DEVELOPMENT OF A NOVEL APPROACH FOR STUDYING THE *IN-VITRO* DRUG RELEASE FROM POTENTIAL NSAID PRODRUGS WITH 2',3',4',5'-TETRAACETYLRIBOFLAVIN CARRIER

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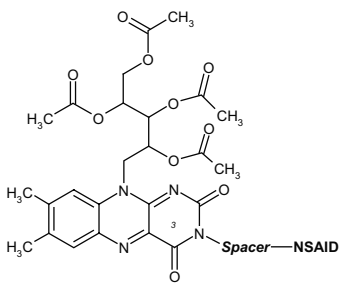
Recently, we have designed and synthesised derivatives of type **X** as potential prodrugs. Structurally, these target compounds are characterised by a NSAID moiety which is linked over a spacer unit to the carrier 2',3',4',5'-tetraacetylriboflavin. Since it is known that NSAID derivatives with masked carboxylic acid function exhibit lower gastrointestinal toxicity than the parent drug, we may expect that our derivatives **X** should possess lower side effects than the corresponding NSAID [1, 2, 3].



(X)

Two different techniques for studying the potential of our target compounds as prodrugs were developed. Although the compounds **X** are bearing several cleavable functions, only investigations concerning the hydrolytic (*i.e.* chemical stability, behaviour in presence of enzymes) drug release were of interest. The first technique involves a separation of the cleavage products using hplc with fluorescence and/or UV detection. Secondly, a complete novel technique was used. The latter is characterised by direct measurement of the fluorescence properties of the incubation mixture. Attempts to employ the fluorescence measurements as an analytical tool for such stability studies will be presented.

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EXAMINATION OF THE CHEMICAL COMPOSITION OF PROPOLIS III. IDENTIFICATION OF DEHYDROABIETIC ACID AND ABIETIC ACID IN PROPOLIS

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Propolis or "bee glue" is a generic name for the resinous hive product collected by bees from various plant sources. Propolis usually contains a variety of chemical compounds, such as polyphenols, terpenoids, steroids, aromatic alcohols, aliphatic acids and esters, sugars, amino acids. Dehydroabietic acid and abietic acid are the major acids of the abietic-type found in different types of resin or they are final products of isomerization of resin acids with conjugated double bonds.

In the present study, HPLC method on C_{18} column with on-line spectrophotometric (abietic acid) and fluorimetric (dehydroabietic acid) detection was used for separation and determination of dehydroabietic acid and abietic acid in propolis samples. The mobile phase for isocratic elution was methanol/water 87/13 containing 0,06% formic acid. The samples were prepared prior the HPLC analysis by liquid extraction. The limits of determination were 100 ng/ml for dehydroabietic acid and 200 ng/ml for abietic acid.

The four samples of propolis from Slovakia were analysed by developed isocratic HPLC method. The comparison of retentions factors of standard solution peaks with propolis peaks and off-line MS were used for identification of dehydroabietic acid in the fraction of propolis tincture. In the mass spectrum of dehydroabietic acid fraction the characteristic molecular ion peak occurred at *m/z* 300, which can be used for identification of dehydroabietic acid in propolis samples. The content of dehydroabietic acid in propolis was different (from 3.7 μg/g to 44.7 μg/g of propolis) depending on the source of propolis and on the vegetation at the site of collection. Abietic acid concentration in all tested propolis samples was below the detection limit of used method.

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DETERMINATION OF ARTEMISININ IN *ARTEMISIA ANNUA* L. BY DIFFERENTIAL PULSE POLAROGRAPHY

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Artemisinin is a potent antimalarial drug against the resistant strains of *Plasmodium falciparum*. It is a sesquiterpene endoperoxide which is isolated from the herb of the Chinese medicinal plant *Artemisia annua* [1].

Analysis of artemisinin is a challenging problem as the compound lacks chromophoric or fluorophoric groups, the concentration in the plant is low, the intact molecule stains poorly and other compounds in the crude plant extracts interfere in its detection. Several analytical methods are suggested (GC, TLC, HPLC, CE, ELISA, SFC) for the determination of artemisinin in the plant [2].

The purpose of the present study was to develop and validate an analytical method for the determination of artemisinin in *Artemisia annua* which ought to be simple, rapid and therefore suitable in routine work. Since artemisinin contains the electrochemically active peroxide (-O-O-) group it can be reduced easily at various electrodes [3]. On the basis of these considerations, the electrochemical behaviour of artemisinin was studied in order to develop a differential pulse polarographic (DPP) method for analysing artemisinin in the plant. Using mercury electrode, the DP polarograms exhibited reproducible peak at -0.0 V vs Ag/AgCl in 0.1 M phosphate buffer pH 5.5/methanol (7:3; v/v). Strict linearity between peak height and concentration of artemisinin was found in a range of 6.16×10^{-7} - 3.18×10^{-5} mol/L (R = 0.9998). LOD was calculated to be 58 ng/mL. The polarographic method was applied to the determination of artemisinin in the traditional Chinese herbal drug of *Artemisia annua* by using the standard addition method.

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MSBA FROM SALMONELLA TYPHIMURIUM AS TEMPLATE FOR A PROTEIN HOMOLOGY MODEL OF POST-HYDROLYTIC P-GP

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In order to investigate the conformational changes of the human membrane-embedded MDR-related ABC-transporter P-glycoprotein, we used the 4.2 Å resolution x-ray structure of the bacterial Lipid A transporter MsbA of Salmonella typhimurium (SalTy) [1] as a template for a comparative homology model of P-gp in the post-hydrolytic state. Like all MsbA proteins SalTy is functional as a homodimer, whereas P-gp represents a single polyfunctional peptide chain. Nevertheless a BLAST query against the PDB retrieves MsbA as first hit with an E-value of 4e-87, a sequence identity of 37% and a sequence similarity of 57%.

After the structural evaluation and refinement of MsbA SalTy, we established a homology model of P-gp using the modelling tool implemented in the MOE software package of the CCG. Finally, the quality of the protein model was improved using molecular mechanics and side chain configuration tools. The model satisfies nearly all structural and stereochemical parameters of computational protein evaluation (e.g. Cα-RMSD of 0.862).

However our model shows unfortunately hardly any good correlations with the experimental post-hydrolytic cross-linking data presented by Loo and Clarke in 2005.

Further studies using molecular dynamics simulations with distant constraints will show whether the MsbA Salty structure is a useful template to visualize physiological properties of P-gp.

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A RAPID AND VERSATILE UPLC METHOD FOR DETERMINATION OF AQUEOUS SOLUBILITY IN EARLY DRUG DISCOVERY

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Aqueous solubility is a major optimization parameter in drug discovery. Usually the compounds (solid or dissolved in organic solvent) are added to aqueous media, filtrated after a certain time and quantified. In this work we present a Ultra Performance Liquid Chromatography ™ (UPLC) method for the determination of dissolved compound in a 96 well format with a cycle time of 1.2 min. Automation of integration and data processing lead to significantly improved and quicker reporting. The described method is suitable for highly polar as well as lipophilic compounds. Impurities and degradation products can be separated with excellent resolution. We used UV detection obtaining sufficient lower limits of quantification. Mass spectrometric detection is possible and would provide additional sensitivity and higher selectivity.

A STANDARD PROTOCOL FOR THE CALIBRATION OF CAPILLARY ELECTROPHORESIS (CE) EQUIPMENT

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In accordance to the EU-GMP guide [1] and several ICH guidelines [2], dealing with the topic of pharmaceutical analysis, calibration of analytical equipment is one of the „must“ topics prior to the validation of analytical methods. In most cases, this takes place after succesfully performed Design- (DQ), Installation- (IQ) and Operation Qualification (OQ). During these phases, it is demonstrated that the equipment meets the user requirements, that the equipment is appropriate installed and it is proven, that the equipment operates within its predetermined ranges. After these basic qualification activities, a performance qualification (PQ) should take place, which shows, that the equipment is able to fulfill its intended use. Part of this PQ procedures could be the first calibration of the instrument leading to a combined calibration/performance qualification report. In addition, calibration is also part of the requalification activities, performed after a defined time period in accordance to the rules of good laboratory practice.

In this contribution, we want to introduce a generalised approach, how CE equipment could be calibrated. Based on a standard operating procedure (SOP), a calibration has been performed, controlling several instrumental parameters, as temperature and current stability, reproducibility of the injection system and standard deviation of peak areas and migration time, with and without internal standard. Contrary to the performance test of most of the suppliers, we have used two different systems to check the above mentioned parameters, e. g. one at low pH, suppressing the EOF nearly completely, one at pH of 9.3, leading to results, which are more comparable to the “normal” operation conditions than the supplier tests.

[1] EudraLex: The Rules Governing Medicinal Products in the European Union [http://pharmacos.eudra.org/F2/eudralex/index.htm]

[2] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [www.ich.org, Guidelines Quality]

THE APTS-DEXTRAN LADDER: A NOVEL TOOL TO DETERMINE THE TIGHTNESS STATUS OF CELL LAYERS

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The development of new drugs determining a directed transport across epithelial and endothelial barriers is one of the major tasks in pharmaceutical sciences. In this regard, it is essential to learn more about the correlation between tightness status of membranes and permeation of drugs. Tightness of cell layers is mainly characterised by the formation of tight junctions. Several studies using different types of molecules with different molecular weight for comprehensive tightness characterisation were carried out [1,2], but still no method for measuring a permeability pattern based on molecular weight of similar, oligomeric molecules exists. Thus, a novel method was developed, which describes the paracellular transport route by a molecular weight ladder. Dextrans were labeled by reductive amination with fluorescent 8-aminopyrene-1,3,6-trisulfonate (APTS). This mixture was used for transport studies using a Caco-2 cell line Transwell model. Samples were analysed by fluorimetry and capillary electrophoresis. Following this approach, a logarithm correlation of $R^2 = 0.9211$ between transepithelial electrical resistance (TEER) and APTS-dextran permeability was shown. Also, a TEER dependent permeability pattern could be observed including each single fraction from free APTS, APTS-glucose up to APTS-dextran consisting of 35 glucose units. All in all, the developed APTS-dextran ladder is a useful tool to characterise cell layer tightness - especially to describe paracellular transport ways and the extent of leakiness of cell layers (for blood-brain barrier or intestinal studies) over time - applying a wide array from smaller to larger molecules at the same time in order to refine TEER, sucrose or Evans blue measurements.

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A BINARY QSAR MODEL FOR P-GLYCOPROTEIN SUBSTRATES AND NON-SUBSTRATES

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In the current drug development research concerning multi drug resistance confered by P-glycoprotein the main interest shifted from inhibitor design to predictive substrate recognition. This concernes mainly cns penetrating and cytotoxic drugs. In this respect we established a model using binary QSAR and the VSA-descriptors developed by Labute [1] as implemented in MOE for a set of 139 structurally and functionally diverse P-gp substrates and non-substrates. Compared to previously published methods based on support vector machines, decision tree analysis or filter rules [2] we obtained similar results with a cross-validated prediction accuracy of 0.78 for the training set and 0.74 and 0.81 for two external test sets. Considering the rapid VSA-descriptor calculation this model may be useful in the preliminary high throughput screening of large compound librairies for P-gp interacting drugs.

[1] Labute P. A widely applicable set of descriptors. J. Mol. Graph. Mod. 2000; 18(4/5):464-477

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SPEICHELFLÜSSIGKEIT ZUR THERAPEUTISCHEN BESTIMMUNG VON MEDIKAMENTEN UND DROGEN UNTER VERWENDUNG EINES NEUEN SAMMELPRINZIPS

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Speichel kann in der klinischen Diagnostik gegenüber Blut oder Urin als Analytenmedium eine Vielzahl von Vorteilen besitzen. Durch nichtinvasive Probennahme stellt Speichel eine stressfreie Alternative zur Blutabnahme dar. Probleme liegen jedoch in einer leichten und reproduzierbaren Gewinnbarkeit und der Speichel kann stark in Menge und Zusammensetzung variieren, hat eine mikrobielle Begleitflora und kann durch Speisereste verunreinigt sein.

Ein neues Speichel-Sammelsystem (Saliva Collection System® von GREINER BIO-One) erlaubt eine standardisierte Speichelsammlung unter Verwendung einer Spülflüssigkeit und verhindert dadurch eine Diskriminierung von Analyten, wie dies bei trägergebundenen Sammelsystemen auftritt. Durch die flüssige Phase und die einfache Handhabung des Systems ist eine Speichelgewinnung auch bei Mundtrockenheit (Xerostomie) und auch in Eigenanwendung möglich. Dieses Speichelsammelsystem wurde für einen möglichen Einsatz in der Drogenanalytik evaluiert: Eine Gruppe von 10 Versuchspersonen nahm eine definierte Dosis Morphin (über Mohnkonsum) auf. Über einen Zeitraum von 8 Stunden wurden Blutproben und Speichelproben bei zwei verschiedenen pH-Werten gewonnen und die Opiatkonzentration mittels eines modifizierten Immuno-Testsystems (CEDIA) gemessen. Es konnte gefunden werden, dass bei allen Probanden die Morphinwerte im Speichel im Verlauf eine gute Übereinstimmung mit den Konzentrationen im Blut haben.

Die Verteilung anderer Drogensubstanzen und Medikamente zwischen Speichel und Blut wird derzeit weiter untersucht.

P_VSA-DESCRIPTORS: A PROPER TOOL TO PREDICT P-GLYCOPROTEIN INHIBITOR ACTIVITY

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P-Glycoprotein (P-gp), an extensively studied ABC-transporter, functions as a drug efflux pump, mediating multidrug resistance and limiting the efficacy of many anticancer drugs. Due to it`s promiscious nature many attempts have been undertaken, covering all areas in computational drug design, to characterize P-gp modulators. However, almost all studies rely on homologous series of compounds and generally applicable models are rare. Within our studies we investigated the applicability of P_VSA descriptors, which are stated to be widely applicable [1].

