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10/09

Mikrobiologie • Biofilme

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Mikrobiologie in der Zahnmedizin, Medizin und Pharmaindustrie: Das Thema «Biofilme» erfordert ein Umdenken!

Keine «Palastrevolution» für das Fach Dentalhygiene – aber noch mehr Verantwortung bei der Beratung der Patientinnen und Patienten.

Gespräch mit Prof. em. Dr. Bernhard Guggenheim, Institut für Orale Biologie, Zahnmedizinisches Zentrum der Universität Zürich, Zürich

Das Gespräch zeigt auf, dass für das Fach Mikrobiologie in den Bereichen Umwelt, Industrie, Pharmaindustrie, Landwirtschaft, Zahnmedizin und Medizin ein Umdenken eingesetzt hat. Es geht überall nicht mehr um einzelne Bakterienarten, sondern um Biofilme, auf Oberflächen räumlich organisierte Gemeinschaften von Mikroorganismen. Diese Tatsache stellt doch einiges auf den Kopf, das bisher und seit den Anfängen der mikrobiologischen Forschung so eindeutig nicht erkannt worden ist.

Swiss Journal for
the Pharmaceutical Industry
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für die pharmazeutische Industrie
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pharmaceutique
Rivista svizzera per l'industria
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SWISS PHARMA SCIENCE DAY 2009

Coldplay and hot discussions – Pharmaceutical scientists meet on 2 September 2009 at the University of Bern for the 2nd SWISS PHARMA SCIENCE DAY.

Prof. Dr. Rudolf Brenneisen, University of Bern, President Scientific Advisory Board (SAPhS)
Prof. Dr. Gerrit Borchard, University of Geneva, University of Lausanne, School of Pharmaceutical Sciences (EPGL)

On September 2, more than 120 pharmaceutical scientists, mostly Ph.D. and Masters students of all pharmaceutical schools in Switzerland took the opportunity to meet with their peers, senior staff and representatives of academia, industry and pharmaceutical organisations. The organizers were pleased to be able to welcome international participants from Mendeleev University of Chemical Technology of Russia representing the Russian-Swiss Science and Education Center for Transfer of Biopharmaceutical Technologies in Tushino/Moscow. A total of 59 posters were presented by young and enthusiastic scientists, showing the diversity of topics in pharmaceutical sciences. This poster session was embedded into a framework of an impressive keynote lecture concerning the roots of the current world-wide financial crisis and invited speakers covering the broad range of pharmaceutical sciences. This combination gave an ideal setting for an attractive day of scientific discussions and social networking.

Addresses of welcome

Prof. Dr. Hans Leuenberger, President SSPhS
Prof. Dr. Peter Eggli, Dean Faculty of Medicine, University of Bern

The day started with the welcome addresses of Prof. Hans Leuenberger, President of the Swiss Society of Pharmaceutical Sciences, and the Dean of the Faculty of Medicine at the University of Bern, Prof. Peter Eggli.

The President opens the 2nd SWISS PHARMA SCIENCE DAY

Lecture 1: Keynote Lecture

Prof. Dr. Franz Blankart, former Swiss State Secretary: "The Impact of the financial crisis on the pharmaceutical research and industry in Switzerland"

The welcome addresses were followed by the keynote lecture "The impact of the financial crises on the pharmaceutical research and industry in Switzerland" presented by Prof. Blankart, former Secretary of State for Foreign Economic Affairs. His long experience enabled him to share the thoughts on the current economic crisis, how the (Swiss) pharmaceutical industry fares in this situation, and what concepts may ensure the thrive of the industry in the near and distant

future. In a variation of Warren Buffett's motto to "beware of geeks bearing formulas", Prof. Blankart warned to "beware of gurus bearing promises". In his mind, the scope of the financial crisis resembled a tsunami. Though sparing the Swiss pharmaceutical industry, after-effects are still expected to come, and the industry will have to adapt to a changed environment, both in terms of the market situation as well as regarding organizational structures. While knowledge growth is nowhere as rapid as in the field of life sciences, the appearance of block buster drugs is getting rare. In order to shorten the time to market, individuals must be empowered, leading to good governance and turning ideas into products faster. The pharmaceutical industry today needs to learn to navigate complexity by trial and error, engaging in "risk networking" by empowered individuals. In this, sharing knowledge might prove to be superior than intellectual property (IP) protection and "hoarding knowledge". "Wikiknowledge", an open source of information, will help to advance the development of new medicines, or, in the words of Prof. Blankart, "Brockhaus is defeated by Wikipedia". At the end of his speech, Prof. Blankart encouraged the young scientists to actively take part in this process of "creative destruction".

Prof. Franz Blankart, Keynote speaker

Lecture 2: Pharmacology

Prof. Dr. Alex Odermatt, University of Basel Department of Pharmaceutical Sciences: "Keeping the balance of glucocorticoid action by modulators of 11ß-hydroxysteroid dehydrogenase – From basic science to therapy"

The keynote lecture was followed by the lecture on "Keeping the balance of glucocorticoid action by modulators of 11ß-hydroxysteroid dehydrogenase – From basic science to therapy" given by Prof. Alex Odermatt of the University of Basel. Picking up on where Prof. Blankart had left, Prof. Odermatt stated that imbalance in both the economic and health field will lead to crisis. In his presentation, he focused on the many regulating pathways glucocorticoids are involved in, both at the systemic and local level. He specifically pointed out a family of enzymes, 11ß-hydroxysteroid dehydrogenases (11ß-HD) as promising drug targets in metabolic diseases, such as obesity, insulin resistance, type 2 diabetes and cardiovascular disease.

Prof. Alex Odermatt, UniBS, speaker

Lecture 3: Pharmacognosy/ Phytochemistry

Prof. Dr. Muriel Cuendet, University of Geneva, School of Pharmaceutical Sciences: "Natural products as cancer chemopreventive agents"

The morning session was concluded with the presentation on "Natural products as chemopreventive agents" by Prof. Muriel Cuendet, newly appointed professor in pharmacognosy at the University of Geneva. She focused on the search for new actives in cancer therapy, derived from natural products using a novel approach for cancer prevention. She pointed out that, following a paradigm shift, cancer is today seen as the result of a chronic disease process, which might be better prevented at the onset. Natural resources are suspected to provide a plethora of compounds to be applied in this "chemoprevention" approach; however, the detection of such compounds is in need of a systematic drug discovery strategy.

During the lunch break, accompanied by the soundtrack of Coldplay's *Viva la vida* album, participants were not only supplied with excellent snacks, but also with the latest results of pharmaceutical research, presented in poster form. Participants flocked to the poster stands, giving the jury selecting posters for awards a hard time to identify outstanding posters among the many excellent contributions.

Prof. Muriel Cuendet, UniGE, speaker

Discussions during lunch: Prof. Hans Leuenberger talking to Dr. Felix Wüst (Editor "Swiss Pharma") and Prof. Robert Gurny/UniGE talking to Prof. Pierre-Alain Carrupt/UniGE

Poster session

Lecture 4: Analytics, Diagnostics

Dr. Joel Rossier, DiagnoSwiss S.A., Monthey: "Microfluidics system for rapid immunoassay"

The afternoon session started off with a lecture on "Microfluidics system for rapid immunoassay" by Dr. Joël Rossier, Chief scientific Officer of DiagnoSwiss S.A. (Monthey), presenting the application of microfluidic technology to medical diagnostics. The chip developed by his company, a technology called ImmuSpeed, allows for the reduction in reagent used for detection of biomarkers, and a reduction in analysis time per sample, thus providing cost benefits to the user in medical diagnostics for endemic diseases and biotechnology screening.

Dr. Joel Rossier, DiagnoSwiss Monthey, speaker

Prof. Gerrit Borchard (UniGE) and Prof. Rudolf Brenneisen (UniBE), organizing committee of SPhSD 2009 trying to solve a PC problem of Dr. Joel Rossier, speaker

Lecture 5: Biotechnology

Dr. Mario Amacker, Pevion Biotech Ltd., Bern:
"Influenza virosomes – a vaccine carrier system: From the preclinic to the product"

Dr. Mario Amacker, Head of Process Development and Manufacturing at Pevion Biotech Ltd. (Bern), gave with his presentation "Influenza virosomes – a vaccine carrier system: from preclinic to the product" an overview on strategies to develop effective vaccines based on his company's virosomal technology. Virosomes ideally combine the antigen itself and the adjuvant material in the same carrier system. Pevion has developed and already put on the market vaccines against hepatitis A and influenza. A vaccine against malaria has successfully completed clinical phase I trials, and will enter into phase Ib. Another vaccine against vaginal infections will enter clinical trials as early as 2010.

Dr. Mario Amacker, Pevion Biotech Bern, speaker

Lecture 6: Pharmacokinetics

Prof. Dr. Theodor W. Guentert, F. Hoffmann-La Roche AG, Basel:
"PK/PD at key milestones in drug development"

To conclude the afternoon session, Prof. Theo Guentert (F. Hoffmann-La Roche AG and University of Basel) presented with his lecture "PK/PD at key milestones" the role of physiology based pharmacokinetic modeling (PBPK) in modern drug development. In his mind, PBPK is an approach often superior to interspecies extrapolation, or allometric scaling, when it comes to translating drug PK from animal experiments to clinical trials. In fact, PBPK has already been used for defining clinical phase I trial plans, and may even be used to predict pharmacodynamic (PD) effects.

Recognitions and Awards

Professor Dr. pharm. Ulrich Honegger and Professor Dr. pharm. Stefan Mühlebach
New Fellows of the Swiss Society of Pharmaceutical Sciences (SSPhS) and new Members of the Swiss Academy of Pharmaceutical Sciences (SAPhS)

After a short coffee break, it was time for the recognitions and awards. Two outstanding scientists, Prof. Ulrich Honegger and Prof. Stefan Mühlebach were honored with the Fellowship of the Swiss Society of Pharmaceutical Sciences, and appointed members of the Swiss Academy of Pharmaceutical Sciences:
Prof. Honegger received his training as pharmacist at the University of Basel, and obtained his Ph. D. from the University of Bern. Until his retirement in 2005, Prof. Honegger held the position of professor in pharmacology at the same university, focusing his research on the mechanism of action of antidepressants. At the same time, he was very active in several committees and the training of pharmacists. His effort and contribution can be expressed with the full length of the following laudatio: "Prof. Dr. pharm. Ulrich Honegger has been designated as Fellow by the Swiss Society of Pharmaceutical Sciences (SSPhS) and Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) for his outstanding engagement in continuing education of pharmacists and physicans, his great efforts to keep the first two pharmaceutical study years at the Universities of Bern and Fribourg and the planning of the derogation for the new Pharmacy Curriculum, respectively."
Prof. Mühlebach, currently CSO of Vifor Pharma Ltd., obtained his diploma in pharmacy and his Ph. D. in pharmacology and toxicology from the University of Bern. Being a former president of GSASA, he held several positions at the Medical Faculties of Bern and Basel, and served as Chief Pharmacist at Kantonsspital Aarau. Prof. Mühlebach has been very active in the training of hospital pharmacists, and was a member of the Federal Commission for the reform of the Pharmacy Curriculum and Head of the Pharmacopoeia unit of Swissmedic. His effort and contribution can be expressed with the following full length of the laudatio: "Prof. Dr. pharm. Stefan Mühlebach has been designated as Fellow of the Swiss Society of Pharmaceutical Sciences (SSPhS) and Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) for his substantial and continuing contributions to hospital pharmacy and Swiss Pharmacopoeia in academia (University of Basel), practice (Kantonsspital Aarau) and administration (Swissmedic). For example he established postgraduate university teaching and training programs and was head of the Swiss delegation of the European Pharmacopoeia Commission."
The Swiss Society of Pharmaceutical Sciences, and its Scientific Council, i. e. the Swiss Academy of Pharmaceutical Sciences, are very proud to count Prof. Honegger and Prof. Mühlebach among their ranks.

Prof. Stefan Mühlebach and Prof. Ulrich Honegger, happy fellows 2009, enjoying society of Vroni Jakob-Alther (Pharmaceutical Society Zürich)

Poster Awards

1st prize
Doris Gabriel
School of Pharmaceutical Sciences, University of Geneva

2nd prize
Simon Heuking
School of Pharmaceutical Sciences, University of Geneva

3rd prize
Srinivas Madduri
Institute of Pharmaceutical Sciences, ETH Zürich

Special prize
Michael Adams
Institute of Pharmaceutical Biology, University of Basel

The poster awards, being sponsored by the Swiss Society of Pharmaceutical Sciences (1st prize), the Opo Foundation Zürich (2nd prize), and the AKB Stiftung zur Förderung des wissenschaftlichen Nachwuchses (3rd prize), were selected based on their scientific quality and clarity of presentation. The jury voted unanimously to award the poster contribution of Doris Gabriel (School of Pharmaceutical Sciences, University of Geneva), titled "Thrombin-sensitive dual modality prodrugs for the simultaneous imaging and treatment of inflamed lesions in rheumatoid athritis" the first prize. Simon Heuking (School of Pharmaceutical Sciences, University of Geneva) was awarded the second prize for his presentation titled "In vitro evaluation of Toll-like receptor-2 (TLR-2) agonist functionalized nanocarriers" The third prize was awarded to Srinivas Madduri (Institute of Pharmaceutical Sciences, ETH Zürich), who presented on "Aug-

mented nerve regeneration by controlled co-delivery of synergistic growth factors from nerve conduits". This year, the jury decided to award a special poster prize for a fourth outstanding contribution. Michael Adams' (Institute of Pharmaceutical Biology, University of Basel) presentation of "Medicinal herbs for the treatment of rheumatic disorders – a survey of European Herbals from the 16th and 17th century" was chosen for this award.

Poster prize awardees 2009 (from left): Dr. Michael Adams, UniBS (special prize); Srinivas Madduri, ETHZ (3rd prize); Doris Gabriel, UniGE (1st prize); Simon Heuking, UniGE (2nd prize)

Thanking … and Invitation to the 3rd SWISS PHARMA SCIENCE DAY on 8 September 2010 in Bern

This, in the minds of all participants, successful 2nd SWISS PHARMA SCIENCE DAY ended with drinks and snacks at the House of the University. The organizers would like to thank all speakers for their excellent presentations, and the Medical Faculty of the University of Bern as the host of this event. The Opo Foundation Zürich, Verlag Dr. Felix Wüst AG Küsnacht ZH, AKB-Stiftung, pharmaSuisse, the Pharmaceutical Society of Zürich, and Galexis are recognized for their continued financial support. The organizers are looking forward to welcome young pharmaceutical scientists to the third edition of the SWISS PHARMA SCIENCE DAY on the 8th September 2010 in Bern (www.sgphw.ch).

2nd SWISS PHARMA SCIENCE DAY 2009 (www.sgphw.ch), Bern, 2nd September 2009 (Keynote Lecture)

Impact of the Financial Crisis on Pharma Research and Industry*

Prof. Dr. Franz Blankart, former State Secretary for Foreign Economic Affairs

A word of warning at the outset

On country lanes I've seen a sign saying: "one curve may hide another". I'd like to paraphrase this warning: "one financial crisis may hide another". The central bank officials announce the "light at the end of the tunnel". Wall Street booms on the specious ground that "after the bubble shares are a safe buy".

At this point one might profitably remember that last year's 'toxic assets' have just been quarantined by a wholesale shift of bad private debt to public treasuries. The toxicity problem has not gone away, however, and the holding tanks may leak and break. The next bubble may be already on the way. But last year's salvage operation through the public treasury can't be repeated, for the credit of governments has been stretched to the limits – or beyond. In short: short-term success in rescuing reckless financial institutions may lead to even more reckless behaviour.

Success in salvaging the world economy does not mean that it has been set on a sounder footing, or that the 'self-healing' virtues of the market have been vindicated after all. Reforms are needed. Financial instruments based on erroneous assumptions have to be retooled or discarded. If the political will to do the necessary evaporates, as it might do if the storm settles, we may find ourselves unable to act when the storm resumes – without credible government guarantees this time.

But let's move on to the subject at hand.

I. The scope of financial crisis

- The effects of the financial crisis have hit 'real economy' like a tsunami – Japan's fourth quarter of 2008 contraction was 12.7%, and in the US it was 6.7%. Not all countries or all sectors have been hit head on in same fashion, or at the same time with same force[1]. So far Switzerland e. g. has been spared the worst. In a globalised economy expect vast ricochet effects. Expect also severe after-shocks as debt obligations come due for refinancing, or asset classes slip from prime to subprime or even junk.
- Jeremy Grantham of GMO estimates[2] that the 'de-leveraging' awaiting the US economy will be twice as severe as that that took place during the Great Depression, but still half the de-leveraging that Japan underwent in the '90s. Yes, it will be bad, but Japan managed the deed without wrecking the real economy. The main question is: can we scale up from Japan's experience to the US, or even the world?
- Much will depend on the ability of Chi-merica (to use a term dear to Niall Ferguson to signify the economic link between China and America) to hang together – lest we all hang separately. The few signs coming out of China we can discern are worrying[3]. Chinese statistics have always been more difficult to read than green tea leaves. Now they have become totally opaque.
- Unemployment has risen sharply in the US[4], to be followed soon by other countries. There is talk of widespread social unrest – a Russian think-tank has even predicted the rapid break-up of the US. Far-fetched as this might sound today, one cannot exclude that the road ahead will not take us through The Great Repression – as governments struggle to retain control over a restless populace that is unprepared for really bad times.

One can spend a gloomy hour prophesising doom – to general applause. We all love scare stories. You can't really predict how a jar will smash – except that it might.

As far as the pharmaceutical industry as a whole is concerned, one may fairly say that it has been spared. This sector is too important to fail, and since the state in many cases is the payer, demand has not suffered excessively.

Let's now move beyond short-term destruction – to restructuring. For, if we get out of this in one piece, we'll do so thanks to much and fast restructuring.

