borderless. At the same time, their quality depends on both competition and cooperation. This natural tendency has become even more pronounced in the age of globalisation. We can see this in Switzerland as well:

- The number of students taking part in exchange programmes is rising steadily;
- The proportion of foreign nationals among teaching and research staff at Swiss higher education institutions has also reached a high level;
- Interest among Swiss researchers in cooperation initiatives within European and international networks is strong. According to a bibliometric study conducted by the State Secretariat for Education, Research and Innovation (SERI), nearly 70% of all research partnerships involving Swiss-based researchers in 2005–2009 were international. Compared to the early 1980s, this corresponds to an increase of seventeen percentage points.

Against this backdrop, the Confederation is intensifying its efforts to internationalise the Swiss ERI sector. Of course, it is up to our autonomous higher education and research institutions as well as to each and every researcher and professor to establish and pursue their own international strategies. Nevertheless, the Confederation will also do what it can to help open international doors to Swiss research institutes and researchers.

In recent years, Switzerland has signed a number of bilateral research agreements with Brazil, China, India, Russia, South Korea and South Africa. All of these countries have made tremendous strides in the area of science and technology. These bilateral agreements are by far the most powerful instrument in support of international cooperation, but are also continental in focus.

It is a fact that participation in EU research framework programmes, which are managed by Brussels, has been one of the highest priorities in Swiss science policy. In early 2013, Switzerland began technical negotiations for full-fledged Swiss participation in "Horizon 2020", which – after FP7 formally comes to a close at the end of 2013 – will become the next programme generation.

We hope to successfully reach an agreement with the EU because we understand just how important it is for Switzerland to secure full-fledged participation in what constitutes the world's largest intergovernmental research funding instrument. Of course, participation costs are certainly not low, around CHF 570 million each year. However, securing the right of researchers from Swiss public institutions and private companies to work alongside Europe's best partners cannot be more promising. Information technologies and nanosciences, issues relating to "health and biotechnology" or "energy and climate protection" – here Switzerland must be present and Swiss researchers must be able to contribute their competences. And through constant competition for European grant funding, Swiss researchers can remain as fit as they are today.

And here I am naturally counting on your commitment, ladies and gentlemen, not because Switzerland's return on investment from "Horizon 2020" should ideally be positive but rather, and primarily, because of what can be achieved through pan-European cooperation in research and development.

I shall now share with you my specific views on the Confederation's current ERI policy. So far, I have only spoken about ERI funding for 2013–2016. Actually, the Confederation's ERI policy is based on a much longer-term vision than a four-year timespan. Our vision is based on certain principles that have proven their merits over time. French statesman Charles-Maurice de Talleyrand was quoted as saying: "People should keep their eyes on the future while remembering the past." Here I am thinking of the successful interplay that exists in Switzerland between intense national competition and partnership-like cooperation whenever such partnerships make sense. As the most innovative country in the world, we should remain true to this basic principle. Other good principles include the autonomy of higher education and research institutions and the time-honoured bottom-up processes within the system. For me "learning from the past" – as I mentioned briefly in my introduction – means never losing sight of the entire system, even when specific parts of that system become overheated. As a case in point, there is currently discussion of what the "right" proportion of baccalaureate holders in Switzerland should be.

I do not believe that we should adopt a Swiss-wide policy on baccalaureates, since the needs of the Canton of Glarus are entirely different from those of the Canton of Geneva. If we heed OECD recommendations and increase the number of baccalaureate holders to the OECD average, then there will be at least two consequences that we should be wary of.

1. Having more baccalaureate holders means a larger number of university students later on. There is no way around this. At what point does the “massive university” phenomenon that we see in so many other countries become a problem? And at what point does university overcrowding have a negative impact on the quality of teaching and research at our universities?
2. At the same time, having more baccalaureate holders also means fewer people undergoing dual-track vocational education and training (VET). This is not without problems. And here I am not thinking primarily about companies. After all, education expenditure is not mainly intended to ensure advantageous conditions for the private sector. It is just as important for us to offer all young people the best possible chances to develop themselves in accordance with their needs and wishes and to gain a foothold in society. And thanks to the permeability of the Swiss education system, more academically inclined young people stand to benefit a great deal from pursuing training within the Swiss VET sector. In addition to the standard vocational qualification, the Federal VET Diploma, such learners also have the option of obtaining the Federal Vocational Baccalaureate either during or after completion of their VET programme. The Federal Vocational Baccalaureate opens the way for subsequent training at Swiss universities of applied sciences (UAS), which are part of the Swiss higher education sector.

Whether a person decides to obtain a baccalaureate followed by a university degree, or a Federal VET Diploma followed by professional education and training (PET), or the dual combination of Federal VET Diploma and Federal Vocational Baccalaureate followed by professional education and training (PET) or studies at a university of applied sciences (UAS) is irrelevant in the end. The most important thing in my opinion is that we provide the general conditions needed to enable everyone in our country to take part in successful lifelong learning.

Ladies and gentlemen, thank you for your attention.

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Gesellschaft der Schweizerischen Industrie-Apotheker
Société Suisse des Pharmaciens d'Industrie
Swiss Society of Industrial Pharmacists

## GSIA in a nutshell

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.

## GSIA auf einen Blick

Die Gesellschaft der Schweizerischen Industrie-Apotheker ist eine Vereinigung von über 600 Pharmazeuten und anderen in der pharmazeutischen Industrie tätigen Life Science Akademikern. Wir bieten unseren Mitgliedern den Aufbau eines Netzwerkes in der pharmazeutischen Industrie sowie interessante Fortbildungen im pharmazeutischen Umfeld. Zudem unterstützen und fördern wir den Nachwuchs.

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### Anmeldung für Mitgliedschaft / Application for Membership:

http://www.gsia.ch/component/option,com_fabrik/Itemid,56/

# SWISS PHARMA SCIENCE DAY 2013

## Poster Abstracts

**P-1**

### A New UHPLC Method to Determine Aloin A and B in *Aloe capensis*

**I. Rosenthal, E. Wolfram, B. Meier**
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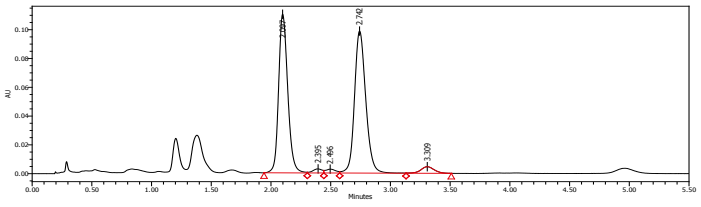
**Introduction:** The current monograph in the European Pharmacopeia for *Aloe capensis* describes a photometric assay based on an adapted Bornträger reaction to determine hydroxyanthracene glycosides, calculated as aloin A. The method is time-consuming, unspecific for aloin A and B and the precision is not adequate for a modern assay. There are several HPLC methods published but their runtime is too long and the resolution for aloin A and B is not satisfactory. So far, there is no validated and robust method existing.
**Aim:** The aim of the present study was to develop a short, robust and validated UHPLC method that meets specific needs of the pharmaceutical industry.
**Method:** About 100 mg of the dried drug are placed in a 100 mL volumetric flask and extracted with 70 mL of methanol for 20 min by sonication. An Acquity UHPLC BEH Phenyl column, 50 x 2.1 mm i. d. and 1.7-$\mu$m particle size, was used. The mobile phase consisted of 17:83 (v/v) acetonitrile/water. The flow rate was 0.5 mL/min, the detection wavelength 355 nm, and the injection volume 3 $\mu$L.
**Results:** The UHPLC method allows to separate aloin A and B (see Fig. 1). Results of several samples are presented on the poster.

Figure 1 HPLC chromatogram of an *Aloe capensis* extract




**Conclusions:** The method developed is simple, robust and precise. The method is also applicable for normal HPLC systems. It is a suitable option to replace the outdated photometric assay described in the European Pharmacopeia.

**Keywords:** UHPLC, *Aloe capensis,* aloin A, aloin B.

**P-2**

### A New HPLC Method to Determine Frangulin A and B as well as Glucofrangulin A and B in Frangulae Cortex

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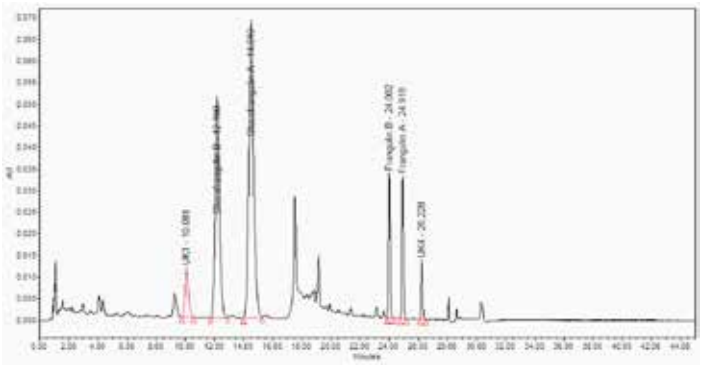
**Introduction:** The current monograph in the European Pharmacopeia for Frangulae cortex describes a photometric assay based on an adapted Bornträger reaction to determine hydroxyanthracene glycosides, calculated as frangulin B. The method is time-consuming, unspecific for frangulines and the precision is not adequate for a modern assay.
**Aim:** The photometric method shall therefore be replaced by a modern HPLC method. There is no HPLC method published in the literature that allows the determination of frangulin A/B and glucofrangulin A/B in Frangulae cortex.
**Method:** About 300 mg of freshly milled drug were extracted for 15 min by sonication. The extraction solution consisted of acetonitrile-water 50:50 v/v and 2 g/L $NaHCO_3$. A RP $C_{18}$ Nucleodur column from Macherey Nagel, 125 x 4 mm i. d. and 3-$\mu$m particle size, was used. The mobile phase A consisted of water (pH of 2.0, adjusted with phosphoric acid). Mobile phase B consisted of acetonitrile/methanol 20:80 v/v. The flow rate was 1.0 mL/min, the detection wavelength 435 nm, the column temperature 50 °C, and the injection volume 20 $\mu$L. The gradient is shown in Table 1.

Table 1 Gradient conditions

| Time (min) | Mobile phase A (%) | Mobile phase B (%) |
|---|---|---|
| 0 | 66 | 34 |
| 15.0 | 66 | 34 |
| 16.0 | 50 | 50 |
| 26.0 | 24 | 76 |
| 26.5 | 0 | 100 |
| 28.5 | 0 | 100 |
| 29.0 | 66 | 34 |
| 45.0 | 66 | 34 |

**Results:** This HPLC method allows to separate the four frangulins sufficiently (see Fig. 1). Results of several samples are presented on the poster.

Figure 1 HPLC chromatogram of a Frangulae cortex extract



**Conclusions:** The method developed is simple, robust and precise. It is a reasonable option for pharmacopeia applications to replace the outdated photometric assay.

**Keywords:** HPLC, Frangulae cortex, frangulin A, frangulin B, glucofrangulin A, glucofrangulin B.

P-3

## New Antiprotozoal Isoflavan Quinones from *Abrus precatorius ssp. africanus*

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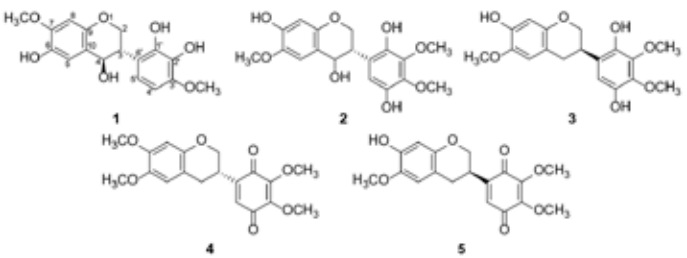
**Introduction:** A library of 207 extracts from selected South African plants was screened *in vitro* against a panel of protozoan parasites. A $CH_2Cl_2$/MeOH (1:1) extract of *Abrus precatorius* L. *ssp. africanus* Verdc. (Fabaceae) strongly inhibited *T. brucei rhodesiense* (1.04 $\mu$M).
**Aims:** To identify the compounds responsible for the antiprotozoal activity of a $CH_2Cl_2$/MeOH (1:1) extract of *A. precatorius.*
**Methods:** Active constituents in two different batches of plant material were tracked by HPLC-based activity profiling, and isolated by normal phase flash chromatography and RP-HPLC. Structures and relative configuration of compounds were established by NMR spectroscopy and HR-TOFMS. The absolute configuration was determined by comparison of electronic circular dichroism (ECD) spectra with calculated ECD data.
**Results:** From the first batch, we isolated five isoflavan quinones and hydroquinones [1]. From the second batch we identified a series of additional compounds. Among these, **2, 3, 4,** and **5** showed strong *in vitro* activity against *T. brucei rhodesiense* ($IC_{50s}$ of 0.11, 0.02, 0.02, and 0.01 $\mu$M, respectively), while **1** was inactive when tested at 30 $\mu$M. Selectivity indices (SI) as calculated from cytotoxicity data in L-6 cells were 508, 374, 1379, and 668.
**Conclusions:** Abruquinones K **(2)**, L **(3)**, A **(4)**, and D **(5)** possess between tenfold to hundredfold higher SI than cynaropicrin, the only plant derived compound with demonstrated *in vivo* activity against *T. brucei rhodesiense* [2]. The compounds are thus good candidates for *in vivo* testing.




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

**References:**
[1] Hata Y, Raith M, Ebrahimi SN, Zimmermann S, Mokoka T, Naidoo D, Fouche G, Maharaj V, Keiser M, Brun R, Potterat O, Hamburger M. Planta Med 2013; 79: 492-8.
[2] Zimmermann S, Kaiser M, Brun R, Hamburger M, Adams M. Planta Med 2012; 78: 553-6.

P-4

## TLR2 Ligation with Peptidoglycan Attenuates Airway Epithelial Barrier Disruption Induced by Pro-Inflammatory Cytokines

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**Introduction:** Airways epithelial barrier function could be perturbed by inhaled pathogens or allergens. The exposure to pro-inflammatory factors involved in the pathogenesis of inflammatory respiratory disorders also leads to impairment of barrier integrity by tight junction disassembly. The stimulation of Toll-like receptor 2 (TLR2) has been shown to increase barrier function in intestinal epithelial cells.
**Aim:** The effect of TLR2 agonist on upper airways epithelium has not been studied yet. This project aimed to develop an *in-vitro* model of inflamed airway epithelium to assess the potential of a TLR2 agonist, peptidoglycan (PGN), to ameliorate airway epithelial barrier function.
**Methods:** Assessment of this bacterial wall component was carried out using Calu-3 cell line. This cell line forms polarized monolayers expressing features of airways such as mucus and cilia. The transepithelial electrical resistance and paracellular permeability assays were used to assess the effect of PGN on healthy and inflamed model of airway epithelium. The inflamed model of airway epithelium was obtained by inducing barrier disruption using pro-inflammatory cytokines: TNF-$\alpha$, IL-4, IFN-$\gamma$ at clinically relevant concentrations. Western blot experiments were performed to investigate the molecular mechanism involved.
**Results:** TEER and paracellular permeability assays have demonstrated an inverse correlation indicating the same effect of pro-inflammatory cytokines and of PGN on the inflamed model. The stimulation of healthy Calu-3 monolayers by PGN has displayed an improvement of barrier function. In the inflamed model, Calu-3 monolayers pre-treated with PGN attenuated the barrier disruption induced by pro-inflammatory cytokines. This observation correlated with a 200-fold increase in claudin-1 tight junction protein expression.
**Conclusion:** TLR2 ligation attenuated the barrier disruption induced by pro-inflammatory cytokines by upregulating the expression of tight junction protein Claudin-1.

**Keywords:** TLR2 ligation, airway inflammation, tight junctions, Calu-3 cell line.

P-5

## Antitrypanosomal Natural Compounds and Epigenetics: A Target-Fishing Approach Involving Sirtuins

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**Introduction:** The silent-information regulator 2 (SIR2)-like proteins, commonly called sirtuins, are nowadays considered as emerging antiparasitic targets. It was demonstrated that nicotinamide, a pan-sirtuin inhibitor, caused kinetoplast alterations and an arrested growth of *Trypanosoma cruzi,* the protozoan parasite responsible for Chagas disease. These observations suggested that sirtuins TcSir2rp1 and TcSir2rp3 may play an important epigenetic role on the parasite-cell cycle. Thus, their inhibition could explain the mechanism of action of natural compounds with a known antitrypanosomal activity.

**Aims:** The construction of three-dimensional models of sirtuins from *T. cruzi* together with the determination of a reliable computational protocol for the isolation of meaningful hits able to potentially inhibit the activity of these specific biotargets.
**Methods:** A homology modeling approach was used for unraveling the three-dimensional features of the sirtuin Sir2rp1 from *T. cruzi.* The apo-form of human SIRT2 (PDB id: 1j8f) and the recently solved structure of human sirtuin SIRT2 in complex with its natural substrate (PDB id: 3zgv) allowed the modeling of the parasite protein in both non-productive and productive conformational states. Molecular docking was then carried out for validating the modeled biotargets. A database composed by fifty natural and diverse compounds, active against this parasite, was finally collected from the literature and virtually screened against TcSir2rp1 and the previously modeled TcSir2rp3.
**Results:** Two conformational states of TcSir2rp1 (non-productive/productive) were modeled and described. Molecular docking of ligands with a known affinity to the target revealed to be meaningful when the productive form of the protein was taken into account for calculations. This specific conformation was then considered for the virtual screening of antritrypanosomal plant compounds against TcSir2rp1 and TcSir2rp3 proteins. The calculations identified a restricted number of chemical scaffolds extracted from *Vismia orientalis, Cussonia zimmermannii, Amomum aculeatum* and *Anacardium occidentale* potentially interacting with both modeled proteins.
**Conclusions:** The study provided the structural basis of sirtuins from *T. cruzi,* through reflections about the possible conformational states. Molecular docking highlighted not only the advantages of performing *in silico* interaction studies on the productive forms of these proteins but also suggested an epigenetic mechanism of action for known antitrypanosomal phytochemicals. This information may be useful in the research of new drug candidates against Chagas disease.

**Keywords:** Chagas disease, sirtuins, natural compounds, molecular docking.

**P-6**

## MLP-Tools – A 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 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 conformation-depended *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.

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**P-7**

## Effects of *E*-Vallesiachotamine and Prunifoleine in Neurodegeneration Targets and in the Viability of Astroglial Cells

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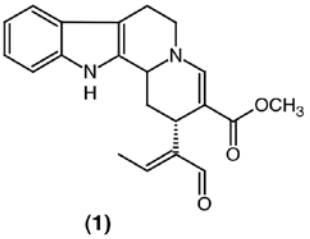
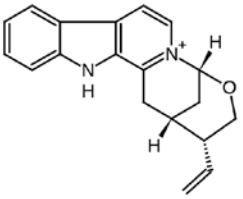
**Introduction:** The monoterpene indole alkaloids (MIAs) *E*-vallesiachotamine **(1)** and prunifoleine **(2),** found in neotropical *Psychotria* species [1-2], have been demonstrated to inhibit cholinesterases (AChE and BChE) and monoamine oxidases (MAO-A and -B) in concentrations ranging from 1 to 100 $\mu$M [2]. Moreover, enzyme kinetics and docking simulations revealed details regarded the modes of AChE, BChE and MAO-A inhibition by **(1)** and **(2)** [2]. Both **(1)** and **(2)** behave as time-dependent MAO-A inhibitors while **(1)** has been shown to inhibit AChE in a noncompetitive way. Cholinesterases and monoamine oxidases play important roles in neurodegenerative diseases, and currently AChE and MAO-B inhibitors are employed in the treatment of the symptoms related to Alzheimer's Disease and Parkinson's Disease, respectively [3].
**Aims:** In the present work, we describe the *in vitro* cytotoxicity of the MIAs *E*-vallesiachotamine **(1)** and prunifoleine **(2)** on primary cortical astrocytes cultures from Wistar rats.
**Methods:** Cells were incubated for 24 h with compounds **(1)** and **(2)** at concentrations corresponding to 1, 10 and 100 $\mu$M and the cytotoxicity of both compounds was evaluated by the metiltetrazolium reduction, neutral red uptake, and propidium iodide exclusion assays.
**Results:** Alkaloid **(1)** decreased astrocyte viability only at 100 $\mu$M as observed by metiltetrazolium reduction (viability corresponding to 8.09 ± 0.75%), neutral red uptake (viability corresponding to 22.15 ± 0.84%), and propidium iodide exclusion. Alkaloid **(2)** did not affect cellular viability at the tested concentrations.
**Conclusions:** In this work, it was demonstrated for the first time that alkaloid **(1)** at 100 $\mu$M is cytotoxic to astrocyte cells, suggesting that high concentrations of this compound could impair astrocyte viability and CNS functions.

**Keywords:** Monoterpene indole alkaloids, cholinesterases, monoamine oxidases, cytotoxicity.




(1)




(2)

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**P-8**

## Novel Biocompatible Hyaluronic Acid-Chitosan Hybrid Hydrogel for Osteoarthritis Therapy

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**Introduction:** Conventional therapies for osteoarthritis (OA) include the intraarticular injection of very high molecular weight hyaluronic acid (HA), requiring repeated, frequent injections [1]. To extend HA visco-supplementation effect, we propose to associate it with another biopolymer, forming a so-called hybrid hydrogel. For this purpose, chitosan (Cs) was chosen due to structure similarity with synovial glycosaminoglycans, antiinflammatory effect and ability to promote cartilage growth [2].
**Aims:** To avoid spontaneous polyelectrolyte aggregation and obtain transparent, homogeneous gels, different excipients were evaluated. The biocompatibility of optimized formulation was assessed in healthy rabbit articulations [3].
**Methods:** Gel transparency and presence of aggregates was evaluated by visual inspection. The gel was formulated with various buffering, isotonic agents and steam-sterilized. Selected formulation was injected intraarticularly in rabbits and animals were evaluated by periodic clinical and ultrasound examination. Biocompatibility was assessed at one month by macroscopic and histological evaluation of surrounding tissues.
**Results:** HA and Cs aggregation was found to be mediated by polyelectrolyte complex formation and could be inhibited in a stable manner by use of cationic ions as calcium, sodium, magnesium, aluminium and dibasic sodium hydrogenophosphate. Sodium chloride formulation containing phosphate buffer was selected on the basis of a previous subcutaneous biocompatibility study in rats. Neither clinical signs of pain nor ultrasound presence of liquid were detected. Macroscopic and histologic scoring of the synovial membrane and cartilage were not statistically higher than after a HA commercial formulation, physiologic serum injection or after no injection.
**Conclusions:** HA added with Cs was formulated into sterile, homogeneous and biocompatible hydrogels. The formulations will be evaluated in an OA rabbit model as novel viscosupplementation therapy agents.