P_VSA descriptors display the amount of Van der Waals surface area (VSA) in a specific property-value P, namely lipophilicity[2], molar refractivity[2] and partial charge[3]. 56 P_VSA descriptors were calculated and QSAR calculations performed using Partial Least Squares (PLS), both implemented in MOE, on a series of 48 heterocyclic P-gp inhibitors. Defining the optimal number of components followed by a stepwise decrease of the number of descriptors led to final models which were validated using Leave-One-Out (LOO) crossvalidated r^2 values and external prediction of 186 propafenone-type inhibitors. For means of comparison, we also performed Hologram-QSAR (HQSAR) [4], implemented in Sybyl. Within both methods, final models obtained show predictive power comparable to published ones.

[1] Labute P. A widely applicable set of descriptors. J. Mol. Graph. Model. 2000; 18(4/5):464.

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A BINARY QSAR MODEL FOR PREDICTION OF HERG POTASSIUM CHANNEL BLOCKERS

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Acquired long QT syndrome caused by drugs that block the human ether-a-go-go-related-gene (hERG) K+ channel causes severe side effects and thus represents a major problem in clinical studies of drug candidates. Therefor, early prediction of hERG K+ channel affinity of drug candidates is becoming increasingly important in the drug discovery process. In light of our studies on in silico models for promiscuous drug-protein interactions we transformed in vitro hERG binding values (IC50) of 169 compounds into a binary (active: IC50 < 1 μM; inactive: IC50 > 10 μM) data format. The data set was split into a training set comprising 80 compounds and a test set. A predictive binary quantitative structure-activity relationship (QSAR) model based on 32 van der Waals surface area (P_VSA) descriptors was derived from the training set. In a leave one out cross validation a total accuracy of 95% and 98% accuracy for prediction of active compounds was obtained. For the external test, we obtained a total accuracy for prediction of actives of 88.9%. Thus, this model represents a versatile tool for alerting potential hERG activity.

TKM is grateful for finacial support by the Austrian Academic Exchange Service

AKTIVITÄT VON 5-AMINO-2-AZA[3.2.2]NONANEN GEGEN PROTOZOEN

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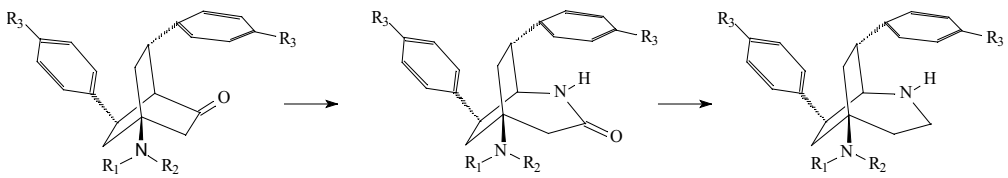
Die bicyclischen 5-Amino-2-azabicyclo[3.2.2]nonane **5** weisen in vitro eine signifikante Wirkung gegen die Erreger von Malaria bzw. Schlafkrankheit auf.[1] Zur Synthese dieser Verbindungen wurden 4-aminosubstituierte Bicyclo[2.2.2]octanone **1** zunächst zu den Azabicyclo[3.2.2]-nonanonen **3** umgesetzt. Diese wurden mit $LiAlH_4$ zu den Azabicyclo-nonanen **5** reduziert.



a: R_1=R_2 = -CH_3
b: R_1+R_2 = -$(CH_2)_4$-
c: R_1+R_2 = -$(CH_2)_5$-
1,3,5: R_3 = H
2,4,6: R_4 = Cl

Auf analoge Weise wurden die Bis-(p-chlorphenyl)derivate 6 hergestellt, die höhere Aktivität gegen Plasmodium falciparum und besonders gegen Trypanosoma rhodesiense aufweisen.. Die am besten wirksamen Substanzen werden in vivo auf ihre Wirksamkeit überprüft. Weiters wird nach noch wirksameren Substanzen mit gleicher Grundstruktur weitergesucht.

[1] Seebacher W., Weis R., Kaiser M., Brun R., Saf R. Synthesis of 2-azabicyclo[3.2.2]nonanes from bicyclo[2.2.2]octan-2-ones and their activities against Trypanosoma brucei rhodesiense and Plasmodium falciparum K1. J Pharm Pharmaceut Sci 8(3):578-585, 2005.

ENTWICKLUNG NEUER *N*-METHYL-2-PYRROLYL-SUBSTITUIERTER [1,2,4]TRIAZOLO[4,3-*A*]CHINOXALINE ALS ADENOSIN-REZEPTOR-LIGANDEN

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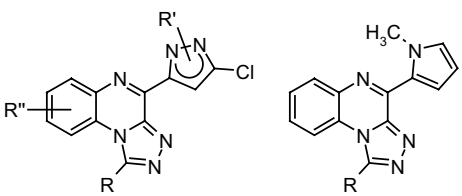
Im Rahmen unserer Untersuchungen zur Synthese neuer pharmazeutisch interessanter Verbindungen mit heterocyclischem Grundkörper gelang es uns, ausgehend von einem mittels Pyridazin → Pyrazol-Ringtransformation erhaltenen chlorpyrazolyl-substituierten Chinoxalin-2-on den Zugang zu einer neuen Klasse von tricyclischen Adenosinrezeptor-Antagonisten (*i.e.* Verbindungen des Typs **I**) zu eröffnen. [1, 2] Darüber hinaus konnten durch Einführung von Substituenten in unterschiedliche Positionen des Tricyclus sowie des Heteroaryl-Restes einerseits Derivate mit hoher Rezeptoraffinität sowie Subtyp-Selektivität und andererseits erste Einblicke in Struktur-Rezeptor-Affinitäts-Beziehungen erhalten werden [3].

In weiterer Folge galt unser Interesse nun der Darstellung von Desaza-Derivaten des Typs **II**. Wir berichten hier über Versuche, das entsprechend substituierte Chinoxalinon mittels alternativer Synthesestrategie zugänglich zu machen, über die weiteren Derivatisierungen dieses Grundkörpers zum Aufbau der Zielverbindungen des Typs **II** sowie über erste Ergebnisse der Adenosinrezeptor-Affinitäts-Untersuchungen.



(I) **(II)**

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N^4-SUBSTITUTED THIOSEMICARBAZONES DERIVED FROM ACYL DIAZINES: SYNTHESIS AND ANTITUMOR STUDIES

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Very recently we described the synthesis and antitumor properties of N^4-azabicyclo[3.2.2]nonane thiosemicarbazones (TSCs) derived from acyl diazines [1]. Although the compounds were potent antiproliferative agents, only a moderate inhibition of tumor growth (40%) was observed and this was accompanied by a high *in vivo* toxicity. In a continued effort to obtain more efficacious compounds, we have synthesized TSC-derivatives bearing N^4-methyl and cycloaliphatic moieties. The cytotoxic activities of the TSCs were evaluated in a panel of human tumor cell lines by the MTT assay. TSCs bearing terminal cycloaliphatic amines and especially a pyrrolidine moiety exhibited potent cytotoxic activity against Burkitt´s lymphoma Ca 46 (IC_{50} = 0.002-0.59 µM), HeLa cervical (IC_{50} = 0.0003-0.057 µM), and Colon HT-29 (IC_{50} = 0.0005-0.47 µM) cells. Several of the highly active TSCs were tested *in viv*o in CD1 nude mice bearing various tumor xenografts. The compound Hexahydro-1*H*-azepine-1-thiocarboxylic acid 2-[1-(3-methyl-2-pyrazinyl)ethylidene}-hydrazide (**EPH 280**) applied at doses of 20-25 mg/kg in mice bearing H460 human lung carcinoma xenograft resulted in a 70% reduction in tumor burden. Treatment of DLD1 colon adenocarcinoma cells with 40 nM of **EPH 280** in resulted in the increase of the phosphorylation of HSP 27 which was determined to occur at the ser-82 site. The synthesis and structure-activity relationships of this class of novel antitumor agents will be presented.

Financial support by the Austrian Science Foundation (FWF) is generally acknowledged (project no. P 09879-MED).

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COMPARISON OF OPIOID RECEPTOR BINDING PROFILES OF 6-AMINO ACID SUBSTITUTED DERIVATIVES OF 14-*O*-METHYLOXYMORPHONE AND OXYMORPHONE

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Currently, treatment of severe pain relies mostly on the use of centrally acting opioid analgesics such as morphine, fentanyl and oxycodone. Clinical use of these opioids is limited by a number of adverse actions (e.g. sedation, tolerance, addiction), which are mediated predominantly *via* the central nervous system. This has led to an active search for novel opioid compounds exhibiting more favourable pharmacological features. A series of 6-amino acid conjugates (e.g. Ala, Phe) of the opioid analgesic 14-*O*-methyloxymorphone was developed in an effort to obtain agonists that would have potentially limited ability to cross the blood-brain barrier. In addition, 6-amino acid substituted derivatives of oxymorphone were chemically synthesized. Binding affinities to opioid receptors were determined using displacement binding assays in rat (μ, δ) or guinea-pig (κ) brain membranes. All derivatives displayed high affinities (K_i: 0.52 – 3.20 nM) at the μ-opioid receptor. The 14-*O*-methyloxymorphone derivatives showed significantly higher binding affinity towards all three opioid receptors compared to the 14-hydroxy substituted counterparts. The newly developed ionisable derivatives could find clinical applications as potent analgesics without the adverse actions of centrally acting opioids.

CYCLOHEXENYLDIHYDROTHIOURACILES AS BUILDING BLOCKS FOR THE SYNTHESIS OF 8,13,15-TRIAZASTEROIDS

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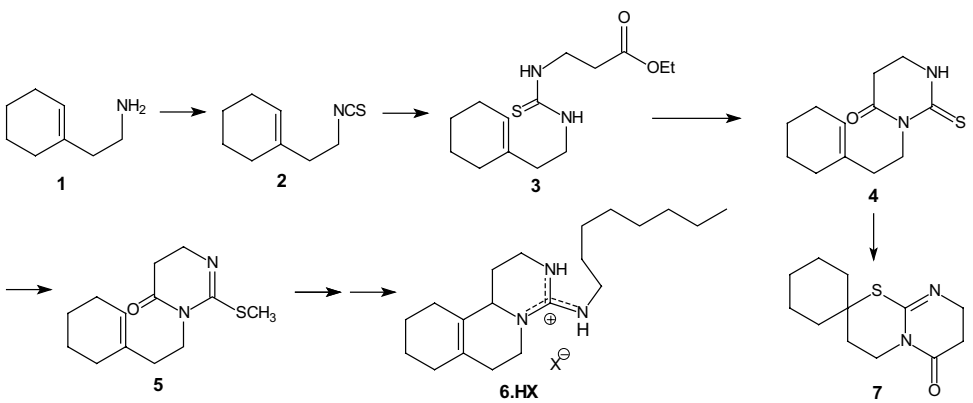
4-Alkylaminodecahydropyrimido[4,3-*a*]isoquinolinium salts of type **6**.HX represent stable tricyclic 8,13,15-triaza-analogues (HEIAs) of steroidal carbocationic high energy intermediates (HEI) generated during the fungal ergosterol biosynthesis [1]. They could potentially inhibit the synthesis of ergosterol and thus act as antifungal agents. Two main routes for a convenient and short preparation of HEIAs **6**.HX starting from cyclohexenylethylamine **1** are under investigation [2].

In this contribution the reaction pathway via the key intermediate N^3-[2-(1-cyclohexenyl)ethyl]-5,6-dihydrothiouracile (**4**) is reported [3, 4]. In the course of the *Bischler-Napieralski*-like cyclization reaction of cyclohexenylethyldihydrothiouracile **4** with polyphosphoric acid an interesting side-product,3',4',7',8'-tetrahydro-spiro{cyclohexane-1,2'-(2'*H*,6'*H*-pyrimido[2,3-*b*][1,3]thiazine)}-6'-one (**7**), was formed.



[1] Gößnitzer E, Punkenhofer A, Amon A, Favre B, Ryder N S. Eur J Pharm Sci. 2003; 19: 151-164

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STRUCTURE-ACTIVITY RELATIONSHIP OF TELMISARTAN REGARDING ITS PPARγ ACTIVITY

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The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors. A series of coactivators/ corepressors are involved in ligand-induced transcription of genes allowing a tissue and ligand specific activation of these target genes by PPARs. The most abundant isoform in adipose tissue, PPARγ, plays an important role in the regulation of insulin sensitivity. Glitazones or thiazolidinediones (TZDs) are high affinity ligands for this receptor and are currently used in the treatment of type 2 diabetes mellitus. Together with an improvement of insulin sensitivity and glucose tolerance, they improve lipid profiles. Given the side effects of glitazones like weight gain, edema, and fluid retention, the characterization of new PPARγ ligands that retain metabolic efficacy without exerting adverse actions plays a central role in the development of new therapeutic strategies for insulin resistance and type 2 diabetes mellitus.

A promising new group of such ligands are SPPARγMs (Selective PPARγ Modulators), compounds that activate only a subset of the functions induced by cognate ligands or act in a cell-type selective manner. Recently, Berger and colleagues [1] described a non-TZD partial agonist (nTZDpa) as a new SPPARγM in preclinical studies. It has been shown by Schupp et al. [2], that a subgroup of angiotensin II receptor type 1 blockers (ARB) induce PPARγ activity.

The aim of this SAR-Study is the identification and characterization of new selective PPARγ activating compounds, based on the structure of Telmisartan, which attends to be the most potent PPARγ activating ARB. We focused our attention on the variation of the position 2 of the 1H-benzimidazol-1'-ylmethyl-[1,1'-biphenyl]-2-carboxylic acid and its role on PPARγ activation.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF MIFEPRISTONE DERIVATIVES CONTAINING A LINKER GROUP

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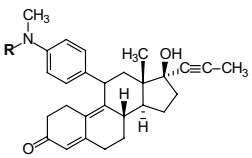
Aim of this work was the synthesis of mifepristone derivatives with high selectivity to progesterone receptors. These compounds have a linker chain attached to the amino-phenyl group in Position 11β of the steroid skeleton. The linker chain contains a functional group useful for the attachment of other molecules. Cancer cells in hormone dependent carcinomas develop an enhanced number of such receptors. Our compounds are useful starting materials for the synthesis of molecules which will bind selectively to these tumor cells. These molecules will be very useful for targeting progesterone receptor positive breast cancer cells.