II. Restructuring in the pharmaceutical industry

1. A world of expanding opportunities

Good news at the outset:

- Human *knowledge* doubles every five years. Nowhere as fast as in the life sciences. This process is still exponential. One only has to point to the promise of cell biology, or the systematic screening of plants and animals for pharmaceutical properties. An 'artificial bacterium' has just been announced. This progress is tempered by the increasing realisation of the diversity of our bodies, which makes the development of 'blockbuster' drugs more challenging.
- New *technologies* are coming on stream – nano-technology is but one area of promise in the pharmaceutical area. We know how to bring such innovations to market faster.
- New ways of doing *research* are emerging, thanks to the self-organising properties of internet (also known as "wikinomics"). Let me quote Richard Barker of ABPI: "On R&D we are moving from vertical integration, with occasional outsourcing, to an open innovation model, where the person or organisation best equipped to perform a step in the R&D process forms part of a virtual network. What do we mean by best equipped? – The best blend of quality, cost and speed for the task. This network model sounds complicated, but it may actually work better than a fully in-house model, which is bedevilled with organisational complexity across multiple sites, often around the globe, by internal politics, by complex resource allocation decisions and by decision-making treacle."[5]

* I thank Dr. Aldo Matteucci for his important input.

1 Factory output is collapsing at the fastest pace everywhere. The figures for the most recent month available are, year-on-year: Taiwan (-43pc), Ukraine (-34pc), Japan (-30pc), Singapore (-29pc), Hungary (-23pc), Sweden (-20pc), Korea (-19pc), Turkey (-18pc), Russia (-16pc), Spain (-15pc), Poland (-15pc), Brazil (-15pc), Italy (-14pc), Germany (-12pc), France (-11pc), US (-10pc) and Britain (-9pc). Norway sails blissfully on (+4pc). http://www.telegraph.co.uk/finance/comment/ambroseevans_pritchard/4884975/We-need-shock-and-awe-policies-to-halt-depression.html

2 https://www.gmo.com/Europe/CMSAttachmentDownload.aspx?target=JUBRxi51IIBfJXb8ASd8%2bfe6xTnek30r%2fSsfGLZdf%2fgBjNfDjVKz9zThOLvkRsKh1jZMssV4OcWW%2fOUiSM4rs67OTavUbkMkH3jel9URyBY%3d

3 http://www.telegraph.co.uk/finance/economics/4799926/China-nears-deflation-trap-as-rail-freight-collapses.html

4 Payroll employment has declined by 3.6 million since the start of the recession in December 2007; about one-half of this decline occurred in the past 3 months. In January, job losses were large and widespread across nearly all major industry sectors. http://www.bls.gov/news.release/empsit.nr0.htm

5 http://www.abpi.org.uk/press/RB_speech_economist_0209.pdf

• *Demand* is strong: as breakthroughs seem close at hand, social conscience is re-awakening. I only need to refer to the Melissa and Bill Gates Foundation and their stimulus to medical research.

I'd conclude that the sector is riding a strong and rising tide of knowledge that has the potential of translating into innovation and growth. Far from worrying that 'block-buster drugs' are getting rarer in the pipeline, I'd expect the potential contribution of the pharmaceutical industry to the improvement of human health to strengthen in the future.

2. A changing regulatory paradigm

Warren Buffett recently quipped: "Beware of geeks bearing formulas", and I may add: "Beware of gurus bearing ideologies". The myth of the benign self-regulating market has been carried to its grave – fittingly Alan Greenspan acted as pall-bearer.

After the recent self-regulatory follies, the lame state and the blind market have agreed that they need each other to move forward. As US President Obama said in his inaugural speech: usefulness, not principle, shall be our guide. And so it is: a principle is congealed experience and a default point of departure, not dogma or patent medicine.

I'd expect a re-invigoration of a regulatory system based on the deliberative dynamics of checks and balances involving both the state and private enterprise. If the state has at times been arrogant and byzantine, the market has also been deceitful, greedy, and self-serving. From thalidomide, to tobacco, to lead… the instances of markets working against the common good are just too numerous to dismiss or ignore. Modern avatars of such practices may just be with us: I'm not sure that the current mass-marketing of cures for alleged conditions like Attention Deficit Hyperactivity Disorder (ADHD) or agoraphobia are warranted by the medical outcome.

Adversary proceedings are not the solution. To bridge the gap *both* sides will need to follow common standards of good governance. 'Good governance' has been translated by 'good procedures and practices' – the endless red tape that – like Ariadne's thread – is supposed to help us out of life's labyrinth. 'Good procedures', however, can become, and often do become, a very autocratic exercise. As an old defender of individual liberty, let me utter here a word of caution. More than on 'good procedures' good governance rests on empowerment of individuals, who alone can translate intentions in outcomes. The 'principal-agent' conundrum is not solved by turning the agent into an automaton. On the contrary – only empowerment will do it. Empowerment does lead to good governance, for it allows individuals to contribute, while achieving respect. And lest you feel I'm getting mystical – the last sentence is a reformulation of The Toyota Way[6]. Toyota is the world's most successful manufacturer. This approach aims to *embrace* change and accountability to the whole.

Let me add a note of realism at the end concerning the pharma industry: if the regulatory process can be made less contentious, and more effective, it can hardly become less expensive. Our knowledge of side-effects of drugs is cumulative, so the check-list of possible side-effects grows longer as the testing procedures become more sophisticated. Also the standards of 'health care comfort' have risen dramatically: patients have become finicky indeed.

3. A changing economic environment

Health care is a growing business that is largely immune to cyclical change. This is why the pharma sector has hardly been touched by the current economic crisis. People are demanding good health as never before.

The market is a peculiar one, with a third (i. e. state) party regulatory process that determines both product quality and prices. In a rush to offer universal coverage, states have adopted tax-based funding, without securing the necessary long-term means – health care is a major budget item today and crowding out other tax-based services and functions. But the peculiarity goes deeper: the scope (if not the effectiveness[7]) of health care expands continuously. The medical procedures on offer today dwarf anything that was available only a few years ago. 1.5, now 3, soon 7 Tesla-strength MRI, CT-scans, the alphabet soup of techniques is bewildering – and costly. Imagine you owned a life insurance company where the *promised* payout of the policies expands yearly.

As the financial crisis sucks the state into involuntary roles like lender of last resort, default share-holder, or receivership administrator and landlord, the pressure to cut health costs – also on drugs – will increase. The days where pricing of drugs could be geared to fund up front the future generation of pharmaceuticals, rather than recover historical investment plus a return on the risk are – I dare say – gone.

Add to this that the market leader – the US – will need to expand health care significantly to come closer to the world standard of universal coverage. Half of the 60+ billion dollars 'down-payment' on health care in Obama's 2009 budget will be funded through *savings* from reduced health care costs. Other states will follow suit.

If many large pharmaceutical companies have entered the current crisis in excellent health this may not be the case for the vibrant sector of the small R&D oriented firms. They represent a major segment of the sector – they specialise in identifying novel active substances, while the big firms manage the regulatory process, production and marketing. Venture capital is drying up. University endowments are faltering – as the much reduced Harvard endowment attests – it has lost about half its value. Vanity funding of chairs and labs may become less certain.

It is in the interest of the sector as a whole that this diversity be sustained. Don't expect assistance from governments – they are good at picking losers, not winners. State resources will go into propping up faltering sectors of the economy, not in creating growth.

I hope the sector will wisely manage its long-term prospects rather that aiming for quarterly results. Concentration and heightened control does not create innovation – just a monopoly. We all know that monopolists are static. 'Synergies from concentration' are mostly bonfires of vanities, as long term studies of M&A show. And when I speak of sector, I ignore national borders: BRIC countries may host a whole new generation of brilliant researchers.

4. A change in business paradigm

Up to the French revolution drill was the mainstay of an army. That's why the Prussian army was best. Battles were set pieces, and essentially static. Napoleon's armies relied on individual initiative, speed and movement. They may have been disorderly, but they surprised the automatons every time.

I may be wrong, but I sense that we are at the tipping point where a new business paradigm is emerging. The world has become complex. Success goes to the firm that first learns to navigate complexity. Such navigation relies on trial and error, rather than plan. This is akin to what happens in nature: the species most adapted to its environment occupies the ecological niche. Other species survive by differentiation, not competition. In this process speed is of the essence.

The traditional firm is based on a 'command and control' structure. Its workers are drilled to fulfil a central business plan. Change comes mainly from the top. By the time the need for change is conveyed to the centre, a new plan is developed and change is ordered from the centre – it is too late. In navigating complexity and risk

6 Jeffrey K. LIKER (2004): *The Toyota Way. 14 management principles from the world's greatest manufacturer.* McGraw Hill, New York; xxii + 330 pp.

7 It might be a perverse effect of the very exuberance of the sector that quality may decline as offer increases. As doctors struggle to keep up with new technologies, resources are wasted on scattershot tests and second opinions.

8 For a discussion of this new paradigm, see: Don Tapscott and Anthony D. Williams (2008): *Wikinomics: How Mass Collaboration Changes Everything.* Portfolio Hardcover; Expanded edition. 368 pp.

About the Author

Mr. Franz Blankart was born on November 27, 1936, and studied philosophy, economics and law at the Universities of Basel, Paris (Sorbonne), Exeter and Bern. In 1964, he was awarded the degree of Doctor of Philosophy (summa cum laude) at the University of Basel.
Following banking experience, he entered the Swiss diplomatic service. He held i. a. the rank of Private Secretary to the Minister of Foreign Affairs and was then a member of the Swiss delegation negotiating the Free Trade Agreements with EEC/ECSC. From 1973 to 1980, he was Head of the Office for European Integration in Bern and negotiated most agreements elaborated with the Communities between 1973 and 1980 (e. g. on insurance). In June 1977, the Swiss Government granted him the rank of Minister, and in 1980, with the rank of Ambassador, he was appointed Head of the Swiss Representation to EFTA, GATT, the UNCTAD and the U. N. Economic Commission for Europe, and Head of the Swiss Delegation to the negotiations on commodities. Mr. Blankart chaired i. al. the EFTA and the UNCTAD Council (TDB) and was spokesman of the Western countries (including USA and Canada) in the ECE/UNO negotiations with the Eastern Block. In May 1984, he was, with the rank of Ambassador, nominated "Delegate of the Swiss Government for Trade Agreements". This function involved his role as Governor of the Inter-American Development Bank as well as bilateral relations with the whole American continent and South Africa. Moreover, he was in charge of GATT-matters and World Trade, including the commercial aspects of OECD. In 1986, he was appointed State Secretary and Director of the Federal Office for Foreign Economic Affairs. He directed in Bern the Swiss negotiating team in the Uruguay Round and was Chief negotiator for the European Economic Area Agreement.
From 2000 to 2004, he was limited partner of the private bank "Mirabaud & Cie" in Geneva. He was also a member of the Executive Council of the Basel University and was a member of the Société Générale de Surveillance's ethics committee. He is a board member of the BHF Bank (Schweiz) AG.
Mr. Blankart, Distinguished Associate of the Atlantic Economic Society, has been rewarded i. a. by the "Prize of the European Economy", the Medal of the Charles University of Prague and the "Prize for Foreign Economy" by the Swiss Multinational Companies.
Mr. Blankart was professor at the Graduate Institute of International Studies in Geneva. He held the rank of Colonel in the Swiss Army (cavalry).
Mr. Blankart ist married with Mrs. Anne de Palézieux Blankart. He has two children (Serge 1968 and Andréa 1970).

networking by empowered individuals bound together by a few procedural rules is superior – every time.[8]
Brockhaus is no more. Defeated by Britannica? No, by Wikipedia – this is a network of empowered volunteers without a master plan. Just a few procedural rules bind these volunteers together as well as the willingness of the individuals to engage voluntarily and without pay.
Toyota has many networking features. Boeing's Dreamliner, the 787, is built on a networking platform. The open software Linux is such a networking platform, to which haughty IBM, Big Blue, modestly contributes by the billion – for free.
The ability to create networks so as to tackle rapidly ever changing tasks is more important than defining the task itself. A broad vision suffices. This may sound counter-intuitive, until we stop to reflect on the essence of a democratic constitution. It is no master plan for the future of the country: it is a set of dry procedural rules – checks and balances – that allows a group to act together over time.
Moving from a command and control toward a networking paradigm has consequences. The wall of separation between the firm and the outside becomes porous. Sustaining such walls is just too costly. IBM contributes to the LINUX platform – for free. How can this possibly work, when effort no longer matches reward? In strange and roundabout ways – it does. IBM earns money on value added services it provides to customers using LINUX. In this new paradigm sharing knowledge – rather than defending it with intellectual property – might be the superior strategy: simply because sharing may yield better and richer opportunities than hoarding.
We are just beginning to grasp the power of networking – after all Hypernet is only ten years old or so. The firms chances of the future will be surprisingly different from those of the past. I'm not even sure that the firm as you and I know it will survive at all. Ronald Coase, Nobel in economics, had drawn attention to the oddity of a 'command and control' structure at the core of the market model. He had justified it with transaction costs – a blemish, not a structural element.

III. Conclusions

Schumpeter has described capitalism as an engine of 'creative destruction'. This process is ongoing, but can at times accelerate and threaten to overwhelm the system. We are in such a convulsive phase.
For the moment the pharma industry has weathered the crisis. Strong demand, and a rising tide of knowledge, may allow it to ride out the perfect storm. But even within the sector, the process of creative destruction will accelerate. Embracing change and jettisoning ideology in favour of trial and error is the best way forward.
Broadly speaking, all factors point into the same direction: a move from competition to networking, collaboration and democratic empowerment. Barriers are too costly to erect and defend – so many opportunities are missed every time we retrench and regroup.
Will we succeed? Who knows, and I'm not in favour of a Whiggish view of history. Life is an experiment with no predictable outcome – always. But what a time for you all to be active.

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Poster Session – Abstracts

P-1

In vitro Evaluation of Toll-like Receptor-2 (TLR-2) Agonist Functionalized Nanocarriers

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The present strategy for the formulation of new plasmid DNA (pDNA) vaccines is to include highly purified synthetic adjuvants, which are able to trigger well-defined elements of the immune system. Moreover, for effective vaccination the adjuvant needs to be co-encapsulated with the pDNA vaccine within the same particulate system [1].
In order to address this necessity, we recently synthesized a novel copolymer *CM-TMC-g-PEG-Pam$_3$Cys,* based on a chitosan polymer [2]. To a new chitosan derivative, 6-*0*-carboxymethyl-*N,N,N*-trimethylchitosan *(CM-TMC),* the Toll-like receptor-2 (TLR-2) agonist, *Pam$_3$Cys,* an adjuvant activating the innate immune system was grafted through a polyethylene glycol (PEG) spacer. The successful synthesis of this novel copolymer was confirmed by means of ^{1}H and ^{13}C NMR, as well as FTIR spectroscopy.
In a second step, *Pam$_3$Cys* decorated nanocarriers were prepared by complexation between *CM-TMC-g-PEG-Pam$_3$Cys* and a model plasmid DNA (pDNA) expressing green fluorescence protein (GFP) [3]. Our results showed that the TLR-2 functionalized pDNA nanocarriers have the ability to complex and to protect pDNA against enzymatic degradation. pDNA nanocarriers (N/P ratio of 3:1) were around 400 nm in size, and displayed a positive zeta potential of 27.9 ± 1.6 mV. Interestingly, TLR-2 agonist decorated pDNA nanocarriers induced IL-8 secretion from differentiated THP-1 macrophages, which was increased by 10-fold compared to non-decorated carriers.
Based upon these promising results, the adjuvant effect of the described nanocarriers will also be evaluated *in vivo.* Moreover, we believe that TLR ligand functionalized polymers, and nanocarriers systems, represent a technology platform to address modulation of TLR activity in a variety of diseases, including autoimmune and inflammatory diseases, and cancer. Studies pertaining to such applications are currently underway in our lab.

References:
[1] D.T. O´Hagan, M. Singh, J.B. Ulmer, *Methods* 2004; 40:10-19.
[2] S. Heuking, A. Iannitell, A. Di Stefano, G. Borchard, *Int. J. Pharm.* 2009; doi: 10.1016/j.jim.2009.03.239.
[3] S. Heuking, S. Adam-Malpel, E. Sublet, A. Iannitelli, A. di Stefano, G. Borchard, *J. Drug. Target.,* submitted.

P-2

Uterine contractility pattern after Bryophyllum pinnatum *in vitro* vs. *in vivo*

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Bryophyllum pinnatum preparations have been used since 30 years for tocolysis. The present study covers the uterine contractility pattern after the addition of *B. pinnatum* on *in vitro* muscle strip preparations (myograph) compared to the changes registered in the cardiotocogram (CTG) of pregnant women with B. pinnatum tocolysis.
Press juice (component of Weleda Bryophyllum Tablets) from *B. pinnatum* leaves or its fractions are given to strips from myometrium obtained during caesarean section for contraction (voltage) measurements by myometry. The influence on the spontaneous uterine contraction pattern is quantified by AUC and the frequency of contractions. The results are compared with the AUC and frequencies in the CTG performed in pregnant women with *B. pinnatum* tocolysis.
The press juice shows *in vitro* a concentration-dependent inhibition of the AUC. Among the fractions whose HPLC data indicate bufadienolides as constituents the AUC is more inhibited than those who presumably contain flavonoids or cinnamomic acid derivatives. With the decrease of the AUC, the frequency of contractions is increasing and is contributing to a saturation mechanism in the press juice. Initial results from the CTG are suggesting a similar pattern also *in vivo.*
B. pinnatum extracts and some fractions inhibit the contractility of myometrium *in vitro* while initial frequency increases. Consequences for mother and child will be discussed.