**Keywords:** Osteoarthritis, hyaluronic acid, chitosan, biocompatibility.

**References:**
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**P-9**

## *Cirsium spinosissimum* Scop., a Forgotten Edible Wild Plant from the Canton of Valais

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**Introduction:** In the course of an ethnobotanical survey on forgotten traditional food plants in the canton of Valais, we came to know about the ancient use of the spiniest thistle *(Cirsium spinosissimum* Scop., Asteraceae). The plant was traditionally eaten by shepherds similarly to an artichoke, after cutting the surrounding leaves to reach the heart of the flower, called receptacle. Despite the fact that it grows abundantly in mountain regions, no information was available on its chemical constituents.
**Aims:** Aims of this study were to analyze the phytochemical profile of *C. spinosissimum,* and to quantify compounds relevant for nutrition in the receptacles. Absence of cytotoxicity was demonstrated on Caco-2 cell line.
**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, polyphenols, and carotenes were obtained according to standard procedures described in the literature. Major flavonoid glycosides, *i. e.* linarin and pectolinarin, were quantified by HPLC-UV. Finally, absence of cytotoxicity was demonstrated on Caco-2 cell line using a reported MTT cytotoxicity assay procedure.
**Results:** A wide range of compounds including flavonoid glycosides, phenylpropanoids, sesquiterpene lactones, fatty acids, and a spermine derivative were identified online or after targeted isolation. The fresh receptacles contained interesting amounts of ß-carotene (1.4 ± 0.1 mg/100 g fresh weight (FW)), potassium (505.8 ± 12.2 mg/100 g FW), and calcium (298.3 ± 3.1 mg/100 g FW). The total phenolic content was determined as 410 ± 33 mg gallic acid equivalents per 100 g FW of in the receptacles. Amounts of 85.5 ± 0.2 and 106.9 ± 0.5 mg/100 g FW were found for linarin and pectolinarin, respectively. The crude ethanolic extract showed no sign of cytotoxicity on the intestinal Caco-2 cell line when tested at concentrations up to 500 µg/mL.
**Conclusions:** Several classes of secondary metabolites were identified in the aerial parts of *C. spinosissimum.* In addition to the absence of cytotoxicity on Caco-2 cell line, no compounds with reported toxicity, or substance classes with known toxicological risks were detected. Based on its chemical composition combined with pleasant gustatory properties, *C. spinosissimum* can be considered as a safe and healthy wild food plant.

**Keywords:** *Cirsium spinosissimum,* alpine plants, food

**P-10**

## *Phyllostachys Edulis* Leaf Extract Reduces TNFα-Induced Release of VEGF and IL-8 in Immortalized HaCaT Cells

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**Introduction:** *Phyllostachys edulis* Carriere (Poaceae) is a bamboo species which is used for multiple purposes such as furniture production, nutrition and in traditional Chinese medicine. Bamboo leaves have received considerable attention in pharmacological research due to their potent antitumor, anti-inflammatory, antimicrobial, and antiulcerogenic activities.
**Aims:** In this study, we investigated the antiinflammatory effects of two leaf extracts (young versus old harvested leaves) prepared from *Phyllostachys edulis* on TNFα-induced overproduction of IL-8, VEGF and IL-6 in immortalized human keratinocytes (HaCaT). These cytokines play important roles in various inflammatory skin diseases, such as psoriasis.
**Methods:** Both leaf extracts were prepared by Soxhlet extraction using water as extraction solvent. The amounts of major flavonoids were quantified using a LC-MS/MS method. HaCaT cells were stimulated for 24 h with the proinflammatory cytokine TNFα without or with the addition of different concentrations of bamboo extract. VEGF, IL-8 and Il-6 were measured in cell supernatants by enzyme-linked immunosorbant assays as biomarkers of antiinflammatory response.
**Results:** Isoorientin was detected as the main flavonoid in both extracts with amounts of 1590 mg/kg (old leaves) and 1440 mg/kg (young leaves). The Soxhlet extract prepared from the young bamboo leaves (SEYL) dose-dependently (25–250 μg/mL) inhibited the release of TNFα-induced IL-8, and VEGF, but not IL-6, in HaCaT cells while the extract prepared from the old leaves (SEOL) had no effect. In addition, isoorientin (ISO; 10–100 μM) dose-dependently reduced the levels of VEGF, IL-8 and IL-6 in TNFα-treated HaCaT cells, comparable to the positive control hydrocortisone (HC; 10 μM). Cell viability was determined by the MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide) colorimetric assay. SEYL and SEOL up to a concentration of 250 μg/mL as well as ISO and HC (10–100 μM, respectively) did not have any toxic effects on HaCaT cells.
**Conclusion:** Taken together, an extract prepared from the young leaves of *Phyllostachys edulis* as well as isoorientin exerted antiinflammatory effects in TNFα-treated HaCaT cells, suggesting interesting cosmetical and pharmacological applications.

**Keywords:** Bamboo, *Phyllostachys edulis,* isoorientin, antiinflammatory, HaCaT cells.

**P-11**

## Phenytoin Therapeutic Monitoring Guidelines in a Teaching Hospital: the Impact of a Proactive, Continuous Pharmacy Service in a Large Ten Years Retrospective Analysis

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**Introduction:** In teaching hospitals a regularly updated and compulsory drug formulary is mandatory for rational, correct, and economic drug use. To minimize medication errors and to assist optimal drug therapy, therapeutic guidelines have to be implemented in a multi-disciplinary approach. The hospital pharmacy service has an obligation to support such guidance adherence to optimize patient care [1]. Critical-dose drugs like phenytoin (PHT) requiring therapeutic drug monitoring (TDM) for an appropriate individual patient regimen represent a special challenge for a patient-oriented pharmaceutical activity. PHT is established and authorized to treat but also to prevent epilepsy. Preventive use is routine in neurosurgical patients. In emergencies a quick and effective i.v. loading dose is necessary but difficult to achieve because of PHT's nonlinear and largely varying inter-individual pharmacokinetics. A rapid, body-weight adjusted i.v. PHT loading regimen (PHENDOSE) using population-based Bayesian forecasting was established and introduced in 1997 in a Swiss 550-beds tertiary acute care hospital [2] serving as a neurosurgery referral center. The hospital pharmacy took the lead to establish, introduce, and continuously educate PHENDOSE with also the therapeutic monitoring guidelines to health care professionals in this hospital. PHENDOSE included information on initial i.v. dosing, correct dilution and administration of PHT, but also on timing of plasma sampling for TDM. The pharmacist on duty took over the lab data interpretation, gave the proposal for the next PHT dosing, and TDM timing with a level forecast. The education program included distribution of pocket cards for physicians, standard operation procedures on PHT medication for nurses, forms to fill for documentation and TDM ordering, and the information on the specific pharmacy support. All PHT level determinations by the lab were transferred by fax to the pharmacy, where the PHT data interpretation service support was available round the clock. In 2005 a change in the direction of the pharmacy caused the neglect of the active outside promotion of the PHT TDM support.
**Aims:** An analysis of the impact of the proactive, multi-tool pharmacy support of PHT TDM was aimed by measuring the frequency and adherence to the PHENDOSE protocol over a long-term period (1997–2007).
**Methods:** A single center, retrospective analysis over 11 years (1997–2007) of all PHT levels determined in the hospital central lab and transferred to the pharmacy.
**Results:** Over 6000 PHT levels were analyzed, ≈2800 for PHENDOSE and ≈3300 for conventionally dosed (CD) patients. PHT target levels (40–80 μM) were achieved more quickly and more sustainably by PHENDOSE compared to CD ($p<0.001$). The proactive pharmacy TDM support increased the PHENDOSE proportion from 24% to 80%, but in absence it declined rapidly to 17% although highly superior performance versus CD was reported [3].
**Conclusions:** Only proactive, continuous, and combined written (internet), oral, and direct interactions with the users by educational and informational meetings will keep an even highly effective medication guidance in use. For the adherence to TDM guidelines of hospital formulary drugs, the active involvement of the hospital pharmacy plays a crucial role.

**Keywords:** Therapeutic drug monitoring, phenytoin, hospital pharmacy, therapeutic guidelines.

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**P-12**

## Quantitative Analysis of the Antiplasmodial Alkaloid Carpaine in Papaya Leaves *(Carica papaya)*

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**Introduction:** Decoctions of papaya leaves have been used in Indonesia as a traditional remedy to prevent and treat malaria. Previous studies reported high antiplasmodial activity *in vitro* and *in vivo* of papaya leaves extracts and of a crude alkaloidal fraction [1]. With the aid of HPLC-based activity profiling, we identified the piperidine alkaloid carpaine as the main active constituent. It had an $IC_{50}$ of

0.21 $\mu$M when tested *in vitro* against *Plasmodium falciparum* (K1 strain), and a selectivity index of 98 in rat myoblast L-6 cells.
**Aims:** To establish a validated quantitative assay for carpaine, and to determine the carpaine content in papaya leaves of different origins and maturity levels.
**Methods:** Papaya leaves were collected from six different locations in Java, Indonesia. In addition, a commercial sample originating from India was analyzed. Extraction of carpaine was carried out by pressurized liquid extraction (PSE), whereby the extraction conditions were optimized with respect to solvent used, extraction temperature, and number of extraction cycles. For quantification of carpaine, a UPLC-MS/MS method was established and validated.
**Results:** Best extraction was achieved by n-hexane, with prior moistening of the dry leaves with 33% (w/v) aqueous ammonia solution, and an extraction temperature of 90 °C. An extraction yield of >98% was achieved with three extraction cycles of 5 min each. The validated UPLC-MS/MS method demonstrated linearity ($R^2$) of 0.9908 over a concentration range of 20–5000 ng/mL. The carpaine content in the leaves samples varied between 0.02% and 0.31%.
**Conclusions:** The carpaine content in the leaves samples varied more than tenfold. We found no direct association of the content with geographic origin and maturity of leaves.

**Keywords:** *Carica papaya,* carpaine, quantitative analysis, pressurized solvent extraction, UPLC-MS/MS.

**References:**
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**P-13**

## Identification of Dihydrostilbenes as a New Scaffold for $GABA_A$ Receptor Modulators in *Pholidota chinensis* Stems and Roots

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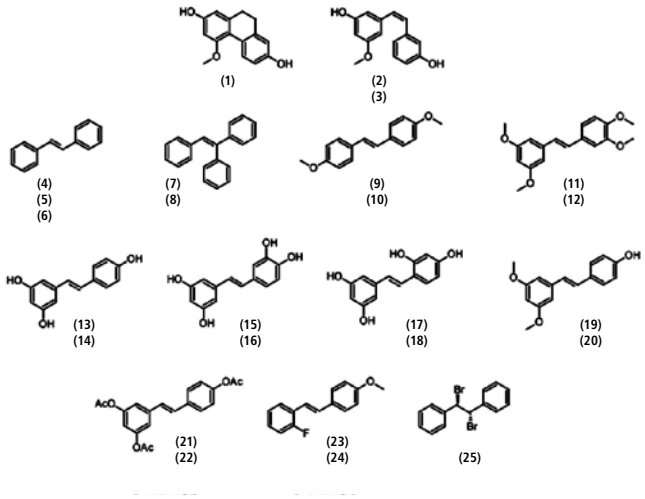
**Introduction:** $\gamma$-Aminobutyric acid type A ($GABA_A$) receptors are the major mediators of fast synaptic inhibition in the central nervous system and the target for many clinically important drugs such as benzodiazepines and other CNS depressants. In a screening of a plant extract library for $GABA_A$ receptor modulatory activity, a dichloromethane extract of stems and roots of *Pholidota chinensis* (Orchidaceae) showed significant activity.
**Aims:** In the present work, we aimed to identify the compounds responsible for the $GABA_A$ modulatory activity of the extract. Furthermore, the importance of flexibility for receptor modulation of stilbenes was to be confirmed.
**Methods:** HPLC–based activity profiling [1] combined with high resolution LC–MS and microprobe NMR was used for isolation and structure elucidation of the active compounds. $GABA_A$ receptor modulatory activity was tested in a two–microelectrode voltage clamp assay [2] using *Xenopus laevis* oocytes transiently expressing the $GABA_A$ receptor subtype $\alpha_1\beta_2\gamma_2$s. Twelve commercially available stilbenes and their corresponding in-house produced semisynthetic dihydrostilbenes were submitted to the oocyte assay in the search for structure-activity relationships.
**Results:** Three structurally related stilbenes, coelonin **(1),** batatasin III **(2),** and pholidotol D **(3),** were identified as responsible for the activity of the extract, with **(2)** showing the highest receptor modulation (max. potentiation of $I_{GABA}$ by 1512.19% ± 176.47%). This suggested conformational flexibility to be crucial for the activity of stilbenoids.
When comparing commercially available stilbenes with their corresponding dihydrostilbenes, the latter showed higher activity in the oocyte assay when tested at a concentration of 100 $\mu$M. The dihydro derivatives of tetramethoxy-piceatannol **(12)** and pterostilbene **(20)** were the most active among this compound series, although their maximal enhancement of the $I_{GABA}$ was lower than that of compound **(2)** (870.7% ± 160.8% and 694.2% ± 86.0%, respectively).
**Conclusions:** Batatasin III was identified as the major active compound of the dichloromethane extract of *P. chinensis,* with an efficiency higher than that of benzodiazepines like triazolam. Dihydrostilbenes were established as a new scaffold for $GABA_A$ receptor modulators. Structural flexibility of stilbenoids was confirmed to play a crucial role in the $GABA_A$ receptor modulation.




**Keywords:** Pholidota chinensis, dihydrostilbenes, $GABA_A$ receptor.

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**P-14**

## High-Throughput Bioanalytical LC-MS/MS Methods Applied to *In Vivo* Animal Snapshot and Full PK Studies in Anti-Trypanosomal Drug Discovery Research

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**Introduction:** In pharmaceutical industry conventional *in vivo* animal PK studies are still routinely performed in a traditional low-throughput manner, and thus remain bottlenecks in drug discovery research. For each candidate compound and animal model used, a bioanalytical LC-MS/MS method has to be validated according to recommendations of international guidelines [1, 2].
**Aims:** In order to reduce time and costs for compound screening we have developed a high-throughput approach by omitting compound validation, and we have applied it to PK studies for human and livestock anti-trypanosomal drug discovery.
**Methods:** A snapshot PK study in mouse plasma (4 mice, 5 time points, pooled plasma resulting in one averaged PK profile per

compound per dosing route) for three potential drug candidates against human sleeping sickness, and a conventional standard full PK study in goat plasma (4 goats, 11 to 24 time points, not pooled plasma resulting in several PK profiles per compound per dosing route) for five potential diamidines against livestock sleeping sickness were done.
**Results:** The results indicate that the minimum requirements for a reliable quantitative LC-MS/MS method are: the selection of a suitable internal standard; at least two sets of seven calibration samples; three quality control (QC) samples at low, medium and high concentration levels; an additional QC for dilution; a carry-over assessment.
**Conclusions:** Even though the full validation of LC-MS/MS methods will remain essential in drug discovery, this high-throughput approach offers a time and cost saving alternative to accelerate compound selection during the screening and profiling phases [3].

**Keywords:** LC-MS/MS, high-throughput, snapshot PK study, sleeping sickness.

**References:**
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[2] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.
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**P-15**

## Rationally Designed Allosteric Activators of *Clostridium difficile* Toxin B Auto-Proteolysis

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**Introduction:** *Clostridium difficile* infection is the most frequent cause of hospital-acquired diarrhea and can cause life-threatening symptoms. Currently recommended treatments consist of antibiotics, which are harmful to the endogenous gut flora and are associated with high relapse rates. Toxins secreted by the bacteria once they have colonized the colon largely mediate disease symptoms. Targeting these toxins could be a viable therapeutic approach to alleviate the burden of the superbug *C. difficile*.
Inositol hexakisphosphate (IP6) binds to the cysteine protease domain (CPD) of *C. difficile* toxin B once the toxin has been endocytosed by colonocytes and thereby induces auto-proteolysis to release the enzymatic warhead into the cytosol. Triggering this self-cleavage step in the colon lumen before the toxin is taken up has the potential to significantly reduce its toxicity. We have observed that at the high concentrations of calcium found in the colon, cleavage activity of the holotoxin is abolished due to chelation of IP6.
**Aims:** To obtain molecules capable of activating toxin cleavage at the high calcium concentrations found in the colon.
**Methods:** We synthesized analogs of IP6 with sulphate groups replacing 3 (IP3S3), 4 (IP2S4) and 5 (IP1S5) of the phosphate groups respectively. We characterized and screened the analogs using calcium binding assays, cleavage assays on recombinant toxin fragments and on holotoxin B, and toxin fragment binding assays.
**Results:** We found that molecules with fewer phosphates had significantly weaker affinities for calcium ions. Substituting phosphates for sulphates only lead to a small loss in activity, as long as at least one phosphate remained. Holotoxin cleavage assays in 10 mM $Ca^{2+}$ showed that IP2S4 induced 31% cleavage of the toxin whereas IP6 only induced 2%.
**Conclusions:** We have developed what are to our knowledge the first IP6 analogs capable of activating holotoxin cleavage at high calcium ion concentrations. These molecules could therefore have therapeutic potential in the treatment of *C. difficile* infection.

**Keywords:** *Clostridium difficile,* hospital infection, antibiotic resistance, bacterial toxin, suberbug.

**P-16**

## Synthesis of Inhibitors of Oncogenic Fusion Protein NPM-ALK for *In Vitro* and *In Vivo* Assays

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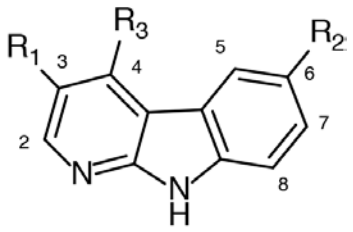
**Introduction:** Anaplastic large cell Lymphomas (ALCL) is a cancer affecting mostly children and young adults. It is driven by the kinase activity of the oncogenic fusion protein NPM-ALK resulting from t(2;5) (p23;q35) chromosomal translocation. Oncogenic fusion proteins involving ALK have been depicted also in a subset of 3–8% of non-small cell lung cancer (NSCLC). A first ALK inhibitor, Crizotinib (Xalkori) reached the market in 2011 with spectacular results. Unfortunately, resistance was rapidly observed, and 100% of the patients experienced disease progression through selection of resistant clones [1]. Over 20 clinically relevant Crizotinib-resistant ALK mutations have been identified to date.
**Aims:** There is therefore an urgent need for second-generation ALK inhibitors. Using structure-based design we have shown that the skeleton pyrido [2,3-b] indole could be a lead scaffold for the preparation of potential tyrosine kinase inhibitors of NPM-ALK. This core, commonly called alpha-carboline, appears in a number of natural products and molecules of pharmacological interest [2].
**Methods:** The objective of this work was to synthesize a library of tyrosine kinase inhibitors active against Crizotinib-resistant ALK mutations starting from the azacarbazole scaffold by modifying it with many different substitutions at position *C*-3 and *C*-6 or *C*-4 and *C*-6. In order to functionalize the aromatic ring, the methodology consists in sequentially using chemo- and regioselective Suzuki-Miyaura, Sonogashira, and Buchwald palladium-catalyzed cross-coupling reactions [3].
**Results:** The medicinal chemistry effort resulted in NPM-ALK inhibitors active *in vitro* and *in vivo*. The achieved *in vitro* activities are comparable or better than the one measured for Crizotinib in particular towards the clinical relevant Crizotinib-resistant NPM-ALK mutant (L1196M).




**Conclusions:** The use of an asymmetric $\alpha$-carboline core allowed us to obtain a wide variety of NPM-ALK inhibitors. The sequential palladium cross-coupling methodology is simple to implement and can be used to generate a broad spectrum of compounds.

**Keywords:** NPM-ALK inhibitors, pyrido [2,3-b] indole, cross-coupling reactions.

**References:**

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**P-17**

## First Comparative Study of Four Immortalized Human Brain Capillary Endothelial Cell Lines, hCMEC/D3, hBMEC, TY10, and BB19, for Studies of Transport Processes Across the Blood-Brain Barrier

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**Introduction:** Reliable human *in vitro* blood-brain barrier (BBB) models suitable for high-throughput screening are urgently needed in early drug discovery and development for assessing the ability of compounds to cross the BBB.
**Aims:** To establish an improved human *in vitro* BBB model, we compared four currently available and well characterized immortalized human brain capillary endothelial cell lines, hCMEC/D3, hBMEC, TY10, and BB19, with respect to barrier tightness and paracellular permeability. Co-culture systems using immortalized human astrocytes (SVG-A cell line) and immortalized human pericytes (HBPCT cell line) were designed with the objective of positively influencing barrier tightness.
**Methods:** Tight junction formation was assessed by Transendothelial Electrical Resistance (TEER) measurements using a conventional epithelial voltohmmeter (EVOM) and an automated CellZscope system which records TEER and cell layer capacitance ($C_{CL}$) on-line. Paracellular permeability was assessed using two fluorescent marker compounds that do not cross the BBB (sodium fluorescein (Na-F) and lucifer yellow (LY)). Conditions were optimized for each endothelial cell line by screening a series of 24-well tissue culture inserts from different providers. For hBMEC cells, further optimization was carried out by varying coating material, coating procedure, cell seeding density, and growth media composition.
**Results:** Highest TEER values and lowest paracellular permeability for Na-F and LY were obtained with mono-cultures of hBMEC cell line when cultivated on 24-well tissue culture inserts from Greiner Bio-one® (transparent PET membrane, 3.0 $\mu$m pore size). In co-culture models with SVG-A and HBPCT, no increase of TEER could be observed, suggesting that none of the investigated endothelial cell lines responded to stimuli from immortalized astrocytic or pericytic cells.
**Conclusions:** In summary, hBMEC cell line appeared to be the most suitable and promising cell line for a human *in vitro* BBB model in terms of barrier tightness in a 24-well mono-culture system intended for higher throughput. This BBB model will be validated with several compounds (known to cross or not to cross the BBB), and will subsequently be used for the assessment of BBB permeation by bioactive natural products.

