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SECTION OF BIOLOGICAL SCIENCES

ALKALOIDS FROM THE HERB OF *GLAUCIUM FLAVUM* CRANTZ AND THEIR IMPACT ON HUMAN CHOLINESTERASES

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Alzheimer's disease, the most widespread neurodegenerative disease, causes decrease of cognitive functions and dementia. The most effective therapeutic approach is the application of central cholinesterases inhibitors, which alleviate cholinergic deficit in brain and thus improve memory.[1] Currently, intensive investigation of new active compounds including natural substances is carried on.[2]

Within the preliminary testing, alkaloid extract from *Glaucium flavum* Crantz herb showed promising inhibition of human cholinesterases, so it was selected for further examination.

The primary alkaloid extract was acquired from dried flowering herb by extraction with ethanol and subsequent liquid extraction with different pH. This extract was treated by preparative thin layer chromatography. The structure of alkaloids was determined by spectroscopic methods (GC-MS, NMR) and their optical rotation was ascertained.

Four alkaloids were obtained, in yellow horn poppy previously detected isoquinoline alkaloids protopine and (−)-norchelidonine and aporphine alkaloids (+)-cataline[3] and (+)-*N*-methyllaurotetatin.[4]

Subsequently, each alkaloid was tested *in vitro* for their inhibition of human acetylcholinesterase and butyrylcholinesterase by modified spectrophotometric Ellman's method.[5]

(−)-Norchelidonine was evaluated as the most potent inhibitor of AChE (IC50 = 35.1 ± 3.9 μM), however, its activity is not significant enough for further investigation. Other isolated alkaloids were considered to be inactive (IC50 > 100 μM).

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PREPARATION OF HAEMANTHAMINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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The plants of the Amaryllidaceae family are known to contain a specific type of compound, namely the Amaryllidaceae alkaloids. One of them is haemanthamine (1), an isoquinoline alkaloid, which exhibits a wide and important range of biological activities, including antitumor, antiviral, antioxidant, antimalarial and anticonvulsant. The recent studies showed that haemanthamine has also apoptopic effect on leukemia cells and strong cytotoxic potential against gastrointestinal cancer cells.[1] Acetylcholinesterase and butyrylcholinesterase-inhibitory activity was also tested (IC50 HuAChE, HuBuChE > 1000 μM).

In the present work, selective modification on the structure of 1 were carried out to study the relationship structure and biological activity. Fifteen derivatives of 1 were prepared and purified using analytic and preparative TLC. The obtained substances were subjected to structural analysis (NMR, MS methods, optical rotatory). Most of prepared compounds were tested on its possibility to inhibit human erythrocytic acetylcholinesterase (HuAChE) and human serum butyrylcholinesterase (HuBuChE). Some of the analogues were also tested for their cytotoxicity against various cancer cell lines. The most promising compound in HuAChE assay was 11-*O*-(3-fluorobenzoyl)-haemanthamine (IC50 AChE = 79 ± 1 μM), and in HuBuChE assay 11-*O*-(3-methylbenzoyl)-haemanthamine (IC50 BuChE = 25.9 ± 2.6 μM). The results suggest that some haemanthamine analogues provide a useful starting point for future experiments.

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ISOLATION AND BIOLOGICAL ACTIVITIES OF ALKALOIDS FROM *NARCISSUS* CV. PROFESSOR EINSTEIN

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The plant cultivar *Narcissus* cv. PROFESSOR EINSTEIN has been chosen for phytochemical study based on result of previous experiments. Twelve alkaloids have been detected by GC/MS and ten of them identified (e.g.: lycoramine, pluviine, haemanthamine, pancracine, homolycorine etc.). Due to the diversity of alkaloids and the fact that summary alkaloidal extract showed interesting human serum butyrylcholinesterase inhibitory activity (IC50 = 49.99 ± 5.38 μg ml^{-1}), this cultivar has been chosen for isolation of Amaryllidaceae alkaloids in pure form and the study of their biological activity.