P-3

Correlating and Modeling Tablet Physical Properties to Radial Die Wall Pressure

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Tablets are complex systems and the behavior of disordered particulates under pressure is not yet clear, especially with high-speed compaction cycles similar to industrial presses. The use of an instrumented compaction simulator in the early stage of development

has a significant benefit for product development process. The key is to search for mathematical models, which are able to predict the performance of a tablet formulation. The aim of this study is to correlate compaction pressure and the physical properties of a tablet to radial die wall pressure. Moreover, mathematical models using radial die wall pressure as a monitoring tool in tabletting are evaluated. Several compaction parameters such as maximum die wall pressure MDP and residual die wall pressure RDP were measured using Presster™, a tablet press simulator. Two materials with different compaction behaviors; Avicel PH 102® and Parteck M200® were used. First results showed that there was a strong correlation ($r>0.90$) ($P<0.05$) between compaction pressure and the RDP/MDP ratio for Avicel PH 102® compacts at different compaction speeds; whereas on using Parteck® M200, the RDP/MDP ratio stayed constant. Mathematical models applying non-linear fitting were suggested in this work using radial die wall pressure as a valuable monitoring tool in high-speed manufacturing.

P-4

Reduction of Safrole and Methyleugenol in Asari Radix et Rhizoma by Decoction

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Asari radix et rhizoma (Xixin, Manchurian Wildginger, Asarum spp) is a herbal drug commonly used as an ingredient in Traditional Chinese Medicine (TCM). Many species of Asarum contain safrole and methyl-eugenol as the main components of their volatile oils [1-3]. However, toxicological studies have shown that safrole may be a hepato-carcinogen and genotoxic leading to concerns regarding the habitual consumption of this herbal drug [4, 5]. An HPLC method was established to assess the levels of safrole and methyleugenol in five batches of Asari radix et rhizoma and two TCM formulae containing this herbal drug as an ingredient. Analysis showed that the content of safrole in the dried herbal drugs tested ranged from 0.14–2.78 mg/g whilst the content of methyleugenol ranged from 1.94–16.04 mg/g.
The present study demonstrated that following a 1-hour decoction, the amount of safrole was decreased by more than 92% resulting in the equivalent of no more than 0.20 mg/g safrole remaining in the aqueous extract. Such a reduction in the content of safrole is regarded as acceptable for therapeutic use. Similarly, the content of methyleugenol was decreased to the equivalent of 0.30–2.70 mg/g. Furthermore, both TCM formulae, after decoction, showed negligible amounts of safrole (maximum, the equivalent of 0.06 mg/g), and only 1.38–2.71 mg/g of methyleugenol. Therefore, the present study shows that a decoction procedure, traditionally used for Chinese herbal preparations, is able to reduce the amount of safrole and methyl-eugenol effectively.

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References:
[1] The State Pharmacopoeia Commission of China (2005) Pharmacopoeia of the People's Republic of China, Vol. 1. Chemical Industry Press. Beijing.
[2] Xiao, P.G. et al. (2002) Modern Chinese Materia Medica, Vol. 3. Chemical Industry Press.Beijing.
[3] Cai, S.Q. et al. Fitoterapia 2008; 79:293-297.
[4] Scientific Committee on Food, European Commission (2001) Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties. http://ec.europa.eu/food/fs/sc/scf/out116_en.pdf.
[5] Scientific Committee on Food, European Commission (2001) Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2-dimethoxybenzene). http://ec.europa.eu/food/fs/sc/scf/out102_en.pdf.

P-5

HPTLC of Citrus Fruit Peels

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Several citrus fruit peels are described in different pharmacopoeia monographs, i. e. European Pharmacopoeia [1], Swiss Pharmacopoeia [2] and Chinese Pharmacopoeia [3]. Tinctures or syrups thereof are monographed as well. Up to now there is no TLC identification test for sweet orange (*Citrus sinensis* Osbeck) and lemon (*Citrus limon* (L.) Burm. fil.) in the pharmacopoeias. Therefore, the aim of the study was to establish a suitable HPTLC test to identify the citrus peel drugs as well as preparations in order to revise the pharmacopoeia monograph. Moreover, for aged tangerine peel (*Citrus reticulata* Blanco), a drug traditionally used in Chinese medicine and called Chenpi, a test to discern it from bitter-orange epicarp and mesocarp was established.
The spraying of an aluminium chloride solution (UV 366 nm) was found to be a suitable HPTLC detection mode to visualize some typical citrus flavanones, e. g. hesperidin or naringin. The resulting fingerprint allows distinguishing orange, lemon and bitter-orange. In order to discern between aged tangerine and bitter-orange, a subsequent derivatization by natural products/polyethylene glycol 400 (NP/PEG) solutions and evaluation in visible light was found to be effective. Whereas bitter-orange shows a prominent red zone of neoeriocitrin, in tangerine this compound is nonexistent. The results show that an identification of different *Citrus* species by HPTLC fingerprint is possible. However, since orange and tangerine have similar flavanone contents [4], the identification solely by HPTLC remains insufficient and has to be complemented by macroscopic and microscopic examination.

Acknowledgments:
We thank Dixa AG and Hänseler AG, Switzerland, for the supply of herbal drug material and herbal preparations, as well as SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support.

References:
[1] European Directorate for the Quality of Medicines (2009) European Pharmacopoeia 6th Edition (6.5). Online-Edition.
[2] Swissmedic (2006) Pharmacopoeia Helvetica 10th Edition. BBL, Vertrieb Publikationen. Berne
[3] The State Pharmacopoeia Commission of China (2005) Pharmacopoeia of the People's Republic of China, Vol. 1. Chemical Industry Press. Beijing.
[4] Peterson J.J. et al. J. Food Comp. Anal. 2006; 19:S66-S73.

P-6

Press Juice of *Bryophyllum pinnatum* (Lam.) Inhibits Oxytocin-Induced Increase of the Intracellular Calcium Concentration in Human Myometrial Cells

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Bryophyllum species are used in the ethnomedicine of the countries of origin and in the anthroposophic medicine. The use of preparations from *Bryophyllum pinnatum* in tocolysis is supported by both clinical (retrospective comparative studies) and experimental (using uterus strips) evidence. We studied here the effect of *B. pinnatum* juice on the response of cultured human myometrial cells to oxytocin stimulation. This preparation prevented in a dose-dependent manner the oxytocin-induced increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) in hTERT-C3 human myometrial cells. Comparable results were obtained with M11 human primary myometrial cells. At least in hTERT-C3 cells this effect was independent of the extracellular Ca^{2+} concentration, and of voltage-dependent Ca^{2+}-channels. To test the effect of *B. pinnatum* juice on voltage-gated Ca^{2+} channels, studies were conducted with SH-SY5Y neuroblastoma cells, which were depolarized with KCl. *B. pinnatum* juice delayed, but did not prevent the depolarization-induced increase in $[Ca^{2+}]_i$. Taken together the data suggested a specific and concentration-dependent effect of *B. pinnatum* juice on the oxytocin signalling pathway. This would explain the rare and minor side-effects in tocolysis with *B. pinnatum* and as well as its high therapeutic index.

P-7

High-Throughput log P Determination by Micromemulsion Electrokinetic Chromatography Coupled with UV and MS Detections

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Precise knowledge of the physicochemical properties of new chemical entities (NCEs) in early steps of drug design and discovery is of prime importance. Among them, lipophilicity is a key parameter involved mainly in pharmacokinetic processes such as absorption, distribution, metabolism, elimination and toxicity (ADMET), as well as in ligand-target interactions. Capillary electrophoresis (CE) in microemulsion electrokinetic chromatography (MEEKC) mode is emerging as an interesting approach for octanol-water partition coefficients (log P_{oct}) determination due to some inherent advantages: (i) small solvent and sample consumption, (ii) universal technique since different detection systems can be used, (iii) automated instrumentation, (iv) ability to handle impure samples, and (v) cost-effective analysis.

In this work, a high-throughput screening (HTS) MEEKC method was developed for log P_{oct} measurements. Two approaches enabled the determination of log P_{oct} values in less than 20 min per compound using a single capillary: (i) a dynamic coated capillary was used to increase the electroosmotic flow (EOF) at low pH, thus allowing the log P_{oct} of acidic compounds to be measured, and (ii) a short-end injection was performed to reduce the capillary effective length. The analytical conditions were optimized to determine the lipophilicity of neutral, basic and acidic compounds with log P_{oct} ranging from 0 to 5. Then the developed method was applied to a well-balanced set of 35 reference compounds to prove its feasibility, and to a set of 21 acidic and 29 basic pharmaceutical compounds. The obtained log P_{oct} values were characterized by a low standard deviation in comparison with the literature values (0.2). Finally, MEEKC was hyphenated with mass spectrometry (MS) allowing the throughput to be increased up to a factor 10 thanks to sample pooling. An atmospheric pressure photoionization (APPI) source was implemented to advantageously replace electrospray ionization (ESI) as it is less affected by non-volatile background electrolyte (BGE) additives, such as borate and sodium dodecyl sulfate (SDS). Furthermore, APPI was particularly appropriate for the ionization of apolar compounds.

P-8

Quantification of Testosterone Phase II Metabolites by UHPLC-QTOF-MS

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Endogenous steroids are mainly excreted in human urine as phase II metabolites (glucuronide and sulfate conjugates). According to the World Anti-Doping Agency (WADA) guidelines, high levels of testosterone glucuronide or metabolites in urine or testosterone glucuronide on epitestosterone glucuronide (T/E) ratio higher than 4 is considered as suspicions of a testosterone misuse. These determinations are commonly performed after hydrolysis of the glucuronide part and derivatization prior to GC-MS analysis. Thus, the information provided by the phase II metabolism is partially lost. Direct quantification of intact steroid glucuronides is still challenging by means of LC-MS. A method has been developed herein to detect simultaneously sulfate and glucuronide conjugates with enough sensitivity. The difficulty remains in the quantification of endogenous steroids ranging from 1 to 10000 ng/mL in urine specimens.

Ultra-high-pressure liquid chromatography (UHPLC) coupled to hybrid quadrupole time-of-flight (QTOF) mass spectrometer is selected for this purpose. On one hand, UHPLC offers high chromatographic resolution by maintaining short analysis time. On the other hand, QTOF mass analyzer allows exact mass determination on molecular and fragment ions. After a selective sample preparation by SPE, a fast chromatographic separation was performed in isocratic mode. To obtain the highest sensitivity, the analytes were detected in the ESI negative mode. Isomeric compounds were separated with resolution higher than 1.5 in less than 10 min.

The lower limit of quantification (LLOQ) ranged from 5 to 10 ng/mL for the glucuronide forms, while it was estimated from 5 ng/

mL for the sulfoconjugates. It is worth to note that androsterone and etiocholanolone were usually observed at high concentration (4000 to 10000 ng/mL) in urine. Thus, the method reflects a compromise to detect testosterone and epitestosterone at the lowest level, while avoiding detector saturation for metabolites excreted at high concentration.
This development reveals the possibility to use UHPLC-QTOF-MS for quantification of intact endogenous conjugated steroids in human urine. Moreover, the identification provided by the exact mass determination open the way to a broader steroid profiling including endogenous metabolites (steroidomics).

P-9

A Test mixture for Cyclodextrin Columns in Chiral Gas Chromatography

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Since the enantiomers of a chiral drug may differ in their pharmacologic, pharmacodynamic and pharmacokinetic profile, the effect of the enantiomers and the racemate *in vitro* and *in vivo* has to be elucidated. For pharmacokinetic and toxicological investigations on contamination, decomposition products or metabolites, special stereoselective and specific analytical methods have to be developed.
To check column quality Grob designed a test mixture for common capillary GC columns [1] and in 1992 the Schurig Test Mixture was published for permethylated β-cyclodextrin coated polysiloxane gas-liquid chromatography phases [2]. The Schurig Test Mixture contains a limited range of functional groups, whereas some compounds are not suitable for all column types. Therefore we designed test mixtures to monitor separation efficiency and column quality for five different chiral capillary columns.
The columns consisted of alkylated-silyl-α-, β- and γ-cyclodextrins dissolved in several polysiloxane liquids. By running a selection of substances from all functional groups, such as alkanes, alcohols, ketones, lactones, esters, amines and acids representing a broad range of evaporation characteristics, suitable substances are chosen for every column as test solutes. The compounds were diluted in a dichloromethane-hexane solution and run with a split mode. The temperature program ranged between 50–200 °C for 75 min. The five columns showed discrete separation properties and the solutes had to be adjusted individually for every column. Highly polar substances such as acids and amines are critical solutes and reflected at first the column impairment by showing a stronger peak tailing. Due to column hydrolysis and decapping of the alkylated-silyl-groups, the peaks shifted to higher retention times. Another general sign for impairment were the smaller peak heights.
The test mixtures show early changes in column quality and therefore column quality can be assured for pharmacokinetic and toxicological studies by running the individual test mixture before and after performing experiments.

References:
[1] Grob K, Grob G, Grob K. Comprehensive, Standardized Quality Test for Glass Capillary Columns. J. Chromatogr. 1978;156:1-20.
[2] Mayer S. A Chiral Test Mixture for Permethylated B-Cyclodextrin-Polysiloxane Gas-Liquid Chromatography Phases: The Schurig Test Mixture. Lc Gc-Magazine of Separation Science 1992;5:58-59.

P-10

Profiling of *Iris germanica* Extracts by LC-PDA-MS and Off-line Microprobe NMR

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The roots of German iris (*Iris germanica* L., Iridaceae) have been traditionally used for various topical applications including the treatment of sores and freckles [1]. Characteristic constituents of the root are isoflavones which reportedly show anti-inflammatory and anti-oxidative properties [1,2]. For these reasons iris root extracts are used as cosmetic ingredients.
Lipophilic and polar extracts of iris root were submitted to a phytochemical profiling by semi-preparative HPLC and off-line NMR measurements in a 1 mm TXI microprobe (active volume 5 µL) [3]. A total of 20 compounds including two new glycosides were purified in sub-milligram to milligram amounts via two successive chromatographic steps on a SunFire column (10 x 150 mm; 5 µM, Waters) with a gradient of acetonitrile in water containing 0.1% HCOOH. The compounds were identified as isoflavones, isoflavone glycosides and acetovanilllone by analysis of on-line MS and PDA, and off-line NMR data including HSQC and HMBC spectra. The activity of the isolated compounds on the proliferation of endothelial cells is currently being investigated. The example demonstrates the applicability of the off-line HPLC microprobe NMR approach as a robust means for a rapid chemical and biological characterization of the constituents of plant extracts.

References:
[1] A.U. Rahman et al. J. Ethnopharmacol. 2003;86:177.
[2] E. Wollenweber et al. Planta Med. 2003;69:15.
[3] J.L. Griffin et al. Analyst 2002;127:582.

P-11

Insect Repellents for Human Topical Use: Formulation and Evaluation Aspects

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Humans and animals are protected against insects and arthropodes to prevent and control insect-borne illnesses like malaria (Anopheles sp.), yellow fever (Aedes sp.) or Lyme disease (Ixodes ricinus, ticks) and to reduce the discomfort and troubles caused by insects and their bites. Beside mechanical body protection, natural and synthesized substances are used to repel (discourage) insects. Due to frequent travelling in different climate zone, to physical outside and leisure activities, repellents are often used consumer health care products. Dermal preparations are competing in their pharmaceutical formulation including skin caring properties, their effectiveness, and in their ease of applications. For convenience once daily application is desirable. As a life cycle product of an established repellent, a long-lasting dermal repellent formulation was targeted. The aim of the study was to develop within the regulatory biocide framework different pilot formulations with more prolonged or expanded repellent activity. These test formulations had to be characterized and checked for their repellent activity. A

simple *in vitro* system should be elaborated to correlate the repellent effect measured in a standard *in vivo* mosquito test. A search in Pubmed and selected specific text books for potential active ingredients and formulation additives was done. Regulatory constraints for biocides were respected. Two hydrogels, two O/W emulsions and four spray solution with DEET (N,N-diethyl-meta-toluamide $C_{12}H_{17}NO$; m.wt. = 191,27 mp -45 °C) or citridiol (main component: p-methane-3,8-diol (PMD), $C_{10}H_{20}O_2$; m.wt. = 172.26, mp 34.5 °C from *Eucalyptus citriodora* (Myrtaceae) essential oil) were prepared usually in a 20% concentration. Transcutol™ (diethylene glycol monoethyl ether) a skin accumulation enhancer, the film forming polymers Dermacryl 79™ (acrylate-octylpropenamide copolymer) and Plasdone S-630™ (acetic acid ethenyl ester) as well as Pemulen TR2™ (alkyl acrylate crosspolymer) as polymeric emulsifier were used as formulation modifiers. Limited stability testing according to usual pharmaceutical preparation monographs was done. The quantification of DEET and PMD was measured by an existing validated capillar GC-FID method using an I.S. (campher and thymol respectively): injection volume 1.0 µl (splitted); column polysiloxane column (Optima™ delta-3 (0.35 µm) 30 m x 0.32 mm ID; Sorbtech); oven temp: 120–300 °C (15 °C/min) and 80–250 °C (10 °C/min), respectively; integration: HP 3365 Chem Station. The Swiss Tropical Institute (University of Basel) tested the repellent effectiveness over max 8 h by a standard conventional "mosquito test". The results were compared to a prototype *in vitro* "evaporation test": the samples were heated 30 and 35 °C (heating plate) over 2 to 6 h in a lab cabinet. At selected time points the content of the active ingredient and the remaining formulation mass was measured to correlate the remaining content with the mosquito test. The results of the mosquito tests demonstrated a dose-effect relation. 10% citriodiol formulation showed a protective effect of ≤ 1 h. Maximal protection time of 4(-8) h was achieved by 20% ingredient concentration; its protection comparable to the existing product with 30%. A large inidividual variation of the effect was seen. The repellent effect occurs only when applied on body surface showing the importances of temperature and total skin cover for such repellents. The results correlated with the evaporation test showing evaporation of the active at >35 °C only; a minimal 10% API concentration was necessary in the formulations. This supports repellent's smell action hypothesis. The different formulations were comparable with regard to their repellent effect. The stress stability testing over two weeks at 40 °C/75% relative humidity was within 100 ± 5%. Skin properties seems to be fulfill the requirements well.