1a-x

1a : R = -CH_3 ; Mifepriston
1b : R = -$(CH_2)_5$-CH_3
1c : R = -H; DMM
1d : R = -$(CH_2)_5$-CH_2OH
1e : R = -$(CH_2)_5$-$CH(OC_2H_4O)$
1f : R = -$(CH_2)_5$-CHO
1g : R = -$(CH_2)_5$-COOH
1h : R = -$(CH_2)_7$-COOH
1i : R = -$(CH_2)_{10}$-COOH
1j : R = -$(CH_2)_5$-$COOCH_3$
1k : R = -$(CH_2)_7$-$COOCH_3$
1l : R = -$(CH_2)_{10}$-$COOCH_3$
1m: R = -CO-$(CH_2)_4$-COOH
1n: R = -CO-$(CH_2)_4$-$COOCH_3$
1o : R = -CO-NH-(4-Cl-C_6H_4)
1p : R = -CS-NH-(4-Cl-C_6H_4)
1q : R = -CO-NH-C_4H_9
1r : R = -CS-NH-C_4H_9
1s : R = -CO-NH-$(CH_2)_5$-COOH
1t : R = -CO-NH-$(CH_2)_5$-$COOCH_3$
1u : R = -CO-NH-$(CH_2)_5$-CONH-Leu
1v : R = -CO-NH-$(CH_2)_5$-$COOCH_3$
1w : R = -$(CH_2)_2$-CONH-(p-Salicylamid)
1x : R = -$(CH_2)_3$-O-CH_2-(p-Salicylamid)

Mifepristone (**1**) was demethylated to desmethyl mifepristone (DMM) (**1c**) and different spacer groups have been attached to the nitrogen of the secondary amino group yielding **1d-x**, see below. The structures of **1b-x** have been established by NMR. The antiprogestine activity was determined by an alkali phosphatase assay using T47-D breast cancer cells and is comparable with mifepristone. These new compound show antiprogestine activity in the nano-molar region, indicating high receptor affinity. From these results we conclude that the compounds **1b-x** are promising starting materials for therapeutic and diagnostic useful drugs for the treatment of breast- ovarial- and indometrium cancer.

This work was supported by Österreichische Krebshilfe/Steiermark, project no.: 03/2001

AN EVALUATIVE SURVEY OF CONFORMATIONAL MODEL GENERATORS

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Following our recent study on Catalyst's conformational space sub-sampling algorithm [1], we investigated the quality of conformational models generated by Catalyst [2], Omega2 [3] and Macromodel [4]. We examined a sample of 768 PDB complexes representing pharmacologically relevant drug targets with reasonable quality and resolution. The ligands were extracted using our pharmacophore management and visualization tool LigandScout [5], and subsequently generated conformational models were retrieved from the respective isomeric smiles. RMS deviation between the best fitting conformer of the generated ensemble and the bioactive conformation stored in the PDB database was used as a benchmark. Several user-adaptable algorithm parameters were analyzed to provide comprehensive user-guidelines for best generator performance.

[1] Kirchmair J, Laggner C, Wolber G, Langer T. Comparative Analysis of Protein-Bound Ligand Conformations with Respect to Catalyst's Conformational Space Subsampling Algorithms. J. Chem. Inf. Comput. Sci. 2005; 45:422-430.

[2] Catalyst, Version 4.11; Accelrys, Scranton Road, San Diego, CA

[3] Omega 2 beta 2. OpenEye Scientific Software, 3600 Cerrillos Rd., Santa Fe, NM 87507

[4] Macromodel v91106. Schroedinger, Portland, 101 SW Main Street, Portland, OR 97204

[5] Wolber G, Langer T. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters J. Chem. Inf. Model. 2005; 45:160-169.

SYNTHESIS OF (+)- AND (-)-8-FLUOR-GALANTHAMINE

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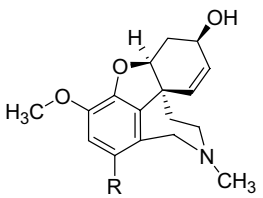
Galanthamine (**1**), an Amaryllidaceae alkaloid, has been used clinically for the treatment of neurological illnesses such as myasthenia gravis [1] or poliomyelitis [2], as an anti-curare agent [3] and as a parasympathomimetic [4]. Later studies proved that Galanthamine acts as a selective, reversible and competitive acetylcholinesterase (AChE) inhibitor [5] as well as an allosteric ligand of nicotine acetylcholine receptors (nAChRs) [3]. It is a approved drug for the treatment of Alzheimer's disease. The synthesis and pharmacology of galanthamine have been reviewed recently and our previous approaches to this molecule and derivatives thereof are summarized therein [6]. Here we report the synthesis of both pure enantiomers of 8-fluorogalanthamine (**2**) using the phenolic oxidative coupling approach.



1: R= H
2: R= F

[1] Chistoni, G.; Guaraldi, G. P. *R*V., *Neuropsichiatr. Sci. Affini* (Parma), 1960, *6*: 53.

[2] Göppel, W.; Betram, W., *Psychiatr. Neurol. Med. Psychol.,* 1971, *23*: 712.

[3] Mayrhofer, O., *South. Med. J.,* 1966, *59*: 1364.

[4] Baraka, A.; Sami Harik, M. D., *JAMA, J. Am. Med. Asso.,* 1977, *238*: 2293.

[5] Sramek, J. J.; Frackiewicz, E. J.; Cutler, N. R., *Expert Opin. Invest. Drugs*, 2000, *9*: 2393.

[6] Marco-Contelles, J.; do Carmo Carreiras, M.; Rodriguez, C.; Villarroya, M.; Garcia, A. G., Chem.Rev.,2006, 106: 116.

NOVEL PYRAZOLO[5,1-B]THIAZOLIDINES VIA CYCLIC HYDRAZINIUM- AND HYDRAZONIUMDITHIOCARBONIC ACID SALTS

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Certain substituted N-amino-1,3-thiazoles show a variety of pharmacological activities, e. g. fungitoxical and antimicrobial potency. In earlier publications we could demonstrate the synthetic potential of cyclic iminiumdithiocarbonic acid diester salts in synthesis of different heterobicyclic ketene-*N*,*S*-acetals or isothioureas. Starting from cyclic N-butoxycarbonylhydraziniumdithiocarbonic acid salts we received novel compounds with a pyrazolo[5,1-b]thiazolidine structure.

N-boc-protected 2-methylthio-3-N-aminothiazolidinium salts can be transformed with C-nucleophiles to the heterocyclic ketene-*N*,*S*-acetals. Heating in toluene with a catalytic amount of p-toluensulfonic acid leads to cyclisation especially if there is a keto- or nitrilo-group in the C-nucleophiles.

While treating hydrazone derivatives of aminothiazolidines, coupled with malonodinitrile or some phenylogous under the same cyclisating conditions described before we obtained a series of compounds as main-products with a new intramolecular C-N bonding after preceded N-N bond cleavage. This reaction is proved by the X-ray structur of one rearranged molecule. Furthermore a novel way in synthesising the little known 2-aminothiazolo[3,2-b][1,2,4]triazoles was found by deprotonation of cyanamide with methanolate and condensation with followed by cyclisating conditions. An advantage of our method is the possibility of a greater and easier attainable variety of substituents. Similar structures are part of fungicides like Tricyclazole©.

The novel compounds were tested against diverse protocoes. Some of them have a moderate depressing effect against different pathogenes in the lower micromolar range. The strongest activity shows a pyrazolo[5,1-b]thiazolidine derivate against the chloroquine resistant strain K1 of Plasmodium falciparum (IC-50 = 1,2 μM) by low toxicity (L6).

OPIOID ANTAGONIST PROFILE OF NOVEL 14-ALKOXY DERIVATIVES OF NALTREXONE

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Selective opioid receptor antagonists are useful tools in defining the receptor type and in evaluating the selectivity of new opioid agonists. In addition to their use as pharmacological probes, selective opioid antagonists have also a therapeutic potential in the treatment of variety of disorders where endogenous opioid peptides are involved. Naltrexone is a competitive antagonist at μ, κ and δ opioid receptors with some preference for μ receptors. For its good oral efficacy and relatively long duration of action naltrexone is still the antagonist of choice for treating opioid addiction and alcoholism [1]. The 14-*O*-methyl and 14-*O*-ethyl derivatives of naltrexone were described to possess similar pharmacological properties [2]. In an attempt to obtain antagonists with higher μ opioid receptor affinity and selectivity, we prepared a series of novel 14-alkoxy analogues of naltrexone. The pharmacological profile of the developed compounds was determined in receptor binding and functional assay. One compound of the series showed remarkable high affinity at the μ opioid receptor, in the subnanomolar range, and better δ/μ and κ/μ selectivity ratios than the parent compounds. 14-Alkoxy substitution enhances the lipophilic properties of naltrexone, which would broaden the therapeutic scope, e.g. use in transdermal systems [3].

[1] Schmidhammer, H. Opioid receptor antagonists. Progress in Medicinal Chemistry 1998, 35, 83-132.

[2] Kobylecki, R.J., Carling, R.W., Lord, J.A.H., et al. Common anionic receptor site hypothesis: its relevance to the antagonist action of naloxone. J. Med. Chem. 1982, 25, 116-20.

[3] Greiner, E., Spetea, M., Krassnig, R., et al. Synthesis and biological evaluation of 14-alkoxymorphinans. J. Med. Chem. 2003, 46, 1758-63.

FORMATION OF C-N- AND C-S-BONDS BY APPLICATION OF ANODIC ELECTROCHEMISTRY

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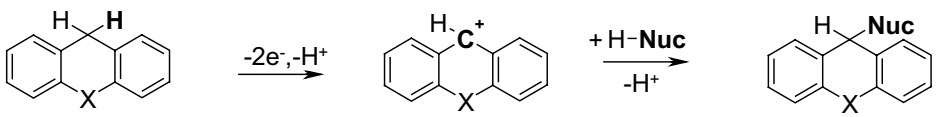
Because of the importance of derivatives of C-N- and C-S-bonds in organic and medicinal chemistry, developement of new methodes for there efficient synthesis is an important challenge. The anodic oxidation is a novel and operationally simple method for the preparation of several types of compounds. After separation of one proton and two electrons, carbenium-ions are obtained which react with nucleophiles in a solution of acetonitrile to yield the compounds, described below.

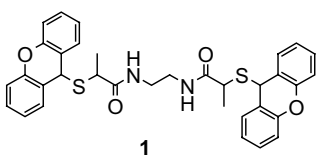
We chose xanthene (X = O) and thioxanthene (X = S) as carbenium-ion-precursors and NH-and SH-acid substrates as nucleophiles.



Reactions of methanesulfonamide, ortho-toluensulfonamide, 3,5-dinitrobenzenesulfonamide and 4-chlor-3-nitrobenzenesulfonamid as N-nucleophiles with xanthene and thioxanthene resulted in the formation of C-N-bonds.

N-hydroxyacetamide, hydroxyurea, N-hydroxyurethane, tert.butyl-N-hydroxycarbamate, N-(benzylcarbonyl)hydroxylamine and benzohydroxamic acid formed via anodic oxidation C-N-derivatives.

For the formation of C-S-bonds, substituted thiophenole and alcyl-and bifunctional derivatives react as S-nucleophiles with the carbenium-ion-precursors.



1

We have found that compound **1** is a potent inhibitor of argenine methyltransferase (39,95% (40µM), 12,10% (10µM), Spannhoff, A., University of Freiburg).

NEUE ALDOSE-REDUCTASE-INHIBITOREN MIT CHINOXALIN-2(1*H*)ON-GRUNDKÖRPER: AUFBAU, CHARAKTERISIERUNG SOWIE BIOLOGISCHE EVALUIERUNG

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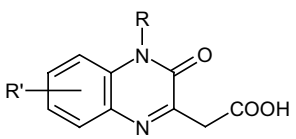
Institut für Pharmazie der Leopold-Franzens Universität Innsbruck
Innrain 52a, A-6020 Innsbruck

Die Hemmung des Schlüsselenzyms des so genannten Polyolstoffwechsels durch Inhibitoren des Enzyms Aldose-Reductase (EC 1.1.1.21) stellt einen Ansatzpunkt zur Minimierung bzw. Vermeidung von Diabetes mellitus-Spätkomplikationen (hierzu zählen insbesondere diabetische Retinopathie, Katarakt, Nephropathie, Neuropathie, diabetisches Fußsyndrom und cardiovaskuläre Erkrankungen) dar [1]. Ein essentielles Strukturmerkmal der Aldose-Reductase-Inhibitoren ist nach heutigem Kenntnisstand eine acide Funktion; diese kann in der dissoziierten Form mit der kationischen Seite des aktiven Zentrums wechselwirken.

Im Rahmen des hier vorgestellten Projektes galt es einen Zugang zu 3-Carboxymethylchinoxalin-2(*1H*)on-Abkömmlingen des Typs **A** und damit zu einer neuen Klasse potentieller Aldose-Reductase-Inhibitoren zu eröffnen. Wir berichten über Versuche zur Darstellung, Derivatisierung und Charakterisierung der Zielverbindungen sowie über erste Struktur-Aktivitäts-Beziehungen.



(A) R = (subst.) Benzyl,
(subst.) Benzothiazol-2-ylmethyl

[1] Costantino L, Rastelli G, Vianello P, Cignarella G, Barlocco D. Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. Med. Res. Rev. 1999; 19: 3-23.