Alkaloidal extract has been prepared from 34.3 kg of fresh bulbs. Separation was initiated by column chromatography and extract was divided into almost 500 fractions some of them were put together based on TLC analysis and finally 27 subfractions were formed. Subfraction 26 was selected for isolation of pure alkaloids. The subfraction was repeatedly divided by preparative TLC to obtain 9-O-demethylhomolycorine; alkaloid of homolycorine structural type. The isolated compound was tested for its acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity against p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

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PRODUCTION OF SECONDARY METABOLITES IN PLANT TISSUE CULTURES

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The goal of this study is to determine the influence of sodium molybdate and sodium tungstate as elicitors on production of scopoletin in cell suspension culture of *Angelica archangelica L.* The culture was grown in a liquid culture medium Murashige and Skoog on a roller apparatus in the dark and light. The content of scopoletin was in cells and in the culture medium determined by high performance liquid chromatography with fluorometric detection. The results show that sodium molybdate served as an elicitor to the production of scopoletin positively, application of sodium tungstate did not increase scopoletin production in any case. The highest production of scopoletin after application of sodium molybdate *versus* the control cells was reached in the suspension culture of *Angelica archangelica L*. cultured in the dark at a concentration of 25.50 mg/l. Scopoletin content increased by 166.7%. After application of sodium tungstate the production always decreased, in the medium of a suspension culture of *Angelica archangelica L*. cultivated in the light at a concentration of 66.00 mg/l, the production decrease was by up 62.5%.

The study was supported by Specific university research SVV 260 292.

ALKALOIDS ISOLATED FROM *NARCISSUS* CV. PROFESSOR EINSTEIN

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Within the Amaryllidaceae family, the genus *Narcissus L*. comprises around a hundred wild species with a center of diversity in the Iberian Peninsula and North Africa. In adition to their ornamental value, *Narcissus* plants have been found to contain many alkaloids with pharmacological activity. Most of the species can hybridize, and a large number of cultivars have been developer with ornamental purposes, with over 27,000 names of *Narcissus* cultivars now registered in the International Register. The *Narcissus* cultivars have advantages for commercial alkaloid production, since they are available in large quantities, but only a few studies on alkaloid profile and content in ornamental *Narcissus* cultivars have been published.[1]

Summary alkaloidal extract obtained from 34 kg of fresh bulbs of *Narcissus* cv. Professor Einstein was separated by column chromatography and gave 27 fractions. For isolation of alkaloids in pure form has been used fraction 6. Preparative TLC and crystallization

have been used for further separation process and two alkaloids of lycorine-structural type were isolated, namely caranine and pluviine. Both compounds were tested for their acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity of both alkaloids agains p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

The study was supported by SVV 260 412.

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THE SELENIUM EFFECT ON SECONDARY METABOLITES PRODUCTION IN *IN VITRO* CULTURES OF MEDICINAL PLANTS

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The subject of this study is the evaluation of secondary metabolites production in *Fagopyrum esculentum* variety Spacinska cultures *in vitro* after elicitor treatment. The aim was to determine if selenium as an abiotic elicitor[1] increases rutin production in *F. esculentum* var. Spacinska cultures *in vitro*. The experiment was carried out in callus and suspension cultures of *F. esculentum* using Murashige and Skoog[2] nutrient medium supplemented with 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid as growth regulator. The elicitor was added in the form of a solution of 3 different concentrations (c1 = 9.012×10^3 mol 1^{-1}, c2 = 9.012×10^4 mol l^{-1}, c3 = 9.012×10^5 mol 1^{-1}), and it was affecting the culture for 6, 12, 24, 48, 72 and 168 hours. The release of secondary metabolites into the nutrient medium was studied as well. The content of rutin was determined by HPLC.

The increasing rutin production after elicitor application was observed in both callus and suspension cultures. However, there were higher levels of rutin content detected in callus culture. The maximum rutin content (0.6 mg g^{-1} DW) was reached in callus culture after 12 h of elicitor treatment of c2 concentration. The maximum rutin production in suspension culture (0.1 mg g^{-1} DW) was detected after 6 and 48 h of elicitor application of c3 concentration. The rutin release into the nutrient medium was not observed. The elicitor selenium is able to increase rutin production in *Fagopyrum esculentum* variety Spacinska cultures *in vitro.*

The study was supported by SVV 260416.

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ALKALOIDS OF AMARYLLIDACEAE FAMILY: ISOLATION, STRUCTURAL IDENTIFICATION, BIOLOGICAL ACTIVITY III.