P-12

Pilot Development of Adaptogenic Drink Solutions for Special Food Use

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Physical and mental fitness are highly ranked in the public health awareness and a major reason to use food supplements. Therefore, there is a commercial opportunity to put evidence-based health products on the market within the regulatory restrictions. In order not to mislead consumers and to formulate state of the art preparations there is a need for scientific evaluation of the ingredients and for appropriate technological development and quality assurance of such formulations. The objective of this study was to develop herbal-based drink solutions as pilot dietary supplements with adaptogenic and performance-enhancing characteristics for use in adults exposed to physical and mental stress. The proposed product should meet prerequisites for a potential industrial manufacture.

A Pubmed search and specific medicinal plant specialized books screening were used to define candidates for herbals and active pharmaceutical ingredients for adaptogenic and performance enhancing food supplements. Commercial *Rhodiola rosea, Schisandra chinensis,* Wild green oat extracts, and chemicals of pharmaceutical or nutrient grade were used. Plant extracts and the final preparations were characterized and quantitated by chromatography: RP-HPLC with UV-DAD detection (Phenomenex, Luna $C_{18}(2)$-HT 2.5-µm column, MP 0.4 ml/min (gradient: phosphate buffer pH 7.0-acetonitrile) using schisandrin and rosavin and salidrosid as reference standards) (A).
TLC for *Schisandra* extract screening was used (CAMAG; 3-7 µl samples (Linomat IV), silica gel 60 F_{254} 20x20 cm, MP: toluene-ethyl acetate-acetic acid (70:33:3); UV and visual detection after H_2SO_4-MeOH (1+9) spraying (B). To show and compare a pharmacological activity of the plant extracts IC_{50} values were calculated from a standardized *in vitro* phosphodiesterase 4 (PDE-4) enzyme assay using 5'[^{3}H] AMP (Amersham, VitaPlant). To develop a drink solution solubility testing, tannin precipitation *(Rhodiola),* flavoring, bitter masking (Frutarom) and stability testing over two weeks at 2–8 °C, room temperature, and 40 °C, respectively, were done according to given standards.
The analytical methods allowed differentiating between high quality pharmaceutical grade and ordinary nutrient grade *Schisandra* extracts by chromatographic method A and B. Careful tannin depletion allowed to keep marker components in the *Rhodiola* extract in similar concentrations, but eliminated a PDE-4 inhibition effect. PDE-4-derived IC_{50}-values were *Rhodiola* < *Schisandra* < Green oat. Two 250-ml formulation proposals with either *Schisandra* or *Rhodiola,* Wild green oat extract, L-carnitin and caffeine, low glycemic carbohydrates (palatinose), stabilizing and flavoring excipients showed promising results to further develop a food supplement for market entry according to the Swiss authority requirements and for use in adults with increased energy and nutrient requirements.
Based on scientific evidence there are attractive (traditional) plants indicating adaptogenic and physical enhancing effects. Commercial extracts vary to a high extent in their composition. The responsible markers in plant extracts show mostly unpleasant (bitter) tasting requiring and challenging good formulations. State of the art technologies in flavoring, bitter masking and product characterization of such special food preparation are needed to guarantee the product quality and safety as well as attractiveness to the consumer. Filling existing gaps in clinical evaluation and still attractive cost of goods represent cornerstones to define potential success in a market.
We thank Mr. M. Schneider from Frutarom Switzerland Ltd. CH-8820 Wädenswil and Dr. K. Berger from VitaPlant Ltd, CH-4108 Witterswil for their technical support.

P-13

Towards a Better Understanding of PET Tracer Affinity Using Molecular Dynamics

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By means of several Molecular Dynamics (MD) techniques, we aim at guiding the synthesis of novel Positron Emission Tomography (PET) tracers designed to monitor Thymidine Kinase (TK) activity in stem cell therapy. For better defining substrate preferences and improving affinity, shedding light on the characteristics of TK dynamics is a prerequisite.
In our work, we distinguish between type I and type II TKs, because they differ in cellular location, structure, length and substrate acceptance. We investigated the dynamics of protein-ligand interactions for both TK types with MD techniques using NAMD2.6 [1]:

1. Classical MD (CMD) was used to assess the enzyme stability and principal component analysis helped to spot the most pronounced TK movements.
2. However, with CMD, large conformational changes between a closed (substrate-bound TK) and an open (apo) form cannot be simulated. Therefore, enzyme opening was achieved with Steered MD (SMD).
3. Possible substrate egress routes were then identified using random acceleration MD [2].
4. In a further SMD, the biologically most sound exit direction (pointing towards the ATP binding site) served as substrate pulling direction out of TK-substrate complexes.

We obtain an excellent correlation of the substrate extraction work (step 4) with experimentally determined affinity data, allowing us to extrapolate potential binding affinities of fluorinated analogues. Moreover, different substrate preferences can be rationalized based on structural evidence and dynamics. Currently, we are awaiting the proof of principle for our approach, namely experimental affinities of some fluorinated analogues with predicted affinity. Our results indicate that *in silico* prediction of PET tracer affinity – prior to synthesis – is a valuable optimization of PET tracer design.

References :
[1] J.C. Phillips et al., J. Comput. Chem. 2005; 26: 1781-1782.
[2] S.K. Lüdemann et al., J. Mol. Biol. 2000; 303: 813-830.

P-14

Stereoselective Serum and Plasma Methadone Analysis for Patients Using the Ph.Helv. Oral Solution

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Methadone (MET) HCl 10 mg/ml oral solution was introduced into the Swiss pharma-copoeia to standardise the pharmaceutical quality of the product, widely used for opioid substitution in addicts. MET is also attractive as a strong, long-acting analgesic. Data on bioavailability of this standard MET oral solution and corresponding kinetics in patients are lacking, but compulsory for safety, efficacy, and interaction assessment. The aim of the study was to provide an assay for therapeutic drug monitoring (TDM) of MET enantiomers.
Solid phase extraction (SPE): 1-ml samples with MET HCl in a concentration range of 0.5–10µg/ml were extracted from aqueous Tris solutions and artificial serum (albumin, IgG, NaCl) by elution through a RP C_8-, C_{18}-, and a mixed-mode C_8-sorbent, strong cation exchanger (SCX)-cartridge, respectively. The N_2-dried residues were dissolved in 200 µl. 50-µl aliquots were analysed with HPLC on a C_{18} (Xterra) 100 mm column with a 1 ml/min isocratic mobile phase of phosphate buffer pH 8 - acetonitrile (45:55); detection at 215 nm (method Ph.Helv.10). Dextropropoxyphene (DPP) (5 µg/ml) served as internal standard (IS). The extraction parameters were strictly controlled. Evaluation was based on recovery, reproducibility of the extraction, and linearity of the measured concentrations.
Stereoselective HPLC assay: After extraction, a stereoselective MET assay on a chiral α-glycoprotein (AGP)-column at 20 °C (50-µl sample) was done. To eliminate interfering peaks, the mobile phase (0.6 ml/min isopropanol in 0.01M phosphate puffer) was varied. Results of spiked samples based on calibration curves with IS and direct MET quantification, with and without extraction, were compared.
Only the C_{18} SPE showed reproducible recoveries: 97–102% of corresponding concentrations. Linearity between 0.5–10 µg/ml correlated with r>0.999 (n=5).
The stereoselective HPLC analysis was best with 12% isopropanol in phosphate buffer pH 6.5 (mobile phase). R- and S-MET had RT of 18.2 and 27.6 min, respectively; DPP at 13.4 min. Recoveries from plasma (FFP) and artificial serum were 89–105% and 95–106%, respectively. Calibration curves showed r>0.999, n=6 each with a small CI, resolutions, and peak symmetries for R- and S-MET were good.
In conclusion, MET in human plasma can be determined sensitively, reproducibly, and with good linearity in therapeutic concentrations using C_{18} SPE and a stereoselective HPLC on an AGP column. DPP is an appropriate IS. The simple extraction method is suitable for serum and plasma samples (TDM), but also for the non-stereoselective HPLC assay (Ph.Helv.10). Control of the extraction conditions is essential.

P-15

Quantitative Analysis of Caffeine in Beverages and Food by HPLC-UV and qNMR

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Numerous food products and beverages contain caffeine, and caffeine is among the most widely consumed natural products. The quality control of caffeinated products usually relies on HPLC-UV and, therefore, the method is well established. Quantitative ^{1}H-NMR (qNMR), on the other hand, is increasingly used for quantification purposes because of its speed, simple sample handling and versatility [1]. There are currently few validated qNMR methods for quality control. Here, we developed a qNMR assay for the quantification of caffeine in beverages (energy drinks, tea and coffee) and solid food products (guarana powder, chocolate). The method uses an internal standard (1,3,5-trimethoxybenzene) and was validated (selectivity, precision, accuracy and robustness). HPLC-UV and qNMR assays were compared with respect to precision (0.7% and 1.2%, respectively) and overall error (3% and 2%, respectively). The comparison shows that qNMR can be an interesting option for quantitative assays of natural products lacking a chromophore and, hence, difficult to analyze by HPLC-UV.

Reference:
[1] S. Bekiroglu et al., J. Pharm. Biomed. Anal. 2008; 47: 958.

P-16

Antioxidant Profiling of New Chemical Entities from Synthetic and Natural Origin

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Antioxidant compounds have become essential to prevent diseases partly induced by oxidative stress, such as cancer or neurodegenerative diseases (e. g. Alzheimer, Parkinson). To further understand and characterize their antioxidant properties, the radical scavenging activity of a large set of reference antioxidants and synthetic compounds was tested against three different radicals by four 96-well microplate assays. The antioxidant activities were ranked by cluster analysis in order to define the antioxidant profile of each compound.
The first assay was realised with a protein, the alkaline phosphatase (ALP) hydrolyzing the 4-methylumbelliferyl phosphate (MUP) to a fluorescent substrate, the 4-methylumbelli-ferone (MU). The marker

of oxidative damages was monitored by decrease of ALP's catalytic activity induced by peroxyl radicals generated by the 2,2'-azobis-(2-methyl-propionamidine) dihydrochloride (AAPH). The second assay, based on the oxygen radical absorbance capacity (ORAC), was still carried out with peroxyl radicals, generated by AAPH. The marker of oxidative damages was monitored by the fluorescence decrease of fluorescein. The two last assays were spectrophotometric, the effectiveness of scavenging activity being monitored by, respectively, the absorbance decrease at 755 nm for 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet}$) and at 515 nm for 2,2-diphenylpicrylhydrazyl radical ($DPPH^{\bullet}$). From the cluster analysis, several antioxidant groups have been constituted and the similarity of the antioxidant profile of each group compared with the antioxidant profile of reference compounds (ascorbic acid, caffeic acid, chlorogenic acid, gallic acid, glutathione, mangiferin, mannitol, melatonin, quercetin, resveratrol, trolox, uric acid). Thus for new chemical entities from synthetic or natural origin, the position in the antioxidant space with respect to the one of reference compounds can be established.

P-17

Preparation and Evaluation of a New Nanoparticulate Delivery System for Cancer Immunotherapy

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Toll-like receptors (TLRs) are known to play a key role in activating the immune system by recognising pathogen-associated molecular patterns (PAMPs). TLRs are expressed in the cells lining the epithelia of the body, representing the first defence barrier against infection. Cancer therapy by activation of T-cells through TLRs is a mechanism of action currently being evaluated. Imiquimod (IMQ) is an agonist of TLR7, whose location on endosomal membranes necessitates cellular uptake of IMQ.
In this study, in order to facilitate the uptake of IMQ, a particulate delivery system has been developed. This system consists of two polymers; the core made of poly (lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer. The surface of the particles was coated with N-trimethyl chitosan (TMC). TMC has mucoadhesive properties and the ability to open the tight junctions between epithelial cells.
Nanoparticles of PLGA coated with TMC containing IMQ and bovine serum albumin (BSA) as a model antigen were prepared by a W/OW emulsion solvent evaporation method. Loading capacity, encapsulation efficiency and release profile of IMQ were determined using HPLC analysis.
The particles' mean hydrodynamic diameter was 400.0 (± 15.0) nm with a polydisper-sity index of <0.31, and a zeta potential of 13.0 (± 1.0) mV. Loading capacity and encapsulation efficiency of IMQ was 0.1 (± 0.13) % and 8,6 (± 0,52) %, respectively. Scanning electron microscopy showed that particles are of spherical shape. Release studies showed a sustained release profile for IMQ at physiological pH and a minimal burst release.
In vitro cell culture studies on the A549 cell line, which was shown by our group to express TLR7, revealed the absence of toxicity for this cell type. TLR7 activation with IMQ free drug and by IMQ nanoparticles was investigated by measuring the concentration of IL8, an inflammatory cytokine produced in response to TLR7 activation, by ELISA. IL8 production in the case of IMQ nanoparticles was found to be 1.6-times higher than for IMQ free drug.
The uptake of the particles was studied by encapsulating a fluorescent label in the particles. According to this experiment, both qualitative evaluation by confocal laser scanning microscopy (CLSM) and quantitative studies by HPLC showed that the use of nanoparticles lead to a higher IMQ cellular uptake than IMQ in solution.
In conclusion, studies on the prepared nanocarrier system containing a TLR7 agonist have shown interesting results. It will therefore further be evaluated for application in cancer immunotherapy.

P-18

Bevacizumab and Ranibizumab in Association with Anti-inflammatory Drugs: An *In-vitro* Stability Study

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Ranibizumab (Lucentis®) and bevacizumab (Avastin®) are monoclonal antibodies that inhibit neovascularization through inhibition of vascular endothelial growth factor. Both protein drugs are widely used for the treatment of age related macular degeneration (AMD) and are generally administered once per month as intravitreal injection. However, because of patient discomfort and risk of complications, a prolonged interval is desirable. This objective could be reached by combining the antibodies with anti-inflammatory drugs, which are also known for their positive effects on AMD. Co-administration *in vivo* has been reported, however possible interactions between antibody and anti-inflammatory drug have never been taken into account.
Since antibodies have a tendency to aggregate and addition of an anti-inflammatory drug could affect the stability of the antibody, it is crucial to characterize the stability profile of the antibody formulation before clinical application. Aggregation should be prevented where possible, since aggregated proteins have shown to cause serious clinical side-effects like immunogenicity, toxicity and a loss in efficacy of the protein drug. The objective of our study was to investigate the *in-vitro* stability profile of ranibizumab and bevacizumab alone and in combination with dexamethasone disodium phosphate and triamcinolone acetonide. The aggregation state of the antibodies was measured by multi-angle light scattering after separation by asymmetrical flow field-flow fractionation.
Our results show that ranibizumab is more stable than bevacizumab *in vitro*, both alone and after association with the anti-inflammatory drugs. The stability of both antibodies is not decreased after addition of dexamethasone disodium phosphate or triamcinolone acetonide suspension. Surprisingly, dexamethasone disodium phosphate even has a stabilizing effect on bevacizumab. Further research will have to point out whether a combined formulation will result in a prolonged injection interval *in vivo*.

P-19

Establishment of a Cellular Model for the Analysis of Kinase-Independent Effects of G-Protein-Coupled Receptor Kinase 2

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The G-protein-coupled receptor kinase 2 (GRK2) phosphorylates activated receptors and thereby initiates receptor desensitization by uncoupling an activated receptor from the heterotrimeric G-protein. In addition to phosphorylation of receptor substrates, GRK2

can also exert kinase-independent effects by direct protein-protein interactions. The impact of phosphorylation-independent effects of GRK2 on cellular signalling processes is not fully understood. To assess kinase-independent effects of GRK2, we generated a cellular model of human embryonic kidney (HEK293) cells stably expressing the kinase-inactive mutant, GRK2-K220R. Cellular expression of the GRK2-K220R protein was confirmed by immunoblotting applying GRK2-specific antibodies. The kinase-independent effects of GRK2 are attributed mainly to the Gbeta/gamma-binding and RGS-domains of GRK2, which blunt Gbeta/gamma- and Gq/11-stimulated signalling, respectively. In agreement with this concept, cells expressing the kinase-inactive GRK2-K220R protein showed a substantially reduced signal generation mediated by prototypic Gq/11-coupled receptors and the Gi-coupled dopamine D2 receptor triggering Gq/11- and Gbeta/gamma-dependent calcium peaks. Down regulation of GRK2 expression by RNA interference partially reversed the GRK2-K220R-mediated signal inhibition confirming GRK-specificity of the observed effects. Altogether, the experiments show that a cellular model was established that is suitable to analyze specifically kinase-independent effects of GRK2.

P-20

Influences of the pH on the Bioactivity of rhBMP-2 in a Chitosan Hydrogel Designed for Bone Repair

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Current bone tissue engineering strategies may take advantage of the potent effects of osteoinductive growth factors on tissue remodeling. Recombinant human bone morphogenetic protein-2 (rhBMP-2) has the ability to induce bone formation at ectopic and orthotopic sites. There is a need for a delivery system that preserves the biological activity of the protein and prevents rapid local clearance from the application site. In the present study, we investigated *in vivo* the effectiveness of a new injectable delivery carrier for rhBMP-2 that forms *in situ* a biodegradable chitosan implant. We evaluated the influence of two different pH values of the carrier on rhBMP-2-induced ectopic bone formation in rats. Chitosan hydrogel (0.2 ml) with rhBMP-2 (145 µg) at pH 6.3 was injected into the right quadriceps of three-month old Sprague-Dawley rats (n=6). The contralateral side was used for formulation at pH 4.8 injections. Ectopic formed bone was assessed by microCT-scan and evaluated by histological analysis. Our results show that, in hydrogel with a pH of 6.3, rhBMP-2 bioactivity decreased compared to pH 4.8. This is attributed to partial aggregation and/or conformational modifications of the protein at pH 6.3. These data underline the importance of a careful carrier design to ensure an efficient growth factor delivery.