**Keywords:** Blood-brain barrier (BBB), *in vitro,* TEER, paracellular permeability, CellZscope.

**P-18**

## Establishment of a Reliable *In Vitro* Human Blood-Brain Barrier Model for Early Screening of Bioactive Natural Products

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**Introduction:** In drug discovery, a major approach for estimating blood-brain barrier (BBB) permeability of new chemical entities is the use of Transwell® systems with tissue culture inserts, on which brain capillary endothelial cells from animal or human origin can be seeded. The drawback of most BBB models consists in the quantification methods used to determine the permeability of compounds across cell layers. LC MS/MS methods validated according to recommendations of international guidelines [1, 2] may overcome these shortcomings.
**Aims:** The purpose of this study was to establish a reliable human *in vitro* BBB model with immortalized hBMEC cells [3]. To verify the reliability of this model, paracellular permeability of ten compounds known to cross (antipyrine, caffeine, DB829, diazepam, and propranolol) or to not cross (cimetidine, epinastine, pentamidine, prazosin, and quinidine) the BBB is assessed using validated LC-MS/MS methods.
**Methods:** Quantitative LC-MS/MS assays for analytes in Ringer HEPES buffer are being developed and validated according to recommendations of current regulatory guidances [1, 2].
**Results:** Bioanalytical methods for four compounds (cimetidine, quinidine, diazepam, and propranolol) in Ringer HEPES buffer have been validated in terms of selectivity, precision, accuracy, and reliability according to EMA/FDA guidances [1, 2]. The remaining six LC-MS/MS methods need to be validated.
**Conclusions:** After verifying the reliability of the human *in vitro* BBB model, natural products showing promising activity against tropical protozoal diseases and CNS disorders will be screened for their ability to cross the BBB.

**Keywords:** LC-MS/MS, blood-brain barrier (BBB), EMA/FDA, natural products.

**References:**

[1] Guidance for Industry: Bioanalytical Method Validation, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research, May 2001.
[2] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.
[3] Stins MF, Badger J, Kim KS. Microb Pathogenesis 2001; 30: 19–28.

**P-19**

## Comparative Study with Two Folic Acid Radioconjugates Showing Differences in Anti-Tumor Efficacy and Kidney Dose Burden

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**Introduction:** The folate receptor (FR) is overexpressed in various cancer types and shows a high affinity to the vitamin folic acid [1]. Therefore it is reasonable to target the FR with folic acid radio-con-

jugates for cancer diagnosis and therapy [2]. However, a drawback so far was the high kidney uptake due to renal FR expression. In view of a therapeutic application using particle-emitting radiation (e.g. $^{177}$Lu) the risk of nephrotoxic side effects arises. To overcome this problem, we recently developed a folate conjugate containing an albumin-binder (cm09) which resulted in a prolonged circulation time and consequently increased tumor-to-kidney ratio [3].

**Aims:** The aim of this study was to compare the therapeutic efficacy and potential kidney toxicity of a conventional DOTA-folic acid conjugate (EC0800) and a novel DOTA-folate conjugate containing an albumin-binder (cm09) radiolabeled with the therapeutic isotope $^{177}$Lu.

**Methods:** The biodistribution of $^{177}$Lu-EC0800 and $^{177}$Lu-cm09 was evaluated by SPECT/CT imaging of KB (FR+) tumor bearing mice. In a therapy study in KB xenografted mice the anti-tumor efficacy of $^{177}$Lu-EC0800 (20 MBq) and $^{177}$Lu-cm09 (20 MBq) was compared. Radionephrotoxicity after the administration of 20 MBq of $^{177}$Lu-EC0800 or $^{177}$Lu-cm09, resulting in estimated absorbed kidney doses of ~100 Gy and ~70 Gy, respectively, was investigated in a long-term study in nude mice over 8 months.

**Results:** The SPECT/CT images revealed a tumor-to-kidney ratio of ~0.1 for $^{177}$Lu-EC0800 and ~1 for $^{177}$Lu-cm09. The increased tumor uptake of $^{177}$Lu-cm09 improved the therapeutic efficacy and resulted in complete tumor remission in 4 out of 5 mice which is superior to a treatment with $^{177}$Lu-EC0800 where the tumor growth was not considerably reduced compared to control animals. The long-term study investigating the kidney toxicity revealed a 3-fold higher creatinine and a 1.3-fold higher blood urea nitrogen plasma value at terminal state for mice administered with $^{177}$Lu-EC0800 compared to animals injected with $^{177}$Lu-cm09. The average survival was reduced to 164 days in the group treated with $^{177}$Lu-EC0800 whereas more than 50% of the animals were still alive at the end of the study (8 months) in the group injected with $^{177}$Lu-cm09.

**Conclusions:** Our results demonstrate that the integration of an albumin-binding entity into the folate conjugate is a strategy to improve the anti-tumor efficacy and to prevent severe nephrotoxic side effects during FR-targeted radionuclide therapy. Therefore, $^{177}$Lu-cm09 is the most favorable radiofolate ever tested for therapy of FR-positive tumors in mice. Therefore, this folic acid radioconjugate will be tested in more detail in future pre-clinical therapy studies.

**Keywords:** Folate receptor, folic acid radioconjugate, albumin-binder, radionuclide tumor therapy, nephrotoxicity.

**References:**

[1] Parker N, Turk MJ, Westrick E. Anal Biochem 2005; 338: 284-93.
[2] Müller C. Curr Pharm Des 2012; 18: 1058-83.
[3] Müller C, Struthers H, Winiger C. J Nucl Med 2013; 54: 124-31.

**P-20**

## Phytochemical and Radical Scavenging HPTLC Profiles of Chilean "Maqui" berries *(Aristotelia chilensis)* at Different Ripening Stages from Cultivated Accessions

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**Introduction:** *Aristotelia chilensis* (Mol.) Stunz is a shrub which grows wild in central to southern Chile and western Argentina. Apart from its traditional medicinal use in Chilean folk medicine, it is also known for producing tasty antioxidant berries, locally called "Maqui". The exploitation of the wild resources has grown extensively.

**Aims:** Selection, domestication and cultivation studies on an agroindustrial scale in Chile are supported by phytochemical and antioxidant activity assessment. On this basis, cultivatable clones shall be selected in order to provide industry with sustainably produced Maqui berries of standardized quality.

**Methods:** HPTLC was chosen for the assessment of phytochemical and antioxidant variations in Maqui berry samples of different genotypes, field cultivation conditions and ripening stages at harvest time. The sample preparation method described in [1] was modified for small sample amounts using mechanically assisted extraction with ceramic beads. A suitable HPTLC method was developed using derivatization with Fast Blue Salt and Natural Product reagents for phytochemicals and DPPH for radical scavenging activity profiles. In addition, a high throughput ORAC assay was used for comparison of antioxidant activity in the samples.

**Results:** The results suggest that differences in ripening stages and cultivation conditions lead to variations in phytochemical and radical scavenging profiles on HPTLC plates supported by antioxidant ORAC data.

**Conclusions:** The study is an example of how HPTLC can be a powerful, rapid and cost-effective tool in both quality control and the support of agronomic research on valuable medicinal and neutraceutical plants.

**Keywords:** *Aristotelia chilensis,* Maqui berry, antioxidant screening, radical scavenging, HPTLC.

**Reference:**

[1] Escribano-Bailón M et al. Phytochem Anal 2006; 17: 8–14.

**P-21**

## A New High-Throughput Screening Test Measuring Human Serum Albumin Dissociation Constant and Artificial Permeability Using One End-Point Measurement

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**Introduction:** Inappropriate pharmacokinetics (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 become a real need to early predict compounds PK properties and in particular their ability to penetrate biological membranes. One of the major protein encounters by NCEs crossing the gastrointestinal track membrane is albumin which represents about 60% of all plasmatic proteins. Most *in vitro* assay being able to predict binding constant such as dissociation ($K_d$) or association constant ($K_a$) are studied in a kinetic way. Whereas the passive permeability can be evaluated with well-known PAMPA (parallel artificial membrane permeability assay) techniques.

**Aim:** The aim of this study was to develop a high-throughput assay able to predict passive permeability through intestinal track and determine the dissociation constant of compounds towards human serum albumin (HSA) using only an one-point measurement.

**Methods:** The assay is based on the PAMPA 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. In

this study, hexadecane has been used as artificial membrane to mimic the passive diffusion through the gastrointestinal track [1]. By adding HSA to the acceptor compartment, this will force a certain amount of compounds to pass the artificial barrier at a certain gradient concentration depending on the affinity it has towards HSA. A mathematical model permitting the calculation of 5 differentials is necessary to determine a $K_d$ value.
**Results:** Passive permeability values obtained were well correlating with the percentage of *in vivo* gastrointestinal absorption. A good correlation was also found between $K_d$ from the literature and the $K_d$ obtained using only one end-point measurement.
**Conclusions:** The ability to have an assay which can predict an equilibrium affinity constant in just one point is a new way to determine $K_d$ without doing a kinetic study. In this study it has been possible to obtained $K_d$ in the range of -3 to -6 of moderate to highly permeate compounds. HSA can be used freely in solution which limits the risk of unavailable active pockets. Therefore, using simple and low-cost PAMPA-based techniques, it is possible to get two information which are nowadays essential in the conception of new drug entities.

**Keywords:** ADME, permeability, PAMPA, human serum albumin.

**Reference:**
[1] Wohnsland F, Faller B. J Med Chem 2001; 44: 923–930.

## P-22

### Hepatocyte Targeting Using Pegylated Asialofetuin-Conjugated Liposomes

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**Introduction:** Incidence rates of hepatic diseases have been increasing steadily over the past decades while available pharmacotherapies have remained insufficient [1]. Delivery of compounds specifically to hepatocytes would be desirable since hepatocytes are important pharmacological targets, especially in chronic viral hepatitis, hepatocellular carcinoma, and genetic disorders where specific delivery of genes would offer interesting therapeutic options. Specific targeting of nucleic acids and drugs to the hepatocytes can be achieved using nanoparticulate drug carriers such as liposomes [2].
**Aims:** The most promising strategy to improve therapeutic efficacy and reduce off-target effects is active targeting through the asialoglycoprotein receptor which mediates uptake of desialylated glycoproteins by receptor-mediated endocytosis. The present work explored a hepatocyte-specific targeting strategy using asialofetuin (AF). AF was thereby attached to liposomes using polyethylene glycol (PEG) as a linker, allowing AF to be flexibly tethered to the liposome surface.
**Results:** Cellular binding and intracellular accumulation of AF-PEG-liposomes were studied *in vitro* using the hepatocellular carcinoma cell line HepG2. Active targeting of hepatocytes and avoidance of unspecific uptake by the reticuloendothelial system were demonstrated *in vivo* in the rat. Different types of encapsulated model compounds, including membrane-linked fluorochromes and quantum dots, were used to demonstrate the feasibility of the targeting strategy.
**Conclusion:** We conclude that the use of AF-conjugated, pegylated liposomes is a promising strategy to avoid the reticuloendothelial system and specifically target hepatocytes via the asialoglycoprotein receptor and thus liver parenchymal cells *in vitro* as well as *in vivo.*

**Keywords:** Nanoparticle, drug delivery, active targeting, hepatocyte, asialoglycoprotein receptor.

**References:**
[1] Poelstra K, Prakash J, Beljaars L. J Control Release 2012; 161: 188–197
[2] Huwyler J, Drewe J, Krähenbuhl S. Int J Nanomedicine 2008; 3: 21–29.

## P-23

### New Sesterterpenoids from *Salvia mirzayanii*, Stereochemical Characterization by Computational Electronic Circular Dichroism

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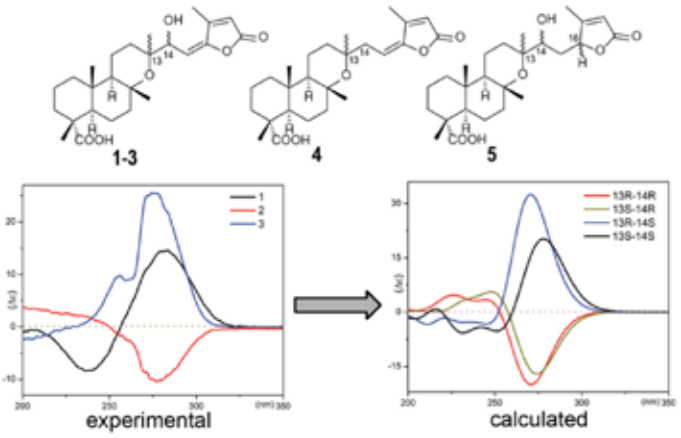
**Introduction:** Sesterterpenes are rare in nature and have been reported most commonly in marine sponges and algae. These compounds exhibit diverse biological properties, such as anti-inflammatory, cytotoxic, anti-biofilm, antimicrobial, and anticancer activities. Among terrestrial plants, *Salvia* species are a rich source for sesterterpenes. *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.
**Aims:** In a project directed at novel bioactive metabolites from endemic Iranian Lamiaceae, we studied *Salvia mirzayanii.*
**Methods:** Phytochemical profiling of a *n*-hexane extract by a combination of normal phase column chromatography and preparative and semi-preparative reversed phase HPLC afforded five new sesterterpenoids. Their structures were established by means of extensive NMR (1D and 2D) and HRESI-MS spectroscopy.
**Results and conclusion:** Structure elucidation revealed that compounds **1–3** differed only in their configurations at C-13 and C-14. Assignment of relative and absolute configurations was challenging due to free rotation around the C-13/C-14 bond, but could be achieved by comparison of experimental and simulated ECD spectra of all possible stereoisomers. Time dependent density function theory TDDFT/CAM-B3LYP/6-31G** with MeOH as solvent, and the "self-consistent reaction field" method (SCRF) with the conductor-like polarizable calculation model (CPCM) were used for calculation [1-2]. Absolute configurations of **4** and **5** were established in a likewise approach.




**Keywords:** *Salvia mirzayanii,* Lamiaceae, sesterterpenes, electronic circular dichroism.

**References:**
[1] Bringmann G et al. Chirality 2008; 20: 628-642.
[2] Moradi-Afrapoli F et al. Phytochemistry 2013; 85: 143-152.

P-24

## Bioreduction of Disulfide-Containing Cationic Delivery Systems

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**Introduction:** The use of disulfide bonds in drug formulations and biomaterials as bioreducible linkers has increased in popularity and functionality in recent years. More specifically, in the field of gene delivery, disulfide bonds are being added to cationic carriers to attach shielding/targeting moieties or to promote biodegradation [1]. Many of these systems were designed under the assumption that the disulfide bond stays intact in the extracellular space and undergoes cleavage inside the cell due to the redox-potential gradient between these two environments. Although it was shown that the introduction of disulfide linkages enhances gene transfer and reduces cytotoxicity of polycationic systems, a significant understanding of the properties of these linkages and the site of reduction during cellular trafficking are still lacking.
**Aim:** The objective of this work was to synthesize a redox-sensitive polycationic model system and to quantitatively investigate its bioreduction in different cell lines. The goal was to clarify the properties of this specific type of disulfide linkage to improve the design and efficiency of future bioreducible gene delivery systems.
**Methods:** A third generation (G3) poly(amido amine) (PAMAM) bearing two boron-dipyrromethene (BODIPY) fluorophores via labile disulfide bonds was prepared and used as a model cationic delivery system. In the intact labeled PAMAM, the fluorescence of the dye molecules was self-quenched due to their proximity to one another. Upon disulfide bond cleavage, fluorescence was restored, thereby allowing a real-time and quantitative spectrophotometric measure of disulfide reduction. The system showed a 10-fold increase of fluorescence intensity after complete cleavage and the bioreduction was then analyzed in four different tumor cells.
**Results:** Detailed characterizations of the system revealed that the polyionic nature of the dendrimer exerted interesting microenvironmental effects on the disulfide bonds. Due to electrostatic interactions with charged reducing agents, disulfide exchange was accelerated, making disulfide bonds in poly-cations highly susceptible to cleavage. Although cell line specific differences were observed, in all cells tested the disulfide bond was already significantly reduced in the extracellular space. The implication of cell surface oxido-reductases and the amounts of cell-secreted thiols could explain these findings. Shielding the disulfide bonds with nucleic acids provided a means to at least partially stabilize the disulfide bonds of such systems.
**Conclusions:** This study provided the first detailed and quantitative analysis of the exchange of disulfides associated with cationic polymers. It was demonstrated that disulfide cleavage of cationic polymers, likely to be used in gene delivery, occurred to a large extent extracellularly. New insights for explaining the reduced cytotoxicity and improved transfection efficacy of disulfide-containing cationic delivery systems are given by these findings [2]. This knowledge is important for the future design of bioreducible gene delivery systems.

**Keywords:** Bioreducibility, dendrimers, disulfide, gene delivery, polycationic.

**References:**
[1] Bauhuber S et al. Adv Mater 2009; 21: 1–21.
[2] Brülisauer L et al. Angew Chem Int Ed 2012; 51: 12454–12458.

P-25

## NMR Spectroscopic Investigations of Porphyrinic Photosensitizers with Nanoparticles as Carrier Systems

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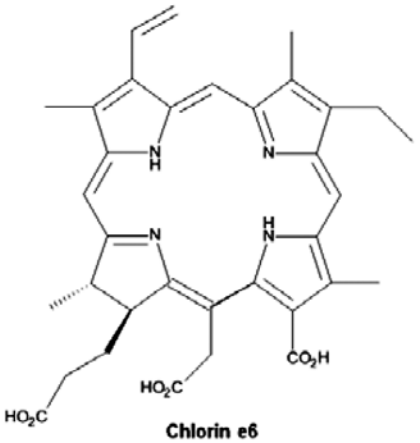
**Introduction:** Photodynamic therapy (PDT) is a cancer treatment modality, which is based on the selective uptake of a photosensitizer (PS) by cancer tissue. Upon excitation with red light the photosensitizer may react with molecular oxygen ($^3O_2$) via energy transfer to generate singlet oxygen ($^1O_2$), which is known to give rise to oxidative reactions triggering cell death. Many porphyrinic PS molecules, however, are hydrophobic and have a strong tendency to aggregate in aqueous solution decreasing their photosensitizing and biological effectiveness. The use of nanoparticles as PS carriers presents a promising approach for enhancing PDT efficacy [1].
**Aims:** To find an appropriate nanocarrier system for selected porphyrinic compounds, which are potential PSs in PDT.
**Methods:** 1D and 2D $^1$H NMR spectroscopic methods were used to probe nano-sized carrier systems such as polyvinylpyrrolidone (PVP) or ß-cyclodextrin for their ability to form complexes with derivatives of the dihydroporphyrin chlorin e6 in phosphate buffered saline.



Chlorin e6

**Results:** Through analysis of the changes in chemical shift and line width of the porphyrin signals the extent of disaggregation in the presence of the nanoparticle could be assessed. Observation of porphyrin ring current induced shifts of the nanoparticle resonances provided information on the porphyrin-nano-particle interaction. Further proof of the intermolecular contact could be obtained by 2D-NOESY and 2D-ROESY experiments. NMR diffusion experiments (DOSY) revealed altered dynamics of the nanoparticles and porphyrins upon complex formation.
**Conclusions:** Providing insight into changes of the chemical environment, NMR spectroscopy has proved to be a powerful tool for investigating if a specific carrier system is able to complex a selected porphyrin and to affect its aggregation. Both PVP and ß-cyclodextrin showed potential to prevent aggregation of the selected porphyrins to a certain extent. Further work will be focused on finding carrier systems with a higher porphyrin disaggregation efficiency and on probing cell uptake of the corres-ponding systems.

**Keywords:** Photodynamic therapy, photosensitizer, porphyrin, nanocarrier, NMR.

**Reference:**
[1] Bechet D et al. Trends Biotechnol 2008; 26: 612-21.

**P-26**

## Quantification by UPLC-MRM ESIMS of Bufadienolides in *Bryophyllum pinnatum* Leaves and Manufactured Products

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**Introduction:** *Bryophyllum pinnatum* (Crassulaceae) is a succulent perennial plant native to Madagascar. It is used in anthroposophical medicine to treat psychiatric disorders, and as a tocolytic agent to prevent premature labour. Besides flavonoids, the plant is known to contain bufadienolides, which reportedly possess sedative and positive inotropic properties, as well as central nervous system related activities. Despite the possible toxicological relevance of bufadienolides, no reliable data are available on their content in plants and phytotherapeutic preparations.
**Aims:** Aim of this study was to quantify bufadienolides in *B. pinnatum leaves* and in manufactured products such as presse juice and dried powder consisting of a 1:9 mixture of dried juice and lactose.
**Methods:** A UPLC-ESIMS assay with multiple reaction monitoring (MRM) was developed and validated for the quantification of the main four bufadienolides, bryophyllin A, bersaldegenin-1-acetate, bersaldegenin-3-acetate, and bersaldegenin-1,3,5-orthoacetate. The separation was performed on a Kinetex 1.7 uXB-C18 column with a gradient of acetonitrile/water containing 1 mM ammonium formiate. Bufalin was used as an internal standard. Reference compounds were previously isolated from the related species *B. daigremontianum.* Leaves and dried powder were extracted with EtOH by accelerated solvent extraction (ASE). For juices liquid/liquid extraction with EtOAc was used.
**Results:** The contents of bryophyllin A, bersaldegenin-1-acetate, bersaldegenin-3-acetate and bersaldegenin-1,3,5-orthoacetate were 7.75, 1.45, 4.89 and 5.17 mg/100 g dry weight (DW), respectively, in leaves from plants grown in Brazil. The contents in these four bufadienolides were significantly lower in plants grown in Germany (1.18, 0.99, 2.08 and 0.94 mg/100 g DW, respectively). The total amount of bufadienolides was 0.066 mg/100 g DW in dried powder, and 1.71 and 0.59 mg/100 mL in press juices obtained from plants cultivated in Brazil and Germany, respectively.
**Conclusions:** This study provides for the first time reliable data on the content of bufadienolides in *B. pinnatum* leaves and manufactured products. The content of these compounds varies between the two analyzed batches of leaves and juices. Analysis of further samples is scheduled to investigate in more detail the influence of geographic origin and harvest time on the bufadienolide content of the plant.