SYNTHESIS, CYTOTOXICITY, CELLULAR UPTAKE AND INFLUENCE ON EICOSANOID METABOLISM OF COBALT-ALKYNE MODIFIED FRUCTOSES

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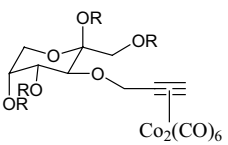
Propargylhexacarbonyldicobalt complexes with fructopyranose ligands (see figure) were prepared and investigated for cytotoxicity at the MCF-7 human breast cancer cell line. The antiproliferative effects depended on the presence of isopropylidene protecting groups in the carbohydrate ligand and correlated with the cellular concentration of the complexes. IC_{50} values of > 20µM demonstrated that the fructose derivatives were only moderate active compared to the references Auranofin and the Aspirin derivative [2-acetoxy-(2-propynyl)benzoate]hexacarbonyldicobalt (Co-ASS). In continuation of our studies on the mode of action of cobalt-alkyne complexes we studied the influence of the compounds on the formation of 12-HHT (COX-1 product) and 12-HETE (12-LOX product) by human platelets as an indication of the interference in the eicosanoid metabolism which is discussed as a target system of cytostatics. Co-ASS was an efficient COX-1 inhibitor without LOX inhibitory activity and Auranofin inhibited both COX-1 and 12-LOX eicosanoid production. The missing activity of the fructopyranose complexes at the 12-LOX and the only moderate effects at COX-1 indicate that COX/LOX inhibition may be in part responsible for the pharmacological effects of Auranofin and Co-ASS but not for those of the fructopyranose complexes. [1]



R= -H, isopropylidene

[1] Ott I, Koch T, Shorafa H, Bai Z, Poeckel D, Steinhilber D, Gust R Synthesis, cytotoxicity, cellular uptake and influence on eicosanoid metabolism of cobalt-alkyne modified fructoses in comparison to auranofin and the cytotoxic COX inhibitor Co-ASS. Org. Biomol. Chem. 2005; 3: 2282-6

SUBSTITUTED IMIDAZO[4,5-C]PYRIDINES AS INHIBITORS OF PESTIVIRUSES

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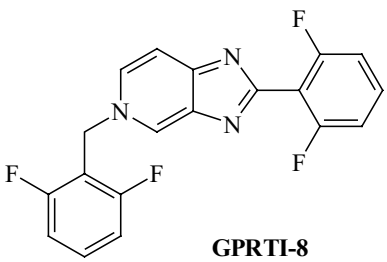
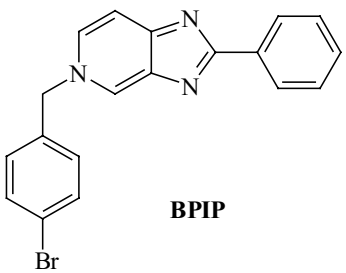
[1]Institute of Pharmacy, University of Innsbruck, Austria
[2]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium
[3]Veterinary and Agrochemical Research Centre, Ukkel, Belgium

BVDV (bovine viral diarrhea virus), a pestivirus, has often been used as a surrogate for hepatitis C. In a broad screening effort, GPRTI-8 was found to exhibit anti-BVDV activity (EC_{50}: 8 µg/ml, CC_{50}: >100 µg/ml) and was chosen as a lead compound. Formal removal of all 4 fluorines resulted in an analogue with improved activity/selectivity (EC_{50}: 0.04 ± 0.03 µg/ml, CC_{50}: 46 ± 5.9 µg/ml). In a second step, substituents were introduced onto the benzyl ring (2-, 3- or 4-fluoro, chloro, methyl, methoxy etc.), resulting in the discovery of BPIP (the 4-bromo analogue) as a highly active and selective inhibitor of BVDV (EC_{50}: 0.006 ± 0.001 µg/ml, CC_{50}: 29 ± 2 µg/ml, SI = 4830).

BPIP is also active against other pestiviruses (e.g. classical swine fever virus: EC_{50}: 0.6 µg/ml), but was inactive against hepatitis C in the subgenomic replicon system.



GPRTI-8



BPIP

BIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF HIGHLY POTENT 14-ARYLALKYLOXY SUBSTITUTED *N*-METHYLMORPHINAN-6-ONES

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A significant drawback of opioid analgesics is a variety of side effects, such as sedation, constipation, nausea, confusion, respiratory depression, and dependence. In an attempt to develop opioid analgesics with fewer adverse effects we synthesized a series of 14-arylalkyloxy substituted *N*-methylmorphinan-6-ones. Their binding affinities to μ, δ, and κ opioid receptors were determined using receptor binding assay in rat (μ, δ) or guinea-pig (κ) brain membranes. The new compounds displayed high binding affinities to the μ opioid receptor, which were comparable to that of the parent compound 14-*O*-methyloxymorphone. In the guinea pig-ileum and mouse vas deferens preparations, these compounds behaved as potent agonists. Antinoceptive potencies of most of the new compounds in the hot-plate test after s.c. administration in mice were considerably higher than the potency of 14-*O*-methyloxymorphone and morphine. In the colonic propulsion test, the most potent analgesic, the 14-benzyloxy substituted derivative, showed negligible constipating activity at the analgesic dose after s.c. administration. The nature of the substituent at position 14 has a major impact on the abilities of morphinans to interact with opioid receptors leading to qualitative and quantitative differences in biological and pharmacological activities.

ENTDECKUNG NEUER 11β-HYDROXYSTEROID DEHYDROGENASE TYP 1 INHIBITOREN MIT HILFE VON PHARMAKOPHORMODELLEN UND VIRTUELLEM SCREENING

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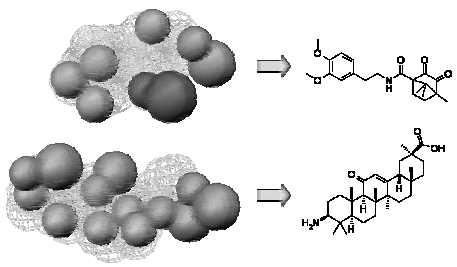
11β-Hydroxysteroid Dehydrogenase Typ 1 (11β-HSD1) katalysiert u. a. die Umwandlung von Cortison in aktives Cortisol. Die Inhibition dieses Enzyms kann bei zahlreichen glucocorticoid-assoziierten Erkrankungen wie Fettleibigkeit, Diabetes, schlechter Wundheilung und Muskelatrophie therapeutische Effekte erzielen. [1]

Basierend auf bekannten, strukturell diversen 11β-HSD1 Inhibitoren wurden mit dem Softwarepaket Catalyst [2] Pharmakophormodelle für selektive und nicht-selektive 11β-HSD1-Hemmer generiert. Mithilfe dieser Modelle wurden aus virtuellen Datenbanken (insgesamt über 1.7 Millionen Substanzen) 30 Kandidaten für eine biologische Testung ausgewählt. Sieben dieser Testsubstanzen zeigten potente 11β-HSD1-Inhibition mit IC_{50}-Werten in z.T. nanomolarem Bereich.



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DEORPHANING ODORANT RECEPTORS

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Humans possess approximately 300 odorant receptors and about 700 pseudogenes, belonging to family A of G-protein-coupled receptors (GPCRs). With this repertoire, humans can distinguish numerous chemical diverse odorants. To unravel the mechanisms underlying this enormous discriminating power, knowledge of receptor agonist pairs is of crucial importance. Up to date, only an infinitely small number of odorant receptors are de-orphaned. The identification of an odorant receptor, that means which ligand activates the corresponding receptor is complicated by the fact that a particular odour impression is obtained from the combinatorial activation of several receptors. This requires large scale odorant screening. Therefore, we developed a theoretical approach to predict ligand specifities for a given odorant receptor. Combining the available predictions of olfactory receptor-specific sequence motifs [1, 2] with known receptor - ligand interaction information, we identified functional requirements of odorant receptors for the recognition of a given odorant. Our model provides rational guidelines for deorphaning experiments of odorant receptors.

Knowledge of large numbers of receptor - ligand - pairs will shed light on how the olfactory family has evolved the ability to recognize such a large variety of distinct chemical structures.

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ANTIMYKOBAKTERIELLE WIRKUNG VON 4-ALKOXYPIPERIDINEN

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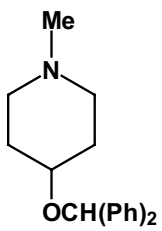
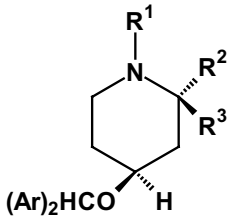
Neben seiner H_1-antihistaminischen Wirkung besitzt Diphenylpyralin auch eine bemerkenswerte Aktivität gegen Mykobakterien während es gegen *E. coli* und *Staph. aureus* als unwirksam einzustufen ist [1]. In Ringposition 2 substituierte Derivate mit unterschiedlicher Konfiguration wurden hergestellt [2] und auf ihre Wirksamkeit gegen *Mycobacterium tuberculosis* $H_{37}R_v$ getestet.





R^1 = Me, Ph, Phenethyl
R^2 = H, Alkyl
R^3 = H, Alkyl

Die Struktur der wirksamsten dieser Verbindungen wurde weiter variiert, wobei die aromatischen Ringe und das Stickstoffatom in Position 1 unterschiedlich substituiert wurden. Auf diese Weise konnte nochmals eine Wirkungssteigerung erzielt werden. Anschließend wurde analysiert, welche Substituenten in welcher Konfiguration die Aktivität günstig beeinflusst haben. Schließlich wurde eine Verbindung synthetisiert, die mehrere dieser wirkungssteigernden Elemente enthält. Diese war aber - vermutlich aus sterischen Gründen - völlig unwirksam.

Zur Synthese der Verbindungen wurden 1-substituierte Piperidinole ausgehend von 4-Piperidinol oder 4-Aminotetrahydropyridin-2-thionen hergestellt. in Abhängigkeit von der Substitution des Stickstoffatoms wurde schließlich nach unterschiedlichen Methoden verethert.

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COVALENTLY BOUND POLYAMINES ENHANCE THE ANTISENSE EFFECT OF BCL-2 TARGETED PHOSPHOROTHIOATES

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Antisense and siRNA oligonucleotides are indispensable scientific tools for transient gene knockdown and the study of gene function. However, their huge promises as therapeutics have not yet been fulfilled. To date, only one product has reached the market and several promising antisense compounds have recently failed in their respective phase III clinical trials. The main problems to be solved are poor cellular uptake and insufficient pharmacokinetic properties. Tethering cationic molecules to oligonucleotides result in zwitterionic molecules with better membrane permeation ability and excellent target affinity. Polyamines are involved in gene function regulation and are counterions of nucleic acids in vivo. Due to their polycationic character and the ideal distance between amino groups, they are perfect ligands for creating zwitterionic oligonucleotides.

Using an easy and versatile procedure for attaching ligands to the 2'-position, the natural polyamines putrescine, spermidine and spermine as well as a chemically prepared pentaamine have been conjugated to phosphorothioate oligonucleotides targeted at bcl-2. CD spectra revealed that polyamine conjugation caused no changes in secondary structure. The thermal duplex stability of all conjugates is slightly lower than that of unconjugated phosporothioate, but increases progressively with increasing polyamine length. In a human melanoma cell culture assay, these conjugates show progressively higher target downregulation ability with increasing polyamine chain length. Conjugates with spermine and the pentaamine have a higher efficiency than the control phosphorothioate oblimersen, a drug currently being used in clinical trials. The higher in vitro efficiency of the pentaamine conjugated oligonucleotide is presumably due to the higher cellular uptake or higher nuclease stability.

QUANTITATIVE ANALYSIS OF PARACETAMOL POLYMORPHS IN POWDER MIXTURES BY FT-RAMAN SPECTROSCOPY AND PLS REGRESSION

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A fast and simple method for the quantitative analysis of powder mixtures of monoclinic (form I) and orthorhombic (form II) paracetamol was developed, based on FT-Raman spectroscopy and PLS regression. Three different spectral preprocessing algorithms, namely orthogonal signal correction (OSC), standard normal variate transformation (SNV), and multiplicative scatter correction (MSC), were applied in order to eliminate light scattering and path length effects. Subsequently PLS regression models were fitted and the models were evaluated on the basis of the root mean squared error of cross validation (RMSECV) over the complete data set. Furthermore, the data were split into two equal-sized training and test subsets by the Kennard-Stone design[1] and the errors of calibration (RMSEC) and prediction (RMSEP) were calculated. It was found that the OSC-preprocessing increases the predictive performance of the PLS regression model (RMSECV=0.500 %, RMSEC=0.842 %, and RMSEP=0.538 %) compared to the SNV (RMSECV=2.398 %, RMSEC=0.911 %, and RMSEP=7.177 %) and MSC (RMSECV=2.7648 %, RMSEC=1.572 %, and RMSEP=4.838 %). In addition, the PLS model fitted to the OSC-preprocessed data is more parsimonious, requiring only a single latent variable, compared to three latent variables required by the models fitted to the SNV and MSC preprocessed data. The proposed multivariate method presents a significant improvement over existing univariate methods for the quantitation of paracetamol polymorphs[2,3]. In general this method seems to be very useful in the quantitative analysis of compounds with similar spectra and not only for polymorphs.

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INTESTINAL EFFLUX PUMP INHIBITION: SAQUINAVIR ABSORPTION IN PRESENCE OF THIOLATED CHITOSAN

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ABSTRACT: It was the aim of this study to investigate the effect of chitosan-4-thiobutylamidine (Ch-TBA) and reduced glutathione (GSH) on the absorption of P-glycoprotein (P-gp) and multidrug resistance protein (MRP) substrate saquinavir. Bidirectional transport studies were performed with freshly excised rat small intestinal mucosa mounted in Ussing type chambers. The functional activity of the efflux pumps in rat intestinal mucosa during the experiment was proven by the efflux ratio of saquinavir, which was 2.1. Ch-TBA and particularly the combination of Ch-TBA with GSH enhanced apical absorption and decreased the secretory transport of saquinavir. In presence of 0.5% Ch-TBA and 0.5% GSH, the uptake of saquinavir was 2.1-fold improved in rat intestinal mucosa.

Results of this study showed that Ch-TBA in combination with GSH can be an interesting tool for increasing the oral bioavailability of actively secreted compounds. This study showed that thiolated polymers, which have been extensively evaluated for their permeation enhancing effects of mainly paracellular transported hydrophilic compounds, are able to significantly enhance absorption of a lipophilic P-gp and MRP substrate.