P-21

Capillary Electrophoresis Evidence of the Stereoselective Ketoreduction of Mebendazole and Aminomebendazole in Echinococcosis Patients

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An assay for the simultaneous determination of the enantiomers of hydroxy-mebendazole (OH-MBZ) and hydroxyaminomebendazole (OH-AMBZ) together with aminomebendazole in human plasma is described for the first time. It is based upon liquid-liquid extraction at alkaline pH from 0.5 mL plasma followed by analysis of the reconstituted extract by capillary electrophoresis (CE) with reversed polarity in presence of a 50 mM pH 4.2 acetate buffer containing 15 mg/mL sulfated β-cyclodextrin as chiral selector. For all compounds, detection limits are between 0.01 and 0.04 µg/mL, and intraday and interday precisions evaluated from peak area ratios are < 6.9% and < 8.5%, respectively. Analysis of 39 samples of echinoccocosis patients undergoing pharmacotherapy with mebendazole (MBZ) revealed that the ketoreduction of MBZ and AMBZ is highly stereoselective. One enantiomer of each metabolite (firstly detected peak in both cases) could be detected only. The CE data revealed that OH-MBZ (mean: 0.715 µg/mL) is the major metabolite followed by AMBZ (mean: 0.165 µg/mL) and OH-AMBZ (mean: 0.055 µg/mL) whereas the MBZ plasma levels (mean: 0.096 µg/mL, levels determined by HPLC) were between those of AMBZ and OH-AMBZ.

P-22

Determination of Ethyl Glucuronide and Ethyl Sulfate in Body Fluids by Capillary Electrophoresis with Indirect Absorbance Detection

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The use of capillary electrophoresis (CE) for the analysis of ethyl glucuronide (EtG) and ethyl sulfate (EtS), two alcohol markers of recent alcohol consumption, in serum and urine were studied. These direct ethanol metabolites remain longer in the body than ethanol itself and can thus be detected hours to days after complete elimination of ethanol. EtG (pKa 3.21) was analyzed with indirect absorbance detection at pH 4.4 using a buffer made of 10 mM nicotinic acid and 6 mM ε-amino-n-caproic acid. Different coatings of the fused-silica capillaries were tested. Best results were obtained with a linear polyacrylamide coating, which does not feature any electro-osmosis, and application of serum that was 1:1 diluted with water. The LOD of EtG was 0.15 mg/L (0.7 µM). EtS (pKa < 1.9) in diluted urine or solid-phase extracts of serum was determined in a bare fused-silica capillary with a pH 2.5 buffer composed of 15 mM maleic acid, 1 mM phthalic acid and 0.05 mM CTAB. LODs for urine and serum were about 1 mg/L (7 µM) and 0.25 mg/L (< 2 µM), respectively. With the investigated assays, EtG and EtS could be recognized in samples of humans that ingested equal or more than 0.4 g ethanol/kg body mass.

P-23

Magnetically Retainable Microparticles for Delivery of an Anti-Inflammatory Drug to the Joint: Efficacy Study in a Mouse Model of Antigen-Induced Arthritis

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Conventional corticosteroid suspensions for the intra-articular treatment of arthritis suffer from limitations such as crystal formation or rapid clearance from the joint. We investigate here an innovative alternative consisting in the encapsulation of a corticosteroid into magnetically retainable microparticles.
PLGA microparticles embedding both superparamagnetic iron oxide nanoparticles (SPIONs) and dexamethasone acetate (DXM) were prepared. We evaluated by *in vivo* imaging the influence of a subcutaneously implanted magnet near the knee on the retention of magnetic microparticles in the joint. The efficacy of intra-articular (i. a.) injection of microparticles was then investigated using a model of antigen-induced arthritis (AIA) in mice. Arthritis severity was assessed by 99mTc accumulation and by histological scoring.
In the mice arthritis model, microparticles embedding DXM and SPIONs induced a diminution in the synovial inflammation 4 days after the i. a. injection. Microparticles could be found in mice joints 3 months following injection. Although the magnetic field resulted in higher microparticle retention in the joint over two weeks, no therapeutic benefit could be shown in the acute AIA model.
This versatile delivery system is promising not only for the targeting of corticosteroids, but also for other substances, such as p38 mitogen-activated protein kinase inhibitors, to reduce the side effects following systemic administration. Future work could be performed using chronic inflammation animal models, for example osteoarthritis, in which the three-month persistence of the microparticles into the joint could represent a real benefit.

P-24

Bioluminescence Imaging for the Evaluation of Active Tumor Targeting of Paclitaxel

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Ovarian cancer is the leading cause of death from all gynecological malignancies [1]. The lethality is closely related with the lack of specific symptoms which results in a late detection of the disease. The current first line chemotherapy consists in the combination of platinum (cisplatin and carboplatin) plus taxanes (paclitaxel and docetaxel) [2]. This regimen is associated with serious side effects and treated patients often relapse within a median time of less than 2 years [3]. Thus, different strategies including active tumor targeting are in development to improve the efficacy of therapy. Active targeting involves the association of targeting moieties to a drug-loaded nanocarrier to recognize and specifically bind to biomarkers over-expressed on cancer cells. More efficient distribution of the drug to tumor tissue, higher efficacy in earlier stages of cancer and higher drug levels in target cells are expected to be reached and maintained for a longer time [4]. Numerous animal models are available for ovarian cancer such as subcutaneous, intrabursal, or intraperitoneal (IP) inoculation of human ovarian cancer cells [5]. IP cell inoculation presents the advantage of allowing intraperitoneal dissemination of the tumors and the development of ascite very close to the clinical features observed in patients. However, while the growth of subcutaneous tumors can be easily monitored by caliper measurements, monitoring of microtumors in the IP model is limited to the observation at a single terminal end point. This problem can be overcome by the use of a novel non-invasive technique, bioluminescence imaging [6]. In this model, animals are inoculated with luciferase-transfected cells which makes the tumors "visible" by bioluminescence after luciferine administration. The present work is focused on the evaluation of a novel paclitaxel formulation, anti-HER2 paclitaxel-loaded immunonanoparticles [7] in an IP disseminated ovarian xenograft cancer model overexpressing HER2 receptors, the SKOV-3 cells. The superior anti-tumor activity of immunonanoparticles compared to free drug was shown by significant regression of tumor size as observed by bioluminescence imaging and a longer survival of mice. Bioluminescence imaging used for tumor detection showed to be a very efficient non-invasive method to monitor tumor evolution in disseminated ovarian cancer model confined to the peritoneal cavity and also to evaluate the preclinical efficacy of novel therapeutics without the need to sacrifice the animals.

References :
[1] Jemal A. et al., Cancer J. Clin. 2008;58:71-96.
[2] Safra T. et al., Gynecol. Oncol. 2009;114:215-218.
[3] Gordon A. et al., Semin. Oncol. Nurs. 2003;19:3-18.
[4] Marcucci F. et. al., Drug Discov. Today 2004;9:219-228.
[5] Vanderhyden B.C. et al., Reprod. Biol. Endocrinol. 2003;1:67.
[6] Jenkins D.E. et al., Clin. Exp. Metastasis 2003;20:733-74.
[7] Cirstoiu-Hapca A. et al., Eur. J. Pharm. Sci., 2009 (accepted).

P-25

Towards a Cell-Free Assay to Investigate Lipid Bilayer Permeation and P-glycoprotein Transport

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Lipid bilayers and efflux transporters like P-glycoprotein (P-gp) represent the most important *in vivo* barriers for therapeutic agents. Driven by ATP hydrolysis P-gp exports structurally diverse hydrophobic compounds from the cell. P-gp expression has also been linked to multidrug resistance (MDR) [1]. The existing models to predict barrier passage of drugs and especially their recognition by P-gp are not satisfactory. To study lipid bilayer permeation and P-gp transport in parallel, P-gp was purified and reconstituted into liposomes. P-gp incorporation was verified by sucrose density gradient centrifugation, ATPase activity was ascertained by an ATPase assay and vesicle tightness was demonstrated by measurement of proton flux after an external acid pulse. Cryo-TEM images showed unilamellar liposomes with a homogenous size distribution below 100 nm. The goal is to combine these proteoliposomes with a liposomal lanthanide-based permeation assay, developed in our group [2]. This will allow to study P-gp transport and passive membrane permeation in a single cell-free assay. The permeation

assay is based on the interaction of intra-liposomal lanthanides with permeating aromatic ligands, resulting in a characteristic luminescence signal. Tetracyclines are used as model compounds in this study; as they have been suggested to be transported by P-gp [3]. Europium(III)-containing liposomes were incubated with the tetracyclines, excited at the appropriate wavelength and the luminescence at 615 nm was recorded. The resulting luminescence time-curves were fitted with a biexponential function and the rate constant of the faster phase was used to calculate the apparent permeation coefficient ($Perm_{app}$). The $Perm_{app}$ of the tetracyclines do not correlate with their lipophilicity or other physico-chemical parameters. These findings are in agreement with previous studies performed with aromatic carboxylic acids [2]. The data obtained strongly emphasize the importance to develop a permeation assay. In a next step, the lanthanide permeation assay will be adapted to be used with proteoliposomes to measure lipid bilayer permeation and P-gp transport in parallel.

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References:

[1] Sharom F. J., Biochem. Cell Biol. 2006;84:979-992.
[2] Thomae A.V., et al., Biophys. J. 2006;89:1802-1811.
[3] Kavallaris M. et al., Biochem. Biophys. Research Comm. 1993;190:79-85.

P-26

Development and Analysis of a CsA Prodrug-Based Topical Ophthalmic Formulation

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Cyclosporine A (CsA) presents interesting activities, however, its nephrotoxicity, hepatotoxicity and hypertensive effect limit its systemic use.
CsA has been determined to be efficient in the treatment of dry eye syndrome and the prevention of corneal graft rejection. For that purpose the topical ocular administration of CsA seems to be the more appropriate treatment. Nevertheless, the high lipophilicity of CsA makes the development of topical ocular formulations a real challenge, and only an oil based formulation and an emulsion are currently on the market.
A novel water-soluble CsA prodrug, OPPH 088, appears to be a promising candidate for the development of a CsA topical ocular formulation for two main reasons: (i) the rapid OPPH 088 biotransformation into CsA under physiological conditions, and (ii) the possibility to develop a patient-friendly aqueous formulation [1]. The developed OPPH 088 formulations were found to be non toxic and well tolerated *in vivo.*
A specific analytical method based on liquid chromatography coupled to tandem mass spectrometric detection (LC-MS/MS) was developed to quantify *in vivo* OPPH 088 and CsA, as well as some intermediate species in tear fluid. Some of the challenges being (i) the extremely rapid biotransformation of OPPH 088 into CsA, (ii) the presence of intermediates with close chemical structures, (iii) the very low volumes and concentrations involved, and (iv) the presence of a complex biological matrix.
The first results indicate the great potential of OPPH 088 formulation for the local treatment of ocular diseases without interfering with vision.

Reference:

[1] Lallemand F. et al., Int. J. Pharm. 2005;295:7-14.

P-27

The Development and Validation of a Protocol for HPLC-Based Activity Profiling of Plant and Fungal Extracts Against Tropical Parasites

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Most of the antiprotozoal drugs on the market today have a lot of side effects and are expensive. The search of bioactive natural products is a time consuming process. HPLC-based activity profiling combined with high-throughput biological screening method is an effective strategy to speed up the discovery of new leads. We established a library of 640 plant and fungal extracts in 96- well format which was screened for antimalarial, antileishmanial and anti-trypanosomal activity. Active extracts were separated by analytical column (SunFire RP-18, 3.5 µm, 3x150 mm, Waters) and thirty-two one-minute fractions were collected into 96 deep well micro titer plates. After parallel evaporation of the microfractions, suitable dilution schemes permitted parallel activity profiling for antimalarial, antileishmanial and anti-trypanosomal activity to identify the active compounds. The protocol was validated with extracts and positive controls such as *Artemisia annua* which were treated likewise. The activity was confined to one or few of the micro fractions, which could be chemically characterized by HPLC-hyphenated methods (HPLC-PDA, -MS^n, -HR-MS, -NMR). Examples for using this protocol for the identification active natural products are shown.

P-28

Chemical Degradation and Protein Stability: Effect of Methionine Residues Oxidation on the Thermal Stability of Recombinant Human Growth Hormone

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Oxidation is one of the major chemical degradations of proteins. It can occur during the storage and production of biotherapeutic drugs, and it can involve different amino acids such as methionine residues. In the oxidation of the methionine residues, the amino acid side chain is added with one oxygen atom, and a sulfoxide group is formed. This sulfoxide group can interact with various portions of the protein, mainly hydrogen bond, and change the conformation of the protein. A conformation change can reduce the physical stability of the protein and induce aggregation. In this study the effect of the oxidation of the methionine residues on the physical stability of recombinant human growth hormone (r-hGH) was investigated. The physical stability was measured with thermal methods by using a Jasco J-815 spectropolarimeter. The oxidized

and non-oxidized protein was analyzed in different buffers, with different pH and salts. Under each condition the melting temperature (T_m) of the two proteins was measured. Our findings show that the oxidized protein is overall less thermally stable than the corresponding non-oxidized. Furthermore, the effect of pH variations and salts is different between the two proteins. These results show the strong influence of methionine oxidation on the conformational stability of r-hGH.

P-29

Development of a New Artificial Membrane to Predict the Passive Permeation Through the Blood-Brain Barrier Using PAMPA

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Inappropriate pharmacokinetic (PK) has been recognized as being one of the major factors leading to the withdrawal of new chemical entities (NCEs) from drug development. Therefore, a large number of compounds has 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 vitro* high throughput methods thus become tools of choice to assess compounds PK properties and in particular their ability to penetrate biological membranes such as the blood-brain barrier (BBB). Parallel artificial membrane permeability assay (PAMPA) is a high throughput 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 this membrane, different biological barriers can be targeted [1]. This technique has been already applied to BBB penetration studies using phospholipids allowing the ranking of compounds in two classes: compounds passively transported (CNS+) or not transported (CNS-) into the brain [2].
In this study, a membrane composed of a mixture of octanol, ortho-nitrophenyl-octylether (o-NPOE) and hexadecane has been evaluated and optimized to predict the passive permeation through the BBB using the PAMPA technique and avoiding the well known drawbacks of the biological material. The permeation coefficients log Pe obtained experimentally for each compound are compared to the ones obtained with a cellular model taken from the literature [3, 4].

References:
[1] Y. Henchoz, B. Bard, D. Guillarme, P.-A. Carrupt, J.-L. Veuthez, S. Martel. Anal. and Bioanal. Chem. 2009.
[2] L. Di, E.H. Kerns, K. Fan, O.J. McConnell, G.T. Carter. Eur. J. Med. Chem. 2003;38: 223-232.
[3] R. Cecchelli, B. Dehouck, L. Decsamps, L. Fénart, V. Buée-Scherrer, C. Duhem, S.Lundquist, G. Torpier, M.P. Dehouck. Adv. Drug. Deliv. Rev. 1999;36: 165-178.
[4] S. Lundquist, M. Rentfel, J. Brillault, L. Fénart, R. Cecchelli, M.P. Dehouck. Pharm. Res. 2002;19: 976-981.

P-30

Medicinal Herbs for the Treatment of Rheumatic Disorders – a Survey of European Herbals from the 16th and 17th Century

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From the 16th up into the 19th century botanical and medicinal knowledge in Europe was documented and spread in magnificently illustrated herbals. For the most part modern science has neglected what is written in them and old remedies have not been systematically evaluated in terms of modern day pharmacological knowledge. In this study Five of the most important European herbals of the 16th and 17th century (Bock (1577), Fuchs (1543), Matthiolus (1590), Lonicerus (1770) and Tabernaemontanus (1687)) which are available at the Swiss Pharmaceutical Museum in Basel were searched for terms related to rheumatic diseases and sixty three plants were found. An extensive search of the scientific data banks Medline and SciFinder scholar was done to find recent results concerning the phytochemistry and possible antiphlogistic activities of the plants. More than half of them have shown *in vitro* or *in vivo* antiphlogistic activities.
European herbals may be a valuable source of information for the selection of plants for focussed screening programmes. Information contained in these herbals should be explored in a systematic manner.

P-31

Triclosan: the Quest for its Real Target in *Plasmodium falciparum*

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Triclosan, a widely used commercial antibacterial agent, has been validated to be specifically active against the blood form of *P. falciparum in vitro*. The discovery that triclosan is a very potent inhibitor targeting an essential enzyme (enoyl-acyl carrier protein reductase; FabI) in the type-II fatty acid biosynthesis pathway of *P. falciparum,* has triggered several drug discovery projects with the aim to develop antimalarials targeting FabI of *P. falciparum.* Although very potent, the derivatives of triclosan lacked correlation between inhibition of the target enzyme and whole-cell activity, hence suggesting the possibility of off-target activity, and raised doubts that FabI really is the primary target of triclosan and analogs thereof. Such results bring forward the question of which the other specific targets for this compound are. To address this question we apply a chemical proteomics approach for the identification of the triclosan target(s) in *P. falciparum* using an integrated approach that consists of computational and *in vitro* techniques. The first part is based on an inverse screening method, while in the second one we use a chemical proteomics methodology based on affinity chromatography. The results of both analyses are presented and compared, and the strategy for a preliminary validation is outlined.

P-32

Hexyl-substituted Polylactide Based Micelles for Cyclosporine A Drug Delivery

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Nanoscopic polymeric micelles have gained increased interest in pharmaceutical research. Amongst other advantages, their specific core-shell structure enables incorporation of poorly water soluble drugs within the micelle core. However, efficient drug loading depends highly on the affinity between the core forming block and the drug itself. Biodegradable and biocompatible polylactides (PLA) have shown some limitations in the incorporation of poorly water soluble drugs, for this reason hexyl-substituted polylactides (hexPLA) with an increased hydrophobicity were developed [1].
Copolymerized with methoxy poly(ethylene glycol) (MPEG), the novel amphiphilic MPEG-hexPLA copolymers self-assemble into unimodal nanosized polymeric micelles above a critical micellar concentration (CMC) of around 8 mg/L. Due to this low CMC, these micelles remain stable upon dilution, which envisage their application in intravenous formulations [2,3]. They show good shelf-life stability at 4 °C and have a comparable haemocompatibility and non-toxicity to MPEG-PLA micelles, both as disassembled single polymers and as self-assembled micellar systems up to a copolymer concentration of 20 mg/mL. The hydrophobic immunosuppressant drug Cyclosporine A (CsA) can efficiently be incorporated within the micelle core, resulting in a more than 100-fold increase of the drug water solubility. For a same CsA concentration, MPEG-hexPLA formulations require 4-times less excipient in comparison to the current surfactant Cremophor®EL applied for intravenous application. In conclusion of the experimental data the presented MPEG-hexPLA formulations have a great potential as drug delivery systems of Cyclosporine A.