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

**P-27**

## Development and Validation of a LC-MS/MS Method for Assessment of an Anti-inflammatory Indolinone Derivative by *In Vitro* Blood-Brain-Barrier Models

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**Introduction:** Previously we identified (E,Z)-3-(4-hydroxy-3,5-dimethoxybenzylidene)indolin-2-one (indolinone) from woad (*Isatis tinctoria* L., Brassicaceae) as a compound possessing histamine release inhibitory and anti-inflammatory properties [1]. Evaluation of the pharmacokinetic properties of the compound, and in particular, the ability to cross the blood-brain barrier (BBB) is necessary.
**Aims:** We aim to screen indolinone in several *in vitro* cell-based human and animal BBB models. For this purpose, we first established a LC-MS/MS assay for the compound according to international guidelines [2,3].
**Methods:** The LC-MS/MS quantification method for indolinone in Ringer HEPES buffer was validated according to EMA and FDA guidances [2,3].
**Results:** The calibration curve of indolinone in Ringer HEPES buffer in the range between 30.0 and 3000 ng/mL was quadratic, and the limit of quantification was 30.0 ng/mL. Diluting samples up to 100-fold did not affect precision and accuracy. The carry-over was within acceptance criteria. Indolinone proved to be stable for 3 h at room temperature, and for 3 successive freeze/thaw cycles. The processed samples could be stored in the autosampler at 10 °C for at least 28 h. Moreover, indolinone was stable for at least 16 days in Ringer HEPES buffer when stored below -65 °C.
**Conclusions:** This validation study demonstrates that our method is specific, selective, precise, accurate and capable to produce reliable results. The method will be used to assess BBB permeability of indolinone in human and animal *in vitro* BBB models.

**Keywords:** *Isatis tinctoria,* anti-inflammatory, LC-MS/MS, blood-brain barrier (BBB).

**References:**
[1] Kiefer S et al. Eur J Pharm Sci 2010; 40: 143-147.
[2] Guidance for Industry: Bioanalytical Method Validation, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research, May 2001.
[3] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.

**P-28**

## 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 of different origins, harvesting dates, and between single and repeatedly harvested plants.
**Methods:** Plants were grown under identical conditions on experimental plots at the Agricultural Field Station of Thuringia in Dornburg, Germany. For the study, plants of six origins were planted, and they were harvested at six time points. In addition, one part of the plants was single and another part repeatedly harvested. 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 70% aqueous

methanol. The NMR spectra were analyzed by multivariate data analysis.
**Results:** The score plots produced by principal component analysis (PCA) revealed differences in the metabolic profile between the harvesting dates. The loading plots showed that the spectral region of carbohydrate resonances was responsible for these differences. In contrast, no major differences were seen in the metabolites of different origins. Partial least square discriminant analysis (PLS-DA) revealed no effect of repeated harvesting on the metabolic profiles.
**Conclusions:** The results demonstrated that the time point of harvesting has an influence on the metabolic profile influencing mainly the carbohydrate metabolites. However, there are no major differences between the origins observed. Furthermore, repeated harvesting showed no effect on the metabolic profile.

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

**Reference:**
[1] Hamburger M. Phytochem Rev 2002; 1: 333–344.

**P-29**

## *In Silico* Modelling of Hydrophilic Floating Tablet Formulations

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**Aims:** The objectives of the study were to model *in silico* the floating behavior and drug release mechanism of a gastroretentive drug delivery system (GRDDS) and compare the results to experimental observations. An attempt towards understanding of behavior of hydrophilic floating tablet formulation was made.
**Methods:** *In silico* dissolution and floatation profiles of the tablet were simulated using the standard three-dimensional cellular automata-based model [1]. The automata rule set was extended to account for medium absorption kinetics by porous compounds. Additionally, the state of every cellular automaton was recorded after every iteration step. Thus, calculation of tablet density during *in silico* dissolution simulation and in consequence evaluation of tablet floatation was possible. For *in vitro* evaluation, we used a custom-built stomach model to simultaneously analyze floating characteristics and drug release.
**Results:** During dissolution simulation under acidic conditions, the virtual floating compact eroded completely while releasing drug substance. The calculated tablet densities increased only slightly higher than 1 g/cm$^3$. In water as dissolution medium, the density of the compact was reaching values higher than 1 g/cm$^3$ after approximately 60 min. This event indicated sinking of the floating tablet.
**Conclusions:** The *in silico* drug release and floatation simulations were in accordance with the experimental results. They support the hypothesis of floating mechanism, i. e. a reaction-based erosion mechanism with polymers as imbibition-inhibiting components. *In silico* dissolution simulation was found to offer a possibility to understand the floating mechanism and drug release of floating dosage forms.

**Keywords:** 3D cellular automata dissolution model, gastroretentive drug delivery system, floatation mechanism.

**Reference:**
[1] Puchkov M, Tschirky D, Leuenberger H. 3-D cellular automata in computer-aided design of pharmaceutical formulations: mathematical concept and F-CAD software. Formul. Tools Pharm. Dev., Woodhead Publishing; 2013.

**P-30**

## Twin-Screw Hot-Melt Granulation for Preparation of Floating Tablet Formulations

**V. A. Eberle[1], J. Schoelkopf[2], D. E. Gerard[2], R. Alles[1], J. Huwyler[1], M. Puchkov[1]**
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**Aims:** Floating drug delivery systems (FDDS) offer a possibility to overcome the problem of limited gastric residence times of oral dosage forms. Due to its high porosity and lamellar surface structure, functionalized calcium carbonate (FCC) was found to be a promising novel pharmaceutical excipient for preparation of floating tablets. The aim of the study was to investigate the applicability of hot-melt granulation using a twin-screw hot-melt extruder for manufacture of FCC-based floating formulations.
**Methods:** Planning and analysis of the experiments was done using the experimental design software *STAVEX*. In first cycle, different excipients and hot-melt extruder settings were studied. The amounts of the excipients selected for preparation of the floating formulations were varied in second cycle. We defined tablet hardness and drug release time of the floating tablets as response variables. *In vitro* floating behavior and drug release were characterized using a custom-built stomach model.
**Results:** Hot-melt granulation and compaction of tablets with a density less than 1 g/cm$^3$ to achieve floatation on the gastric fluids were possible for all formulations. In cycle 1, the highest tablet breaking strength was obtained with FCC in combination with the excipients paraffin and Polyox WSR-301 prepared at temperature profile 80 °C and a high hot-melt extruder throughput rate. In cycle 2, we selected an optimal formulation containing 15% (w/w) caffeine as model drug substance, 40% (w/w) FCC, 40% (w/w) paraffin, and 5% (w/w) Polyox WSR-301. The resulting tablets had a hardness of 32 N. After being placed on the surface of the dissolution medium, the compacts floated immediately. Complete caffeine release was obtained after 11 h; a floating tablet core remained after 100% drug release.
**Conclusions:** The usability of the twin-screw hot-melt extruder for hot-melt granulation of FCC-based floating formulations was demonstrated. Compaction of tablets with a density less than unity and at the same time sufficient hardness was possible. Floating tablets had no floating lag time and consequently a reduced risk for unpredictable premature emptying from the stomach. We obtained drug release times within the desired range of 8 to 24 h.

**Keywords:** Gastroretentive drug delivery system, floating tablet, hot-melt extrusion, stomach model.

**P-31**

## Evaluation of Novel Thymine Derivatives as Potential Positron Emission Tomography Probes for HSV1-tk Gene Expression Imaging

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**Introduction:** The expression of a transfected therapeutic gene in certain cells can be noninvasively evaluated *in vivo* using a reporter system. Herpes simplex virus type 1 thymidine kinase (HSV1-tk)

gene is a reliable reporter gene. The translation product, HSV1-TK, can accommodate a wide spectrum of nucleoside analogues as substrates and selectively phosphorylate them [1,2].
**Aims:** This project aims at synthesizing pyrimidine analogs with an unsaturated alkyl chain as a sugar mimicking moiety in the C-6 position and biochemically characterising them in order to select the best candidate for developing PET tracers.
**Results:** Several C-6-substituted analogs were synthesized and structurally characterized. Their phosphorylation pattern was monitored in presence of HSV1-TK or hTK using a protocol based on HPLC-UV/DAD separation and detection method [3]. Synthesized compounds were phosphorylated in presence of HSV1-TK but not in presence of hTK. Diphosphorylation by HSV1-TK of non-natural substrate was observed for the first time. The crystal structures of HSV1-TK in complex with the compounds were solved by molecular replacement using the data collected at the SLS synchrotron. The electron density maps calculated at the beginning of the refinement showed the presence of the compounds inside the binding site of HSV-1 TK. The proliferation of HSV1-TK transduced HEK293-cells was selectively inhibited by one of the compounds with an $IC_{50}$ value comparable to the one of ganciclovir. PK15 NTD cell lines expressing human equilibrative and concentrative nucleoside transporters have been used according to the published protocol [4] to assess compounds transportability. The experiments to determine $IC_{50}$ and Ki values of these compounds are in progress and will be shown.
**Conclusions:** The results obtained indicate that one of the synthesized compounds could be potentially a new PET reporter probe and for that purpose *in vivo* PET imaging studies will be performed.

**Keywords:** HSV1-TK, C-6-substituted pyrimidine derivative, positron emission tomography, nucleoside transporters.

**References:**
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**P-32**

## Screening of Alpine Plant Extracts from the Canton of Valais as Protective Agents Against UV-Induced Skin Damage

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**Introduction:** In Switzerland, incidence of skin cancer has increased in the last few years. High sun protection factors (SPF) in suncare products allow the consumer to expose himself to sunlight for longer periods, but they do not completely protect the skin from UV-related damage. Therefore, it is important to develop novel cosmetic formulations which can efficiently fight the production of reactive oxygen species, subsequent cell damages, and ultimately skin cancer. Alpine plants used in medicine and nutrition represent an attractive source for the development of new products with skin-protective effects. Plants growing at high altitudes have developed chemical defense mechanisms such as the synthesis of secondary metabolites protecting against exposure to high UV doses.
**Aims:** The objective of this study is to develop a cosmetic ingredient issued from alpine plants to protect skin against damages resulting from UV exposure.
**Methods:** More than 30 extracts from alpine plants growing in the Swiss Valais region were screened for antiradical and antiinflammatory activities *in vitro*. Plants were extracted with ethanol using accelerated solvent extraction (ASE). Cyclooxygenase-2 inhibitory activity was measured through COX-2 catalyzed prostaglandin synthesis in Mono Mac 6 cells. The formation of 6-keto $PGF_{1a}$ was determined by ELISA. Absence of cytotoxicity was confirmed by assessing cell mitochondrial activity (resazurin and MTT bioassays) in Mono Mac 6 cell line. Antioxidant activity of the extracts was assessed with the DPPH radical.
**Results:** Several extracts exhibited significant activities. The strongest COX-2 inhibition was observed for the extracts of *Pritzelago alpina* (Brassicaceae), *Athamanta cretensis* (Apiaceae), *Satureja montana* (Lamiaceae) and *Sisymbrium irio* (Brassicaceae) with 21.9, 28.6, 21.8 and 22.5% inhibition at 100 µg/mL, respectively. With regard to the free radical scavenging activity, *Geum montanum* (Rosaceae) ($IC_{50}$ 17 µg/mL) was the most active, followed by *Helianthemum nummularium* (Cistaceae) ($IC_{50}$ 49 µg/mL), *Salix reticulata* (Salicaceae) ($IC_{50}$ 53 µg/mL), and *Satureja montana* ($IC_{50}$ 65 µg/mL).
**Conclusions:** Alpine plants have been identified which showed interesting COX-2 inhibition and antioxidant activity. The most promising extracts have currently being submitted to HPLC-based activity profiling in order to identify the active constituents. This should enable to select the best candidates as cosmetic ingredients for suncare formulation.

**Keywords:** Alpine plants, cosmetics, COX-2 inhibition, free radical scavenging.

**P-33**

## Synthesis and Evaluation of Novel Cannabinoid Type 2 Receptor Traers for PET Imaging

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**Introduction:** The cannabinoid receptor type 2 (CB2) has a very low expression level in brain tissue under basal conditions, but it is upregulated in diverse pathological conditions such as neuro-inflammation and neurodegenerative diseases including Parkinson's and Alzheimer`s disease.
**Aims:** Our goal is to develop a suitable brain PET tracer with high specificity towards CB2 and high selectivity vs. CB1.
**Methods:** We evaluated the potential of a promising 4-oxo-quinoline lead structure from literature – designated KD2 – as an imaging agent for CB2 sites. Within an optimization program with focus on lowering lipophilicity and plasma protein binding, several new KD2 derivatives were synthesized and their binding affinities towards hCB1/2 were determined in *in vitro* competitive binding assay experiments using [$^3$H]-CP-55940. Furthermore, various novel CB2 ligands from a novel structural class were synthesized and tested in functional and binding assays.
**Results:** Preliminary *in vitro* and *in vivo* studies showed that [$^{11}$C] KD2 is a promising PET tracer for CB2. *K*i values for the CB2 receptor for both series of novel compounds with improved properties ranged from 0.7 to 1220 nM, with a selectivity hCB2 over hCB1 from 10 to >10'000.
**Conclusions:** Several new compounds show very promising *in vitro* properties to become suitable CB2 brain PET tracers. A small selection of compounds will be radiolabeled with $^{11}$C or $^{18}$F isotopes for further *in vitro* and *in vivo* evaluation.

**Keywords:** Cannabinoid receptor type 2 ligand, neurodegenerative, radiolabeling, autoradiography, positron emission tomography (PET).

**P-34**

## Does Alteration of Adenine Purine Levels Lead to a Loss-of-Fitness Phenotype in *Trypanosoma brucei* ?

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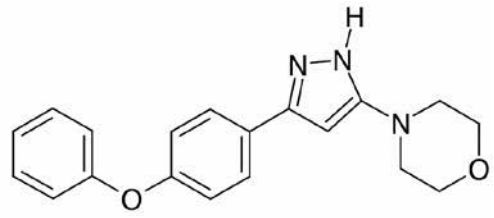
**Introduction:** Human African Trypanosomiasis (HAT), also known as sleeping sickness, is caused by the parasites *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Available treatments for this disease still remain unsatisfactory, needing parental administration, are toxic, or parasites have acquired resistance. Thus safe and potent new drugs are needed to treat sleeping sickness. *Trypanosoma brucei* adenosine kinase (*Tb*AK), an enzyme involved in the purine salvage pathway, was identified by chemical proteomics to be the intracellular target of 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine (compound 1, Fig. 1). This molecule exhibits very good antitrypanosomal activity with an $IC_{50}$ of 1 $\mu$M, and biochemical analysis revealed it to be a strong activator of *Tb*AK [1,2].
**Aims:** It is goal of the current project to understand the mechanism of action of compound **1** at the cellular level.
**Methods:** Changes in adenine and guanine purine levels of trypanosomes induced by compound **1** were measured by means of an ion-pair RP-HPLC/UV method. Several strains of *Trypanosoma brucei* were tested, e. g. (i) wild type strain, (ii) a tetracycline-inducible ak overexpression strain, hypothesizing that the presence of compound 1 is similar to overexpression of *Tb*AK), and (iii) a tetracycline-inducible null mutant ak overexpression strain to gain insight into the effect of non-physiologically high levels of *Tb*AK.
**Results:** Compound **1** is interacting with adenine nucleotide pools, and overexpression of functional and non-functional *Tb*AK leads to alterations in adenine nucleotide levels.
**Conclusions:** Growth phenotype induced by compound **1** cannot fully be explained by the observed alterations of adenine purine levels. Therefore, additional metabolites need to be investigated and/or additional potential targets of compound **1** need to be discovered. Overexpression of functional *Tb*AK has a similar but not the same effect on parasite growth and nucleotide pool levels compared to compound **1.** In addition, overexpression of non-functional *Tb*AK has pointed to an additional role besides converting adenosine and ATP to AMP and ADP of this enzyme.

Figure 1 Chemical structure of compound 1




**Keywords:** *Trypanosma brucei,* ion pair RP-HPLC/UV, adenine nucleotides, guanine nucleotides.

**References:**
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**P-35**

## Drug Loading into Porous Calcium Carbonate by Solvent-Evaporation

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**Introduction:** Porous drug carriers were shown to control and improve *in vitro* drug release of poorly water-soluble drugs. However, drug loading of porous carriers by adsorption is challenging since the impregnation method lacks reproducibility and efficiency for certain drug carriers and active substances. Functionalized calcium carbonate (FCC) is a novel pharmaceutical excipient [1] and has potential as drug carrier as it exhibits small particle size ($d_{50}$ = 17.9 $\mu$m), relatively large pore size (up to 1 $\mu$m), high intraparticle porosity (70% v/v), and large specific surface area (43.9 $m^2$/g).
**Aims:** The aim of this study was to evaluate an efficient drug loading method based on solvent-evaporation and crystallization, which is applicable on FCC or comparable calcium carbonate particles, and various drug substances.
**Methods:** Four model drugs with different solubility and permeability properties were selected for investigation of drug loading. In brief, ibuprofen (IBU), nifedipine (NP), losartan potassium (LK), and metronidazole benzoate (MBZ) were dissolved in acetone or methanol. After dispersion of FCC the solvent was removed under reduced pressure in a rotary evaporator. For each model drug, a series of drug loads was produced ranging from 25% to 50% (w/w) in steps of 5% (w/w). Loading efficiency was qualitatively analyzed by scanning electron microscopy (SEM) using separate drug crystals as indicators of poor loading efficiency. The particles were further characterized by mercury intrusion, energy-dispersive X-ray spectrometry and specific surface area measurements. The crystal state of the loaded drug was analyzed by differential scanning calorimetry, the drug load was quantified by HPLC-UV, and *in vitro* drug release from drug-loaded FCC (dl-FCC) was studied by standard paddle dissolution method (USP II). Mixtures of drug and FCC without specific loading strategy served as reference samples (dm-FCC).
**Results:** SEM analysis revealed high efficiency of pore-filling up to a drug load of 40% (w/w). Above a drug content of 40% (w/w), number of agglomerates and separate crystals were significantly increased indicating poor loading efficiency and reaching of drug loading capacity. Specific surface area and intraparticle porosity were decreased after drug loading due to the effect of pore-filling. Analysis of elemental composition of dl-FCC also proved drug deposition within the pores. Dissolution rate of MBZ- and NP loaded FCC was enhanced compared to dm-FCC, mainly due to surface enlargement, because only small fractions of amorphous drug (12.5%, w/w, and 8.9%, w/w, respectively) were found by thermal analysis.
**Conclusions:** Drug loading of FCC by the solvent-evaporation method is suitable for drugs with various physicochemical properties since the four model drugs showed excellent loading efficiency up to a drug load of 40% (w/w). The solvent-evaporation method allows precise drug dosing and provides possibility for scale-up, making it competitive to the impregnation method.

**Keywords:** Drug loading, porous drug carrier, calcium carbonate, solvent-evaporation.

**Reference:**
[1] Stirnimann T et al. Pharm Res 2013; 7: 1915–1925.

P-36

## Pepcan Localization and Role in Pain

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**Introduction:** The activation of cannabinoid receptors CB1 and CB2 has shown to positively affect pathological conditions in the CNS and in the periphery. Interestingly, in the CNS CB1 activation may lead to detrimental effects, like memory and learning deficits and hyperalgesia under certain conditions [1,2]. In addition to the classical endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG), which have been studied extensively, our group recently identified a new class of endogenous peptides (Pepcans), which show negative allosteric modulation at the CB1 receptor [3].
**Aims:** We are interested in the role of Pepcans in the CNS, and we particularly want to investigate their modulatory properties in pain. Furthermore we want to determine the exact localizations of Pepcans in the CNS.
**Methods:** We are currently assessing the effects of Pepcans *in vivo* in mouse models of hyperalgesia (increased pain sensitivity) by subcutaneously injected capsaicin to induce secondary hyperalgesia and also an inflammatory pain model by subcutaneous zymosan A injection. Subcutaneous capsaicin evokes a progressive and long-lasting secondary hyperalgesia around the injection site. The hyperalgesia is a result of centrally released endocannabinoids that retrogradely activate homosynaptic or heterosynaptic CB1 receptors, causing a depression of GABAergic inhibition in the spinal cord. Paw-withdrawal thresholds in response to mechanical stimulation were measured with #7 electronic von Frey filaments after intrathecal injection of Pepcan 12 (PC12, 12 indicates the number of amino acids), CB1 agonist CP55,940 (10 nmol) or CB1 antagonists AM251 (0.5 nmol). To determine the localization of Pepcans in the brain, we have generated suitable monoclonal antibodies and optimized immunohistochemical staining procedures for brain tissue sections of mice and rats.
**Results:** Our preliminary results in the capsaicin model of hyperalgesia confirmed that the intrathecally injected CB1 receptor antagonist AM251 significantly reverses mechanical sensitization. Intrathecally applied PC12 almost reached significance at a concentration of 10 $\mu$g/kg as expected by its potential action as a negative allosteric modulator of CB1 in the presence of released endocannabinoids.
In our second model of zymosan A-induced inflammatory pain we see opposing effects depending on the concentration of PC12 applied. Intrathecal injection of the CB1 agonist CP55,940 significantly reverses mechanical hyperalgesia, whereas a simultaneous injection of 1 $\mu$g/kg PC12 and CP55,940 reduced its antihyperalgesic effects. When we applied 100 $\mu$g/kg PC12 together with CP55,940 the threshold appeared to increase instead
Free-floating 40-$\mu$m cryosections were stained with immunofluorescence or immunoperoxidase (3,3'-diaminobenzidine (DAB)). Preliminary results show comparable staining patterns in both sexes of mouse and rat (infant and adult) with peptidergic projections throughout the brain and spinal cord. Both species stain for Pepcans to a high extent in the forebrain and brainstem. Furthermore, DAB staining allowed us to detect Pepcans in the white matter of the spinal cord as well as in the dorsal horn.
**Conclusions:** As it has been shown *in vitro,* Pepcans also have potential as a negative allosteric modulator of CB1 receptors *in vivo.* Their numerous appearances all over the CNS suggest a possible importance in physiological conditions. We will assess this by continuing the pain tests with both lower and higher dosages of PC12. Interestingly, the antihyperalgesic effects of CP55,940 in our inflammatory pain model appeared to increase when applied together with a high concentration of PC12 (100 $\mu$g/kg) indicating that Pepcans administered at higher doses might be antihyperalgesic, possibly acting through CB2 receptors.