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The family of Amaryllidaceae plants consist of about 75 genera, whose 1100 species are widely distributed in tropic and warm temperature region of the world. In history plants of this family have been used in traditional herbal medicine. The family contain significant substances called Amaryllidaceae alkaloids, which was created *via* specialized metabolism. These alkaloids are known to show a wide range of biological activities, included acetylcholinesterase inhibitoty, cytotoxic, antiviral, antibacterial, antigungal, antimalarial and analgesic activity.[1] The aim of the research was to isolate Amaryllidaceae alkaloids from fresh bulbs of *Narcissus* cv. Professor Einstein, and to evaluate their biological activity connecting with Alzheimer's disease and cytotoxicity. Summary alkaloidal extract was prepared from 34 kg of fresh bulbs and separated by column chromatography. Preparative TLC and crystallization were used for the isolation of substances from subfraction 6. Two pure alkaloids (lycoraminone and narwedine) of galanthamine structural type were obtained and tested for their acetylcholinesterase, butyrylcholinestease and prolyl oligopeptidase inhibition activity. The cytotoxicity of both alkaloids agains p53-mutated gastrointestinal cancer cell lines (Caco-2 and HT-29 colorectal adenocarcinoma) has been also measured.

The study was supported by SVV 260 412.

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IRON-CHELATING PROPERTIES OF FRUIT EXTRACTS OF VARIOUS ELDERBERRIES

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Inorganic iron is the major food source of iron in humans. It plays role in many biochemical reactions. Thus, iron metabolism disorders can lead to different diseases as-

sociated with lack of iron or iron overload. One of the possible treatment modalities for the latter represents the administration of iron chelators.

Interest in anthocyanins has increased immensely during the past decade. Anthocyanins may play an important role in health promotion in terms of obesity prevention, cardiovascular health, anti-inflammatory and anti-cancer effects. Elderberry, *Sambucus nigra L.* (*Adoxaceae*), has been used in traditional medicine. The fruits of elderberry are a rich source of cyanidin-based anthocyanins as the main component. There are important differences, both in chemical and physical properties between several cultivars of elderberry. Anthocyanins might interact with metals in the gastrointestinal tract by formation of chelates. However, data on metal interactions with anthocyanins are sparse. The main aim of this study was to perform the analysis of interaction of iron with elderberry fruit extract as a rich and cheap source of anthocyanins with cyanidin as the aglycon.

In this *in vitro* study ten purified and standardized ethanolic elderberry fruit extracts were tested for iron chelating activities under different (patho)physiologically relevant pH conditions. Spectrophotometric method based on ferrozine as an indicator was used for the quantitative comparison.

All extracts were able to chelate iron, however, there were marked differences between extracts from different varieties which might be transformed in dissimilar biological effect. It was found that chelation activity of all tested extracts was increased with increasing pH. The extract of 'Haschberg' was the most potent iron chelator, both of ferrous and ferric ions.

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ANALYSIS OF GENOMIC REGIONS BOUND AND REGULATED BY ATAXIN-3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is dominantly inherited polyglutamine neurodegenerative disease. In SCA3, the disease protein ataxin-3 (ATXN3) contains an abnormally long polyglutamine (polyQ) tract encoded by CAG repeat expansion. The ATXN3 binds DNA and interacts with transcription regulators pointing toward a direct role for ATXN3 in transcription. It is conceivable that mutant ATXN3 triggers multiple, interconnected pathogenic cascades leading to neurotoxicity, however, the principal molecular mechanism remains elusive. PCR analyses of 16 ATXN3-bound genomic regions were performed. CCAAT/enhancer binding protein delta (CEBPD), period circadian clock-2 (PER2), phosphatase and tensin homolog (PTEN), alpha 2 antiplasmin (SERPINF2) and thrombospondin-1 (THBS1) were selected for further study. To investigate the putative regulatory effect of the ATXN3 on subcloned genomic regions, luciferase reporter constructs were generated. Subsequently, wild type (WT) and hetero-

zygous ATXN3-knockout human neuroblastoma cell line (SH-SY5Y) were transfected and luciferase assays were performed. To further analyze the effect mediated by ATXN3, the luciferase reporter constructs were co-transfected with expression plasmids encoding human full length normal (Q13) and mutant (Q77) ATXN3 into the WT SH-SY5Y. Differences in ATXN3-dependent luciferase activity were observed in CEBPD and THBS1 suggesting the repressor effect of ATXN3 in both cases.

The analysis of normal and mutant ATXN3 regulatory effect on subcloned regions showed differences between luciferase activity in CEBPD, THBS1 and SERPINF2 genomic regions revealing potential connection with SCA3 disease.