MOISTURE-INDUCED POLYMORPHIC TRANSITION OF ORTHORHOMBIC PARACETAMOL

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The metastable orthorhombic (form II) paracetamol (PCM) was crystallized from the melt as well as from ethanol solution by seeding[1]. The samples were characterized by powder X-ray diffraction (PXRD), optical microscopy, thermogravimetric analysis coupled with FTIR spectroscopy (TG-FTIR), and dynamic moisture sorption. The moisture-induced transformation to the stable monoclinic form (form I) was monitored by time-resolved PXRD at high relative humidities (RH) of 85% and 90%, and with a novel gravimetric moisture sorption analyzer (SPS11). It was found that crystals grown from solution are contaminated with nuclei of form I that grow during filtration and drying[2]. Time-resolved PXRD at 85% and 90% RH revealed that the transformation rate increases with RH as well as by grinding of the crystals. Moisture sorption/desorption studies show that the transformation of solution-grown form II is connected with a mass loss of 0.1-0.6% w/w. The extent of this mass loss is inversely proportional to the initial monoclinic content of the samples. Mass loss starts at 60% RH and accelerates at higher RH until form II completely transforms into form I at 95% RH, which was verified by PXRD. Karl-Fischer titration confirmed the absence of water in the solution-grown orthorhombic PCM samples, while TG-FTIR proved the presence of ethanol as residual solvent, which is released during the moisture-induced transition to form I. Residual ethanol is not removable by grinding, indicating the formation of a solid solution[3] between ethanol and PCM form II and not a physical solvent inclusion. Highly pure, melt-grown form II showed no transformation at 90% RH. Thus, it can be concluded that the moisture triggers a residual ethanol-mediated growth of existing form I nuclei but does not induce the nucleation of form I.

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THIOLATED POLYMERS AS EFFECTIVE INHIBITORS OF INTESTINAL MRP2 EFFLUX PUMP TRANSPORTERS

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The degree to which orally administered drugs are absorbed by the intestinal epithelium is regulated, among others, by specific membrane transport systems, so called efflux pump transporters, located to the apical membrane of the cell. These transporters, which include P-glycoprotein and multidrug resistance protein 2 (Mrp2), extrude compounds back to the intestinal lumen limiting their absorption into blood [1]. By inhibiting efflux pump transporters the bioavailability of many orally administered, poorly absorbed drugs, acting as substrates for intestinal efflux pumps can be improved [2]. Within this study an inhibitory activity of poly (acrylic acid)-cysteine conjugates on Mrp2 efflux pump transporter was investigated using sulforhodamine 101 and Penicillin G as model compounds for substrates. In order to determine the influences of the molecular mass and the degree of thiolation of the polymer conjugates, poly (acrylic acid)s of different molecular mass and increasing degree of thiolation were tested.

The highest impact on inhibition of Mrp2 exhibited poly (acrylic acid) of 250 kDa with 355.9±39 µmol thiol groups per gram polymer showing 4.67-fold improvement in permeation of sulforhodamine 101 compared to unmodified poly (acrylic acid) used as control. Using the same thiolated polyacrylate a 1.7-fold improvement in permeation could be observed for Penicillin G in comparison to the control.

The study demonstrates that thiolated poly (acrylic acid) acts as an inhibitor of Mrp2 mediated transport. The extent of inhibition depends on the molecular mass and degree of thiolation of the polymer.

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EVALUATION OF A NEW MUCOADHESIVE PATCH SYSTEM BASED ON CHITOSAN-GLUTATHIONE

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Several gastrointestinal patch systems provide mucoadhesion, drug protection and unidirectional release [1]. It was the aim to develop a patch system based on thiolated chitosan, known as thiomer, providing improved mucoadhesive and permeation enhancing properties.

Thiomers were shown to exhibit improved mucoadhesive, controlled release, permeation enhancing and enzyme inhibitory properties [2]. In order to combine both promising strategies Chitosan-Glutathione (Ch-GSH) was used as multifunctional layer with the protective coating of a patch system.

For mucoadhesive studies patch systems with Chitosan-Glutathione (Ch-GSH) were compared to patch systems with chitosan as a carrier matrix. Patch systems based on Ch-GSH conjugate remained even after 180 hours of incubation attached to mucosa. In contrast, the corresponding control detached from mucosa within 24 hours

Permeation studies with Ch-GSH/GSH patch systems demonstrated significant enhanced absorption in comparison with chitosan patch systems. A Ch-GSH/GSH patch system resulted a 2.1-fold higher transport of FD_4 in comparison to patch systems with chitosan and unbound GSH.

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IN VITRO EVALUATION OF CHITOSAN-EDTA CONJUGATE POLYPLEXES AS NANOPARTICULATE GENE DELIVERY SYSTEM

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OBJECTIVE: The purpose of this research was to evaluate the use of different molecular-weight and modification-rate chitosan-EDTA conjugates for preparation of nanoparticulate gene delivery system.

METHODS: Chitosan-EDTA conjugates were synthesized as described previously [1]. Chitosan control particles were prepared by mixing of 250 µl pDNA solution (100 µg/ml in distilled water) with 400 µl 0.02% (w/v) chitosan solution pH 4. Chitosan-EDTA particles were prepared by mixing 250 µl pDNA solution with 80 µl 0.4% chitosan-EDTA solution pH 4 and 570 µl distilled water. Generated particles were characterised by analysing size and zeta potential via photon correlation spectroscopy. Cytotoxicity of these nanocomplexes was determined with the lactate dehydrogenase test using Caco-2 cell line. Transfection efficiency of nanocomplexes on Caco-2 cells was measured via beta-galactosidase activity after transfection with pSV-β-Galactosidase Control Vector®.

RESULTS: Chitosan-EDTA conjugate produced from low viscous chitosan with a degree of modification of 68% showed the highest complexing efficacy resulting in complexes of 70 nm mean size. The cytotoxicity was below two percent over a time period of four hours. Chitosan-EDTA nanoplexes showed improved transfection efficiency compared to unmodified chitosan nanoparticles.

CONCLUSION: Chitosan-EDTA might be a possible alternative to chitosan as gene delivery system used at low pH values.

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ORAL GENE DELIVERY: DESIGN OF POLYMERIC CARRIER SYSTEMS SHIELDING TOWARDS INTESTINAL ENZYMATIC ATTACK

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The gastrointestinal tract sets a varietis of morphological and physiological barriers that can limit internal absorption of therapeutic gene [1]. In order to improve the efficacy of orally given gene delivery systems by providing an improved stability towards the harsh intestinal fluid, it was the aim of this study to generate and characterize a nanoparticulate gene delivery system being based on chitosan and DNase inhibitor. As inhibitory agent aurintricarboxylic acid (ATA) was chosen, as it has already been used as nuclease inhibitor in the GI tract [2] in order to enhance the transfection rate in gene delivery [3]. Chitosan-ATA/pDNA nanoparticles showed a size of 98.5 ± 26 nm and a zeta potential of -13.26 ± 0.24 mV (n=3-4). Stability studies with salt solution, lysozyme, DNase, and freshly collected porcine intestinal fluid showed that chitosan-ATA/pDNA nanoparticles are significantly ($p<0.05$) more stable than unmodified chitosan/pDNA nanoparticles. A part from improved stability, chitosan-ATA/pDNA nanoparticles showed a 2.6-fold higher transfection rate than chitosan/pDNA nanoparticles in Caco-2 cell line, thus being promising carrier for orally administered therapeutic genes.

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CRYSTAL POLYMORPHISM OF BUPIVACAINE HYDROCHLORIDE

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Bupivacaine hydrochloride (BPVCN-HCl) is a local anaesthetic drug of the amide type. The monohydrate is specified the European and United States pharmacopoeia. The present study presents a comprehensive characterization of the solid state properties of the hydrochloride salts of the racemate and the S-enantiomer applying a variety of analytical techniques such as thermal analysis, IR and Raman spectroscopy, moisture sorption analysis and X-ray diffraction methods.

The monohydrate is stable in a dry atmosphere (25°C) and desolvates not below 115°C. The anhydrous mod. A (T_{fus}: ca. 257°C under decomposition) is obtained by dehydration of the monohydrate or by lyophilisation. A highly crystalline material of this form can be produced by crystallization from an acetonitrile-solution. Two new anhydrous forms were obtained by crystallization from chloroform (mod. B) and ethylacetate (mod. C), which was confirmed by powder X-ray diffraction. Furthermore, a hemi-ethanol-solvate for which the crystal structure is already known [1, 2] was reproduced and characterized by various methods. Additionally, the single crystal structures of methanol-, 1-propanol- and 2-propanol solvates were determined. The comparison of the crystal structures (all monoclinic, $P2_1/c$) of these solvates shows that they are isostructural. It is likely that this type of structure can also host other solvents such as butyl alcohol, acetone or even lower alkanes. Upon thermal desolvation mod. A, mod. C or mixtures of these forms are obtained.

The (S)-isomer of BPVCN-HCl, which shows less cardiotoxicity than the racemate, was present as mod. II in commercial samples. On heating, a spontaneous phase transition to a new form (mod. I, T_{fus}: 254.5, $\Delta_{fus}H$: 24.3 ± 1.0 kJ/mol) can be observed at 89.0°C ($\Delta_{trs}H$: 4.9 ± 0.5 kJ/mol), which is associated with strong movement and jumping of the crystals due to a fast volume expansion. Mod. I transforms back to mod. II at 85°C on cooling. The two forms are enantiotropically related.

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FEUCHTESORPTION VON CALCIUMPHOSPHAT

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In einer früheren Arbeit [1] wurde gezeigt, dass als Tablettierfüllstoff deklarierte Calciumphosphatproben verschiedener Hersteller aus unterschiedlichen kristallinen Formen zusammengesetzt sein können. Etwa die Hälfte der untersuchten Proben entsprachen nicht der vom Hersteller angegebenen Zusammensetzung. Neben Dicalciumphosphat Anhydrat (Monetit) und -Dihydrat (Brushit) können Monocalciumphosphat-Anhydrat und -Monohydrat sowie Tricalciumphosphat (Withlockit) und sogar Hydroxylapatit sowie Mischungen aus diesen Phasen vorliegen. Diese hinsichtlich Zusammensetzung (Pulverröntgendiffration, Elementaranalyse) und Partikeleigenschaften (Helium-Pyknometrie, spezifische Oberfläche, Schütt- und Stampfvolumen, Böschungswinkel, Partikelgrößenverteilung) sehr gut voruntersuchten Proben wurden im Rahmen der vorliegenden Arbeit tablettiert und die Pulver sowie die Tabletten auf ihr Feuchtesorptionsverhalten hin untersucht.

Aus den Pulvermassen (n=25) wurden mit einer instrumentierten Presse biplane Tabletten mit einer Masse von 1,0 g und einem Durchmesser von 13 mm mit einer Presskraft von 15 kN hergestellt (n=5). Die Bestimmung der Porosität und Porenverteilung der Komprimate erfolgte mit Hg-Porosimetrie und zusätzlich wurde die Gesamtporosität auch aus der Masse, dem Tablettenvolumen sowie der wahren Dichte der jeweiligen Probe berechnet. Die gemessenen und berechneten Porositätswerte zeigen eine gute Übereinstimmung. Die Sorptionsisothermen (Sorptionsprüfschrank SPS11-10µ) der Komprimate zeigten im Vergleich zu den Pulvermassen einen ähnlichen Verlauf, aber eine generell höhere Feuchteaufnahme und Hysterese zwischen Sorption und Desorptionszyklus. Dies ist vermutlich auf Kapillarkondensationseffekte zurückzuführen. Tabletten aus hydroxylapatithaltigen Proben zeigten auffällig kleinere Porenradien (20 bis 40 nm). Unabhängig davon zeigen diese Proben auch das mit Abstand größte Feuchtesorptionsvermögen von etwa 5-10% m/m (andere Proben meist <1% m/m). Ferner wurden auch feuchteinduzierte Phasenumwandlungen beobachtet. Die Untersuchungen demonstrieren klar die Rohstoffproblematik von Hilfsstoffen in der pharmazeutischen Produktion.

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EVALUATION OF THIOLATED POLY(ACRYLIC ACID) 450 KDA WITH RESPECT TO ITS COUPLING RATE

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The objective of this study was to examine the mucoadhesive and permeation enhancing properties of thiolated 450 kDa poly(acrylic acid) with six different amounts of L-cysteine covalently attached to it (PAA I 53.0 ± 1.8; PAA II 113.4 ± 1.6; PAA III 288.8 ± 9.7; PAA IV 549.1 ± 4.2; PAA V 767.0 ± 14.6 [µmol thiol groups/g polymer]).

The obtained conjugates were characterized in vitro by quantification of immobilized thiol groups, by their mucoadhesive properties on freshly excised porcine mucosa and by their permeation enhancing properties on a Caco-2 cell culture monolayer system.

In order to evaluate the mucoadhseive properties, 30 mg tablets of the six different thiolated polymers were attached to freshly excised porcine mucosa, which was spanned on a stainless cylinder and rotated in pH 6.8 phosphate buffer [1]. Results showed that the higher the amount of L-cysteine covalently attached to the thiomer is, the longer the residence time on the mucosa. The thiomer PAA V exceeded PAA I by a factor of 15.5 [2].

Permeation studies revealed that unlike the mucoadhesive properties, permeation enhancing effects are not directly proportional to the coupling rate. Apparent permeability coefficients display the influence of the different thiomers on the permeation of sodium fluorescein: PAA III 11.1 ± 0.7 > PAA II 10.1 ± 0.1 > PAA I 9.8 ± 0.2 > PAA IV 8.9 ± 0.4 PAA V 8.2 ± 0.2 > control 6.4 ± 0.2 [$P_{app} * 10^{-6}$, cm/s]

Because of these results poly(acrylic acid) 450 kDa conjugates are suggested to be used with high amounts of immobilized L-cysteine if high mucoadhesive properties are essential.