**Keywords:** CB1 receptor, peptides, pain, immunohistochemistry.

**References:**
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## Automated Micro Extraction and Large Volume Injection with Gas Chromatography

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**Introduction:** Gas chromatography (GC) coupled to a mass spectrometric detector (MSD) is a common used method for the analysis of low concentrated and volatile samples. For drug and metabolite analysis, where volumes are small (1–2 mL) and solutes often are dissolved in liquids like serum, blood or buffer, prior sample preparation is indispensable. Solid phase extraction (SPE) provides a technique for extraction and enrichment of the analytes. The miniaturized form of SPE, the Micro Extraction by Packed Sorbent (MEPS) can be easily automated and is suitable for small volumes with elution volumes of a few microliters. MEPS is effectuated by a syringe with a disposable needle, containing a miniaturized liquid chromatography column (C18), called BIN (Barrel Insert and Needle). The elution volume of the extract is directly introduced into the GC system, using generally a Programmed Temperature Vaporization injector (PTV). This GC injector is designed for Large Volume Injections (LVI, 10–150 $\mu$L) through its capacity to evaporate and eliminate large solvent volumes.
**Aims:** We intended to develop a fully automated GC method to analyze low concentrated samples of various different matrices.
**Methods:** Analytes dissolved in buffer solutions, at concentrations of 1–10 ng/mL, were automatically extracted following a defined MEPS protocol. The extracted analytes were directly injected into a GC-MSD system equipped with a PTV injector and vaporized according to the newly developed LVI method with back flushing option for high boiling compounds. With the MS detector all analytes were detected and quantified.
**Results:** The MEPS protocol reveals efficient extraction of different molecules and enrichment on the MEPS BIN with analyte concentrations of 1 ng/mL. The number of MEPS enrichment cycles is directly proportional to the corresponding peak area in the chromatogram. The full factorial designed LVI method vaporizes and eliminates the solvent of the injected 30 $\mu$L. The analytes are successfully transferred to the column without any loss.
**Conclusions:** We developed a new method for GC separation of MEPS extracted compounds with LVI. Low concentrated (ng/mL), high boiling molecules are fully automatic extracted from buffer solutions and analyzed with the LVI-GC-MSD method. The MEPS syringe with disposable needle is reusable for up to 400 extraction cycles without any impairment of extraction capacity or any carryover. Solvent elimination of LVI is successfully optimized with back flushing the GC pre-column to prevent damage of the analytical column's stationary phase caused by excessive solvent amounts. This analytical procedure allows an automated, highly sensitive and robust determination of numerous drugs and metabolites.

**Keywords:** Gas chromatography, MEPS, Large Volume Injection, PTV.

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## Chemically Modified Nucleic Acid Conjugates for Local Delivery to the Colon

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**Introduction:** Nucleic acid drugs have an enormous potential in the biomedical field, because in principle, any problematic gene can be targeted with high efficacy and sequence-specificity. However, they suffer from susceptibility to enzymatic degradation and poor cellular uptake. Chemical modification of nucleic acids can increase resistance to degradation, and conjugation of lipids has been shown to enhance their uptake [1]. However, this uptake is not tissue selective and is therefore more suitable for local delivery. Oral delivery of nucleic acid drugs to the colonic mucosa would be highly desirable for the treatment of inflammatory bowel disease and colonic cancer, since it can minimize the dose required to achieve therapeutic effect and prevent side-effects resulting from systemic exposure to the drug. We propose a novel colon specific delivery platform based on the local delivery of chemically modified nucleic acid-lipid conjugates to the colon. The proposed system is based on single-molecule conjugates, therefore is chemically well-defined and does not rely on potentially toxic carriers.
**Aims:** Synthesis of lipid-antisense oligonucleotide (AON) conjugates and evaluation of their *in vitro* silencing activity upon carrier-free transfection.
**Methods:** Standard phosphoramidite solid-phase synthesis conditions were used for the synthesis of all nucleic acids. Corresponding lipophilic moiety was conjugated via an amino-hexanol-linker to the 5'-end of oligonucleotides. Final products were purified by reverse phase HPLC.
The lipid-AON conjugates were examined for their bcl-2 mRNA inhibition efficacy on human colon cancer cell lines (Caco-2 and HT-29 cells) in serum-free medium after overnight incubation. Seventy-two hours post-transfection, the gene expression levels of bcl-2 were assessed by qRT-PCR, as described previously [1].
**Results:** Cholesterol- (CHL) and docosanoyl- (DSA) AON conjugates were assessed for their delivery ability by screening bcl-2 mRNA inhibition efficacy in Caco-2 and HT-29 cells. It was found that derivatives featuring a DSA (saturated $C_{22}$ fatty acid) moiety exhibited higher knockdown efficiency compared to CHL. Therefore further experiments were conducted with DSA-AON conjugates. When DSA was conjugated to non-modified phosphorothioate DNA, the resulting conjugate failed to knockdown bcl-2 mRNA even at 1 $\mu$M. In contrast, substitution of some of the DNA bases with 2'-fluoro-arabinonucleic acid (2'F-ANA) led to significant reduction of bcl-2 expression. Interestingly, "altimer" pattern showed higher efficacy than "gapmer", pointing out the importance of AON modification position on the silencing activity of the conjugate. The most potent conjugate, DSA-2'F-ANA altimer, exhibited concentration-dependent silencing, with 70% of targeted mRNA knockdown at a concentration of 500 nM. Moreover, the control conjugates containing scrambled AON did not decrease the bcl-2 mRNA level, confirming the sequence-specificity of the target knockdown.
**Conclusions:** The chemically-modified lipid-AON conjugates demonstrated good silencing activities without the use of transfection agents. The proposed delivery system may provide a platform for exploring and evaluating new therapeutic targets in various bowel diseases.

**Keywords:** Antisense oligonucleotide, chemically modified nucleic acids, lipophilic moiety, gene silencing, carrier-free transfection, colonic delivery.

**Reference:**
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## TIC – Tablet-In-Cup Drug Delivery Device

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**Introduction:** Release modification is a common task in pharmaceutical technology in order to increase drug efficacy or to reduce side effects, dose frequency or toxicity. Conventional approaches are predominantly based on release control by diffusion through matrix or membrane systems. Such systems often require intensive formulation and process development because critical factors like surface area and osmotic activity are changing during dissolution of the dosage unit. With the special geometrical design of the newly developed tablet-in-cup (TIC) device, disadvantages of conventional drug release systems can be over-come by keeping the surface exposed to the dissolution media constant over time.
**Aims:** The aim of this project was to develop TIC drug delivery devices and to elaborate their potential for drug release modification. A second aim was to simulate TIC devices with a software tool in order to predict and optimize dissolution profiles.
**Methods:** Caffeine and duloxetine were used as model drugs to develop TICs with different geometries. Factorial design experiments were carried out with the help of DOE software in order to optimize the TIC design. Core-tablets and cups as well as the final TIC devices were produced on a Medelpharm Styl'One single-punch press with multiple-layer option and dry-coating device. Dissolution was carried out on a SOTAX AT7 dissolution tester (USP apparatus II) with online UV spectrophotometrical analysis. *In silico* simulations were done using F-CAD software, which is based on three-dimensional cellular automata and massively parallel computing [1].
**Results:** The design of TIC devices is characterized by a non-dissolving, non-swelling, non-porous and inert outer "cup" surrounding flat core tablets made of pure drug. TICs with caffeine showed zero order kinetics with fairly constant release rates from beginning to end. Dissolution speeds depended on the intrinsic dissolution rate of the drug and its surface exposed to the medium. It was possible to modify release rates by changing the core-tablet diameters without changing the formulations of the cores or the cups. With multiple-layer core tablets, TIC devices with complex release kinetics were developed: delayed release with adjustable lag-times, multiple pulsatile release and end-accelerated release. End-accelerated TIC devices were simulated with F-CAD showing good correlation to *in vitro* experiments, which allowed successful optimization of the dissolution kinetics *in silico.* Trials with duloxetine showed constraints of the design: interactions between dissolution medium and drug led to partial gel formation, which influenced the dissolution rate and caused non-linear release kinetics.
**Conclusions:** The TIC drug delivery device represents a promising alternative to conventional oral drug delivery systems. TICs with complex release kinetics can be developed in relatively short time and optimized with the help of simulation software. Investigations on applicability to other drugs and producibility on larger-scale equipment are suggested.

**Keywords:** Modified release, pulsatile release, dissolution rate, release profile simulation, drug delivery.

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P-40

## Spray-Drying of Nanosuspensions: An Optimization Through Design of Experiments

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**Introduction:** Nowadays, 70% of the drug molecules arising from synthesis and high throughput screening programs are poorly soluble in aqueous media. Thus, conventional formulation approaches are usually not suitable for such drug compounds. Nevertheless, there are some options for poorly water soluble drug substances, the most direct being the generation of a salt. If the compound is not ionizable, usually size-reducing or oil based formulation techniques are used [1]. The formulation of nanosuspensions by media milling is an economic and scale up ready technique. However, the formulation of active pharmaceutical ingredients as nanosuspensions involves several challenges, one of them being the long-term stability. A possible way to solve the stability problems is the conversion of the nanosuspensions into solid products (e. g., by spray drying technique). To elucidate the optimal process conditions of spray drying, the design of experiments (DoE) should be applied in order to understand the interactions between the process and formulation parameters, and desired outcome [2].
**Aim:** The aim of this work was screening of the spray drying process and related formulation parameters with the help of DoE in order to maximize the yield of the powder.
**Methods:** The comminution of poorly water-soluble budesonide with particle size around 6 $\mu$m was carried out in Dyno®-Mill Multi Lab (Willy A. Bachofen AG). The resulting nanocrystals were stabilized by D-$\alpha$-tocopherol-polyethylene glycol 1000 succinate (TPGS). The spray drying was carried out with the Mini Spray Dryer B-290 (Büchi Labortechnik AG). A two-level fractional factorial design with five factors complemented with central composite face-centered design was used to evaluate the influence of the process parameters on the yield. The DoE setup and evaluation was done using Modde v. 9.1.1, (MKS Umetrics AB). The residual moisture content, the particle size and morphology of the spray-dried powders were determined.
**Results and conclusion:** The milling of budesonide led to a decrease in median particle size to about 300 nm. Within the screening of the spray drying, powders with secondary particle sizes from about 6 $\mu$m to 29 $\mu$m were created. The powders contained less than 1% of moisture. The maximal yield of 68.3% was achieved within the experiments. A good model with $R^2$ of 79.4% and $Q^2$ of 53.9% was achieved. Two factors and two interactions of factors showed significant positive influence on the yield.

**Keywords:** Nanomilling, spray drying, design of experiments.

**References:**
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P-41

## Novel Quality by Design Tools for Concentrated Drug Suspensions: Surface Energy Profiling and the Fractal Concept of Flocculation

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**Introduction:** Quality by design (QbD) is an important tool to embed quality in the product by thoroughly characterizing materials' and processes' quality [1]. Highly concentrated lipid based suspensions are often used nowadays, especially in encapsulation, and thus their manufacturing should comply with QbD standards. Such formulations, in fact, require a careful design to manage the suspension's viscosity and, consequently, machinability.
**Aim:** Our work aimed to introduce surface energy profiling and fractal flocculation analysis as novel QbD tools to understand and predict the rheological properties of concentrated lipid-based suspensions.
**Methods:** The model drug, mebeverine hydrochloride, was studied by means of advanced inverse gas chromatography (iGC-SEA, Surface Measurement Systems Ltd). Several other physicochemical properties were also investigated, such as crystallinity, true density, enantiomeric excess, particle size, and particle shape. The hydrophilic drug was formulated as a lipid-based suspension, manufactured in a laboratory-scale homogenizing vessel (MI-MOLTO, Krieger AG) under vacuum, introducing homogenization intensity as a variable process parameter. Flow curves were recorded using a cone-and-plate rheometer (Bohlin Gemini, Bohlin Instruments Ltd) and the extrapolated yield stress was used to calculate the fractal dimension of particle flocculates [2]. The viscosity values were fitted with a mathematical model [3] as a function of the drug volume fraction.
**Results:** We detected with most methods no significant differences in the physicochemical properties between the two drug batches. However, the surface analysis showed significant differences both in specific surface area and in surface energy distribution. We found a significant difference also in the suspensions' viscosities, which correlated to the different surface energy profiles. The fractal analysis revealed different particle structures depending on manufacturing conditions and used drug batch. Finally, we could fit a model equation for viscosity change as a function of drug concentration. This model was also able to discriminate between different manufacturing conditions.
**Conclusions:** Surface energy and specific area proved to be critical attributes of the powder for suspensions' viscosities. Moreover, the fractal flocculation analysis was shown to be a valuable QbD tool to understand the suspension's rheological characteristics.

**Keywords:** Quality-by-Design, fractal dimension, inverse gas chromatography, surface energy, concentrated suspension.

**References:**
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[3] Mooney M. J Colloid Sci 1951; 6: 47-71

P-42

## Structural Features for HDAC6 Inhibitory Specificity of *Salvia corrugata* Diterpene $\alpha$-Hydroxyquinones

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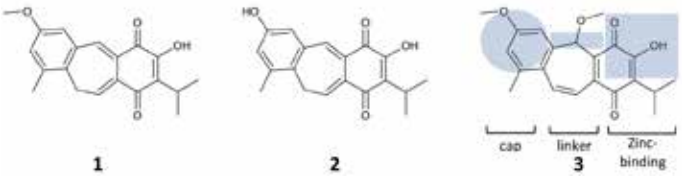
**Introduction:** Histone deacetylases (HDACs) are enzymes that deacetylate lysine residues from histones as well as from several other nuclear, cytoplasmic and mitochondrial non-histone proteins. In mammals, 11 zinc-dependent HDACs have been classified into three classes: Class I (HDACs 1 to 3 and HDAC8), class II which is subdivided into classes IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6

and 10), and class IV (HDAC11) [1]. Because of its central role in memory, protein aggregate elimination, neuronal oxidative stress and mitochondrial functions, HDAC6 appears as a promising target for Alzheimer's disease [1, 2]. However, developing new selective HDAC6 inhibitors is a challenge and their design is usually based on structural modifications on the cap portion, which is separated from a zinc-binding group by a linker portion [2].
**Aims:** To search for potential structural features within natural scaffolds which are able to provide isoform inhibitory specificity for HDAC6 compared to class I HDACs.
**Methods:** 7 diterpene α-hydroxyquinones isolated from the dichloromethane exudate of *Salvia corrugata* aerial parts were tested for HDAC inhibition on nuclear extracts from HeLa cells. The inhibitory specificity was assessed on HDACs 1-3 and 6 isoforms.
**Results:** The results showed that **1, 2** and **3** were the most active among the 7 tested compounds. To the best of our knowledge, this is the first report of the inhibition of zinc-dependent HDACs by α-hydroxyquinones. However, the hydroxyquinone moiety is known to complex with zinc [3], which might explain the activity of such compounds on HDACs. In order to check HDAC selectivity, the activity of **1–3** was tested against various isoforms. The inhibitory profile of **3**, with a selectivity against HDAC6, was different from the ones of **1** and **2**. The presence of a methoxy group in the 7-membered ring is the main feature for the HDAC6 selectivity of inhibitor **3.**
**Conclusion:** These results suggest that changes in the linker portion (part adjacent to the zinc binding group) of HDAC inhibitors may be important for the selectivity towards HDAC6. This appeared as a new information compared to the usual cap modification.




**Keywords:** HDAC6, HDAC inhibitors, *Salvia corrugata,* Alzheimer's disease.

**References:**
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[3] Yamada K et al. Chem Eur J 2004; 10: 2647-60.

**P-43**

## Expression and Purification of Calcium-Dependent Protein Kinase 2, a Potential Target of Triclosan in *Plasmodium falciparum*

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**Introduction:** Calcium-dependent protein kinases (CDPKs) are a group of serine-threonine kinases involved in many cellular processes of *Plasmodium falciparum.* By means of computational methods, we recently identified one of them, PfCDPK2, as a potential target of Triclosan (TCL), a biocide able to kill *in vitro* cultures of *Plasmodium falciparum* with an $EC_{50}$ of less than 1 μM [1]. PfCDPK2 is considered to be essential for the parasite, but its function is not yet known.
**Aims:** To obtain PfCDPK2 in quantity and purity suitable for further biochemical characterization, and to perform the assays needed for confirmation of PfCDPK2 as target of TCL.
**Methods:** PfCDPK2 was recombinantly coexpressed in *E. coli* cells with Lambda phosphatase and purified in three steps by FPLC. Inhibition by TCL and its derivatives was assessed by means of a radioactive assay.
**Results:** PfCDPK2 was obtained in quantities (8 mg/L of culture) and purity (>95%) suitable for biochemical characterization and evaluation of inhibitors [2]. Biochemical parameters were determined for ATP and the non-natural substrate myelin basic protein (MBP). TCL was shown to be a non-competitive inhibitor of PfCDPK2 with an $IC_{50}$ of 48 μM. A small library of TCL derivatives was subsequently used to perform a structure-activity relationship (SAR) study, showing that the B-ring could be modified in order to improve activity.
**Conclusions:** This study represents a first step towards the understanding of the antiplasmodial effect of TCL in *P. falciparum* and a starting point for the development of new and potent PfCDPK2 inhibitors.

**Keywords:** *Plasmodium falciparum,* PfCDPK2, Triclosan, SAR.

**References:**
[1] Perozzo R et al. J Biol Chem 2002; 277: 13106-13114.
[2] Lauciello L et al. Prot Expr Purif 2013; 90(2): 170-177.

**P-44**

## Biomarker Analysis in Dried Blood Spots

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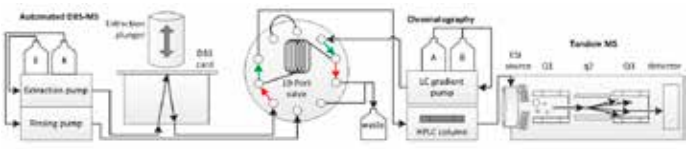
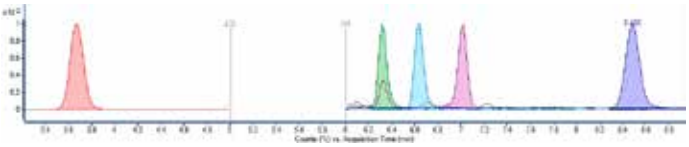
**Introduction:** Dried Blood Spot analysis (DBS) is an alternative method of sampling biofluids, where a blood sample is spotted on a filter paper and dried. This technique is minimally invasive and cost-effective in terms of sample collection, shipment and storage. Most analytes show a high stability during storage without refrigeration [1].
**Aims:** The focus of this project is the DBS analysis of healthcare biomarkers, such as vitamins D and E, in human blood. A fully automated method has to be developed to qualify and quantify the biomarkers directly from a DBS card. The method should be used to study micronutrient supply to enable evidence-based recommendations for individual diet improvements.
**Method:** The DBS-MS16 is a fully automated card extraction system developed by CAMAG [2]. The DBS cards are moved to the extraction unit, where a plunger seals a circular hole in the card. The extraction solvent is pumped through the card and loaded into a loop (Fig. 1: red arrows). By switching the 10-port valve, the loop volume is connected to the HPLC-MS/MS flow path (Fig. 1: green arrows) and guided to the column and the tandem MS after separation. Meanwhile, the extraction head is cleaned by a rinsing cycle to avoid carry over.

Fig. 1 Flow scheme of the automated DBS-LC-MS/MS approach



**Results:** A combined method for the determination of both vitamin D and E derivates was developed via the automated DBS-LC-MS/MS approach. The separation was achieved in less than 9 min using a Zorbax C8 column with methanol/water as mobile phase. The LC/MS/MS chromatogram (Fig. 2) shows (with increasing retention time) 25-Hydroxy vitamin D3 (1), vitamin D3 (2), γ-Tocopherol (3), α-Tocopherol (4) and α-Tocopherol acetate (5) in spiked dried blood spots. Further optimization and validation of the method is in progress.

Figure 2 LC-MS/MS chromatogram



**Conclusions:** The recent development of various validated DBS methods in different fields of application indicates the high potential of DBS analysis. The technique can be used for pharmacokinetic and toxicokinetic studies as well as for many other applications, like clinical chemistry. However, further investigations in terms of analyte stability have to be done.

**Keywords:** DBS, vitamin D and E, LC-MS/MS, whole blood.

**References:**

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[2] http://www.camag.com/en/dbs/what_is_dried_blood_spot_sampling.cfm

**P-45**

## Inhibition of Porcine Detrusor Contractility by a Flavonoid Fraction of *Bryophyllum pinnatum* – A New Possible Treatment for Overactive Bladder Syndrome

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**Introduction:** Patients with overactive bladder syndrome (OAB) suffer from urgency with or without incontinence, frequency, and nocturia [1]. Antimuscarinic agents are used as a first-line pharmacotherapy with a proven clinical benefit but also anticholinergic side effects. Up to 20% of the patients fail to respond adequately. Therefore, we recently investigated the inhibitory effect of *Bryophyllum pinnatum* leaf press juice on porcine detrusor contractility *in vitro* [2].
**Aims:** The aim of this study is the identification of the responsible ingredients of *B. pinnatum* for the inhibitory effect on the porcine detrusor contractility. We tested a flavonoid and a bufadienolide fraction against the reference substance oxybutynin (competitive muscarinic receptor antagonist) on the contraction of the porcine bladder strips.
**Methods:** In a previous metabolite profiling of the MeOH extract of *B. pinnatum* leaves, 9 different flavonoid glycosides and 2 phenolic acid derivatives have been isolated and identified. In addition 4 bufadienolides have been detected. For the present experiments the MeOH extract of *B. pinnatum* leaves was partitioned between $CH_2Cl_2/H_2O$ to separate the flavonoids from the lipophilic bufadienolides. The $H_2O$-phase was further applied to a Diaion HP-20 CC (eluted with MeOH and $H_2O$) to obtain an enriched flavonoid fraction.
Detrusor muscle strips used for the contractility experiments were prepared from porcine bladders. In an organ bath chamber (6 mL Krebs solution) we investigated the effect of the purified flavonoid fraction (0.17–1 mg/mL), bufadienolide fraction (0.1–40 $\mu$g/mL) as well as of oxybutynin ($10^{-8}$–$10^{-6}$ M) on the contraction of the bladder induced by Electric Field Stimulation (EFS, 40 V and 32 Hz).
**Results:** The flavonoid fraction at a concentration of 0.83 and 1 mg/mL inhibited the induced contraction of bladder strips in a time-dependent manner by 47.5 ± 8.5% and 65.0 ± 7.5%, respectively, as compared to the contraction measured before treatment (100%). Concentrations > 1 mg/mL showed an irreversible alteration of the muscle vitality. The bufadienolide fraction was not able to inhibit the contraction of the muscle strips. Oxybutynin $10^{-7}$ M and $10^{-6}$ M inhibited the detrusor contraction by 70.3 ± 3.3% and 87.3 ± 2.3%, respectively.
**Conclusions:** The flavonoid fraction isolated from *Bryophyllum pinnatum* inhibits the porcine detrusor contraction in a time-dependent manner. The flavonoids seem to be the active ingredients of *B. pinnatum* with an inhibitory effect on the detrusor contractility. The bufadienolides do not have any effect on the contraction. Further investigations are ongoing to study the effect of the different fractions on human detrusor strips.