References:
[1] Trimaille T., Gurny R., Möller M., J. Polym. Sci. Part A: Polym. Chem. 2004;42: 4379-4391.
[2] Trimaille T., Mondon K., Gurny R., Möller M., Int. J. Pharm. 2006;319: 147-154.
[3] Mondon K., Gurny R., Möller M., Chimia 2008;62: 832-840.

P-33

An Injectable Polymer for Sustained Release Formulations – Hexylsubstituted Poly(lactide)

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Poly(lactide) (PLA) and copolymers thereof e. g. with poly(glycolide) are polymeric excipients often used for parenteral sustained release formulations. They are entirely biodegradable by hydrolysis of the ester bonds, avoiding the removal of the excipient after therapy. The polymers and degradation products are biocompatible and were generally regarded as safe by the FDA. Furthermore, the rate of degradation and hereby the release profile can be modified by varying the molecular weight or the ratio of lactic acid to glycolic acid. Poly(lactides) and poly(lactide-*co*-glycolides) have a solid aggregation state, allowing to prepare e. g. micro- and nanoparticles, medical screws, sutures, and implants. But, the solid aggregation state also limits the use of these polymers, because their injection is difficult. To inject implants needles with a large diameter are needed, which are patient unfriendly. Micro- and nanoparticles have to be reconstituted prior to injection or high ratios of irritating solvents have to be added to obtain liquid and injectable formulations.
To overcome these difficulties we synthesized a polymer based on the PLA polyester backbone, to preserve degradability, but with modified side-chains. Substituting the methyl groups of the PLA by hexyl groups resulted in the semisolid and viscous hexyl-substituted poly(lactide) (hexPLA) excipient. The hexyl groups act as internal plasticizers, decreasing the glass transition temperature and making the material liquid at room temperature.
With hexPLA it is possible to prepare formulations by simply mixing this new excipient with the active compound, without using any further excipients or solvents. The formulations prepared are suspensions, which are directly injectable at room temperature. To prevent sedimentation of the active compound in hexPLA it can either be micronized or the formulation can be cooled, because the viscosity of hexPLA is higher at low temperatures, thus preventing sedimentation. Despite its liquid aggregation state hexPLA forms stable drops in hydrophilic solutions because of its high lipophilicity. Although hexPLA is semisolid and PLA is solid, polymers of equal molecular weight show a similar profile for the molecular weight decrease and weight loss. The degradation profile of hexPLA can be modified by using polymers with different initial molecular weights, as hexPLA with a high molecular weight shows slower weight loss than hexPLA with shorter chains. The incorporated drug is being released from hexPLA formulations continuously after a burst release period of less than three days.
Hexylsubstituted poly(lactide) is a potential excipient for solvent free and directly injec-table formulations with sustained release characteristics, whereby degradation and release can be modified as a function of the molecular weight.

P-34

Rapid Log *P* Determination of Natural Products in Crude Plant Extracts from UHPLC-TOF-MS Profiling Data – An Additional Parameter for Dereplication and Bioavailability

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Lipophilicity, described by log *P*, is a key-parameter involved in many pharmacokinetic and pharmacodynamic processes, as absorption, distribution, metabolism and excretion (ADME) or ligand-target interactions are parameters widely used in drug discovery. One of the most used techniques for log *P* determination is RP-LC (reversed phase liquid chromatography) which is known to be a fast and low-sample consuming technique, based on the analyte's retention behaviour [1].
With the concomitant introduction of ultra high pressure liquid chromatography (UHPLC) systems and new generation of columns packed with sub-2µm particles with very stable chemistry, the determination of lipophilicity on synthetic libraries has been considerably improved in term of throughput and pH range [1]. UHPLC can be coupled with high resolution mass spectrometers such as the Time-Of-Flight systems (TOF-MS). This represents a powerful platform to analyse crude plant extracts used as a reservoir of putative leads for drug discovery. Based on this technology, an efficient dereplication of natural products (NPs) [2] can be obtained, and this method is potentially applicable to the rapid estimation of their physico-chemical properties for early ADME predictions.
In the present study, generic UHPLC profiling gradients data were exploited for rapid and robust log *P* determination of a representative library of NPs, selected by cluster analysis using different mo-

lecular descriptors. The relations log *P* – log *k* have been established at different pH and using various UHPLC conditions, compatible with crude extract profiling studies.
This strategy is expected to provide both on-line structural information and rapid estimation of NPs lipophilicity of crude plant extract metabolites prior isolation, and thus notably improve the efficiency of bioactivity-guided isolation procedures.

References:
[1] Henchoz Y. et al. J. Med. Chem. 2008;51: 396-399.
[2] Wolfender J.L. et al. Exp. Opin. Drug Discovery 2006;1: 237-260.

P-35

Modeling of Microspheres Coating Process

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Coating is a widely used technology in the pharmaceutical industry. It serves to modify release, mask odor or bad taste, protects actives against reactive environment like oxygen, light and humidity and improves the flowability of products. The aim of this study was to investigate the microspheres Kollicoat MAE 100P film-coating process in fluidized bed. Effects of process-related variables such as flow rate of feed liquid and concentration of polymer, and fluidized air temperature was investigated. The following materials were used: microspheres of micro cellulose 700 μm as the coating support and an aqueous dispersion of Kollicoat MAE 100P as coating liquid. Coating experiments were carried out in a batch fluidized bed Hüttlin Mycrolab. At the end of each experiment the following characteristics of a product were defined: particle size distribution, coating efficiency, residual moisture content, real and bulk density, flowability, homogeneity and roughness of enteric film. A mathematical model was developed using statistical methods. This model allows to quantitatively calculate optimal coating process parameters.

P-36

Rapid Capillary Electrophoresis Frontal Analysis (CE/FA) for the Study of Drug-Protein Binding

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Reversible interactions play a key role in many biochemical and physiological processes that form the basis of living systems. The assessment of these interactions in terms of affinity constants (K_a) and stoichiometry is an important part in describing and understanding such systems. In this regard, drug – plasma protein binding is a critical feature because of its significant impact on numerous parameters, including drug pharmaco-kinetics, drug-drug interactions, blood-brain barrier penetration, determination of margins in safety assessment studies and adjustment of the optimum therapeutic dose of a drug. Although equilibrium dialysis is considered as the reference method, it suffers from many drawbacks: (i) bias by osmotic dilution, (ii) nonspecific adsorption on the dialysis apparatus, (iii) incompatibility with samples of low purity, (iv) tedious and time-consuming methodology. In this work, a CE/FA (capillary electrophoresis frontal analysis) method based on Kraak's work [1] was developed for the rapid determination of K_a values. First, the following model system was evaluated to assess the method validity. Binding of bovine serum albumin (BSA), a relatively cheap protein with properties analogous to human serum albumin, the most representative plasma protein, and warfarin (acidic) and lidocaine (basic), two well-known drugs with very high (>95%) and very low (<20%) binding to BSA respectively, was studied. The results obtained were in good agreement with those obtained by conventional methods in our laboratory. Secondly, the developed CE/FA method was modified to improve the throughput using a short-end injection. Therefore, the analysis time per run was reduced from more than 10 min to less than 3 min for acidic compounds and to only 1 min for basic drugs. These examples demonstrate the ability of CE/FA to assess drug-protein binding for highly and weakly bound drugs to proteins such as BSA, thus making CE/FA a powerful tool that permits rapid analysis of bimolecular interactions.

Reference:
[1] Kraak J.C., Busch S., Poppe H., J.Chromatogr. 1992;608: 257-264.

P-37

Trans-Fatty Acids: Do They Influence APP Processing in a Cell Culture Model?

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Reducing cleavage of amyloid precursor protein (APP) at its alpha-cleavage site may increase the generation of the neurotoxic Aβ a signature peptide found in neuronal plaques of Alzheimer's disease (AD) patients. As the alpha-cleavage position is located in close vicinity of the membrane surface [1,2], we hypothesize that dietary fatty acids may influence alpha cleavage via their effects on the membrane properties. With liposomes as model membranes we found an influence of the lipid bilayer thickness on the hydrolysis kinetics at the alpha-cleavage site by trypsin [1]. To study the effect of fatty acids on APP processing, we supplemented human neuronal SH-SY5Y cells with *trans*-vaccenic acid and elaidic acid, respectively. We quantified the soluble APP cleavage products sAPPα and sAPPβ in the culture medium with ELISA. We found a slight but non-significant increase of the sAPPα:sAPPβ ratio. This trend indicates a possible influence of the supplemented fatty acid on APP cleavage. To further investigate on the impact of dietary fatty acids, we are currently testing saturated fatty acids.

References:
[1] Marenchino M., Williamson P.T.F., Murri S., Zandomeneghi G., Wunderli-Allenspach H., Meier B.H., Kramer S.D. Biophys. J. 2008;95: 1460-73.
[2] Beel A.J., Mobley C.K., Kim H.J. Tian F., Hadziselimovic A., Jap B., Prestegard J.H., Sanders C.R. Biochem. 2008;47: 9428-46.

P-38

Toll-Like Receptor Modulation in a Cell Culture Model for Respiratory Inflammation

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Toll-like receptors (TLRs) are a class of membrane proteins, expressed by immune as well as epithelial cells, which play a key role in the innate and adaptive immune system. The role of these receptors, as modulators of epithelial permeability, has only recently been described.
To evaluate the role of epithelial barrier dysfunction in inflammatory diseases, the effect of TLR regulation on tight junction (TJ) barrier function of an *in vitro* model of inflammation was studied. For this purpose, an *in vitro* co-culture system was used to examine the effect of a TLR2 agonist, Pam3Cys (PSCK), on modulating the TJ between the inflamed airway epi-thelial cells. To assess transepithelial permeability, measurement of transepithelial electrical resistance (TEER) and flux of sodium fluorescein (NaF) was done.
For the co-culture experiments, human acute monocytic leukemia (THP-1) cells were differentiated to macrophage-like cells by treatment with 200 nM phorbol myristrate acetate (PMA) for 48h. The conditioned medium (CM) of PMA stimulated THP-1 cells, which contains pro-inflammatory cytokines such as IL8, TNFα and other soluble factors, was used to induce an inflammatory state in human bronchial epithelial cell monolayers (Calu-3). Calu-3 cells were seeded in cell culture inserts on a semipermeable collagen coated supports. Cells were grown to confluency over a time period of 16–18 days required to obtain highest TEER values. The cell monolayers were incubated with CM at the basolateral side and TEER and NaF flux were measured. After three hours of CM incubation, PSCK was added to the apical side, and permeability was measured at time intervals of 6, 24 and 48h after addition of PSCK.
Our results showed a significant drop in TEER accompanied by an increase in permeability values for the cells incubated with CM, as compared to control. At the same time, TLR2 agonist showed a significant transient positive effect on TEER values of inflamed Calu-3 cells. To determine whether these changes in barrier function are related to any change in the levels of junction proteins, we carried out preliminary q-PCR studies, which showed a significant difference in the expression of mRNA of individual tight junction proteins between inflamed cells, control and TLR2-treated cells. These proteins included ZO-1, JAM-1, occludin, claudin-1 and claudin- 2, which are considered to be major tight junction proteins. Further studies, e.g. Western blotting and immunolabeling for individual TJ proteins, will be done to further examine the underlying mechanism of modulation of epithelial TJ by TLR ligand PSCK.
In conclusion, incubation of airway epithelial cells with macrophage CM constitutes an *in vitro* model system, which is useful to evaluate the effects of pathological modulators on TJ barrier function. In addition, our observation that TLRs are implicated in modulation of TJ barrier function indicated possible novel therapeutic approaches that can improve existing strategies.

P-39

A Click Chemistry Approach for the Development of ^{18}F-labelled Peptides to Image Angiogenesis via Positron Emission Tomography (PET)

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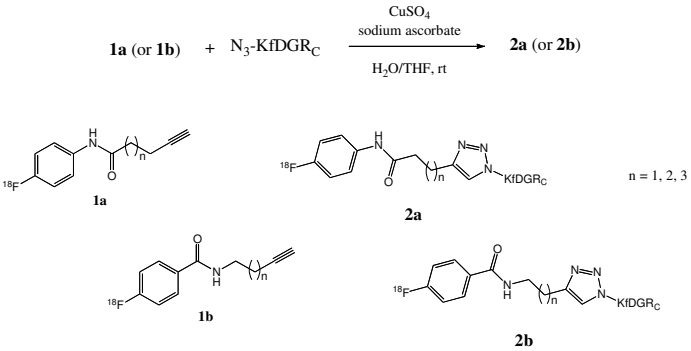
We herein report the developement of a novel sytemic strategy to radiolabel peptides with the positron emitting radionuclide F-18 ($T_{½}$ = 109.7 min) via click chemistry. Angiogenesis plays a pivotale role in tumor growth and proliferation and, as result, chosen as therapeutic target despite the lack of established methods to predict tumor response to antiangiogenic drugs. Thus, we have successfully applied our method to a RGD-based peptide for monitoring tumor integrin $\alpha_v\beta_3$ and/or $\alpha_5\beta_1$ expression by PET imaging. Two series of alkyne containing prosthetic groups (1a and 1b) have been synthetized and subsequently attached to an azido-RGDfK$_C$ peptide through Cu(I)-catalyzed Huisgen cycloaddition. Attachment of the prosthetic group was performed under mild conditions, yields and times appropriate for preparation of ^{18}F-labeled radiopharmaceuticals.



P-40

A Validated Phenytoin Analysis from CNS *ex vivo* Brain Microdialysis Samples, Blood and Saliva by GC/MS

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To study pharmacokinetics of critical dose drugs in biological samples from neurosurgical patients, specific and sensitive analytical methods are mandatory. Phenytoin (PHT), an acidic lipophilic drug, is routinely used to prevent and treat epilepsia. Therapeutic drug monitoring for PHT with its non-linear kinetics and the small therapeutic index is standard; data on PHT in CNS fluid of neurosurgical patients are lacking. The aim of this study was to establish a GC/MS method for PHT and its metabolites from brain microdialysates and biological samples. A suitable sample extraction procedure and the robustness of the analytical procedure (validation) were also targeted.
A GC/MS method with an internal standard (IS) was defined. A quadrupole MS in the full scan mode was used. MassLib™ spectral search software allowed correlating MS by similarity of spectra. Artificial CNS fluid, preserved (CPDA) blood (Blood Donor Centre Berne), saliva (from an author) and CNS samples (provided by the Kantonsspital Aarau from *ex vivo* microdialysis of tumor tissue after surgery and stored deep frozen (-20 °C) before processing) were spiked with PHT for the method evaluation. 100-µL samples were assayed. Method validation was according to ISO 17025. Na-PHT injection solution (Phenhydan® Desitin Pharma, Switzerland), PHT (>96% Fluka) and a Ph.Eur CRS were used. 5-(p-Methylphenyl)-5-phenylhydantoin (MPPH) >99% from Sigma Aldrich served as IS (conc. 1200ng/mL). The PHT calibration curve ranged from 50 to 1200ng/mL (n=12 samples; 6 concentrations). A solid phase C_{18} extraction was performed. The final elute in acetone was evaporated to dryness (50 °C; N_2), redissolved and methylated with trimethylsulfone hydroxide for GC/MS analysis using a nonpolar capillary column (DB-5ms 0.25 mm x 30 m x 0.25µm, Agilent). 2-µl sample volumes were injected. A 10 °C/min temperature gradient (120–325 °C) was applied.

MPPH was an appropriate IS; RT: PHT =15.12, MPPH =16.15 min. The biological sample clean-up with solid phase extraction using acetonitrile-citrate buffer pH 5 showed a recovery >90% in spiked samples. The dried samples were stable for ≥4 weeks at room temperature. Calibration curves were linear (r^2 ≥0.998, n=6). The recovery at >100ng/ml was ≥91%. The limit of detection (LOD) was 50ng/mL and the limit of quantification (LOQ) 100ng/mL. The SD_{rel} of the QC measurements was <15%. The extended measurement uncertainty was ≈ 56ng/mL.
In conclusion, the method presented is suitable for PHT determination in therapeutic concentrations from CNS microdialysate and biological samples tested and appropriate for pharmacokinetic modeling. The validation data meet ISO 17025.

P-41

Stereoselective Block of hERG1 Channel by Bupivacaine Scrutinized at Molecular Level

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In the heart, the hERG1 voltage-gated K^+ channel mediates the I_{Kr} current, which is crucial for the duration of cardiac action potential. Undesired block of the channel may prolong the QT interval with increased risk of malignant ventricular arrhythmias [1]. Although the molecular determinants of hERG1 block have been studied thoroughly, stereoselectivity has been poorly studied. (S)-bupivacaine was the first drug reported to have higher affinity for hERG1 than its enantiomer [2].
This study aims at understanding the principles underlying the stereoselectivity of bupivacaine block with the help of both electrophysiology experiments and molecular modeling simulations. Patch-clamp recordings using cells expressing hERG1 confirmed that (S)-bupivacaine blocked the wild-type (WT) channel more potently than (R)-form. Stereoselectivity was reversed in mutant F656A and abolished in Y652A. Putative binding modes of (S)- and (R)-bupivacaine inside an open form model of hERG1 channel [3] were predicted by docking simulations, allowing a clear depiction of ligand-protein interactions. Estimated binding energies for both enantiomers in WT and mutants Y652A/F656A are in line with electrophysiology measurements.
These results may be considered as a confirmation at the molecular level of bupivacaine stereoselective behavior towards hERG1. Moreover this information lays the foundations for a structural guideline to filter out potentially cardiotoxic drug candidates *in silico*.