STUDY ON THE ROLE OF ABC EFFLUX TRANSPORTERS IN CELLULAR RESISTENCE TO BRAF INHIBITORS COBIMETINIB AND DABRAFENIB

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ABCB1 (P-glycoprotein, MDR1) and ABCG2 are well-known members of ATP-binding cassette (ABC) transporter family. Overexpressed in cancer cells they efflux a wide variety of structuraly unrelated anticancer drugs out of the cells and thereby represents one of the tumor deffence mechanisms against anticancer therapeutics leading to the development of multidrug resistance (MDR) and treatment failure.

BRAF protein plays an important role in MAPK/ERK pathway affecting cell division, differentiation and secretion. Mutations of BRAF lead to overactivity in MAPK/ERK pathway in many cancer cells and can be therefore targeted by anticancer therapy.

Cobimetinib and dabrafenib are used in melanoma treatment with BRAF mutations. Cobimetinib targets MEK kinase, a component of MAPK/ERK pathway, while dabrafenib inhibits directly BRAF kinase.

The aim of this project was to investigate whether the efflux transporters ABCB1 and ABCG2 could confer MDR to cobimetinib and dabrafenib. Using the XTT assay, we studied the antiproliferative effect of these drugs to MDCKII cell lines overexpressing ABCB1 and ABCG2 and to the transporter-expressing human epithelial A431 cells. Antiproliferative IC50 values in transporter expressing cells were determined and compared with the results from control cell lines.

Our results indicate that presence of ABCB1 can play a role in the cellular resistance to cobimetinib in A431 cell line, nevertheless, the effect could not be observed in the canine MDCKII-ABCB1 cells. Expression of ABCG2 did not affect proliferation of the ABCG2-expressing MDCKII or A431 cells when compared to their respective controls, indicating that ABCG2 is not able to confer MDR to cobimetinib. Interestingly all the cell lines showed high resistance to dabrafenib exhibited no antiproliferative effect in tested concentration scale ($IC50 > 50\ \mu M$). To conclude, ABCB1, but not ABCG2 might slightly affect sensitivity of cancer cells to cobimetinib. Further studies would be needed to evaluate clinical significance of this finding.

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DEVELOPMENT OF CRISPR-CAS9 BASED TECHNOLOGY FOR GENETIC MODIFICATION OF *LACTOCOCCUS LACTIS*

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Lactococcus lactis subsp. cremoris, also known as Lactic Acid Bacteria, is an important microorganism widely used in fermentation of cheese products, but it also became a first genetically modified microorganism used alive for therapeutic reasons.[1] The aim of this study is to develop technology that allows modifying *Lactococcus lactis'* genome using Clustered Regularly Interspased Palindromic Repeats – Cas9 system, that will become faster, easier and relatively cheap tool for genetic engineering of this bacterium.

First part of the project is designed to test cells containing two plasmids, and how efficiently Cas9 expressed from one plasmid is cutting a targeted gene on another plasmid. For this I implemented the erythromycin resistance gene and designed CRISPR-Cas9 system aimed to disable this gene and measured activity of Cas9 protein by growing cells with designed plasmids in different medium (with or without antibiotic) and comparing their optical density.

The second part of the project was based on genetic modification of cell's chromosome using homologous recombination with the fragment on the plasmid and then applied CRISPR-Cas9 as a tool for eliminating cells which remained unchanged.

For both experiments, I used Nisin Controlled gene Expression system.[2] Plasmid genes expression was induced by nisin added into the growth medium.

Experiments showed some promising results although the genetic design of plasmids and the protocols of cell growth still require some further changes and adjustments.

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PHARMACOLOGICAL CHARACTERIZATION OF NOVEL P2X3 RECEPTOR'S LIGANDS

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P2X3 receptor takes a part in transferring painful signals through the neuronal and non – neuronal cells along the neuraxis. ATP, the agonist of P2X3 receptor, is released from afferent neurons or from the damaged cells and non-neuronal peripheral tissue by stimuli. Before ATP is being degraded, it may activate P2X3 receptor at nociceptive cells endings and stimulate pain pathway. So, it seems to be hopeful to discover potential P2X3 receptor's antagonists which may help in future in treatment of relieving severe pain in cancer or in chronic pain disorders. Therefore, completely new allosteric antagonists were synthesized and tested *in vitro* at human astrocytoma cell line 1321N1 expressing human P2X3 receptor. Some of them have shown promising activity.