**Keywords:** *Bryophyllum pinnatum,* flavonoids, bufadienolides, overactive bladder.

**References:**

[1] Abrams P et al. Neurourol Urodyn 2002; 21: 167-78.
[2] Schuler V et al. Phytomedicine 2012; 19: 947-51.

**P-46**

## A Novel Type of Stirred Bead Mill (Nanomill) for the Size Reduction of Pharmaceutical Products

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**Introduction:** Wet milling with stirred bead mills is a well-established method to produce nano-scale particles in an industrial production scale for various applications. In recent years also applications for pharmaceutical products have been established successfully. Ideally, an industrial production scheme should be versatile, simple, robust and cost-effective. Up to now, the large scale production of nano-sized pharmaceutical products requires high pressure loading and high shear rates. This entails the disadvantage of generating mechanical and thermal stress at the same time.
**Aims:** To develop a novel type of nanomill dedicated to the production of liposomes and to determine the influence of the main operating parameters necessary to form liposomes. Furthermore to prove, that this novel type of mill is also fully applicable for the nanomilling of poorly water-soluble drug substances.
**Methods:** The design of this mill underwent a radical change: the process chamber is vertically arranged, ready for gravimetrical filling with grinding beads, suspension flow from bottom to top and conversely, top down unloading of grinding beads. The chamber has been optimized with regard to the flow conditions both in milling mode and in emptying mode. Complete emptying is ensured by the patented WAB DYNO accelerators which generate a reverse flow inside the process chamber strong enough to carry all grinding beads out of the closed system. Furthermore multi-lamellar large vesicles were stressed in the novel mill and crystalline pharmaceutical substances were milled down to the nano-scale range.
**Results:** Core of the "Concept Mill", patent pending, (Willy A. Bachofen AG, Muttenz, Switzerland) is an entirely new process chamber, which is integrated into a milling unit ready for production on the kilogram-scale. The milling unit remains entirely closed, even also when the unit is being prepared for another batch. Comparing

the novel mill with standard mills with regard to their milling results it has been revealed that the novel mill provides very similar or even better results.
The influence of the main operating parameters necessary for the size reduction of liposomes down to approximately 100 nm indicates that the result of size reduction with this type of mill can be described by the concept of the stress number (reduced number of stress events, SNr) which, consequently, can be used to control the size reduction of soft organic particles [1, 2].
**Conclusions:** The novel mill meets the requirements of liposome production [1] but also the requirements of the pharmaceutical industry with regard to the nano-milling of poorly water-soluble drug substances.

**Keywords:** Nanomill, nano-milling, stirred media mill, liposomes, pharmaceutical process engineering.

**References:**
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**P-47**

## Finding a Needle in a Haystack: Identifying Early Drug Leads by Screening DNA-Encoded Compound Libraries

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**Introduction:** The plethora of potential drug targets in the post-genomic era makes necessary the development of novel techniques for the rapid and inexpensive discovery of protein ligands. DNA-encoded compound libraries (DECLs) are emerging as a promising approach with this respect because they allow screening proteins in parallel in an one-pot procedure requiring minimal amounts of the protein targets. DECLs are compound collections in which each structure is unambiguously defined by an unique nucleic acid barcode. Similar to phage-display technology, panning these libraries against immobilized target proteins provides enriched binders. High-throughput sequencing of the corresponding DNA-barcodes enables the identification of these structures.
**Aims:** We designed and developed a structurally compact DECL of high purity and chemical diversity. We further established the efficacy of these DECLs to identify early drug lead structures by screening them against selected protein targets.
**Methods:** A $10^5$-compound DECL was assembled by a novel solid/liquid-phase synthesis strategy comprising combinatorial pairs of 670 structurally diverse building blocks. Affinity binders were identified by panning the DECLs against immobilized proteins followed by high-throughput sequencing of the corresponding DNA-barcodes. Identified hit structures were resynthesized and dissociation constants determined by fluorescence polarization.
**Results:** The developed DECL enabled the one-pot discovery of protein ligands. Positive control screening experiments provided the anticipated hit structures. Previously unknown small-molecules could be identified, which bind the target proteins with high affinity. Association of these ligands to the proteins required the synergistic interactions of both building blocks with the protein. Furthermore, simple structure-activity trends were discernible directly from the high-throughput sequencing results.
**Conclusions:** DECLs enable the rapid and cost-effective identification of hit compounds and this method bears considerable promise as a potential tool for drug discovery in the future.

**Keywords:** DNA-encoded library, lead discovery, combinatorial chemistry, screening, protein ligands.

**P-48**

## Synthesis and Characterization of Highly Cytotoxic Ruthenium Complexes

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**Introduction:** Over the last decades, ruthenium-based anticancer drugs have become the focus of research, since they are known to cause fewer side effects than platinum drugs [1]. In recent years, we have designed different hexacationic hexaruthenium assemblies that can encapsulate various guest molecules, which makes them useful for drug delivery to cancer cells. We have also synthesized three series of thiophenolato-bridged dinuclear *p*-cymene ruthenium complexes of the general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(SR)_3]^+$, which proved to be highly cytotoxic against human ovarian cancer cells A2780 and their cisplatin resistant mutant A2780cisR, the $IC_{50}$ values being in the nanomolar range [3].
**Aims:** The aim of our work is to assess the reactivity of the hexacationic hexaruthenium assemblies and the thiolatobridged dinuclear ruthenium complexes and to determine their biological targets under physiological conditions.
**Methods:** We have investigated their possible mechanism of action using different NMR techniques as well as ESI mass spectrometry.
**Results:** Our results show that the assemblies undergo disassembly in the presence of amino acids, but remain rather stable in the presence of nucleotides and DNA. Surprisingly, these complexes are substitutionally inert and we have found them to act as highly efficient catalysts for the oxidative conversion of GSH into GSSG. Furthermore, a correlation between cytotoxicity, lipophilicity and Hammett's constants of the corresponding thiol ligand could be evidenced [3].
**Conclusions:** For the hexacationic hexaruthenium assemblies, proteins rather than DNA are the most probable cellular targets [2]. On the other hand, thiophenolato-bridged dinuclear *p*-cymene ruthenium complexes are highly cytotoxic, but rather inert. We have recently observed precipitation of proteins induced by these complexes, which appears to be a novel mode of action for a metal-based anticancer compound.

**Keywords:** Anticancer drugs, ruthenium, NMR.

**References:**
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[3] Giannini F, Süss-Fink G, Furrer J. Inorg Chem 2011; 50: 10552-54.

**P-49**

## Biological and Computational Evaluation of Alkaloids from *Psychotria* as Sirtuin Inhibitors

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**Introduction:** Epigenetic targets such as histone deacetylases (HDACs) play a crucial role in the development of aging related dis-

eases such as neurodegeneration [1]. Among the eighteen isoforms found in human, class III HDACs, also known as sirtuins, seem to be promising targets for treating neurodegenerative conditions [2]. Recently, monoterpene indole alkaloids of *Psychotria* were reported for their inhibitory properties against cholinesterases (BChE and AChE) and monoamine oxidases (MAO-A and B) [3].

**Aims:** Search for new inhibitors of the human sirtuin isoform 1 (SIRT1) in order to elucidate mechanisms of action related to neurodegeneration. Natural compounds are of main interest.

**Methods:** Inhibition properties of alkaloids isolated from *Psychotria* were evaluated on SIRT1 human recombinant enzyme using a fluorimetric assay. The three inhibitors displaying the highest activity on SIRT 1 were further investigated through a molecular docking approach.

**Results:** Three compounds were found to inhibit human SIRT1 in the range of nicotinamide, the compound of reference ($\mu$M range). All *Psychotria* compounds were able to bind the C-site of SIRT1. The position of their ß-carboline scaffold in the SIRT1 pocket was conserved.

**Conclusions:** Among ten compounds isolated from *Psychotria,* three inhibited SIRT1 isoform at the $\mu$M range. Molecular docking reveals that all these compounds occupy the C-site of SIRT1 and bind to it in the same way.

**Keywords:** SIRT1, neurodegeneration, molecular docking, natural compounds, monoterpene indole alkaloids.

**References:**

[1] Jakovcevski M et al. Nature Med 2012; 18: 1194-204.
[2] Duan W. CNS Drugs 2013; 27: 345-52.
[3] Kumar R et al. Plos One 2013; 8: e61560.

**P-50**

## A Hydrolysis-Resistant Fluorescent Endocannabinoid Analogue to Investigate Cellular Endocannabinoid Uptake and Trafficking

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*[2]Universidad Computense Madrid, Madrid, Spain*

**Introduction:** Endocannabinoids (ECs) are key mediators involved in many physiological and pathological conditions in CNS and peripheral tissues where they exert biological activities by interacting with extracellular and intracellular targets. By definition, ECs are endogenous molecules which activate primarily cannabinoid $CB_1$ and/or $CB_2$ receptors. Anandamide (AEA), *N*-arachidonoyl dopamine (NADA) and 2-arachidonoyl glyceryl ether (noladin ether; 2-AGE) are functionally more selective for $CB_1$; virodhamine appears to prefer $CB_2$ while 2-arachidonoyl glycerol (2-AG) is equipotent at both receptor subtypes [1]. ECs effects are regulated by cellular biosynthesis, release, re-uptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about ECs biosynthetic and metabolic pathways, their cellular re-uptake mechanism is not fully elucidated yet. The best experimentally supported theory relies on a passive membrane transporter-mediated mechanism. One of the main issues in elucidating the uptake process is the tight interplay between ECs plasma membrane movement and their rapid and almost complete cellular cleavage mainly dependent on FAAH and MAGL activity. Recently, we have shown that all ECs compete for the same putative membrane transporter (EMT) independently of their intracellular fate (trafficking and enzymatic inactivation) [2].

**Aims:** To generate and characterize a hydrolysis-resistant EC analogue in order to investigate cellular EC uptake and trafficking.

**Methods:** We characterized the fluorescent endocannabinoid analogue properties by performing the classical radioactivity-based method, the analytical quantification and real-time fluorescent cytometry.

**Results:** We have generated a fluorescent analogue of noladin ether (NBD-2-AGE), the only hydrolysis-resistant EC. The results show that fluorescently-tagged 2-AGE possesses the same biochemical features as 2-AGE in terms of CB receptor binding, cellular uptake and release and enzymatic cleavage resistance. We made use of this new tool compound to study EC uptake and release kinetics in different cell types by FACS measurement. In addition, we also describe the movement of NBD-2-AGE from pre-loaded cells towards unloaded cells. Both processes could be selectively inhibited by the classic EMT inhibitors. When NBD-2-AGE was co-incubated with AEA, 2-AG or 2-AGE a selective competition in cellular uptake was detected. Other *N*-acetylethanolamines did not show any significant effect on the uptake as previously shown for the main endocannabinoids AEA and 2-AG [2-4].

**Conclusions:** Altogether, our data suggest that NBD-2-AGE is a very useful probe to investigate ECs cellular uptake and trafficking kinetics with a sensitive and radioactivity-free based method. Unlike the other ECs, NBD-2AGE is resistant to the fast and very efficient FAAH- and MAGL-mediated hydrolysis, which is a well-known confounding factor for studying cellular uptake and trafficking of AEA and 2-AG. Finally, NBD-2-AGE will allow monitoring the ECs distribution in different cell types when applied to complex matrices.

**Keywords:** Endocannabinoid, noladin ether, cellular uptake, endocannabinoid release, hydrolysis resistant.

**References:**

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[4] Jacobsson SO, Fowler CJ. Br J Pharmacol 2001; 132: 1743-1754.

**P-51**

## New PET Tracer: $^{44}$Sc Labeled Somatostatin Analogue Peptide

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**Introduction:** Different neuroendocrine tumors overexpress somatostatin receptors. Targeting these receptors with somastostatin analogues (e. g. octreotide) labeled with radioactive isotopes is a well established diagnostic method for detecting metastases and staging the disease. Classically pentetreotide (OctreoScan®; DTPA-octreotide), a kit for $^{111}$In labeling, is used and patients diagnosed by SPECT scan. Using instead a PET tracer (e. g. $^{68}$Ga-DOTA-octreotate) improves sensitivity and is currently widely used. There is no commercial kit formulation available. Therefore, only well equipped radiopharmaceutical labs following GMP guidelines are allowed to produce the compound for clinical application. The relatively short half life of 68 min prevents it from being distributed within a significant range. $^{68}$Ga is available from generators only. These provide a limited amount of activity, are expensive and not yet licensed. The better availability of the $^{177}$Lu labeled therapeutic product ($^{177}$Lu-DOTA-octreotate) requests that a PET labeled octreotide is available in an equal manner. With its half life of 4 h, $^{44}Sc^{3+}$ is a promising ß$^+$-emitter [1] which has similar affinity to the DOTA chelator as $^{68}Ga^{3+}$, $^{177}Lu^{3+}$ and $^{90}Y^{3+}$. Also $^{44}$Sc may be produced at small cyclotrons (15→9 MeV) by irradiation of enriched $^{44}$Ca. $^{44}$Sc is currently available from the PSI cyclotron.

**Aims:** Suitability of $^{44}$Sc production by irradiation for clinical use in labeled DOTA peptide injection solution shall be verified. Key parameters are radiochemical purity and maximal specific activity as well as endotoxin levels of the final product. An optimized labeling procedure on an automated system shall be developed together with robust analytical methods for routine quality control. The final aim is to provide methods for centralized labeling of DOTA peptides exemplified on DOTA-octreotate with $^{44}$Sc for diagnostic PET scans. Chemical and radiochemical purity of the final injection solution shall be explored. The formulation of the final product shall provide a shelf life period suitable for the intended distribution range.
**Methods:** Radionuclide purity after $^{44}$Sc production was tested with gamma spectroscopy (HPGe detector). Automated labeling: $^{44}$Sc- DOTA-octreotate was synthesized using the Eckert & Ziegler Modular-Lab PharmTracer (with Modular-Lab 4.2.1.0 software) and a C4-Lu177-00 synthesis cassette. 35 nmol (50 $\mu$g) DOTA-octreotate were used. The reaction was performed at 95 °C for 30 min followed by a Sep-Pak® C-18 purification. Quality control of the radiopharmaceutical product: the chemical and radiochemical purity was verified by HPLC. The endotoxin levels were evaluated by chromogenic determination (Endosafe®-PTS™).
**Results:** The radionuclidic purity was >99.0%. The overall radiolabeling yield after synthesis was 84% with a specific activity of 7.9 GBq/$\mu$moL peptide. The radiochemical purity was >98%. Endotoxins are expected to be under the limit of 175 EU/patient as experienced from the $^{177}$Lu synthesis with a similar synthesis scheme. Extended stability data are under investigation.
**Conclusions:** The optimized production of $^{44}$Sc has proved its suitability for clinical use. Synthesis of $^{44}$Sc-DOTA-octreotate showed very promising results with a good overall yield and an expected specific activity. $^{44}$Sc-DOTA-octreotate is an interesting candidate to be used as diagnostic tracer in the evaluation of patients for radionuclidic therapy of different neuroendocrine tumors. The new octreotate PET tracer will be clinically evaluated after the full GMP synthesis is achieved. It is proposed to compare its value with the well established $^{68}$Ga-DOTA-octreotate.

**Keywords:** GMP production, $^{44}$Sc-DOTA-octreotate, Nuclear Medicine Imaging.

**Reference:**
[1] Krajewski S et al. Radiochim Acta 2013; 101: 333–338.

**P-52**

## Improved Therapeutic Potential of L-Asparaginase by Modification with Comb-Shaped Polymers

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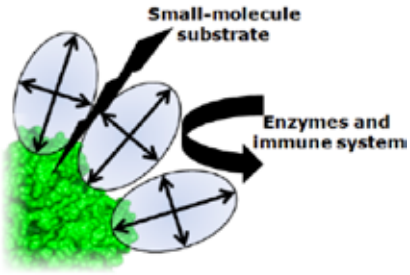
**Introduction:** L-Asparaginase (ASNase) is an important therapeutic protein used to treat acute lymphoblastic leukemia (ALL), the major form of malignancy in children. ALL has a high incidence in Switzerland. This enzyme works by depleting blood asparagine, which can be produced by normal cells but not by these cancer cells [1] However, ASNase is highly immunogenic in its native form. The immunogenicity is often associated with severe side-effects and loss of activity.
**Aims:** In this work, we aim to improve the pharmacological behavior of ASNase by modifying it with a biocompatible comb-shaped polymer – poly(oligoethyleneglycol monomethylether methacrylate) (pOEGMA). When grafted to the surface of a protein, this polymer can create a "molecular sieving" effect: small molecules can access the protein while large molecules are blocked [2]. As asparagine is a small molecule, molecular sieving ASNase–pOEGMA conjugates may have higher activity and lower immunogenicity than either native ASNase or a clinically-relevant ASNase–(linear PEG) control.
**Methods:** A library of 15 well-defined ASNase–pOEGMA conjugates was prepared. The molecular sieving effect was optimized by analysis of both the conformation of pOEGMA and the bioactivity of the conjugates. The *in vitro* immunoreactivity of ASNase and the conjugates was assessed in an ELISA with anti-asparaginase antibodies. The pharmacokinetics was characterized in mice.
**Results:** Selected ASNase–pOEGMA conjugates possessed 30–50% of the catalytic activity of native ASNase, but were >1000-fold less immunoreactive *in vitro*. The ASNase–(linear PEG) control had 60% of the activity of native ASNase, but was only 30-fold less immunoreactive than the native protein *in vitro*. The circulation lifetime of ASNase modified with pOEGMA substantially longer than that of native ASNase. Immunogenicity studies *in vivo* are ongoing.



Molecular sieving effect

**Conclusions:** By harnessing an emerging "polymer engineering" approach for tuning the bioactivity of protein–polymer conjugates, an active and well-protected form of ASNase could be engineered. *In vitro* and ongoing *in vivo* experiments are promising, and may provide additional understanding and guidelines for exploiting specific properties of polymers to optimize the therapeutic potential of ASNase and other protein drugs.

**Keywords:** L-Asparaginase, protein-polymer conjugates, PEG, comb-shaped polymer.

**References:**
[1] Crowther D. Nature 1971; 229: 168–171.
[2] Liu M et al. Adv Funct Mater 2013; 23: 2007–2015.

**P-53**

## Determination of Risk Factors for Drug-Related Problems – A Multidisciplinary Triangulation Process

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**Introduction:** Detecting patients at risk for drug related problems (DRPs) may help pharmacists to apply intensive pharmaceutical care where it is needed most.

**Aims:** The aim of this study was to determine evidence-based risk factors for DRPs. The results shall serve as a basis for the development of a prospective patient risk-assessment tool.
**Methods:** We used a triangulation process:
a) We conducted a multidisciplinary expert panel using the method of the nominal group technique (NGT). The panel consisted of 10 healthcare providers: one clinical pharmacologist, 3 senior hospital physicians (geriatrics, emergency, internal medicine), 1 general practitioner, 2 nurses (acute hospital care, home care), 2 community pharmacists and 1 clinical pharmacist. During a structured discussion, all participants had to write down as many risk factors as possible from their professional experience and rank them by their importance.
b) The subsequent discussion was audio-taped and we retrieved additional factors from a qualitative analysis.
c) We performed a literature search in PubMed and Embase. Titles and abstracts were screened for the terms "risk factors", "high risk", "predictors" combined with "drug-related problems" or sub terms of its definition.
Finally, we compiled a questionnaire to validate the risk factors with the Delphi technique. We addressed the same participants as in our first expert panel.
**Results:**
a) The first expert panel resulted in 33 items.
b) Fourteen additional risk factors were extracted from the qualitative analysis of the NGT-discussion.
c) Literature research resulted in 39 additional items.
From this total of 86 risk factors we excluded 40 factors that where compliant to exclusion criteria (such as factors mentioned in only one publication, set in the lowermost quartile of our NGT's ranking list, representing an unpredictable event or circumstance, interventions to improve seamless care, issues of seamless care). We eliminated 5 synonyms and split one risk factor into two components. Thus, 42 factors where implemented in the Delphi questionnaire.
Panellists judged 28 risk factors as "important" or "rather important". The consensus list contains patient-associated (e. g. dementia) as well as drug-related (e. g. anticoagulants) and disease-related (e. g. visual impairment) risk factors.
**Conclusions:** The multidisciplinary triangulation process served as an efficient and valid method to gather risk factors for DRPs. Results will enable a well-funded basis for the development of a risk assessment tool.

**Keywords:** Drug related problems, assessment tool, patients at risk.

**P-54**

## Multi Tracer Use Automated System (MTUAS)

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**Introduction:** Positron Emission Tomography (PET) is an evolving non-invasive diagnostic tool in the field of nuclear medicine (e. g. oncology and neurology). To date, the most prominent PET radiotracer is still $^{18}$F-labeled 2-deoxy-2-fluoro-*D*-glucose ($^{18}$F-FDG), developed in 1977 [1]. The availability of medical cyclotrons in Nuclear Medical Centres allows the continuous $^{18}$F production. Modern PET/CT and PET/MRI scanners have spurred research resulting in more specific and selective radiotracers. Fluorine-18 is the most often used PET radionuclide with the advantage of a relatively long physical half-life of 110 min which allows distribution to regional clinics. The production of radiopharmaceuticals for early phase clinical trials is typically done using dedicated automated radiosynthesis modules. Each automated system in the GMP facility is installed in a clean room, in a grade C lead-shielded, so called "hotcell", and often only produces a single tracer. To gain flexibility with respect to different radiotracers and to decrease required time for translation of preclinical radiotracers to clinical settings as well as to save expenses, a multi tracer use automated system (MTUAS) is needed.
**Aims:** To develop a MTUAS which is equipped to perform all relevant production steps for $^{18}$F-labeled radiotracers. The MTUAS should be compliant with current codes of good manufacturing practice (GMP) as well as with the radiation protection rules.
**Methods:** The MTUAS was built from small, sealed, stainless steel modules (Eckert & Ziegler, Germany). The versatile and flexible unit operation modules were stacked and connected by process tubing and by a single electric cable. An intuitive graphical interface (Modular-Lab software) was used to design and control the radiosynthesis process. To allow MTUAS applicability for the production of a variety of different $^{18}$F-radiotracers all relevant radiosynthesis steps were accounted for by a team of experienced scientists involved in the hardware design. An automated cleaning procedure was set up based on former experience gained with dedicated automated systems. Cleaning validation was performed to demonstrate the cleaning effectiveness and absence of cross-contamination. Taking into account the product specifications acceptance criteria were defined for visual inspection, bioburden (microbiological contamination) and chemical purity (by HPLC and GC). Cross-contamination with other isotopes presented no risk since $^{18}$F was the only radioisotope used in this facility.
**Results:** The MTUAS contains two reactor modules which enable heating and cooling for the radiochemical synthesis part. The purification unit is equipped with a semi-preparative HPLC system and several connections for cartridge purification of the drug substances (APIs). Activity, pressure and temperature sensors were installed and are used as in-process-controls. A MTUAS was established for two recently developed radiotracers. A single cleaning procedure proved to be effective after production of both radiotracers. The MTUAS was visually clean and API residues from previous radiosynthesis were <LOD (<0.03 $\mu$g/mL,radiotracer A) and <0.04 $\mu$g/mL (radiotracer B), respectively. Additionally, toxicological data showed that API residues were below the No Observed Adverse Effect Level (NOAEL). The content of residual solvents was factor 10 below the maximal limit given by the Ph. Eur [2]. Microbiological contamination was <10 CFU/mL. Both radiotracers can successfully be produced alternately without cross-contamination.
**Conclusions:** The MTUAS concept proves positive when two different radiotracers were involved. The utilization of the production space was doubled. Considering that only small amounts of starting materials are introduced into the system during the radiosynthesis as well as a very low final product concentration, such MTUAS can expand to a wider number of projects. Progress in this direction is ongoing in our facility.