References:
[1] Rossenbacker T. and Priori S. Rev. Esp. Cardiol. 2007; 60: 675-682.
[2] Gonzalez T. et al. Brit. J. Pharmacol. 2002; 137: 1269-79.
[3] Farid R. et al. Bioorgan Med. Chem. 2006; 14: 3160-3173.

P-42

SAR Studies of Synthesized Valerenane Derivatives on the $GABA_A$ Receptor

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Valeriana officinalis L. (valerian) is one of the most ancient medicinal plants of Europe. Its preparations are used as mild sedatives and anxiolytics [1] and these effects are assumed to be the result of the synergistic action of a multitude of compounds. Valerenic acid (VA) is one of the major constituents of *Valeriana officinalis* rhizome and is routinely used as an analytical marker for the standardization of valerian extracts, but until recently has not been implicated in the pharmacology of *Valeriana* preparations.
VA, which was first isolated by Stoll and Seebeck in 1957 [2], is a sesquiterpene carboxylic acid with an unusual carbon skeleton [3] that has been designated as the valerenan type [4]. VA has recently been shown to act as a positive allosteric modulator on the $GABA_A$ receptor complex with nanomolar affinity [5, 6]. It functions as a partial agonist on the 5-HT_{5a} receptor with micromolar affinity [7].
Up to now two stereoselective syntheses of VA have been reported in literature, one by Ramharter and Mulzer [7] and the other by our own group [9]. We have already shown promising effects of valerenol and VA *in vivo* [6] and we have now gone on to elaborate the SAR of the interactions of the $GABA_A$ receptor complex with a variety of analogs of VA. In this contribution we report on the synthesis of these derivatives and their biological activity *in vitro*. The derivatives were analyzed at recombinant $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor expressed in *Xenopus* oocytes using electrophysiological techniques.

References:
[1] European Scientific Cooperative on Phyotherapy (ESCOP) Monographs, 2nd ed., Thieme, Stuttgart-New York, 2003, 539.
[2] A. Stoll, E. Seebeck, Ann. Chem. 1957;603: 158-168.
[3] G. Buchi, T. L. Popper et al., J. Am. Chem. Soc. 1960;82: 2962-2963.
[4] G. I. Birnbaum, J. A. Findlay, J. J. Krepinsky, J. Org. Chem. 1978;43: 272-276.
[5] S. Khom, I. Baburin et al., Neuropharmacol. 2007;53: 178-187.
[6] D. Benke, A. Barberis, S. Kopp, K.-H. Altmann, M. Schubiger, K. E. Vogt, U. Rudolph, H. Mohler, Neuropharmacology 2009;56: 174-181.
[7] B. M. Dietz, G. B. Mahady et al., Mol. Brain Res. 2005;138: 191-197.
[8] J. Ramharter, J. Mulzer, Org. Lett. 2009;11: 1151-1153.
[9] S. Kopp, B. Schweizer, K.-H. Altmann, Synlett. 2009;11: 1769-1772.

P-43

Augmented Nerve Regeneration by Controlled Co-Delivery of Synergistic Growth Factors from Nerve Conduits

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Functional regeneration of transected human peripheral nerve remains to be improved. Several studies have aimed at improving the performance of nerve conduits (NC) by delivering neurotrophic factors (NTFs) to the site of nerve defect, but the clinical outcome remained generally unsatisfactory, because of aberrant axonal growth. This may be attributed to the poorly known or understood parameters such as optimal NTF dose, temporal and spatial require-

ments (i.e., release kinetics), and the needs for multiple factors rather than single factor mostly applied so far. We have recently observed the synergistic effect of GDNF and NGF on axonal growth (Madduri et al. 2009). Thus, we were interested to exploit their synergistic effect on peripheral nerve repair. Therefore, we have developed collagen (native or cross-linked) nerve conduits (NCs) delivering glial cell line-derived neurotrophic factor (GDNF) alone or in combination with nerve growth factor (NGF), and tested their effect on nerve regeneration in rats. Controlled and combined delivery of GDNF and NGF promoted nerve regeneration to a greater extent than delivery of GDNF alone. These results are in agreement with our previous *in vitro* observations, suggesting the synergistic action of GDNF and NGF on axonal outgrowth.

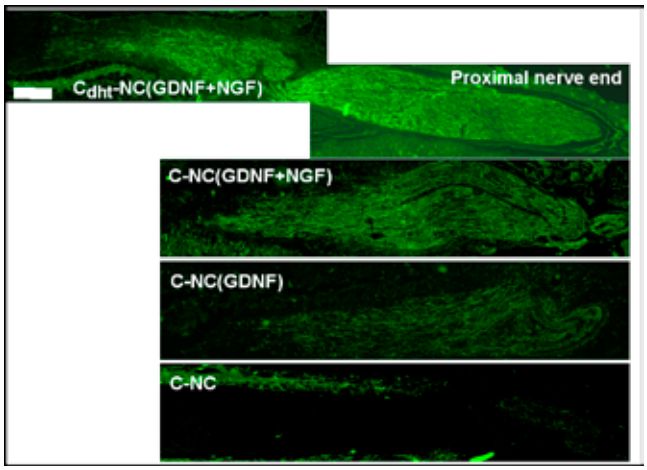

Fig. 1: Regenerating axons immunostained with PGP9.5 labeled with Alexa488. Scale = 650 µm.

C_{dht}-NC(GDNF+NGF) = Cross-linked collagen nerve conduit loaded with both GDNF and NGF.
C-NC(GDNF+NGF) = Collagen nerve conduit loaded with both GDNF and NGF.
C-NC(GDNF) = Collagen nerve conduit loaded with GDNF alone.
C-NC = Collagen nerve conduit without growth factors.

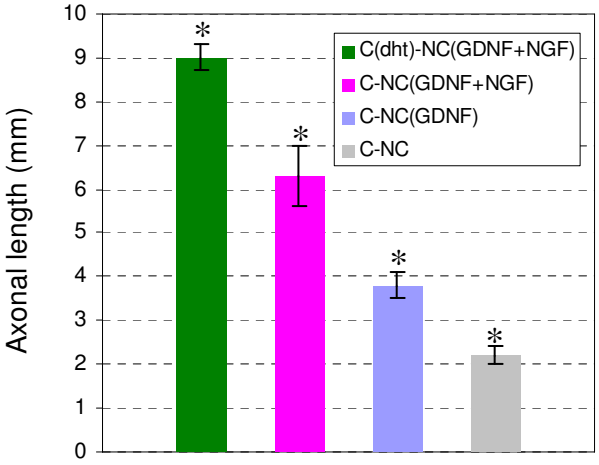

Fig. 2: Axonal growth within NCs implanted in a 10 mm sciatic nerve gap. The bars represent the means ± SD, n = 3. Significant differences at p<0.001* are indicated in comparison to all other treatment groups

P-44

Single-Run Separation of Closely Related Cationic and Anionic Compounds by CE-ESI-MS: Application to the Simultaneous Analysis of Melamine and its Analogs

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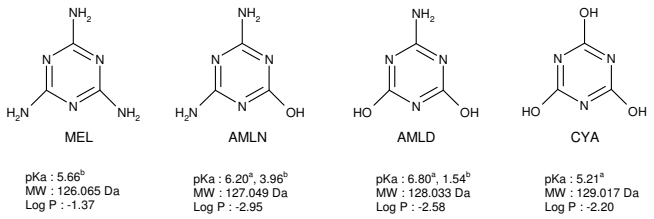
Nowadays, capillary electrophoresis (CE) is recognized as a powerful separation technique for polar compounds due to several advantages such as high efficiency and low sample consumption, while mass spectrometry (MS) detection provides high sensitivity and selectivity. In the context of polar compounds analysis, the coaxial sheath-flow interface is currently the most suitable source for the electrospray ionization (ESI). Acidic background electrolyte (BGE) and ESI in the positive mode are commonly used for cations analysis, while basic BGE is used for anions analysis in ESI negative mode. Thus, simultaneous analysis of both cations and anions by CE-ESI-MS remains challenging and BGE and sheath liquid conditions have to be optimized to afford the best electrophoretic selectivity while maintaining a good ionization yield.
This issue was encountered in the context of melamine analysis. Melamine (MEL) is a nitrogen-rich industrial compound that was illegally added in infant formulas in China at the end of 2008 to misrepresent protein levels. By-products such as ammeline (AMLN), ammelide (AMLD) and cyanuric acid (CYA) can be generated during the manufacturing process of MEL. The combination of MEL with CYA leads to crystal formation in kidneys, resulting in kidney stones and renal failure. Renal damages can also be produced by AMLN and AMLD in combination with the formers [1]. MEL and related compounds possess close physico-chemical properties in terms of pKa and molecular weights. Therefore, a very selective analytical tool has to be implemented to distinguish the four species. In this study, MEL and its degradation products were for the first time analyzed in a single-run by CE-ESI-MS with the use of optimized generic CE and ESI conditions.



[a] : acidic
[b] : basic

Reference:
[1] Melamine and Cyanuric acid : Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food, World Health Organization, 25th September 2008, updated 30th October 2008

P-45

Synthesis of 5-Substituted-Anilino-Quinazolines for the Prediction of Treatment Response to EGFR/HER-2 Inhibitors by Positron Emission Tomography (PET) Imaging

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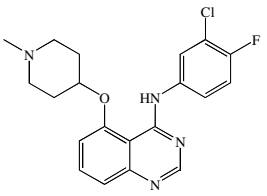
The deregulation of the EGFR and HER-2 tyrosine kinases is associated with a variety of cancers. The small molecule inhibitors that could control the tyrosine phosphorylation event are of therapeutic value as potential antitumor agents. We herein report multistep synthesis of 5-substituted-anilino-quinazolines, as potential PET tracers to predict EGFR/HER-2 targeted therapy. The compounds were synthesized starting from 5-fluoroquinazolone by introducing the N-methyl-piperidinyl group at position 5, followed by conversion of the quinazolone into 4-chloro- or 4-thiomethyl-quinazoline, and subsequent attachment of aniline or substituted benzyloxy-anilines. The biological evaluation of these compounds using cancer cell lines harboring varying degrees of EGFR and HER-2 expression or mutational status is underway.



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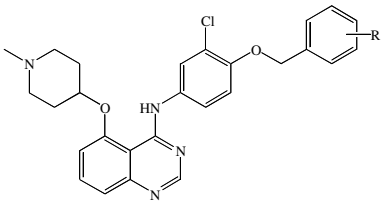

2 (R= 3-F)
3 (R= 4-F)

Reference:
[1] Seimbille Y. et al. J. Label. Compd. Radiopharm. 2005; 48: 829-843.

P-46

Biomarker Discovery in the Urine of γ-Irradiated Fisher 344 Rats Using Metabolomics of GC/MS Combined with Random Forests Machine Learning Algorithm

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A major threat of modern civilisation is accidental (e. g. hazardous incident in a nuclear power plant) or intentional (e. g. terrorist attack with a dirty bomb) exposure to ionizing radiation. The exposure of an unknown number of inhabitants to an unknown dose of radiation might be the consequence. The need is obvious for the development of high-throughput, noninvasive and rapid biomonitoring tools for the screening of mass populations in the event of such a catastrophe. Radiation metabolomics employing mass spectral technologies represents a plausible means for this purpose. A simplified metabolomics protocol is described that employs general-purpose gas chromatography-mass spectrometry and open source software including random forests machine learning algorithm to uncover latent biomarkers of 3 Gy gamma-radiation in rats. Urine was collected from 6 male Fisher 344 rats and 6 sham-irradiated controls for 7 days, 4 prior to irradiation and 3 post-irradiation. Water and food consumption, urine volume, body weight, and sodium, potassium, calcium, chloride, phosphate and urea excretion showed major effects from gamma-irradiation. The metabolomics protocol uncovered several urinary metabolites that were significantly increased: glyoxylate (lipid peroxidation), threonate (oxidation of ascorbic acid), thymine and uracil (oxidative deamination of cytidine) and p-cresol (from tyrosine during protein degradation). Decreased metabolites related to radiation encompassed citrate, 2-oxoglutarate, adipate, pimelate, suberate and azelaate as a result of radiation exposure (changes in renal tubular cells). Gas chromatography-mass spectrometry (GC/MS) is a promising platform on which to develop further the field of radiation metabolomics and to assist in the design of instrumentation for use in detecting biological consequences of environmental radiation release.

Reference:
Lanz, C., Patterson, A. D., Slavík, J., Krausz, K. W., Ledermann, M., Gonzalez, F. J., Idle, J. R. Rad. Res. 2009;172: 198-212

P-47

A Novel Drug Carrier System for the Delivery of Cyclosporine A to the Eye

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Biodegradable hexylsubstituted poly(lactides) (hexPLA) are novel polymers, which in combination with polyethylenglycole (PEG), as diblock copolymers (PEG-hexPLA) self-assemble in aqueous environment into micelles with a very low critical micellar concentration. The hexyl substituents increase the polylactide hydrophobicity and thereby improve the incorporation of poorly water soluble drugs into the hydrophobic micelle core. We will present a strategy to deliver Cyclosporine A (CsA) into and through the cornea using polymeric micelles as an effective drug carrier system for Dry Eye treatment. CsA loaded micelles were prepared with different drug concentrations and characterized by TEM, DLS and HPLC. The prepared micellar formulations are perfectly transparent, with a particle hydrodynamic diameter of around 50 nm. Ocular tolerance was evaluated on rabbit eyes by CLSO and the test showed that the micelles are well tolerated. The kinetics of the formulation was studied *in vivo* with drug delivery system containing CsA and a fluorescent dye (Nile Red) after topical instillation. The results show that PEG-hexPLA micelles are able to penetrate the cornea, accumulating in the epithelium and in the stroma. Fluorescent micelles were also observed in the endothelium, showing that micelles are able to penetrate into all corneal structures. These encouraging results demonstrate that CsA/PEG-hexPLA micelle formulations are promising novel drug delivery system for ophthalmic applications.

P-48

Investigations on the Lyophilization of PEG-hexPLA Micelle Formulations

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Polymeric micelles based on poly(ethylene glycole) and hexylsubstituted poly(lactides) (PEG-hexPLA) are promising carriers for hydrophobic drugs. The micellar formulations have shown to be stable in size and drug loading over several months. However, the stability of these colloidal systems could be further improved by freeze-drying towards long term stability and drug loading, if particular conditions of stress occur during transportation and storage. Therefore, different freeze-drying procedures were studied in order to obtain solid formulations that could be recon-stituted to colloidal formulations. Moreover, several excipients with different properties (lyoprotectants, cryoprotectants, isotonic agents, bulking agents) were

screened in the freeze-thawing tests, to determine their influence on the micelle sizes and shapes.

P-49

Isolation and Purification of Two Isoflavones (Tectoridin and Iridin) from *Belamcanda Chinensis,* Iridaceae

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Belamcanda chinensis (Iridaceae) is distributed in China, Japan, Korea, Thailand and India and in these regions is used as a valued medicinal plant for its demonstrated analgesic, antibacterial, antifungal, antiallergic, antiinflammatory and expectorant properties. Chinese Materia Medica (Pharmacopoeia of Traditional Chinese Medicine) recommends to use this extracts in "laryngitis inflammation" and productive cough.
Several chemical studies demonstrated that a variety of compounds, such as isoflavonoids, stilbenes, xanthones and iridal-type triterpenoids, are present in this medicinal plant.
Our previous investigations on *B. chinensis* were focused on isolation and purification of the main xanthone (mangiferin), which was successfully completed. Isoflavonoids are identified as the major phenolic constituents in *Belamcandae* rhizoma, so we subsequently decided to both evaluate their content, and assess the antioxidant potential in different assays.
Here, we present the method of chromatographic isolation and purification of two isoflavonoid glycosides (tectoridin, and iridin) from *B. chinensis,* a monocotyledoneous perennial species, acclimated in moderate Central Europe and cultivated in Poland.
The exhaustive liquid/liquid extraction of a MeOH extract with BuOH was followed by SPE on ODS gel and reversed-phase flash chromatography. The isolation was monitored using TLC on silica gel and C_{18} plates. The identity of the compound was verified by TLC and HPLC-ESI/MS and the structure confirmed by H- and C-NMR. We are planning to examine antioxidant properties of these two compounds. Currently, we can confirm that the applied methodology provides means for isolation of these pharmacologically active isoflavonoids and other polyphenols from an alternative, locally grown plant crop for use both in research and in herbal medicine. The method used is simple, efficient and easily reproducible.

P-50

Total Synthesis and Structure Elucidation of Haliclamide

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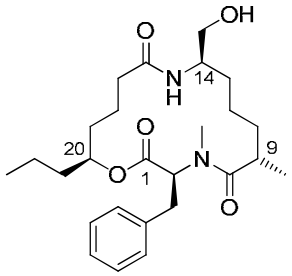
Marine sponges are well known producers of bioactive natural products. In 2001, Randazzo et al. [1] reported the isolation and structure elucidation of a new secondary metabolite from *Haliclomа sp.,* a marine sponge from Vanuatu, and termed it haliclamide (1). Haliclamide was shown to exhibit *in vitro* antitumor activity against the human bronchopulmonary non-small-cell-lung-carcinoma cell line NSCLC-N6 with an IC_{50}-value of 4 µg/ml. The structure of (1) showed a 16-membered cyclic depsipeptide with similarities to other bioactive depsipeptides such as e. g. spongidepsin, jasplakinolide, chondramide C or the very potent cytotoxic doliculide. Randazzo et al. however could not determine the absolute configuration of the stereocenters at C-9 and C-20. In an effort to identify the absolute configuration of these stereocenters we synthesized the four possible diastereoisomers of haliclamide in a convergent approach. Comparison of the resulting ^{1}H- and ^{13}C-NMR spectra with those reported for natural haliclamide, the structure of the natural product could be established as (1). The cytotoxic, antifungal and antibiotic activity of (1) and it diastereoisomers was investigated.