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EFFECT OF PRENYLATED FLAVONOIDS ON BIOTRANSFORMATION ENZYMES *IN VITRO*

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Prenylated flavonoids xanthohumol (XH), isoxanthohumol (IXH), 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN) are flavanones and chalcones occurring in hops and their characteristic feature is prenyl chain present on A ring.[1] Prenylation, addition of isoprenoid functional group, significantly increases their estrogenic and anticancer activity as well as bioavailability in organism. The aim of our study was to find out, whether XH, IXH, 6PN, and 8PN have any impact on cytosolic carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C subfamily (AKR1C). Viability test revealed that prenylated flavonoids in lower concentrations do not affect or even increase the viability of primary rat hepatocytes, but higher concentrations are toxic. CBR1 and AKR1C activity was not affected after 24 h treatment but an increase in expression of AKR1C3 in IXH and 6PN treated samples was observed using immunoblotting, decrease in XH and 8PN samples. Expression of CBR1 was at the detection limit. Using qRT-PCR was found that XH caused significant increase in gene expression of CBR1, and significant decrease in AKR1c14 expression in rat hepatocytes.

Based on the results, prenylated flavonoids XH, IXH, 6PN and 8PN do affect the activity of CBR1 and AKR1C subfamily, gene expression of enzymes is significantly affected only by XH.

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THE EFFECT OF A PARASITE ON THE ACTIVITY OF SELECTED INTESTINAL ENZYMES OF THE HOST

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Hymenolepis diminuta, known as a rat tapeworm, is commonly used in science as a model of Cestoda for studying physiology, biochemistry and drug metabolism.[1] Recently, *H. diminuta* has been studied for helminth-based therapy for inflammatory bowel disease.[2] The aim of our study was to determine how *H. diminuta* influences the activity of detoxification enzymes of the host. At first 6 male rats (Wistar breed) were infected by cysticercoids of *H. diminuta* previously isolated from the beetles *Tenebrio molitor* (intermediate host).[3] At the same time the physiological saline solution was administered for the control group of 6 male rats. All the rats were housed for 2 months in air-conditioned animal quarters with a 12 h light/dark cycle. Thereafter, the tapeworms were removed from the intestines. Intestinal mucosa containing metabolic active enzymes was isolated. Subsequently, the subcelullar fractions were prepared and used for *in vitro* experiment. The activity of enzymes was measured by spectrophotometry and spectrofluorimetry. The results show that *H. diminuta* is able to affect the activity of biotransformation enzymes. It can be assumed that the activity of reductases or some isoforms of cytochrome P 450 can differ. The activity of conjugation enzymes seems to be higher in intestine infected by *H. diminuta*. Concerning the enzymes of oxidative stress, the activity of catalase was increased apparently.

The study was supported by the Charles University research project SVV 260 416.

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EFFECT OF NANOPARTICLES ON PLANT PROTEOME

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The environment is contaminated by increasing amounts of different types of xenobiotics. One of the potential polutants are nanoparticles of heavy metals. Effective method used for decontamination of the environment is phytoremediation which is based on the deposition of contaminants in plants. The plant is suitable for phytoremediation when it can absorb, metabolise and store contaminants without impact on its function. Influence on the function and construction of the plant can be detected at the proteome level.

In our research we focus on effect of nanoparticles on plant proteome in a model organism, which is *Arabidopsis thaliana*. A few weeks old plants were exposed to various types of copper particles (nanoparticles, bulk and salt) for different times. As a next step, proteins were isolated from plant material and electrophoresis and analysis was realised. The changes in the amount of structural proteins, proteins involved in photosynthesis, energy metabolism, carbohydrate metabolism and plant defence were detected. Modulation of the amount of protein was characterized by modified intensity of the spot on protein map. Toxic effect on plants is manifested for example by reducing the amount of structural proteins or proteins involved in photosynthesis. Conversely, increase in the content of proteins involved in stress response may indicate that the plant is able to fight against xenobiotics. One day treatment by bulk copper oxide caused decrease of intensity of six protein spots and four spots were increased.