**Keywords:** Automated system, cleaning validation, fluorine-18.

**References:**
[1] Gillings N. Magn Reson Mater Phy 2013; 26: 149-158.
[2] European Pharmacopoeia, Edition 7.8, Chapter 5.4.

**P-55**

## Medicinal Cannabis: *In Vitro* Validation of Vaporizers for the Smoke-Free Inhalation of Cannabinoids

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**Introduction:** The interest in the medical use of *Cannabis sativa* L. has increased over the past years because cannabinoids have proven to be effective to alleviate and treat various symptoms such as spasticity, pain, nausea, anorexia, and depression in a variety of diseases. Main indications for the use of cannabinoids are multiple sclerosis,

amyotrophic lateral sclerosis, chronic pain and cancer. Cannabis contains more than 80 different cannabinoids, among which $\Delta^9$-tetrahydrocannabinol (THC) is the primary psychoactive constituent. However, growing evidence has shown that in addition cannabidiol (CBD) has important pharmacological activity that contributes significantly to the clinical effects of Cannabis. Since THC and CBD occur in the plant as their biologically inactive acids (THCA-A and CBD-A) high temperatures above 200 °C are needed to deliver neutral THC and CBD. Therefore, Cannabis is usually smoked in combination with tobacco. An alternative is the use of vaporizers, which release THC and CBD into the gas phase without pyrolysis of the plant material, avoiding therefore the formation of harmful combustion products.

**Aims:** The aim of this study was to assess the suitability of the four commercially available vaporizers Volcano and Plenty (Storz & Bickel, Tuttlingen, Germany), Arizer Solo (Arizer Tech, Waterloo, Canada) and DaVinci (Organicix LLA, Las Vegas, USA) for the inhalation of cannabinoids from *Cannabis sativa* L. for medical purposes. Consequently, an *in vitro* validation of four vaporizers was performed.

**Methods:** Two *Cannabis sativa* L. varieties were used (50 mg), one with 4.6% of total THC (THC type; 90.4% as THCA-A) and the other with 2.6% of total CBD (CBD type; 85.8% as CBD-A). In addition, pure THC and CBD standards in ethanol (2 mg) were vaporized using the same experimental design. The temperature of the vaporizers was set to 210 °C. The vapor was aspirated through a SPE cartridge (Li Chroprep RP-18) and eluted with methanol-chloroform 9:1 *(v/v).* The mouthpiece, heating chamber and connection tube were rinsed with the same solvent and the fractions collected. The samples were evaporated to dryness under $N_2$, reconstituted in methanol-chloroform 9:1 and diluted with methanol. The residue was extracted with the same solvent. GC/MS was used to quantitate THC, CBD and CBN (decomposition product of THC) with deuterated internal standards on a DB-1ms column (30 m x 0.25 mm i.d., 0.25-$\mu$m film). THCA-A and CBD-A were determined by HPLC-PDA on a Spherisorb ODS I column (125 x 4 mm i.d., 3-$\mu$m particle size).

**Results:** The four devices efficiently decarboxylate acidic THC and CBD (> 98%) and release neutral THC and CBD from Cannabis into the vapor (54–80% and 51–74%, respectively). The recoveries are similar to those obtained for the standards with 41.6–71.1% and 53.6–84.3% for THC and CBD, respectively. Only trace amounts of THCA-A and CBD-A were found in the vapor, proving that adequate amounts of active THC and CBD are available to the patient. Less than 15% of the total cannabinoids remain in the residual plant material using the Solo and the Volcano vaporizer, whereas the release into the vapor is complete with the two other devices (remaining cannabinoids < 5%).

**Conclusions:** The four temperature controlled vaporizers revealed efficient decarboxylation of the biologically inactive THCA-A and CBD-A, releasing active neutral THC and CBD into the vapor. Non-pyrolytic inhalation of Cannabis by vaporizers might therefore be a promising alternative to the oral administration of cannabinoids for medical use.

**Keywords:** *Cannabis sativa,* cannabinoids, vaporizers, *in vitro* validation, non-pyrolytic inhalation.

**P-56**

## Guineensine is a Potent CNS-Active Inhibitor of Endocannabinoid Uptake and Cyclooxygenase-2 Showing Analgesic and Anti-inflammatory Effects

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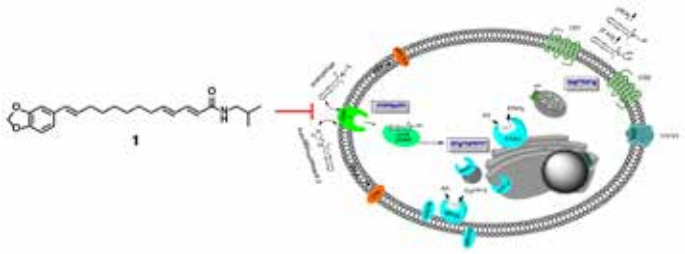
**Introduction:** The *Piper* plant genus is a rich source of novel bioactive scaffolds. Besides its popularity as spice pepper is used in TCM and Ayurveda for medicinal purposes and several curative principles have already been suggested [1]. In a bioactivity-guided screening for endocannabinoid uptake inhibition we have identified extracts of *Piper nigrum* L. and *Piper longum* L. as positive hits revealing a novel highly potent and selective secondary metabolite, which showed prominent CNS activity and anti-inflammatory properties.

**Aims:** Modulation of the endocannabinoid system provides a promising therapeutic strategy during pathological conditions. Potentiation of anandamide and 2-arachidonoylglycerol signaling in neuropathic and inflammatory pain as well as in anxiety and mood related disorders was shown to be beneficial in rodents [2]. Therefore we aim to identify and develop novel inhibitors of endocannabinoid reuptake using bioactivity-guided screenings, compound profiling and *in vivo* validations to identify novel drug scaffolds and potential tool compounds.

**Methods:** Medicinal plant extracts were prepared by accelerated solvent extraction with EtOAc. Compounds were isolated using standard bioactivity-guided fractionation by HPLC and identified by multidimensional NMR and MS. EC uptake experiments were performed in U937 and HMC-1 cells and LSC. Enzyme activities were assessed in cellular and mouse brain homogenates or purified COX-1/2. The tetrad test and endotoxemia model were carried out in BALB/c mice (n = 7–14 per group).

**Results:** Guineensine (1) isolated from *Piper nigrum* L. was identified as a novel nanomolar inhibitor of endocannabinoid uptake (EC50 = 288 nM (95% CI = 190-437 nM) in U937). It did not inhibit EC degrading enzymes (FAAH, MAGL/ABHD) nor interact directly with CB1 or CB2 receptors. Further, no binding to FABP5, a cytosolic AEA carrier protein, could be demonstrated. These properties characterize guineensine as an inhibitor of high selectivity for the putative endocannabinoid membrane transporter. Further, a COX-2 inhibitory component of the compound could be detected. *In vivo* guineensine triggers a full tetrad in BALB/c mice whereas catalepsy and analgesia could be abolished with rimonabant (SR141716A), a CB1 selective antagonist. In a mouse model of endotoxemia, guineensine potently inhibited the acute expression of TNF-$\alpha$ and IL-10.




**Conclusions:** Guineensine is the first plant product shown to selectively inhibit endocannabinoid uptake. *In vivo* it indirectly activates CB1 receptors and is a surprisingly potent anti-inflammatory, analgesic and CNS active polypharmacophoric agent. Therefore, guineensine has high potential for further drug development and contributes novel insights to the broad bioactivity spectrum of *Piper* species.

**Keywords:** Guineensine, endocannabinoid reuptake inhibitor, analgesic, anti-inflammatory.

**References:**

[1] Ahmad N et al. Asian Pacific J Trop Biomed 2012; 2: 1945–1953.
[2] Di Marzo V. Nature Rev Drug Discov 2008; 7: 438–455.

P-57

## OATP2B1 as Possible Mediator in Atorvastatin Induced Cellular Effects

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**Introduction:** The implantation of drug eluting stents is associated with the incidence of restenosis mainly mediated by proliferation and migration of smooth muscle cells from the intimal layer of the vessels. Statins, commonly used in treatment of hypercholesterolemia are assumed to interfere with smooth muscle and endothelial cell proliferation.
**Aims:** It was aim of the herein reported study to compare the cellular effects of statins in endothelial cells and smooth muscle cells in order to understand the mechanisms contributing to observed differences.
**Methods:** All *in vitro* assays were performed in human coronary artery endothelial cells [HCAEC] and – smooth muscle cells [HCASMC]. The influence of five relevant statins on proliferation of both cell types was measured by BrdU incorporation. Uptake studies were carried out with radiolabeled [$H^3$]-atorvastatin. Protein expressions of human organic anion transporting polypeptide (OATP2B1), 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) and specific cell markers were analyzed by Western Blot. OATP2B1 was overexpressed using an adenovirus encoding for OATP2B1.
**Results:** Atorvastatin significantly inhibited smooth muscle cells proliferation compared to endothelial cells. However, quantification of the primary drug target HMGCR revealed no difference in expression levels. Subsequent uptake studies showed significantly higher accumulation of [$H^3$]-atorvastatin in HCASMC, most likely driven by significantly higher expression of OATP2B1, a transporter known to be involved in pharmacokinetics of statins and expressed in the human heart [1]. In accordance with the assumption of enhanced cellular uptake and efficacy of atorvastatin we were able to show that adenoviral overexpression of OATP2B1 significantly increased anti-proliferative activity in HCASMC.
**Conclusions:** Knowledge on the contribution of OATP2B1 to the smooth muscle cell specific effect of atorvastatin might be basis of the identification of novel compounds which could be used for cell-specific activity on vascular stents.

**Keywords:** OATP2B1, atorvastatin, proliferation.

**Reference:**
[1] Grube M et al. Clin Pharmacol Ther 2006; 80: 607–620.

P-58

## Transcriptional Regulation of the Urate Transportosome Member SLC2A9

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**Introduction:** Renal tubular handling of urate is realized by a network of uptake and efflux transporters including members of drug transporter families such as *solute carrier* proteins (SLC22, SLC17), and *ATP-binding cassette* transporters (ABCG2, ABCC4). The *solute carrier family 2, member 9* is also part of this so called "urate transportosome".
**Aims:** The aim of our study was to understand the transcriptional regulation of SLC2A9 and to test whether identified factors might contribute to a coordinated transcriptional regulation of the transporters involved in urate handling.
**Methods and Results:** *In silico* analysis, and cell based reporter gene assays identified an HNF4α *(hepatocyte nuclear factor 4 alpha)* binding site in the promoter of SLC2A9 isoform 1 whose activity was enhanced by transient HNF4α overexpression, while mutation of the binding site diminished activation. HNF4α overexpression induced endogenous SLC2A9 expression *in vitro.* The *in vivo* role of HNF4α in modulation of SLC2A9 gene expression was supported by findings performing quantitative real-time RT-PCR analyses. Indeed, mRNA-expression of SLC2A9 and HNF4a in human kidney samples was significantly correlated. We also show that in renal clear cell carcinoma down-regulation of HNF4α mRNA and protein expression is associated with a significant decline in expression of the transporter.
**Conclusions:** Taken together, our data suggest that the nuclear receptor family member HNF4α contributes to control of the transcriptional activity of SLC2A9. Since HNF4α is one of the transcription factors previously described in literature for transcriptional regulation of several urate transporters our findings support the notion that there could be a transcriptional network providing synchronized regulation of the functional network of the urate transportosome.

**Keywords:** SLC2A9, HNF4α, transporter network, nuclear receptor.

P-59

## Insulin Resistance In Mice Conceived By *In Vitro* Fertilization

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**Introduction:** Assisted reproductive technologies (ART) involve the manipulation of embryos at a very sensitive point of development (James Barker's theory). Some studies have shown a higher risk of cardiovascular diseases in children conceived by ART. Moreover it is well known that there exists a link between cardiovascular and metabolic dysfunction.
**Aim:** The hypothesis tested in this master thesis was that *in vitro* fertilization (IVF) in mice leads to insulin resistance.
**Methods:** In order to exaggerate a possible metabolic dysfunction, Control and IVF mice were fed with two different diets, normal chow (NC) vs. high fat diet (HFD). Insulin resistance was assessed

by performing insulin resistance quantification (HOMA-index) and hyperinsulinemic euglycemic clamps studies. We also assessed glucose tolerance by intraperitoneal glucose tolerance test (IPGTT).
**Results:** The three different tests did not reveal any difference between IVF and Control groups fed with NC. The HOMA-index was significantly higher in the IVF HFD group than in the three other groups. For the clamp study, both HFD groups showed a higher insulin resistance than the NC groups, however, the IVF HFD group was significantly more insulin resistant than the Control HFD. Regarding the IPGTT, both HFD groups showed a higher glucose intolerance than the NC groups. Nevertheless, the IVF HFD group trends to be more intolerant than the Control HFD group.
**Conclusions:** Healthy mice conceived by IVF did not seem to exhibit insulin resistance, however, the addition of a HFD feeding exacerbated metabolic dysfunction thereby revealed the ability of IVF mice to develop metabolic disorders. This phenomenon could be explained by the IVF culture medium composition and the incubation time (depending on the implantation stage) or the surrogate mother mouse. Further research should allow to determine if the metabolic disorders observed could be transmitted to the second generation. It would also be interesting to perform the same tests in older mice. To conclude, it would be important to organize a follow-up care of children born by IVF.

**Keywords:** IVF, ART, mice, insulin resistance.

**P-60**

## Method Transfer from HPLC to Middle Pressure Liquid Chromatography (MPLC) for Efficient Large Scale Isolation of Natural Products

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**Introduction:** In natural product (NP) research bioactive compounds are generally identified in complex natural extracts by bioactivity-guided fractionation. Such an approach is rather time consuming and usually requires multiple chromatographic steps. For performing bioactivity screening on a large set of targets mg amounts of NPs are needed.
**Aims:** Develop an efficient one step isolation procedure for obtaining mg amounts of pure NPs directly from grams of crude plant extracts.
**Methods:** The proposed method takes advantage of HPLC modeling based on generic linear gradients at the analytical level to maximize the separation of compounds of interest in an extract. This step is performed with an HPLC column (250 x 4.6 mm i. d.) packed with the same C-18 material than MPLC. The gradient is geometrically transferred to the MPLC level (920 x 49 mm i. d. column) after system characterization by chromatographic calculation. For a monitoring of the larger possible number of constituents UV and ELSD detections are used at the analytical and preparative scale. MS monitoring is performed by post-chromatographic high throughput UHPLC-TOF-MS profiling of the the aliquots of all fractions in the 96 well microtiter plate format. A rapid evaluation of the performance of the preparative separation is obtained after fusion of all LC-MS profiles (UHPLC x MPLC plot).
**Results:** An example of one step separation of a crude plant extract *(Solanum torvum)* is presented and the possibilities and limitations of the approach are discussed. The crude methanolic extracts of *S. torvum* was used as a model for this study since it contains a series of closely related non-UV active glycosylated triterpene isomers. The monitoring for the separation at both analytical and preparative scale was performed using ELSD. The use of ELSD for MPLC required adequate splitting of the flow rate and subsequent post-column dilution. In order to localize the compounds of interest by MS, all MPLC fractions were analyzed with high throughput UHPLC-TOF-MS. After sampling of each fraction in a 96-well plate, the fingerprints were recorded in 5 min. Software was developed to combine all LC-MS traces in a 2D MPLC x UHPLC map for precise post chromatographic monitoring of the preparative separation and the precise localization of the compounds of interest.
**Conclusions:** The strategy presented combines the initial microfractionation of an active extract in a 96-well plate. The 96-well microfractions obtained are subjected to *in vivo* bioassays which allows an early stage correlation between biological activity and given LC-peaks in the crude extract chromatogram. The UHPLC-TOF-MS analysis provided preliminary information for the dereplication of the active compounds. The high throughput post-column UHPLC-TOF-MS monitoring enabled the precise localization of the active compounds among hundreds of fractions. This rational approach is generic and can be applied to the isolation of natural products that can be profiled by reversed phase chromatography.

**Keywords:** Natural products, isolation, MPLC-UV-ELSD, UHPLC-TOF-MS.

**References:**
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[2] Wolfender JL et al. Chimia 2011; 65:400–406.

**P-61**

## Establishment of a Treatment Protocol for the Combination of Doxorubicin or Methotrexate and Mild Hyperthermia – An *In Vitro* Study

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**Introduction:** Hyperthermia, an established adjuvant in cancer treatment, potentiates the effects of anticancer drugs and synergetic combination can be obtained, improving the therapeutic outcome. Consequently, reduced doses of drug could be used leading to lower systemic toxicity. In particular, the increased cytotoxic effects of several anticancer drugs – such as doxorubicin (DOX) or methotrexate (MTX) – when combined with mild hyperthermia (40 to 42 °C) were reported [1, 2].
**Aims:** To study the combination of hyperthermia and chemotherapy (DOX or MTX) *in vitro* on the human prostate adenocarcinoma cell line (PC3), in term of viability and oxidative stress, in order to establish a treatment protocol that could be used *in vivo.*
**Methods:** PC3 cells line were incubated (100'000 cells/mL) either at 37, 40, 42 or 44 °C during 20, 40 and 60 min of exposure and their viability was assessed using the XTT proliferation assay and the blue trypan coloration. The cells were also incubated with DOX and MTX during 24 h using a range of concentrations between 1 to 75 $\mu$g/mL allowing the determination of the $LD_{50}$. The optimal conditions obtained in mono-therapy were performed in combination to investigate the potential interaction of thermo-chemotherapy. Thus, the effects of the combination on the $LD_{50}$ and on intracellular targets such as the reactive oxygen species (ROS) and the activity of superoxide dismutase (SOD) were determined.
**Results:** Among the different temperatures and exposure times evaluated, cell counting and XTT test showed the optimal conditions for hyperthermia. The temperature of 44 °C did not give significant different results from 42 °C and it has the disadvantage of being more difficult to achieve *in vivo* because of the thermoregulatory system. It seems that the most interesting conditions to combine with chemotherapy are an exposure time of 40 min and temperatures of 40 or 42 °C. The combination of MTX with hyperthermia at

40 °C/40 min of exposure, regardless of the treatment, successive or simultaneous, no significant change in cell viability was observed. However at 42 °C/40 min of exposure for successive treatment, synergy was noted. The $LD_{50}$ decreased from 75 μg/mL to of 68.2 μg/mL. The combination at high concentrations of DOX with hyperthermia at 40 °C/40 min of exposure, demonstrated for simultaneous and successive treatments a $LD_{50}$ of 47.9 μg/mL. On the other hand, at low concentrations for simultaneous treatment, the $LD_{50}$ of DOX is reduced to 4.5 μg/mL. This drastic decrease in $LD_{50}$ – by a factor of 10 – is a strong indication of the effectiveness of the combination of DOX and hyperthermia under these conditions. The combination of DOX with hyperthermia at 42 °C/40 min of exposure showed that at high concentrations for successive treatments, the $LD_{50}$ decreases from 47.9 μg/mL to 36 μg/mL, while at high concentrations for simultaneous treatment, resistance appeared [3].

**Conclusions:** The establishment of a treatment protocol for the combination of thermotherapy and chemotherapy is very complex because it depends on many factors like the active ingredient, the dose, the treatment sequence, the temperature, the exposure time, and finally the cell line used. In this study, the conditions that seem to be most optimal for thermo-chemotherapy are as follows: low concentrations of DOX or MTX with hyperthermia at 40 °C/40 min exposure for simultaneous treatment and high concentrations of DOX or MTX with hyperthermia at 42 °C/40 min of exposure for successive treatment.

**Keywords:** Hyperthermia, doxorubicin, methotrexate, PC3, superoxide dismutase, oxidative stress.

**References:**

[1] Mohamed M, Borchard G, Jordan O. J Drug Delivery Sci Tech 2012; 22: 393–408.
[2] Issels RD. Eur J Cancer 2008; 44: 2546–2554.
[3] Hahn GM and Strande DP. J Nat Cancer Inst 1976; 57: 1063–1067.

**P-62**

## Local Combination of 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(methylmethacrylate) (PMMA) cements are relevant formulations already used in vertebroplasty. They can be loaded with both an anticancer agent – such as doxorubicin (DOX) – and silica superparamagnetic beads (SSB) and/or PMMA superparamagnetic beads (PSB) both embedding superparamagnetic iron oxide nanoparticles (SPIONs) for combining chemotherapy and hyperthermia, the latter being an effective adjuvant in cancer therapy [1–2].

**Aims:** To develop acrylic cement formulations carrying SSB and/or PSB, DOX and zirconium oxide (ZO) as radiopacifier. 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 synergetic effect may be reached improving the therapeutic effects of the implant.

**Methods:** Cements were prepared through an exothermic polymerization reaction (EPR) by mixing PMMA and its monomer in presence of an initiator and an activator. SSB at 24% or 30% (w/w) with PSB or ZO at 10% (w/w) and DOX at 2.5% (w/w) were loaded within the cements. The EPR was followed in term of temperature increase in function of time and parameters such as setting time (ST) and maximum temperature (MT) achieved were determined. Heating capacity was assessed by measuring cement temperature increase under an external alternating magnetic field (3 and 6 mT and 150 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 cell. Young modulus was determined by compression of Ø6x7 mm cylinders. Finally, the cements were injected within human vertebra. The radiopacity was measured using a micro CT-scan.