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COMPARISON OF TWO TYPES OF ARTIFICIAL NEURAL NETWORKS USED FOR VALIDATION OF PHARMACEUTICAL PROCESSES

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Two types of **A**rtificial **N**eural **N**etworks (ANNs), a **M**ulti-**L**ayer **P**erceptron (MLP) and a **G**eneralized **R**egression **N**eural **N**etwork (GRNN), have been used for validation of a modified fluid bed granulator. The training capacity and the accuracy of these two types of networks were compared. Sucrose was granulated using glucose syrup. The variations of inlet air temperature, liquid-binder spray rate, atomizing air pressure, air velocity, amount and concentration of binder solution and batch size were taken as input variables for training the MLP and GRNN. The properties of size, size distribution, flow rate, repose angle, bulk and tapped volumes of granules produced, were measured and used as output variables.

Qualitatively, the two networks gave comparable results, as both pointed out the high importance of the atomizing air pressure to the granulation process. However, the averaged absolute error of the MLP was ten times higher than the averaged absolute error of the GRNN. Furthermore, the correlation coefficients between the experimentally determined and the calculated output values, the corresponding prediction accuracy for the different granule properties as well as the overall prediction accuracy using GRNN were better than using MLP. The product quality parameters of fluid bed granulation can be correlated with the instrumental parameters by various types of ANNs, as non-linear dependencies occur rather often in such processes. The comparison of two different networks (MLP, a so-called feed-forward back-propagation network and GRNN, a so-called Bayesian Neural Network) showed the higher capacity of the latter one for validation of such granulation processes.

DEVELOPMENT AND IN VITRO EVALUATION OF THIOLATED CHITOSAN AS GENE VECTOR

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The objective of this work was to develop and evaluate chitosan-thiobutylamidine as a novel tool for gene delivery. Middle viscous chitosan was depolymerised and modified with 2-iminothiolane. The resulting conjugate, displaying 299.02 ± 11.48 µmol free thiol groups per gram polymer, formed coacervates with pDNA at a mean size of 125 nm and a zeta potential of + 9 mEV. Thiol groups immobilised on the polymeric backbone of chitosan are susceptible for oxidation thereby introducing the property to form reversible disulfide bonds [1]. In order to address the requirements of extracellular stability and intracellular pDNA release [2], the physical stability of these nanoparticles was investigated in various physiological salt solutions. Electrophoretic mobility analysis revealed that the integrity of chitosan-thiobutylamidine-DNA nanoparticles was not significantly harmed under simulated physiological conditions, whereas particles comprising unmodified chitosan disintegrated. Release studies proved a complete pDNA release from chitosan-DNA particles exposed to artificial intestinal fluid within 10 hours, while only 12% were released from the thiolated polymer based particles. The improvement in stability seemed to be directly linked to the formation of disulfide bonds. At pH 7 the amount of thiol groups was rapidly and significantly ($p<0.05$) decreased by more than 25% within 6 hours. In contrast, in a reducing environment as found intracellularly, chitosan-thiobutylamidine-DNA nanoparticles dissociated continuously, liberating approximately 50% of pDNA within 3 hours. Transfection studies performed in a Caco 2 cell culture evinced the highest efficiency for chitosan-thiobutylamidine-DNA nanoparticles in combination with a glycerol shock solution. The combination of improved stability, enhanced pDNA release under reducing conditions, and higher transfection efficiency identifies chitosan-thiobutylamidine as a promising new vector for gene delivery.

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MOLECULAR CALCULATIONS ON CYCLODEXTRIN AND CYCLODEXTRIN INCLUSION COMPLEXES

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The application of molecular calculations in the area of cyclodextrins (CDs) has been strongly limited up to now. The reason for this is that cyclodextrins and cyclodextrin complexes are rather large systems and that they are flexible molecules with numerous local conformational minima. Moreover, experimental data are strongly connected with the influence of the solvent, mostly aqueous solutions. With increasing computational power it is nowadays possible to apply more accurate ab initio or DFT calculations to determine the geometries of the inclusion complexes and the corresponding interaction energies. Ab initio and DFT methods provide the most accurate approximations in molecular calculations including electronic properties. However, beyond very long calculation times they need large amounts of other computer resources, depending on the methods and the basis sets used. Combination of such accurate methods with less expensive procedures may be applied also, as implemented in several QM/MM methods. The reliance of semiempirical methods, which should be also able to calculate electronic properties is generally not very high, as some results, e.g. on hydrogen bond properties, depend strongly on the method used. Force Field methods are widely used for the calculations on CDs, as these methods are convenient for large molecular ensembles, which means, that also water molecules could be incorporated. These force fields are also the basis for techniques, which allow to describe the mobility of the association complexes or the conformational space. Molecular Dynamics, Monte Carlo simulations as well as Molecular Docking are widely used methods, which are mainly based on suitable force fields. The advantages and the disadvantages of several techniques will be demonstrated on particular examples. The geometries of some selected drug/CDs complexes will be compared, also taking into account some information about the interaction energies and the driving forces of the complexation reaction. Another important topic for testing various methods is the determination of the discrimination of chiral compounds by calculating the interaction energies of the individual optical isomers.

APPROACHES TO SOLVE THE PROBLEM OF UNEVEN TISSUE DISTRIBUTION ON CONSTRUCTS IN TISSUE ENGINEERING OF BONE

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One strategy employed in Tissue Engineering of bone is to seed bone marrow stromal cells (MSC) on biodegradable carrier systems and differentiate them in vitro towards bone-like tissue. These living constructs will offer typical extracellular matrix (ECM) and growth factors accelerating bone formation and remodelling after implantation [1]. But one main problem performing 3D cell culture is a nutrient gradient appearing on the constructs resulting in insufficient formation of bone tissue in the inner regions. For this reason, sponge-like scaffolds made of poly(DL-lactic-co-glycolic acid)(PLGA) [2] with 3 different pore sizes were tested in vitro under different culture conditions in order to improve ECM formation, osteogenic differentiation and tissue distribution. We used rat-MSC obtained by centrifugal isolation and proliferated for 8 days. After trypsinisation, 4 x 10^6 cells per scaffold were seeded in spinner flasks for 24h. The scaffolds (pore size: 100-300µm; 300-500µm and 500-750µm) were transferred in 6-well plates and cultured up to 25 days with standard differentiation medium either without agitation (static group) or on an orbital shaker (dynamic group). A third group (perfusion) was cultured in a self-constructed device allowing for an even cultivation of 4 scaffolds in one perfusion chamber. The perfusion rate was adjusted to 2.6ml medium flow per min per chamber pumped in a closed loop. On the whole, the dynamic culture showed considerable advantages over perfusion and static control. The agitation of the medium by the shaker probably results in an enhancement of chemotransport and fluid shear forces. The latter is known to stimulate osteogenic differentiation imitating the physiological interstitial fluid flow in bone [3] and seems to be more pronounced in dynamic than in perfusion culture. Hence, we observed an earlier onset of differentiation (ALP activity and mRNA expression level of BSP, Osteocalcin) and consequently a higher amount of calcium and glycoprotein (BSP, Osteonectin) deposition as markers for the late differentiation in the dynamic group compared to the others. However, looking at the immunohistochemically stained cross sections of the scaffolds with small pore sizes after 25 days of differentiation, we observed a well distributed extracellular matrix after

perfusion culture whereas in the dynamic group tissue formation was focused on the edges of the constructs. This problem can be eliminated by increasing the pore size of the scaffolds to improve nutrient supply and, hence, to enhance bone like tissue formation in the interior of the constructs.

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INCLUSION COMPLEXATION OF VARIOUS TAUTOMERS AND PROTONATION STATES OF MELOXICAM

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Meloxicam, a new non-steroidal anti-inflammatory drug (NSAID), exhibits a high potency in animal tests for potential anti-arthritic action, and has a wider spectrum of anti-inflammatory activity, combined with less gastric and local tissue irritation than othe NSAIDs. Meloxicam is a twobasic acid with a series of tautomers in each protonation state with different intramolecular hydrogen bonds. Under physiological conditions the anionic form of meloxicam is the predominant structure.

The inclusion complexation of meloxicam with various cyclodextrins (CDs) is generally used to improve the solubility and consequently the bioavailability of the drug. Overall equilibrium constants and the corresponding thermodynamic parameters ΔG, ΔH and ΔS were measured for various pH values on the different protonation states. Equilibrium constants and thermodynamic parameters are different for various CDs, depending on the hydrophobicity and the stereochemistry of the cavities and also on the flexibility of the host molecules.

The interaction of the tautomers with the CDs cavities is different. Generally, structures with a higher dipole moments, like zwitterionic forms, are more stabilized in aqueous solution than in the more hydrophobic environment. Therefore, the tautomeric equilibria as well as the protonation and deprotonation steps are shifted.

By molecular calculations on ab initio of DFT level the energy differences between all tautomeric forms of meloxicam can be determined at least qualitatively, without taking into account the influence of the solvent. Moreover, the affinities of the individual tautomers and conformers to CDs can be roughly estimated.

These experimental and theoretical methods.should give some insight into the behaviour of this in fact rather complicated reaction system.

CHARACTERIZATION OF THREE PHENOBARBITAL SOLVATES

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Phenobarbital (PBTL, 5-Ethyl-5-Phenyl-2,4,6(1H, 3H, 5H)-pyrimidinetrione) is widely used as sedative and as anticonvulsant in the treatment of epilepsy. The barbiturate can crystallize in at least eleven polymorphic modifications [1, 2] which is probably the highest number of known polymorphs among small organic molecules. Additionally a hydrate and a dioxane solvate have been identified so far [3]. In the course of a reinvestigation of this complex polymorphic system, two new solvates were found which were characterized along with the known dioxane solvate by X-Ray diffraction, different thermal analytical methods as well as infrared and Raman spectroscopy.

The dioxane solvate (S_{Dox}) was found to crystallize in the monoclinic crystal system ($P2_1/c$), whereas the acetonitrile (S_{AcN}) and nitromethane (S_{NiMe}) solvates are isostructural and belong to the triclinic system (P-1). The order of the calculated densities ($S_{NiMe} > S_{Dox} > S_{AcN}$; 1.376, 1.327 and 1.257 g/cm^3 respectively) correlates with the order of the melting points (126, 100.5, 92 °C) and thermal stabilities. Thermal analysis revealed that the desolvation at elevated temperatures results in mixtures of modification (mod.) III and II. The mixture ratio depends on the heating rate. At low relative humidities (0-30% RH) all solvates transform to mod. III but in aqueous media they transform quickly to the hydrate. This transition was monitored by FT-Raman spectroscopy in order to detect possible intermediate forms. Storage of the solvates at 98% RH either results in the hydrate or different polymorphic forms. This complex transformation behavior can be only explained by considering the thermodynamic stabilities of the various polymorphic forms and the hydrate as well as the interplay of the molecular interactions in the different crystal structures.

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ELASTASE UND BENIGNE PROSTATAHYPERPLASIE – ERSTE ERGEBNISSE ZUR BRENNNESSELWURZEL

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Zubereitungen aus den Wurzeln von *Urtica dioica* und *U. urens* (Brennnessel) stellen wichtige Phytopharmaka zur Behandlung der benignen Prostatahyperplasie (BPH) dar. Als pharmakologisch relevante Drogeninhaltsstoffe gelten Lektine und Polysaccharide, wobei entsprechende Extrakte zusätzlich eine ausgeprägte Inhibition des Enzyms Elastase zeigten [1,2]. Da die Ätiologie der BPH noch nicht eindeutig geklärt ist, und neben anderen Faktoren auch entzündliche Prozesse eine Rolle spielen dürften, wird eine Beteiligung der Elastase an der Entstehung der Erkrankung diskutiert [3].

Die für die Elastase-Inhibition verantwortlichen *Urtica*-Inhaltsstoffe sind bislang noch nicht bekannt. Es war daher Ziel dieser Arbeit mittels „bio-guided fractionation“, ein entsprechender *in-vitro* assay wurde etabliert und wird kurz beschrieben, die aktiven Inhaltsstoffe in der Brennnesselwurzel zu finden bzw. die aktive Region einzugrenzen. Wie die ersten, hier präsentierten Ergebnisse zeigen, dürfte es sich dabei um relativ polare, hochmolekulare, ionische Verbindungen handeln.

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ACHILLEA MILLEFOLIUM S.L. – CHOLERETIC AND SPASMOLYTIC ACTIVITY OF THE PHENOLIC COMPOUNDS

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Yarrow (*Achillea millefolium* s.l.) is traditionally used in the treatment of digestive complaints such as gastro-intestinal and hepato-biliary disorders or lack of appetite [1]. While the antiinflammatory activity is mediated by sesquiterpenes [2], the choleretic and spasmolytic principles of the drug are still unknown. Candidates for those effects are the phenolic compounds being also present in yarrow.

Hence, a 20% methanolic extract of a commercial sample of yarrow was purified by solid phase extraction yielding two fractions enriched in dicaffeoylquinic acids and flavonoids, respectively. HPLC analysis revealed a total amount of 48.8% dicaffeoylquinic acids in one fraction and 10.2% flavonoids in the other. The pharmacological activity was assessed in two *in vitro* test systems: We investigated the choleretic activity of the dicaffeoylquinic acid fraction in the isolated perfused rat liver using cynarin as positive control [3], whereas the antispasmodic effect of the flavonoid fraction was investigated on isolated terminal guinea-pig ilea in comparison to different flavonoids and their metabolites [4]. We could show both, the choleretic activity of the dicaffeoylquinic acid fraction as well as the spasmolytic activity of the flavonoid fraction, which justifies the traditional application of yarrow for the respective indications.

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POWERFUL LC-SPE-NMR IDENTIFICATION OF FARNESYLPHENOLS AND THEIR ISOLATION FROM *ALBATRELLUS SUBRUBESCENS*

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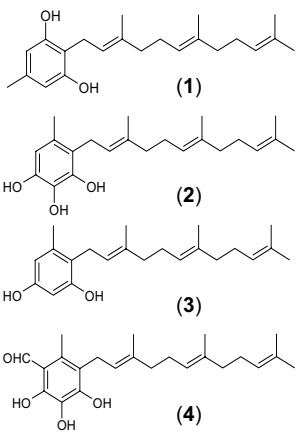
Although *Albatrellus subrubescens* is known as a local, edible mushroom, occasional poisoning is mentioned in literature [1]. A thourough phytochemical investigation of *A. subrubescens* is however missing. Our intention was to identify its main constituents and to isolate them for pharmacological investigations.