Proteome influenced by copper oxide nanoparticles shows decrease of eight spots and increase of only three spots. Longer influence of both copper oxide nanoparticles and bulk form caused a decrease of almost all spots. After short acting of copper salt a lot of protein spots were increased. On proteome exposed to copper salt for four days were detected only a few spots with increased intensity, because the proteins were damaged by effect of copper ions. By comparing the results with literature, it was found that the copper ions are the most toxic for plant. They are followed by nanoparticles of copper oxide and the least toxic is bulk form of copper oxide.

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DETERMINATION OF SELECTED microRNA – POTENTIAL CARDIOTOXICITY BIOMARKERS

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Cardiotoxicity is adverse reaction of chemotherapy that causes damage to the heart. Monitoring of potential biomarkers of cardiotoxicity could have a positive effect on the elimination of drug's toxicity on heart tissue. Therefore, at present, interest in the potential microRNA (miRNA) as biomarkers for cardiotoxicity is rising. MiRNA is very stable short noncoding RNA that has the ability to post-transcriptionally regulate gene expression. From bioinformatic analyses miRNAs are able to regulate more than half of human genes. Various studies have shown miRNAs to be much more specific and rapid diagnostic biomarkers in comparison with troponins. MiRNAs as biomarkers are not established in

routine clinical practice yet. So far, all studies of miRNAs are in the process of search and methods optimization.

In my project selected miRNAs for detection of doxorubicin (DOX) cardiotoxicity *in vivo* in mouse cardiac tissue samples and *in vitro* on rat cardiomyocytes were investigated. RNA isolation from biological samples and reverse transcription, using Stem-loop RT primer, was performed. Primers were designed for the quantitative determination of selected miRNAs using real-time PCR. The levels of expression of monitored miRNAs were compared to a control group of samples that were not affected by DOX. Significant changes in expression of selected miRNAs were detected. When comparing murine and rat samples elevated expression of various miRNAs after DOX treatment were found.

THE EFFECT OF FLUBENDAZOLE AND MEBENDAZOLE ON EPITHELIAL-MESENCHYMAL TRANSITION

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Epithelial-mesenchymal transition (EMT) is a process in which a non-motile epithelial cell switches to motile mesenchymal phenotype. This phenomenon is typical for multiple biological processes including cancer metastases. It could be induced by transforming growth factor-β (TGF-β) which is produced by cancer cells and which regulates its key transcription factors including SNAIL, ZEB and TWIST. EMT involves a series of defined events mediated by microtubules, including changes in cell shape and migration into the surrounding tissue. Based on these findings we suppose that the inhibition of tubulin polymerization could prevent cell migration, therefore the EMT process and also cancer metastases. In our experiments, this effect was triggered by anthelminitic drugs flubendazole (FLU) and mebendazole (MBZ) in dysplastic oral keratinocytes *in vitro*.

Expression of molecules involved in EMT process was examined on microRNA and mRNA level using RT-PCR. The protein level of EMT markers was determined using western blot analysis.

FLU treatment significantly decreased mRNA levels of some mesenchymal markers such as N-cadherin, MMP2, MMP9 and TWIST, in comparison with TGFβ treated cells. Also microRNA from miR-200 family, miR200b and miR200c, involved in EMT processes was considerably increased after FLU, in comparison with TGFβ. The level of miR21, responsible for cancer progression, was significantly decreased after FLU treatment. MBZ was not significantly effective at any tested concentrations.

In total, FLU significantly inhibited expressions of mesenchymal markers in EMT induced cells, which could possibly prevent cancer metastatic process.

The study was supported by the program PRVOUK 37/01.

CHARACTERISATION OF THE METASTATIC AND NON-METASTATIC BREAST CANCER CELL

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Cancer is the second most frequent cause of death worldwide, while breast cancer is the most common type of cancer in women. According to Institute of Health information and statistic of Czech Republic, more than seven thousand new cases of breast cancer is diagnosed every year and approximately two thousand women die every year because of this type of cancer. The big problem is the treatment of the metastatic breast cancer. The goal of this study was characterization of metastatic and non-metastatic breast cancer cells.

Three breast cancer cell lines were used for the experiment, specifically non-metastatic MCF-7 cells, and two metastatic cell lines – MDA-MB-231 and BT474. The mRNA level was determined using RT-PCR and the protein level using western blot analysis. There was also examined the ability to migration of cell lines using the real time analysis, by the system X-celligence. As well as the effect of cytostatic paclitaxel (PTX) on antiproliferative activity of the cells was determined using the method WST-1.