**Results:** PMMA cements were able to generate heat in a range of therapeutic temperatures 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 150 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. Cements 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 formulations are radiopaque allowing to follow the injection as well as to localize the cements within bone. No leakages were observed during the *ex vivo* injection and the cements showed a spherical distribution in bone tissue. The thermometry of the injected cements showed that cytotoxic temperatures are maintained up to 20 mm far from the cements surface allowing a high volume of hyperthermic treatment around the cements. Finally, addition of SSB, PSB, ZO and DOX kept working time (i. e. the ST) within clinically acceptable values and ensured safe non-necrotic polymerization (i. e. MT achieved during the EPR), allowing the use of these formulations by the clinicians.

**Conclusions:** Acrylic cement was successfully loaded with doxorubicin 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.

**References:**

[1] Mohamed M et al. J Drug Delivery Sci Tech 2012; 22: 393–408.
[2] Le Renard PE et al. Int J Hyperthermia 2009; 25: 229–239.

**P-63**

## Interactions Between RBFOX-2 and Pre-MicroRNA-20b Terminal Loop

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**Introduction:** MicroRNAs (miRNAs) are ~ 21-nucleotide long, non-coding RNAs that regulate gene expression in a broad variety of organisms including humans. MicroRNAs are key factors in post-transcriptional regulation and are furthermore commonly associated with human cancer. Multiple RNA binding proteins (RBPs) have been identified in recent years that are involved in the biogenesis and stability of miRNAs in addition to their previously described functions (e. g. hnRNP A1, KSRP, SF2/ASF, LIN28). Human RBFOX-2 is a member of the evolutionary conserved Fox-1 homolog protein family. RBFOX-2, a known regulator of alternative splicing in metazoans specifically binds to GCAUG motifs in the pre-mRNAs to regulate inclusion or exclusion of flanking exons.
**Aims:** The aim of this study was to investigate if RBFOX-2 is capable of binding miRNA stem loops based on its sequence specificity for GCAUG motifs. We hypothesised that putative binding of miRNA stem loops by RBFOX-2 might affect their processing, stability or subcellular localisation.
**Methods:** We used surface plasmin resonance (SPR) to measure the binding affinities of recombinant RBFOX2 RRM domain to several chemically synthesized pre-miRNA hairpins. HeLa cells were transfected with miRNA mimics, synthetic precursor-miRNA and miRNA expression plasmids. RBFOX-2 protein levels were measured using Western blotting. The effects of these treatments on RBFOX-2 3'UTRs were assessed using luciferase constructs. We performed RBFOX-2 CLIP in order to identify *in vivo* binding candidates.
**Results:** As determined by surface plasmon resonance (SPR), the recombinant RRM domain of RBFOX-2 interacted with the syn-pre-miR-20b with moderate affinity (3.6 $\mu$M). Mutation of G5 residue, which is essential for RBFOX-2 binding to GCAUG completely abrogated the binding. The overex-pression of miR-20b by transfection of its chemically synthesized precursor or by a pri-miRNA plasmid suppressed RBFOX-2 protein levels. Upon G to A mutation in the loop, RBFOX-2 suppression activity was lost. Luciferase reporter assays showed activity of miR-20b on RBFOX-2 3'UTRs.
**Conclusions:** Our data provides evidence for a role of RBFOX-2 in miRNA processing or stability. We show that RBFOX-2 specifically binds to a non-conserved GCAUG motif situated in the terminal loop of human pre-miRNA-20b *in vitro* and *in vivo.* Upon knockdown of RBFOX-2 the levels of miR-20b (5p) were reduced. Overexpression of miR-20b by chemically synthesised mimics, precursors or by plasmid overexpression reduced RBFOX-2 protein levels. Furthermore, presence of non-conserved but active miR-20b 5p sites in the RBFOX-2 3'UTR suggests a feedback loop in RBFOX-2 regulation.

**Keywords:** MicroRNAs, RNA binding protein, RBFOX-2.

**P-64**

## Root Bark of *Morinda tomentosa* B. Heyne, a Source of NAD(P)H: Quinone Oxidoreductase 1 Inducing Anthraquinones

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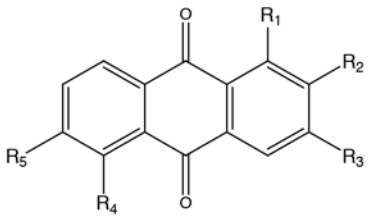
**Introduction:** NAD(P)H: quinone oxidoreductase 1 (NQO1) is a phase II enzyme involved in cancer chemoprevention. Its induction may prevent carcinogenesis by reducing electrophilic compounds such as quinones [1]. Phytochemicals are an important source of new scaffolds described as NQO1 inducers such as flavonoids, steroids and isothiocyanates. Such inducers can be mono- or bifunctional. Monofunctional inducers are regarded as the most interesting for cancer chemoprevention because they selectively induce phase II enzymes and do not interfere with phase I enzymes [1]. Various anthraquinones with good NQO1 inducing activity have been previously isolated from *Morinda citrifolia* without determining if they were mono- or bifunctional [2]. Moreover, *Morinda sp.* are known for their anticancer activity [3].
**Aims:** To test the NQO1 inducing activity of 15 isolated anthraquinones and determine if they are mono- or bifunctional inducers.
**Methods:** The compounds were isolated by RP $C_{18}$ MPLC from a methanolic extract of the root bark of *Morinda tomentosa* (Rubiaceae) and their ability to induce NQO1 was tested on two murine hepatoma cell lines, i. e. the wild-type Hepa1c1c7 and c35, a mutant cell line defective in a functional AhR.
**Results:** The most active anthraquinones have structures similar to 1-hydroxy-2-methylanthraquinone **(14)** and have the capacity to induce NQO1 at the low micromolar level, while 1,6-dihydroxy-2-methylanthraquinone **(13)** does not have activity at all. These results led to the identification of the parts of the molecule important for the activity.
**Conclusion:** Because some of these anthraquinones are found in the diet, it could be viewed as a convenient strategy for cancer chemoprevention.




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | OH | $CH_2OH$ | OGlcAra | H | H |
| **2** | OH | $CH_3$ | OGlcAra | H | H |
| **3** | $OCH_3$ | $CH_2OH$ | OH | H | H |
| **4** | $OCH_3$ | $CH_2OH$ | H | H | H |
| **5** | $OCH_3$ | OH | H | H | H |
| **6** | OH | $CH_3$ | H | OH | OXylGlc |
| **7** | $OCH_3$ | $CH_3$ | OH | H | H |
| **8** | H | H | COOH | H | H |
| **9** | H | $CH_3$ | OH | H | H |
| **10** | OH | $CH_2OH$ | $OCH_3$ | H | H |
| **11** | H | $CH_3$ | H | H | H |
| **12** | OH | $CH_3$ | H | OH | OH |
| **13** | OH | $CH_3$ | H | H | OH |
| **14** | OH | $CH_3$ | H | H | H |

**Keywords:** Cancer chemoprevention, NQO1 inducers, *Morinda tomentosa,* anthraquinones.

**References:**
[1] Cuendet M et al. J Nat Prod 2006; 69: 460-3.
[2] Deng Y et al. J Nat Prod 2007; 70: 2049-52.
[3] Brown AC. Phytother Res 2012; 26: 1427-40.

**P-65**

## Double Chambered LbL Nanoparticles Coated with Viral Proteins as a Novel Intracellular Delivery System

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**Introduction:** Layer-by-Layer technology (LbL) offers a versatile technique to build sophisticated structures from simple building blocks, with the ability to fine-tune even nanosystems. The full po-

tential of this technology is not reached yet and more and more applications are being developed.
**Aims:** In this work we aim to explore a new dimension of application of LbL technology for the purpose of intracellular drug delivery. Nanoparticles with two compartments are designed to deliver a model small molecule and a model protein. To enhance cellular uptake, the outer surface is decorated with influenza virus protein haemagglutinin (HA). System characterization, as well as *in vitro* proof of concept studies in cell lines are carried out.
**Methods:** The proposed design of nanoparticles consists of a calcium phosphate core, loaded with a dye as a model small molecule (first compartment). This is followed by a total of 7 alternating oppositely charged layers, where a cationic protein is alternating with an anionic polyelectrolyte (second compartment). Finally, a lipid bilayer carrying influenza HA is deposited on the outer surface.
The size and surface charge of produced particles were measured and particles of the smallest size with sufficient surface charge to successively adsorb polyelectrolytes were chosen as core particles. For the LBL buildup, a total of 7 layers were deposited on the surface of the particles and the layer deposition was followed by measuring the surface potential of the particles. Then, a lipid bilayer was coated by adsorption and spreading of small unilamellar lipid charged vesicles carrying HA on their surfaces. All steps of LBL buildup were first studied thoroughly using a Quartz Cell Microbalance (QCM) to determine best conditions of layer deposition in terms of pH and salt concentration and imaged using atomic force microscopy (AFM). MDCK cell line was used to investigate the ability of the formulated particles to enter epithelial cells. The cellular uptake of the proposed formulation was investigated using fluorimetric techniques as well as confocal laser scanning microscopy (CLSM).
**Results:** QCM preliminary results helped in deciding on the best conditions to deposit alternating layers of protein/polyelectrolyte. AFM images showed gradual buildup of thickness with each deposited layer. To calcium phosphate cores (<400 nm), layering was applied and successful deposition was proven by monitoring the reversal of surface charge after the addition of each layer including the outer lipid bilayer. Cellular uptake studies showed a greater degree of internalization compared to controls, with no significant toxicity to the cells within the experimental time period.
**Conclusions:** LbL technology enabled the fabrication of double compartment nanoparticles showing efficient delivery properties to both payloads intracellularly. This report sheds some light on the potential of LbL technology to design tailored solutions to challenges in drug delivery.

**Keywords:** Layer-by-Layer (LbL), double compartment nanoparticles, protein delivery.

**P-66**

## Swallowing Difficulties with Oral Drugs Among Polypharmacy Patients Attending Community Pharmacies

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**Introduction:** Swallowing difficulties are common and can affect patients' ability to take solid oral dosage forms, thus compromising medication adherence. Strategies developed by patients to overcome such difficulties while taking medicines have seldom been described.
**Aims:** We conducted a survey investigating difficulties in swallowing solid oral dosage forms in patients attending a community pharmacy, strategies developed by the patients to overcome the difficulties while taking medicines, and health professionals' awareness of these problems.
**Methods:** Community pharmacies in Basel area (German speaking) and Lausanne area (French speaking) were randomly recruited between March and May 2010. A 16-item survey was developed in French and translated in German. Each interviewer spent 4 h consecutively in each pharmacy and asked each consecutive patient (18 years and older) with a prescription for at least 3 different solid oral forms.
**Results:** Among 122 pharmacies, 59 (48%) accepted to join the study and 410 patients were enrolled. Thirty-seven patients (9.0%) reported ongoing swallowing difficulties, while 55 patients (13.4%) reported past difficulties. For the majority of patients, difficulties occurred at each single dose (83.7%), with a single medication (59.8%) and lasted for less than 12 months (53.8%). Number of tablets was not the main trigger. Swallowing difficulties impaired extremely daily life in 12% of the patients. Intentional non-adherence (23% of patients) and altering the oral dose formulation were the most common and potentially harmful strategies used by patients to overcome their swallowing difficulties. According to the patients, pharmacists and physicians rarely inquired about their swallowing difficulties.
**Conclusions:** We report a fairly high prevalence of swallowing difficulties in polypharmacy patients attending their community pharmacies. Pharmacists have to interview patients on their swallowing difficulties in a more systematic way, support patients in finding solutions and refer them to their physician if necessary to ensure continuity in care.

**Keywords:** Swallowing difficulties, dysphagia, prevalence, history, community pharmacy.

**P-67**

## Anisatin: A Neurotoxin Occurring in Various Star Anise Species

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**Introduction:** The neurotoxin anisatin is a natural product found in several *Illicium* species which may induce severe side effects such as epileptic convulsions.
**Aim:** It is of prime importance to have rapid and accurate analytical methods able to detect and quantify anisatin in samples that are purportedly edible star anise.
**Method:** The developed sample preparation combined an automated accelerated solvent extraction with a solid supported liquid-liquid purification step on Extrelut®. Samples were analysed on a porous graphitic carbon HPLC column and quantified by tandem mass spectrometry in negative ionisation mode.
**Results:** The quantification range of anisatin was between 0.2 and 8 mg/kg. High levels of anisatin were measured in *Illicium lanceolatum, I. majus* and *I. anisatum.*
**Conclusion:** The applicability of this validated method was demonstrated by the analysis of star anise *(Illicium verum)* samples

purchased on the Swiss market as well as various related *Illicium* species [1].

**Keywords:** Anisatin, neurotoxin, star anise, quality control, HPLC-MS/MS.

**Reference:**

[1] Mathon et al. Food Additives & Contaminants: Part A. 2013. 1-8.

**P-68**

## Analytical Strategy for the Screening of Botanicals in Food Supplements

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**Introduction:** Safety, quality and composition assessments of food supplements based on botanical ingredients are of major concern, as they have usually not been through a rigorous testing process. To properly delimit botanicals which can be integrated in food derived products, the Federal Office of Public Health (FOPH) has built up a guidance listing over 300 edible plants [1].
**Aim:** Develop an efficient multi-targeted method to screen selected botanicals of interest in herbal food supplements (HFS).
**Method:** Botanicals were characterised by means of appropriate biomarkers, which were unambiguously identified by LC-MS/MS. During this procedure, product ion scans of targeted analytes were generated and compared with an in-house library of MS/MS spectra acquired from reference standards.
**Results:** More than 100 HFS were analysed. A majority of the declared plants were detected (76%). The false negative results concern plants not detected and nevertheless listed on the label. This could simply be due to a lack of sensitivity of the method, which is also linked to the actual amounts of individual botanicals. Another explanation might be that the plants were absent due to an omission or a confusion between species or a fraud. Most of the analysed HFS were not compliant to the Swiss guidance of botanicals established by the FOPH.
**Conclusion:** This generic method enables identification and quantification of 122 biomarkers intended to characterise 91 selected plants [2].

**Keywords:** Food supplements, safety, botanicals, screening, LC-MS/MS.

**References:**

[1] Federal Office of Public Health of Switzerland. Swiss guidance on the categorization of botanicals intended for use as therapeutic preparations or food supplements. 2012. Version 1.4.
[2] Mathon C et al Food Chem 2013; 138: 709-717.

**P-69**

## Enhanced Protein Stability by Non-Covalent PEGylation

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**Introduction:** The advent of recombinant DNA technology has led to a worldwide zeal to develop protein pharmaceuticals over the past decades. However, one of the most challenging tasks in the development of protein pharmaceuticals remains creating a stable formulation, which prevents the proteins from aggregating.
**Aims:** By way of non-covalent PEGylation we aim to improve the shelf-life stability of a model protein (human recombinant G-CSF), while obtaining a higher receptor affinity compared to the non-PEGylated version. Our protein was modified with an N-terminal peptide sequence forming a super secondary structure via a coiled coil interaction with a PEGylated peptide.
**Methods:** Modified rhG-CSF was expressed with a his-tag in *E. coli* and purified by immobilized metal ion affinity chromatography (IMAC). The increase in $\alpha$-helix content due to the formation of coiled coils between PEGylated peptide and modified rhG-CSF was measured by circular dichroism (CD). The increase in protein size due to non-covalent PEGylation was measured by dynamic light scattering (DLS). Binding kinetics between PEGylated peptide and modified G-CSF was analyzed by isothermal calorimetry (ITC). Protein aggregation was monitored with the help of Nile red fluorescence for 5 days in a 96 well-plate at an excitation wavelength of 579 nm and an emission wavelength of 630 nm.
**Results:** CD spectroscopy revealed an overall increase of 24% in $\alpha$-helical content upon addition of PEGylated peptide to modified protein. However, the PEGylated peptide alone showed a $\alpha$-helical signal as well, indicating formation of peptide homodimers via coiled coil formation. The addition of PEGylated peptide to the modified protein was accompanied by an increase from 8.3 (±1.4) nm to 19 (±1.8) nm diameters in particle size measured by DLS. ITC revealed a binding event with a $K_d$ of 3.9 x $10^{-6}$ M. The titration of peptide into buffer resulted in a signal as well, suggesting a dissociation event of peptide homodimers upon dilution. Monitoring protein aggregation with Nile red fluorescence revealed no rise in Nile red fluorescence in presence of PEGylated peptides for modified G-CSF whereas there was a significant increase of Nile red fluorescence for native G-CSF in presence of PEGylated peptides.
**Conclusions:** The results suggest that the PEGylated peptide is binding to the modified protein in the presence of peptide homodimer formation, leading to a dynamic equilibrium between peptide dimers, peptide monomers and peptide bound to the protein. Initial results from stability studies suggest that the PEGylated peptides can specifically attenuate aggregation of modified G-CSF by non-covalent PEGylation.

**Keywords:** PEGylation, protein stability, protein characterization.

**P-70**

## Nanotoxicology: Tunable Silica Nanoparticles and Their Influence on Different Cells

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**Introduction:** Despite their widespread use, our knowledge about possible risk factors associated with nano-engineered materials is limited. Current standard toxicity assays do not specifically address nanoparticulate properties. Thus, it is important to adapt existing toxicity tests and to validate these assays using standardized nanoparticles with defined physical and chemical properties.
**Aims:** We chose amorphous silica nanoparticles as a reference system for relating the physico-chemical properties of 12 different particles with the outcome of three different assays.
**Methods:** We produced 12 different spherical silica nanoparticles as a reference material. The silica nanoparticles were tested regarding viability and oxidative stress at three different time points in

a phagocytotic cell line (THP-1) and a non-phagocytotic cell line (HepG2). Interference of nanoparticles with the readout system, namely adsorptive, optical, and catalytical interference were tested. Furthermore, the hemolytic property of each particle type was assessed.
**Results:** The viability decrease due to silica nanoparticles was surface-charge dependent, where a negative charge exhibits the strongest effect. A decreased viability was observed for particles with a high specific surface area compared to the ones with a low specific surface area. Additionally, the viability decrease and hemolysis was concentration dependent. In THP-1 cells, the viability loss was more pronounced for all particle types. In the given setup, no oxidative stress was detected. Negligible interference was observed.
**Conclusions:** The viability of both cell lines is reduced at high concentrations and is most pronounced for particles with a high specific, negatively charged surface area.

**Keywords:** Nanotoxicology, viability, silica, THP-1.

**P-71**

## Rapid and Efficient Identification of Antifungal Compounds in Crude Plant Extracts

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**Introduction:** Invasive fungal infections have dramatically increased over the last 20 years and they became a major cause of nosocomial infections in developed countries making urgent the need of new antifungal drugs [1]. In this way, natural resources have an interesting potential.
**Aims:** Develop a method to rapidly identify antifungal compounds present in crude plant extracts and use it to target efficiently their isolation.
**Methods:** An efficient strategy combining a sensitive bioautography assay and HPLC microfractionation for the rapid identification of minor antifungal compounds in crude extracts has been developed. The method relies on HPTLC bioautography with an engineered strain of *Candida albicans* (DSY2621) for the screening of crude extracts and detection of bioactive natural products present in low amounts. Microfractionation methods and MPLC were used to isolate the bioactive compounds that were characterized by spectroscopic methods.
**Results:** Two extracts revealing the strongest antifungal activities in the bioautography screening (methanol leaves extract of *Scheffera systilla* and the dichloromethane extract of the root of *Swartzia simplex*) were selected for bioactivity-guided isolation (case 1 and case 2). Using this method, active zone in the HPLC-PDA of the crude extracts could be identified and active pure compounds were obtained. This approach has permitted in one step the identification of alpha-hederin as the antifungal compound in the methanolic leaves extract of *S. systilla* (case 1) and the identification of five minor active diterpenes in the extract of *S. simplex* (case 2)
**Conclusion:** An efficient strategy combining a sensitive bioautography assay and HPLC micro-fractionation for the rapid identification of minor antifungal compounds in crude extracts has been developed. The study of the mode of action of the most interesting isolated compounds on a large panel of fungal strains is underway.

**Keywords:** *Candida albicans,* bioautography, engineered strain, antifungal.

**References:**
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[3] Favre-Godal, Queiroz EF, Wolfender J-L. J AOAC 2013; in press.

**P-72**

## Anti-Insulin Receptor Antibody 83-14 Conjugated Polymersomes for *In Vitro* Targeting of the Blood-Brain Barrier

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**Introduction:** The blood-brain barrier (BBB) remains an obstacle for many drugs to enter the brain. A strategy to overcome the BBB is to modify nanocarrier systems (e. g. liposomes, micelles, nanoparticles) with targeting ligands which bind to endogenous receptors expressed at the BBB and mediate receptor-mediated endocytosis and transcytosis.
**Aim:** The aim of the present study was to investigate the potential of polymersomes (PS) composed of the amphiphilic di-block copolymer poly(dimethylsiloxane)-block-poly(2-methyloxazoline), PDMS-b-PMOXA, for active BBB targeting. Therefore, we conjugated the anti-human insulin receptor antibody 83-14 MAb to the PDMS-b-PMOXA polymersomes and studied their uptake by brain capillary endothelial cells.
**Methods:** PDMS-b-PMOXA polymersomes were coupled to the fluorescently labeled 83-14 MAb using a rapid conjugation method. A thin film containing N-hydroxysuccinimidyl-functionalized PMOXA-b-PDMS polymers was prepared. PS-8314-MAb conjugates were formed upon addition of the antibody solution to the dried film during the rehydration process.
**Results:** Transmission electron micrograph imaging and dynamic light scattering revealed the self-assembly of the polymers to 200 nm vesicles after extrusion. Fluorescence correlation spectroscopy measurements confirmed the successful antibody coupling to the polymersome surface. Binding and uptake of the PS-8314-MAb conjugates were shown using the human BBB *in vitro* model hCMEC/D3 expressing the human insulin receptor. Competitive inhibition with an excess of free 83-14 MAb demonstrated the specificity of cellular binding and uptake.
**Conclusions:** These results indicated that PS-8314-MAb conjugates are potential carriers for brain drug delivery.

**Keywords:** Blood-brain barrier, polymersomes, receptor-mediated endocytosis, anti-insulin receptor antibody.

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