HPLC analysis of the ethylacetate soluble fraction of the methanol extract revealed four major compounds (**1**-**4**). By LC-MS and UV detection they could be identified as farnesylphenolic constituents with molecular masses of 328, 344, 328, and 372, respectively. Due to partial degradation of compound **2,** the extract was subjected to LC-SPE-NMR analysis, which resulted in a complete and rapid structure elucidation of all four analytes, namely grifolin (**1**), ovinol (**2**), neogrifolin (**3**) and scutigeral (**4**) [2]. For evaluation of the toxic profile, compounds **1**, **3** and **4** were isolated using Sephadex LH-20 CC and semipreparative HPLC.



LC-SPE-NMR turned out as method of choice to get structural information prior to a time-consuming isolation procedure.

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ISOLATION OF SESQUITERPENE LACTONES FROM A *PETASITES HYBRIDUS* RHIZOME EXTRACT

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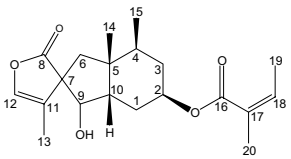
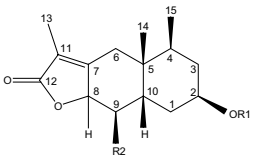
Petasites hybridus rhizomes are traditionally used against diseases of the respiratory tract like bronchial asthma, to prevent migraine attacks and as a spasmo-analgesic remedy in the gastro-intestinal tract [1]. Recently we found a direct inhibition of cyclooxygenase-2 (COX-2) *in vitro* and a prevention of p42/44 MAP kinase activation in rat primary microglial cells by several commercial butterbur extracts. Those effects were independent of the content of petasins [2]. During the search for the active principles, several sesquiterpenoids of the eremophilane type could be isolated and were identified by NMR experiments and comparison with literature data [3, 4]. Besides seven known constituents, we identified the following new compounds: 2-isobutyryl-8α-H-eremophilanolide (**1**), 2-methacroyl-8α-H-eremophilanolide (**2**), 2-methacroyl-8β-H-eremophilanolide (**3**), 2-Tig-8α-H-eremophilanoldie (**4**), 2-Tig-8β-H-eremophilanolide (**5**), 2-Sen-8α-H-eremophilanolide (**6**), and 8β-H-9β-hydroxy-petasitolide-A (**7**).



1



2 8α-H; R1 = 2-methacroyl-; R2 = H
3 8β-H; R1 = 2-methacroyl-; R2 = H
4 8α-H; R1 = 2-tigloyl-; R2 = H
5 8β-H; R1 = 2-tigloyl-; R2 = H
6 8α-H; R1 = 2-senecioyl-; R2 = H
7 8β-H; R1 = 2-angeloyl-; R2 = OH

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PHYTOCHEMICAL INVESTIGATION OF *MYRICARIA LONGIFOLIA* EHRENB. – A PLANT USED IN TRADITIONAL MONGOLIAN MEDICINE

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In an ongoing project we focus on the investigation of plants used in traditional Mongolian medicine to treat liver disorders such as inflammation or malignant tumours. The goal is to find new compounds for therapeutic approaches of chronic and malignant diseases of the liver. The crude water extract of one of these plants, *Myricaria longifolia* EHRENB., has shown a slight inhibition on the cell growth of liver carcinoma cells ($HepG_2$), moreover the growth of breast cancer cells (MCF-7) was clearly inhibited [1, 2]. This cytotoxic effect was confirmed by the fact that the aqueous extract caused damage in the isolated rat liver during perfusion experiments [1]. Therefore, phytochemical analyses were carried out in order to characterize and identify the main constituents that might be responsible for these effects.

In comparison with authentic substances the compounds β-sitosterol, rhamnetin, quercetin, isoferulic, caffeic and syringic acid were identified. Furthermore, characteristic fragments in the mass spectra pointed to the presence of flavonoids and their glycosides which occur as multiple substituted sulphates. Data in literature about *Myricaria longifolia* are poor, but flavonoids of this type have been described [3]. In this contribution we present all techniques that were employed (one- and two dimensional TLC, HPLC-DAD-UV and LC-MS) and all data that were collected to characterise the main compounds of the aqueous extract.

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ANTIMYCOBACTERIAL ACTIVITY OF *LASERPITIUM SILER L.* ROOTS

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The widespread re-emergence of tuberculosis and prevalence of multidrug-resistant (MDR) mycobacterial strains requires the development of new effective agents against this dangerous disease. Plants are an excellent source for a variety of new lead compounds. After an antimycobacterial pre-screening using a minimum inhibitory concentration (MIC) assay, the roots from *Laserpitium siler L. (Apiaceae)* were chosen for further investigations as the hexane and dichloromethane extracts of the roots exhibited good activities against fast-growing mycobacteria (MIC = 64 µg/ml).

Dereplication for polyacetylenes and unsaturated fatty acids was carried out by GC-MS [1,2]. Bioassay-guided fractionation produced active fractions with two-fold decreased MICs which revealed that more compounds might act synergistically. Continuos fractionation led to the isolation of two compounds. The structure of the main compound (**1**) was determined by 1D and 2D NMR and was identified as isomontanolide, which is a known compound of *L. siler* [3]. It showed reduced bacterial growth at 128 µg/ml. The minor compound (**2**) was active at 64 µg/ml. GC-MS analysis of the most active fraction let us assume that falcarinol and other polyacetylenes might also contribute to the antimycobacterial activity of the underground parts of *L. siler*. The structure elucidation of (**2**) is still in progress.

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ANALYTIK DER FLAVONOIDE UND PHENOLCARBONSÄUREN AUS *THYMUS VULGARIS*

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Thymus vulgaris und daraus hergestellte Extrakte werden vor allem zur Behandlung von Atemwegserkrankungen (Bronchitis, Keuchhusten und Katarrhe der oberen Luftwege) verwendet.

Zur Beurteilung der Qualität der Arzneidroge wird im Europäischen Arzneibuch nur das ätherische Öl herangezogen, das expektorierende und antimikrobielle Wirkung zeigt [1]. Die ebenfalls enthaltenen nichtflüchtigen phenolischen Inhaltsstoffe (Flavonoide, Phenolcarbonsäuren) können wesentlich für die bronchospasmolytische [2] als auch antiphlogistische Wirksamkeit [3] verantwortlich gemacht werden, weshalb eine entsprechende Analytik dieser Stoffgruppen zur umfassenden Qualitätsbeurteilung der Arzneidroge und daraus hergestellten Extrakten notwendig ist.

Unter Verwendung einer speziellen stationären Phase (Phenomenex® Luna Phenyl-hexyl) konnte eine HPLC-Methode zur Trennung der nichtflüchtigen phenolischen Inhaltsstoffe aus Thymianextrakt entwickelt werden. Alle Hauptkomponenten konnten mittels Festphasenextraktion und präparativer RP-HPLC isoliert werden, die Strukturaufklärung gelang mit Hilfe von UV-Spektren, Massenspektrometrie und NMR-Spektroskopie. Auf diese Weise konnten sieben Flavonoide und zwei Phenolcarbonsäuren identifiziert werden.

Zur Quantifizierung wird Homoprotocatechussäure als interner Standard verwendet, damit steht eine HPLC-Analysenmethode zur qualitativen und quantitativen Beurteilung von Thymiantrockenextrakten hinsichtlich der Flavonoide und Phenolcarbonsäuren zur Verfügung.

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CUTICULAR STRIATION IN *ILLICIUM VERUM* AND *I. ANISATUM*

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Recently a pharmaceutical company was supposed to have bought up about 90% of the harvest of the *Illicium verum* Hook. f. (Chinese Star anise) for the isolation of shikimic acid as starting material for the synthesis of Oseltamivir [1]. The resulting shortage may cause adulterations with the morphologically similar but toxic *I. anisatum* L. (Shikimi fruit). Neither known morphological nor anatomical characters seem to have the potential to serve as reliable markers for a quick differentiation [2]. We tried to evaluate the structure of the cuticular striation, which lately is said to be different between the mentioned species [3].

7 samples of *I. verum* and 4 of *I. anisatum* have been analysed by means of light microscopy, fluorescence microscopy, SEM, TLC and GC/MS. All samples showed the respective typical composition of the essential oil [4]. The structure of the cuticular striation (Fig. 1) shows even on several fruitlets within an follicetum a considerable variability. Our investigations suggest that the cuticula does not provide reliable characters for the differentiation of *I. verum* and *I. anisatum*.

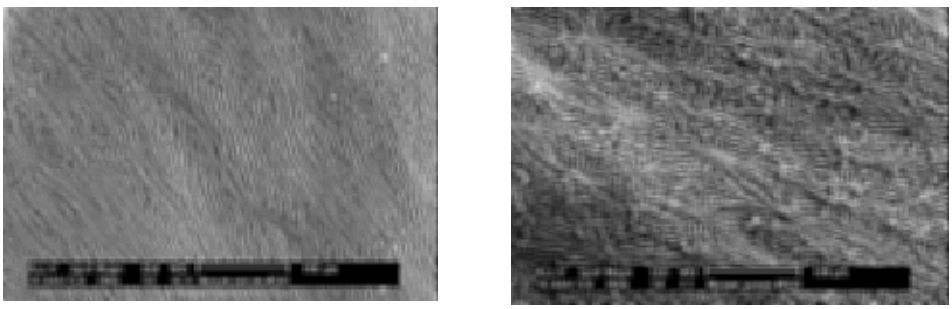

Fig. 1: Examples of the cuticular striation on the follicles of *Illicium verum* (SEM)

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PHYTOCHEMICAL INVESTIGATION OF *GENTIANA BARBATA* FROEL. – A PLANT USED IN TRADITIONAL MONGOLIAN MEDICINE

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Since ancient time Mongolian people have treated liver diseases with many herbs and plants of the genus *Gentiana*, especially *Gentiana barbata* Froel. which takes the main position among them [1]. More than 20 species of the genus *Gentiana* (Gentianaceae) grow on Mongolian territory. In literature xanthone derivatives have been reported as constituents of *Gentiana barbata* and other genera of the Gentiana family [2, 3]. Experiments with aqueous extracts of the aerial parts of the plant showed an increase of the bile flow in the perfused rat liver [4] and, therefore, prompted the phytochemical investigation of *Gentiana barbata*.

The plant material (20g) collected in summer of 2003 was extracted consecutively with petroleum ether (ultra sound, room temperature, 20 minutes) and dichloromethane (reflux, 40°C, 30 minutes). The two extracts were fractionated by Solid Phase Extraction on RP-8 material with 40%, 60%, 80% methanol and pure methanol as eluents. The fractions were checked by TLC and yielded three compounds which were obtained by crystallization. One further compound resulted from the 60% methanolic fraction after purification by CC (stationary phase: silica gel; mobile phase: chloroform). The four substances were characterized by R_f-values, their reaction with different detection reagents on TLC, UV, MS (EI-MS, ESI-MS) and IR-spectroscopy. Two of them were identified as β-sitosterol and dihydroxy-dimethyl-xanthone. Furthermore, a HPLC method was developed in order to compare different extracts and plants from different origin.

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AMOUNT OF ANTIOXIDATIVE CONSTITUENTS IN FLOWERS OF *SAMBUCUS NIGRA* L. GROWING AT DIFFERENT ALTITUDES

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In modern phytotherapy elder flowers (*S. nigra* L.) act as diaphoretic and bronchospasmolytic ingredients in herbal teas treating common cold [1]. In literature the activity of the extracts is mainly accredited to its content of flavonoids and hydroxycinnamic acids, substances with the potential of acting as strong radical scavengers. However, there is only limited information about the variation of antioxidative active compounds in *S. nigra* at different altitudes.

For our investigation, we selected the Naturpak Sölktäler in Upper Styria (Austria) in order to have comparable climatic conditions. The variation of the main flavonol glycosides, rutin and isoquercitrin and the main hydroxycinnamic acids (chlorogenic acid among others) in *S. nigra* L. were investigated in two consecutive growing periods. The air-dried flowers obtained from samples collected at 670m and 1000m above sea level were subjected to accelerated solvent extraction (ASE) and subsequently analysed using RP-HPLC/PDA [2, 3] and LC-MS. Among the hydroxycinnamic acids determined at 320nm wavelength, we found no significant difference between the two groups investigated. Specimen collected at 670m above sea level showed significantly higher amounts of rutin and significantly lower values of isoquercitrin (quantification at 350nm) in both years. Radical scavenging activity was assessed by reaction with the stable radical DPPH (diphenylpicrylhydrazyl) [4]. The IC_{50} values of the extracts ranged from 10,5 to 23,1 µg/mL.

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TASPINE – A POTENT ACETYLCHOLINESTERASE INHIBITING ALKALOID FROM *MAGNOLIA SOULANGIANA*

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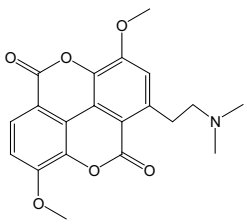
Neurodegenerative impairments, like Alzheimer's disease (AD), are preferentially treated with acetylcholinesterase (AChE) inhibitors because of the direct correlation of cholinergic deficit in the patients' brain and the severity of dementia. Natural products have already proven to be a promising pool for the discovery of AChE inhibiting agents, e.g. galanthamine and huperzin A. The aim of this study was to explore plant extracts in order to discover new lead structures from nature.

In the course of a extract screening using a microplate enzyme assay with Ellman's reagent [1, 2] the methanol leave extract of *Magnolia soulangiana* Lennei showed a 89 % AChE inhibitory activity (c= 1 mg/mL extraxt). Chromatographic separation and bio-guided fractionation resulted in the isolation of a highly active alkaloid, which was identified by 1D and 2D-NMR experiments as taspine. In the enzyme assay taspine showed a dose dependent and long-lasting inhibitory effect which was determined as 10 times more potent (IC_{50} 333 ± 70 nM) than that of the reference substance galanthamine.