Especially, in metastatic cancer cell lines BT474 were observed higher level of mesenchymal markers MMP-2, MMP-9 and N-cadherin as well. On the other hand E-cadherin as the suppressor for metastasis was observed in lower levels in MDA-MB-231 and BT474 metastatic cell lines. The highest migration potential was found in MDA-MB-231 cells. On the other hand, as expected, MCF-7 cells were not able to migrate. Testing of cell proliferation revealed MDA-MB-231 as a most sensitive cells to PTX treatment; in the BT474 cells was the effect weakest. In total, significantly higher metastatic potential was found in BT-474 cells, as well as reduced sensitivity to PTX treatment, in comparison with MCF7 and MDA-MB-231 cells.

The study was supported by the program PRVOUK 37/01.

CO^{2+} LOADED BLOCK COPOLYMER MICELLES: PREPARATION AND THEIR UPTAKE INTO MACROPHAGES

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Cobalt is a biogenic trace element, however in its inorganic form may cause immunological reactions in the human body. The immune response can be inflammatory or anti-inflammatory and both are responsible for specific actions, regarding macrophages activation and cytokines release.

The aim of this study was to provoke such response in macrophages and therefore to possibly control the inflammatory process. Nevertheless, free cobalt ions in certain concentrations may be toxic. In order to find the suitable and safe way of cobalt administration, the triblock terpolymer PEO-b-PAGECOOH-b-PtBGE was synthesized and formed into micelles in water.[1] Eventually the micelles were loaded with cobalt chloride. The properties of these nanoparticles were further studied and their structure, size, shape, appearance and net charge were determined, as well as the amount of cobalt inside.

After the synthesis and characterisation, the micelle uptake into macrophages was investigated and it was found out, that the uptake was increased with increasing micelle concentration in the cell culture medium and that the process was probably not carried out by receptor-mediated endocytosis. Further assessment revealed that the vitality of the cells was not significantly affected by the micelles. The cytokine release measurement suggested that the macrophages could have been activated into M2 anti-inflammatory state. The difference in release of IL-10 between cobalt loaded micelles and $CoCl_2$ solution proves that the micelles are potentially suitable drug delivery system.

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BASIC CHARACTERIZATION OF HUMAN ENZYMES DHRS7B AND DHRS7C

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Human enzymes of the short-chain dehydrogenase/reductase (SDR) superfamily play important roles in the biochemical pathways.[1] They are involved in metabolism of lipids, saccharides, amino acids, steroid hormones, retinoids and prostaglandins etc. Besides physiological processes they take part in development of several serious diseases, e.g. hormonedependent cancer, metabolic syndrome, diabetes mellitus. Moreover, SDR enzymes contribute to the biotransformation and therefore to the detoxification of xenobiotics. Unfortunately, 30% of SDRs remain completely uncharacterized. Dehydrogenase/reductase SDR family members 7B (DHRS7B) and 7C (DHRS7C) are poorly characterized members of the SDR superfamily.[2] According to in silico predictions both are membrane bound enzymes and involved in reductive reactions. The aim of this study was to determine their basic biochemical properties and verify above mentioned predictions.

The results show that both enzymes interact with membrane of endoplasmic reticulum. DHRS7B faces cytosol whereas DHRS7C is oriented to the lumen of endoplasmic reticu-

lum. Reducing activity was detected towards e.g. estrone, prednisone, glucose, ketotifen or 1,2-naphtoquinone for both enzymes.

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EVALUATION OF ANTIMICROBIAL EFFECT OF QUATERNARY AMMONIUM SALTS

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Nosocomial infections in healthcare facilities lead to high morbidity and mortality in devoloped countries. Some type of quaternary ammonium salts (QAS) are already used as disinfactants in practice (benzalkonium, benzoxonium etc.). Structure of these chemical compounds is quite similar to the cell membrane. So, they influence the permeability of the cell membrane and cause it's disrupt. We have tested three homologues according the side alkyl chain length (12, 14 or 16 carbons). We have performed microdilution broth method to prove their antimicrobial effect. Tested substances were dissolved and diluted into the binary dilution. The method was optimized according to the used strains. Several nosocomial strains of gram positive and negative bacteria (obtained from Faculty Hospital Hradec Králové) were tested. Benzalkonium salts were used as a standard for comparison of efficacy. QAS show sufficient effect againts nosocomial bacteria. Their microbicidal effect was better against gram positive bacteria as expected. They were still effective against gram negative bacteria, however in higher concentrations compare to gram positive.