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Reference:
[1] A. Randazzo, C. Debitus, L. Gomez-Paloma, Tetrahedron 2001; 57: 4443.

P-51

Controlled Iontophoretic Delivery of Kojic Acid

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Antioxidants such as ascorbic acid and kojic acid are used to treat various dermatologic disorders that originate as a result of the accumulation of excessive epidermal pigmentation. Hyperpig-mented lentigenes include melasma, age spots or liver spots, and sites of actinic damage. Hyperpigmentation can be treated by blocking key enzymes involved in melanin production, e. g., mammalian melanocyte tyrosinase. Kojic acid (KA) is a potent inhibitor of this enzyme (IC_{50} 6 µg/ml) and is a gold standard for evaluating the activity of novel agents *in vitro.* Unfortunately, its *in vitro* potency at the cellular level does not translate into *in vivo* efficacy in the clinic since its hydrophilicity severely limits its entry into the lipid-rich stratum corneum and prevents it from reaching the melanocytes. Moreover, systemic administration is not feasible since KA is both hepato- and nephrotoxic. Thus, a controlled topical delivery system for KA that could exploit its hydrophilicity would be highly desirable. Here, we describe the results of a feasibility study using iontophoresis to deliver KA into porcine skin *in vitro.*
Dermatomed porcine skin was clamped in three compartment vertical flow-through diffusion cells. Cathodal iontophoresis was performed where the cathode (containing 25 mM HEPES – 133 mM NaCl buffer at pH 7.4) was connected in series with the donor compartment via a salt bridge (SB) assembly (3% agarose in 100 mM NaCl) to minimize the effect of competing ions. After equilibrating the skin for 40 min with buffer, 1.5 ml of KA solution (in Tris buffer pH 8) was placed in the donor compartment. The anodal and receiver compartments were filled with 1.5 and 6 ml of HEPES – NaCl buffer (pH 7.4), respectively.For experiments involving both anodal and cathodal delivery, the same set-up was used except that after equilibration buffer 1.5 ml of KA solution (in 133 mM NaCl pH 6.5) was placed in the anodal compartment.The results of the permeation experiments comparing cathodal iontophoresis of 1% w/w KA solution at i_d = 0.3 mA/cm^2 with passive permeation showed that iontophoretic delivery was clearly superior with a 16-fold improvement over passive diffusion after 3 h (69.60 ± 12.80 vs.

4.24 ± 0.20 µg/cm², respectively). Since the target compartment for KA action is the skin, it was decided to focus on the amount of KA deposited within the membrane rather than the extent of permeation. In the next set of experiments (again at i_d = 0.3 mA/cm²), the duration of current application was reduced from 180 to 30 min. Under these conditions, increasing KA concentration in the solution from 1 to 2% resulted in an ~2-fold increase in skin deposition of KA (28.17 ± 6.14 and 46.52 ± 9.79 µg/cm², respectively). Subsequently, the duration of current application was further reduced to 15 min and the effect of increasing current density from 0.3 to 0.5 mA/cm² (using a 2% KA solution) was investigated. This increased delivery from 38.55 ± 7.92 to 76.30 ± 24.05, respectively. It was then decided to investigate combined iontophoretic delivery of KA from both anodal and cathodal compartments. For these experiments, 2% (w/w) KA was iontophoresed at i_d = 0.5 mA/cm² for 15 min (cathode pH / anode pH 6.5). Since KA has a pKa of 8, 50% of the molecules are negatively charged in a solution at this pH. Thus, electromigration is the predominant transport mechanism for cathodal iontophoresis. In contrast, at pH 6.5 the molecule is essentially unionized and anodal iontophoresis depends upon electroosmosis for delivery. The results confirmed that combining anodal and cathodal iontophoresis improved the delivery efficiency of the system.

P-52

Cloning, Expression and Purification of Recombinant Human Basic Fibroblast Growth Factor Using E. Coli as Expression Host

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Basic fibroblast growth factor (bFGF; 146 amino acids) belongs to a large family of structurally-related proteins that affect the growth, differentiation, migration and survival of a wide variety of cell types. Potential therapeutic applications of bFGF include tissue regeneration, wound healing and angiogenesis. The objective of this study was to develop a fast and reliable method to produce bFGF using a prokaryotic pET expression system. Here, we report on the development of a stable expression plasmid (pET100) carrying the human *bfgf* gene for the recombinant expression of bFGF in *E. coli* BL21(DE3) Star as expression host. Primers were designed to enable cleavage of the His-tag using thrombin. The primers used in the present studies were: forward primer (5')-*CAC C***CT GGT GCC GCG CGG CAG C**AT GGC AGC CGG GAG CAT CAC CAC GCT GCC CGC C and reverse primer (5')-TCA GCT CTT AGC AGA CAT TGG AAG AAA AAG. The first four base pairs of the forward primer were complementary to the destination plasmid, while the bases in bold were used for coding the thrombin cleavage site. The protein was expressed in LB media at 37 °C. Protein expression was induced by addition of IPTG (1 mM final concentration) to an overnight culture. Cells were harvested after 4 h of induction. Soluble protein was purified in a two stage process. In the first step, the protein was isolated and purified by Ni-affinity chromatography followed by gradient elution using buffer A (20 mM Tris, 500 mM NaCl, 10% glycerol; pH 8) and buffer B (buffer A supplemented with 500 mM imidazole) at a flow rate of 2 mL/min. The desired protein started to elute at about 30% of buffer B in buffer A. The His-tag was subsequently cleaved by incubation with thrombin (10 U enzyme per mL of protein solution) for 16 h at 16 °C. As a second step, the protein was further purified by size exclusion chromatography (Superdex-75 10/300 GL) using buffer C (25 mM HEPES). The procedure routinely yielded 6–8 mg of pure (> 95%) soluble bFGF per liter culture. The final product was characterized by SDS-PAGE, and identity was verified and confirmed by ESI mass spectrometry after trypsin digestion and by MALDI-TOF mass spectrometry (molecular weight expected: 22988.9 Da; molecular weight found: 22987.5 Da).

P-53

Homology Modeling of Human Tyrosinase Active Site, Dicopper Center Optimization and Docking Studies

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Tyrosinases (EC 1.14.18.1) are involved in the melanin biosynthesis process which is responsible for skin or hair color in mammals. Tyrosinase inhibitors are used to treat human hyperpigmentation and are interesting for cosmetic and food industries [1]. In order to steer inhibitor discovery, biostructural information about human tyrosinase would be of great interest but this protein has not been crystallized so far. Consequently, the active site of a human tyrosinase (SwissProt accession number P14679) model was built using the SWISS-MODEL/DeepView workspace [2]. An optimization protocol for the tyrosinase dicopper center was elaborated using the template structure (pdb ID: 2ahk) [3] and applied to the model. For the sake of indirect validation of the model, docking simulations were performed using Gold 4.0. The same ligand was first docked into the tyrosinase chain of the 2ahk complex including copper ions and explicit solvent. The same computation was performed into the model active site containing relevant water molecules. In both cases, an identical binding mode, consistent with the crystallographic information was returned.
This is a decisive step forward towards validation and then use of this homology model for target-based protocols to design tyrosinase inhibitors.

References:
[1] Parvez S. et al., Phytother. Res. 2007; 21: 805-816.
[2] Arnold K. et al, Bioinformatics 2006; 22: 195-201.
[3] Matoba Y. et al., J. Biol. Chem. 2006; 281: 8981-8990.

P-54

Determination of the Lipophilicity of Cyclosporin A by LC

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Lipophilicity, a key parameter in the study of pharmacokinetic properties of new chemical entities (NCE), has to be evaluated for a large number of compounds in the early stages of drug discovery. Therefore the development of high throughput methods to determine partition coefficients is an important challenge in pharmaceutical research. In this context, RP-LC methods, based on correlation between lipophilicity and retention factors, have been largely used for the determination of log P_{oct} of neutral, acidic and basic compounds presenting moderate lipophilicity ($0<\log P_{oct}<5$).
However, these methods remain limited for highly lipophilic compounds due to high analysis time. Recently, a method based on ultra-high pressure liquid chromatography (UHPLC) has permitted the determination of partition coefficients with a drastic decrease

in analysis time [1]. Moreover this technique appears to be a promising way to determine high log P_{oct} values. Different stationary phases and experimental conditions, in both RP-LC and UHPLC, were therefore tested on highly lipophilic compounds (log P up to 8) [2].
The three best methods, one in RP-LC and two in UHPLC, were then applied to a series of peptides and especially to the lipophilic Cyclosporin A (CsA) which has shown different lipophilicity behaviours depending on the method used.

References:
[1] Henchoz Y., Guillarme D., Rudaz S., Veuthey J.L., Carrupt P.A. J. Med. Chem., 2008;51: 396.
[2] Guillot A., Henchoz Y., Moccand C., Guillarme D, Veuthey J.L., Carrupt P.A, Martel S. Chem. Biodiversity 2009, in press.

P-55

Formation of Hydrogen Sulfide from N-Acetylcysteine in Man: New Mode of Action of an Old Compound

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N-Acetylcysteine (NAC) is used in the treatment of hepatic failure induced by paracetamol where it acts as a cysteine-donor for the synthesis of glutathione which in turns detoxifies the reactive metabolite of paracetamol. NAC has also been shown to be effective in non-paracetamol induced hepatic failure. In addition, it protects against renal injury associated with contrast media and is widely used as a "mucolytic" agent in chronic bronchitis. It is unlikely that NAC acts as a precursor of glutathione or as a reducing agent in these instances. It could, however, act as a precursor of hydrogen sulfide (H_2S). H_2S, besides NO and CO the third gaso-transmitter, activates potassium channels, thereby relaxing blood vessels and bronchial smooth muscle. Among other pharmacological effects it also protects against ischemia-reperfusion injury and modulates inflammatory processes. Most of these effects have been shown *in vitro*, and it is controversial whether pharmaco-logically relevant concentrations of H_2S are present *in vivo.*
To test the hypothesis that NAC generates H_2S we have developed a method to measure H_2S in breath. Exhaled air is drawn through a trapping solution containing N-ethylmaleimide (NEM). The NEM-H_2S-adduct is then extracted into ethyl acetate and quantitated by LC-MS monitoring ion 285, corresponding to $(NEM\text{-}S\text{-}NEM)^+$. Following the ingestion of 1.8 g NAC (Fluimucil®) by a healthy volunteer the exhalation of H_2S increased from 30 pmol/L to 1907 pmol/L at 14 min, whereupon the concentration decreased in parallel to the plasma concentration of NAC to 446 pmol/L at 30 min.
This is the first demonstration of the formation of H_2S from therapeutic doses of NAC in man. Due to its relatively high water and lipid solubility H_2S rapidly permeates the alveolar membrane, and a near perfect equilibrium between blood and alveolar air would be expected. Based on the physico-chemical properties of H_2S the concentration in exhaled air corresponds to approximately 200 pM H_2S in blood in the basal state and to approximately 13 nM following NAC. Although below the concentrations that have generally been found to be pharmacologically active *in vitro* the >50-fold increase in H_2S may still lead to an increased sulfhydration of target proteins that has recently been proposed as the mode of signalling by H_2S.

P-56

Thrombin-Sensitive Dual Modality Prodrugs for the Simultaneous Imaging and Treatment of Inflamed Lesions in Rheumatoid Arthritis

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Despite considerable advances in the management of rheumatoid arthritis, approximately 30% of the patients receiving the currently approved therapeutic agents do not respond or fail to maintain their response over time. In clinical practice, these cases are considered for radio-isotopic or surgical synovectomy. Both techniques aim to prevent damage of vital joint structures by the invasively growing synovial tissue. Photodynamic therapy (PDT) has been proposed as a minimally invasive alternative to the currently available synovectomy techniques. It combines the simultaneous use of a photosensitizer (PS), oxygen, and light to locally generate cytotoxic reactive oxygen species (ROS), causing the eradication of pathologic tissues. Here we report on the development of a novel polymeric photosensitizer prodrug (T-PS) which is activated by thrombin, a protease up-regulated in synovial tissues of rheumatoid arthritis patients, for transdermal, minimally invasive photodynamic synovectomy. In T-PS, multiple photosensitizer units are tethered to a polymeric backbone via short, thrombin-cleavable peptide linkers. Photoactivity of the prodrug is efficiently impaired due to energy transfer between neighbouring photosensitizer units. T-PS activation by thrombin induced an increase in fluorescence emission by a factor of 16 after *in vitro* digestion and a selective fluorescence enhancement in arthritic lesions *in vivo,* in a collagen-induced arthritis (CIA) mouse model. Moreover, using a multi-spectral imaging system, we were able to distinguish the prodrug and the cleavage product pheophorbide a-peptidyl-fragment *in vitro* and *in vivo,* based on their different fluorescence emission spectra. *In vitro* studies on primary human synoviocytes showed a phototoxic effect only after enzymatic digestion of the prodrug and light irradiation, thus demonstrating the functionality of T-PS induced PDT. The developed photosensitizer prodrugs combine the passive targeting capacity of macromolecular drug delivery systems with site-selective photosensitizer release and activation. In conclusion, lesions associated with enhanced proteolytic activity can both be selectively visualised and treated using these dual-modality prodrugs.

P-57

Bacteria Capture Iron from Heme by Keeping Tetrapyrrol Skeleton Intact

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Because heme is a major iron-containing molecule in vertebrates, the ability to use heme-bound iron is a determining factor in successful infection by bacterial pathogens. Until today, all known en-

zymes performing iron extraction from heme did so through the rupture of the tetrapyrrol skeleton. Here, we identified 2 *Escherichia coli* paralogs, YfeX and EfeB, without any previously known physiological functions. YfeX and EfeB promote iron extraction from heme preserving the tetrapyrrol ring intact. This novel enzymatic reaction corresponds to the deferrochelation of the heme. YfeX and EfeB are the sole proteins able to provide iron from exogenous heme sources to *E. coli*. YfeX is located in the cytoplasm. EfeB is periplasmic and enables iron extraction from heme in the periplasm and iron uptake in the absence of any heme permease. YfeX and EfeB are widespread and highly conserved in bacteria. We propose that their physiological function is to retrieve iron from heme.

P-58

Constituents of Ginger Rhizome (Zingiber officinale Roscoe) Inhibit Interleukin-1β Maturation and Secretion in Stimulated Human Monocytes by a Phospholipase A2 Dependent Manner

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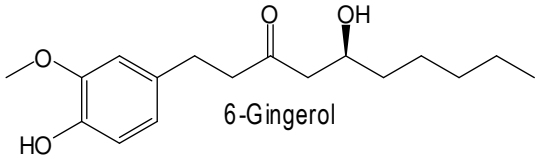
Ginger (*Zingiber officinale* Roscoe) is widely used against emesis and inflammatory diseases [1] but the exact mode of action has still to be elucidated. Several publications reported for example a functional inhibition of NF-κB and MAP kinases [2] *in vivo* or of COX-2 [3] *in vitro*. The main constituent of ginger rhizome (6-gingerol) is often believed to be the dominant active compound but PGE2 inhibition [4] indicates that several other chemically related compounds seem to be involved in the immunomodulatory effects.



We could demonstrate by Cytometric Bead Array and Western Blot that in isolated human monocytes the $iPLA_2$ dependent maturation and the $cPLA_2$ dependent secretion are reduced depending on assay conditions to 63% and 65% respectively (at 10 µg/ml) whereas transcription/translation was unaffected. A flow cytometry method [5] showed that ion fluxes are not affected.
We established a fluorescence coupled phospholipase A2 preferring assay to measure i- and c-PLA_2 inhibition of the dominant phenolic compounds in ginger oleoresin. Gingerols and shogaols (10 µM) were able to inhibit i- and c-PLA_2 by 37 to 71% and 36 to 67%, respectively.
The suppression of secreted mature Il-1β a key player in inflammatory diseases and the inhibition of phospholipases A2 may contribute to the anti-inflammatory potential of ginger preparations.

References:
[1] Srivastava K.C. et al. Med. Hypotheses 1992;39: 342-348.
[2] Kim S.O. et al. Biofactors 2004;21: 27-31.
[3] Tjendraputra E. et al. Bioorg. Chem. 2001;29: 156-163.
[4] Jolad S.D. et al. Phytochemistry 2004;65: 1937-1954.
[5] Jursk C.. JIM 2007;325: 67-77

P-59

Norbergenin Derivatives from the Stem Bark of *Diospyros sanza-minika* (Ebenaceae) and Their Radical Scavenging Activity

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Many species of *Diospyros* (Ebenaceae) are used in African and Chinese traditional medicinal systems for the prevention of gastric ulcers, inflammatory disorders and hepatotoxicity [1]. Recent studies show that some of these species possesses antitumor, antidiabetic and antioxidant effects [2]. The methanol extract from the stem bark of *Diospyros sanza-minika* showed radical scavenging activity against DPPH with IC_{50} values of 1.33 mg/mL. This extract was exhausted with hexane, dichloromethane and ethyl acetate. The strongest active fraction (ethyl acetate, 1.18 mg/mL) was subjected to activity-guided purification to give norbergenin and 4-O-galloylnorbergenin. These compounds were isolated for the first time from *Diospyros sanza-minika*. Norbergenin and 4-O-galloylnorbergenin, the main compounds of the plant, showed DPPH scavenging activities with IC_{50} values of 1.12 and 0.61 mg/mL, respectively, which were comparable to that of quercetin (0.74 mg/mL).

References:
[1] Mallavadhani U.V., Panda A. K. and Rao Y.R. Phytochemistry 1998;49: 901-951.
[2] Chen X.N., Fan J.F., Yue X., Wu X.R. and Li L.T. J Food Sci 2008;73: C24-C28.

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