Interestingly, taspine did not fit in our evaluated pharmacophore model [2]. Docking studies suggested that taspine binds in an alternative binding orientation than galanthamine. While galanthamine is located in close vicinity to catalytic amino acid residues, taspine binds as a "plug" at the end of the hydrophobic channel that leads in the catalytic center of the enzyme.

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NMR BASED METABONOMICS IN COMBINATION WITH LC-SPE-NMR SUPPORTED METABOLITE CHARACTERIZATION AS A NOVEL ANALYTICAL PLATFORM TO CLASSIFY BIOLOGICAL COMPLEXITY: CHEMOTAXONOMICAL INVESTIGATIONS OF *LEONTOPODIUM* (EDELWEISS) AS CASE STUDY.

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Characterizing the secondary metabolite profile of *Leontopodium alpinum* Cass. (Asteraceae), the Edelweiss, in phytochemical and pharmacological terms has been a major focus of our research within the last years [1-3]. Investigations showed a rather complex secondary metabolite pattern, not only in the European, but also in the more than thirty Asian *Leontopodium* species. In fact, the highly complex secondary plant metabolite pattern of the genus *Leontopodium* seems not be subsumable with a single analytical technique like HPLC, GC or CE. To overcome this problem, the classic phytochemical process was replaced by a NMR-based metabonomic approach combined with LC-SPE-NMR as structure elucidation tool. NMR based metabonomics of 12 different *Leontopodium* species allowed to characterize chemosystematical differences within <25% of the time needed for the conventional HPLC-DAD approach. The structure of the chemotaxonomical discriminator of *L. souliei* Beauverd, one of the most outstanding species, was elucidated from an extract of ~5g plant material. LC-SPE-NMR experiments with this extract (about 1 mg used) allowed obtaining a complete set of 1D and 2D NMR spectra within two days. The found analyte, 12-acetoxymodhephene, is a novel natural product.

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IN VITRO-VERMEHRUNG VON RUNDBLÄTTRIGEM SONNENTAU IN FLÜSSIGKULTUR

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Drosera rotundifolia L., der Rundblättrige Sonnentau, wird seit Jahrhunderten arzneilich eingesetzt. Neben 1,4-Naphthochinonen (v.a. 7-Methyljuglon) sind auch Flavonoide für die antibakteriellen, spasmolytischen und entzündungshemmenden Eigenschaften von *Drosera*-Extrakten verantwortlich [1]. Nachdem Hochmoore als die natürlichen Habitate von *D. rotundifolia* stark dezimiert sind, wurden auch andere Sonnentau-Arten eingesetzt [1] – letztlich änderte das jedoch nichts an der schwierigen Beschaffbarkeit der nötigen Mengen an Arzneidroge. Als Alternative zur ohnehin problematischen Wildaufsammlung würde die Kultivierung auch die Möglichkeit der Produktion von Droge einheitlicher Qualität bieten. Über in vitro-Kultur vermehrte Pflanzen können dabei als Setzlinge dienen [2, 3]. Darüber hinaus liesse sich in einem entsprechend effizienten in vitro-System auch Frischdroge für die direkte Verwendung zu homöopathischen Zwecken herstellen, zumal Gehalt und Zusammensetzung der Wirkstoffe in vitro höher sein können als in vivo [3].

Der Einsatz von flüssigen Nährmedien anstelle der üblichen festen Nährböden bietet eine Reihe von Vorteilen, welche letztlich in niedrigeren Produktionskosten resultieren [4]. In der vorliegenden Studie wurde in einem 2-Stufen-System die Vermehrung von *D. rotundifolia* in Flüssigmedium optimiert, wobei neben kontinuierlicher Submerskultur auch mit Temporärimmersion gearbeitet wurde. Vor allem letztgenannte Systeme ermöglichen eine deutlich höhere Vermehrungsrate und erlauben im Vergleich zum Einsatz von festen Nährböden eine einfachere und kostengünstigere Produktion von Sonnentau-Pflanzen.

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HPLC-BESTIMMUNG DER HAUPTSAPONINE VON *SOLIDAGO GIGANTEA*

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Die vier Hauptsaponine von *Solidago gigantea,* sind Bisdesmoside des Bayogenins, mit 9 (Giganteasaponin 1 und 2) bzw. 10 Zuckern (Giganteasaponin 3 und 4), die sich nur geringfügig in ihrer Zuckerkette unterscheiden [1]. Aufgrund dieser großen strukturellen Ähnlichkeit konnten sie bisher weder auf Umkehr- noch Normalphasen ausreichend getrennt werden, zur HPLC Quantifizierung war daher die Kombination beider Materialien und damit die Durchführung dreier Analysenläufe zur Erfassung aller vier Saponine erforderlich [2]. Mit der hier vorgestellten Methode ist es durch den Einsatz von Aquasil C-18, einer „polaren Umkehrphase", möglich, alle vier Hauptsaponine in einem einzigen Lauf zu trennen und mittels internem Standard zu quantifizieren.

Die getrockneten und pulverisierten oberirdischen Teile von *Solidago gigantea* (0,5g) wurden mit 80% Methanol (v/v, 50ml) eine Stunde unter Rückfluss extrahiert und anschließend über eine C18 Kartusche vorgereinigt. Der interne Standard Digitoxin wurde im Zuge dieser Vorreinigung zugesetzt (1,35mg/100ml Methanol 40% v/v) und mit den Saponinen gemeinsam eluiert. Die anschließende HPLC-DAD Analyse erfolgte an Aquasil C18 mit Acetonitril-Wasser (pH 5) in einem Gradientensystem, detektiert wurde bei 210nm. Die den vier Hauptsaponinen entsprechenden Peaks waren zuvor durch LC-MS Kopplung identifiziert worden. Analysiert wurde Drogenmaterial von verschiedenen Standorten, zur Überprüfung der Reproduzierbarkeit wurden jeweils mehrere Extraktionen durchgeführt, die wiederum mehrfach bestimmt wurden.

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IN VIVO NITRIC OXIDE (NO) MONITORING : THE ROLE OF NO IN M1 ACETYLCHOLINE RECEPTORS(M1 ACHRS) MEDIATED EFFECTS IN THE NUCLEUS ACCUMBENS (NAC)

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The NAc is a brain region with highest densities of M1 receptors. Cholinergic interneurons form interfacing contacts between dopaminergic and glutamatergic afferents. ACh release is generated by glutamatergic input terminals via NO. However little is known about the effects and exact function of ACh in NO mediated DA GLU and ACh release. Our aim was to develop a method to monitor NO release in the NAc with parallel quantification of neurotransmission (NTM). We studied the significance of M1 AChR in the NAc for NO formation and NTM in dependence on NO synthesis. An eventual crosstalk between the M1 and NMDA receptor to induce NO release was furthermore investigated. NO was continously monitored in the accumbens of the anaesthetized rat by a newly designed cannula that combines the push-pull technique with an amperometric NO sensor and allowed NO measurement in a nanomolare range. The technique was validated with TTX and the NOS inhibitors 7-NINA and L-NNA. Superfusion of this nucleus with these drugs or i.p. injection of the inhibitors substantially decreased accumbal NO release. NMDA increased the NO response in dose dependent manner while the M1AChR agonist McN-A-343 induced enhancement of NO decreased with increasing concentration of the agonist suggesting a cumulative M4 effect. These effects were abolished by the nonselective NOS-inhibitor L-NNA. Prolonged stimulation of the M1 receptor resulted in an enhancement of NMDA mediated NO production. Therefore M1 receptors might promote NMDA dependent NO formation and subsequent synaptic plasticity in the NAc. McN-A-343 also raised the accumbal levels of GLU, DA and ACh which were abolished or greatly reduced by the selective nNOS inhibitor 7-NINA. These experiments provide the first direct evidence that M1 receptors mediate NO production in the NAc indicating a fundamental role for the control of NTM and neurotoxic processes in association with NMDA receptor stimulation.

ROLE OF M1/M4 ACETYLCHOLINE RECEPTORS (ACHRS) IN AMPHETAMINE (AMPH)-INDUCED NEUROTOXICITY STRIATUM (ST) AND PREFRONTAL CORTEX (PFC)

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Oxidative stress has often been implicated in dysfunctional states and neurodegeneration in ST and PFC. In the rat model of acute AMPH neurotoxicity (5mg/kg ip; 3x in time intervals of 2h) an increase of striatal ACh is associated with elevated nitric oxide (NO) synthesis and raised generation of lipid peroxidation (LPO) in PFC and ST. The interrelationship of these processes is not well understood. In our study, the rise in NO (determined by electron paramagnetic resonance) and LPO production (determined from thiobarbituric acid reactive substances) by AMPH was prevented in PFC and ST by M1/M4 AChR blockade with pirenzepine (30µg icv) and/or pre-treatment of the ST with microinjected MT7 (2µg), a selective and irreversible M1 antagonist. Activation of M1/M4 AChRs with McNA-343 (200µg icv) enhanced generation of NO by about 70% and LPO about twofold in the striatal and cortical tissue. The increases in these parameters of oxidative stress were prevented by pirenzepine (30µg), L-nitroarginine (100mg/kg ip) and quinacrine (40mg/kg ip). These results indicate that striatal M1/M4 AChRs stimulate NO synthase and phospholipase A2 (PLA_2), inducing oxidative stress leading to phospholipide breakdown. M1 AChRs via NO synthesis and PLA_2 activation play an important role in the neurotoxic effect of AMPH in ST and PFC. Probably the hypercholinergic state induced by AMPH leads to the M1 AChR activation. The described neuronal damage-promoting pathway may be of importance in neurological disorders such as dementia, Parkinson's disease and addiction.

BIOLOGICAL ACTIVITY OF MONO- AND DINUCLEAR ALKYLAMINE PLATINUM (II) COMPOUNDS ON HUMAN LYMPHOMA CELLS

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Cisplatin is used in combination with other agents as an effective salvage regimen for patients with relapsed and refractory non-Hodgkin's-lymphoma (NHL). Treatment success, however, is hampered by intolerable side-effects and the occurrence of inherent and acquired resistance. A variety of multinuclear platinum complexes have been designed to circumvent these limitations. Most of them show potent activity in tumor and leukemia cell lines and in human tumor xenografts. An effect on lymphoma cell lines, however, has not been described yet. Our studies identified the mononuclear chloro[meso-1,2-bis(4-fluorophenyl)ethylenediamine][hexylamine]platinum(II) chloride (**HACl**) and the dinuclear di[meso-1,2-bis(4-fluorophenyl)ethylenediamine]dichloro(μ-1,n-diaminoalkane-N:N´)diplatinum(II) dichloride complexes **DAHCl** (n=6), **DANCl (**n=9) and **DADCl** (n=12) with different alkyl chain length n as promising candidates for extended in vitro testing. We investigated the effects on the proliferation and apoptosis of NHL and chronic myeloid leukemia (CML) cell lines and compared these results with those of cisplatin. All compounds showed concentration-dependent activity on the NHL cell lines U-937 and RAJI, while the proliferation of CML cells was decreased only in the highest used concentration of 20 μM. The antiproliferative effects on the NHL cell lines were accompanied by an increase in apoptosis induced by **DANCl**, **DAHCl**, **HACl** and cisplatin. **DADCl** tended to induce necrosis, suggesting toxicity because cell viability decreased. Similar effects were observed when bone marrow cells from a patient with high grade B-NHL were incubated with the platinum complexes.

The data presented here show that these mono- and dinuclear platinum complexes exert specific activity on human NHL cell lines that is similar to that of cisplatin. The mode of action is unclear. However, it is very likely that the complexes attached to different targets in the tumor cells, because cellular uptake studies have shown that NHL and CML cells incorporate the compounds in similar amounts.

INTERACTIONS OF VALERIAN EXTRACTS WITH ADENOSINE A1- AND GABAA-RECEPTORS

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Several lines of evidence support that adenosine is an endogenous sleep factor. Its level increases during waking and decreases during sleep in the brain. Valerian (valeriana officinalis L.) is known as a mild sleep-inducing, anxiolytic and depressant agent. The fixed combination of valerian/hop (25:6, w/w) Ze 91019 and the valerian extract therein [1] exhibited partial agonistic activity at the adenosine A_1-receptor (A_1R). Moreover, caffeine acts on the A_1R as antagonist and induces CNS arousal that can be blocked by Ze91019 [2].

In this study the A_1-agonistic effect of Ze 911, the valerian part of Ze 91119 macerated with methanol/water, was evaluated and compared with other valerian extracts, macerated with ethanol/water 70% w/w (C1) or with ethylacetat (C3). Therefore intracellular recordings with microelectrodes were obtained from cortical pyramidal cells in layer V in rat brain slices. Synaptic potentials (PSPs) were elicited by rectangular pulses using a bipolar electrode.

Ze 911(1-10mg/ml) inhibited concentration dependently the amplitude of the PSPs. Additionally, high concentrations of Ze 911(5-15mg/ml) induced membrane depolarisation. The inhibition induced by Ze 911 (10mg/ml) was antagonised by the A_1R antagonist DPCPX (0.1µM), suggesting the activation of A_1R. C1 (10mg/ml) did not significantly influence the PSPs. Comparable to Ze 911 it depolarized the cell membrane. However, the valerian extract C3 (10mg/ml) increased the PSPs. This effect was antagonised by the $GABA_A$-receptor antagonist picrotoxin (100µM). The results confirm an activation of A_1R when a hydromethanolic valerian extract is used. In contrast, the ethylacetat extract seems to interact with $GABA_A$-receptors. The results may contribute to elucidate the sleep-inducing effect of Ze 91119**.**

[1] Müller C E, Schumacher B, Brattström A, Abourashed E A, Koetter U. Interactions of valerian extracts and a fixed valerian-hop extract combination with adenosine receptors. Life Sci 2002; 71:1939-1949.