In general, longer carbon chains show higher antimicrobial effect. There was no significant antimicrobial effect compare to the benzalkonium standard.

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VALIDATION STUDY OF PREDICTIVE EQUATION OF RESTING ENERGY EXPENDITURE DURING PREGNANCY

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Nowadays, there are a few methods used for expression of resting energy expenditure (REE) during pregnancy: First, REE measurement by indirect calorimetry, very precise, but expensive and time-consuming method. Second, Harris-Benedict equation, which uses a formula to determine the REE, but is aimed to men/non-pregnant women, therefore it is not precise for pregnant women. None of methods present in the field of medicine, predicts the REE in pregnant women with sufficient precision and simplicity. One equation[1] was found and published in 2009: P REE = 346.43943 + 13.962564 · W + 2.700416 · H – 6.826376 · A.

The goal of this study was to verify its validity after almost eight years. A total of 70 randomly recruited healthy pregnant Czech women (non-smokers, not users of chronic medication or abusers of alcohol or drugs, normoglycemic, euthyroid and not anaemic) were divided into three cohorts by the length of gestation and measured by indirect calorimetry. Their REE was also calculated by Harris-Benedict equation and predicted by P REE equation. The results of these measurements were statistically analyzed by correlation analysis and Blend-Altman test. It turned out there was no significant difference between measured and calculated REE.

Results of validation study to confirm possibility of clinical application of P REE predictive equation for exact REE prediction without the need of using expensive technology or invasive examination that are highly important for the settings of proper nutrition during pregnancy.

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GNOTOBIOTIC MICE MODEL AND EXPERIMENTAL INFECTION WITH *FRANCISELLA TULARENSI*

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Francisella tularensis, the causative agent of a disease called tularemia, is a facultative intracellular Gram-negative bacterium. Because of its very high virulency and mortality rate (if untreated) it is included in the Category A of bioterrorism agents by the Centers for Disease Control and Prevention, USA. Since there is no vaccination available for the general public yet, the research nowadays focuses mostly on a vaccine development. However, the experimental infection by *F. tularensis* also plays a significant role in understanding the host-pathogen interactions, serving as an important model of an infection caused by intracellular bacteria.

The aim of this study is to examine the virulence mechanisms of *Francisella tularensis* using the gnotobiotic mice model. The term “gnotobiotic” comes from the Greek words

"gnotos" and "bios", meaning "known life", indicating the limited presence (or absence) of microorganisms in such animal. The gnotobiotic animal model, as a strictly defined system, minimizes the influence of the organism's microbiota on the results of the study. It is, therefore, widely used in immunology and other biomedical sciences, providing great options for vaccine development and studies of the immune system, especially the host-pathogen relationship.

Our long-term goal is to compare the innate immune response of germ-free and specific pathogen free mice after intraperitoneal infection with two different strains of *Francisella tularensis*. By protein fractionation, we have prepared various bacterial samples suitable for the recognition of its immunoreactive proteins and the detection of specific antibodies found in the mice serum – using 2D SDS-PAGE electrophoresis, Western blotting and immunodetection. The different infectivity of the used strains, together with the dissemination of *F. tularensis* into the lungs, spleen and liver of the infected mice were observed as well.

The study was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense, Czech Republic.

THE INFLUENCE OF ANAESTHESIA ON THE DEGREE OF DNA OXIDATIVE DAMAGE

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Oxidative damage is one of the most frequent types of cell components damage leading to oxidation of lipids, proteins and the molecule of DNA. As a consequence, there is a higher occurrence of several pathologies such as atherosclerosis, neurodegenerative diseases, cancer, and diabetes.[1]

In our study, influence of whole body anaesthesia during minor surgery on the level of DNA damage was examined using comet assay technique. The basic principle of this method is fixing the cells (lymphocytes) in agarose, their lysis for the removal of membranes, incubation with the specific enzymes and electrophoresis of the released cell nuclei.[2] During the electrophoresis, free low-molecular weight and negatively charged fragments of DNA move towards anode which causes the formation of the typical comet cell shape. Finally, the gels are stained by ethidium bromide (DNA intercalating dye) and visualized.[3] We have observed single strand breakages (SSBs) and, with the use of modified assay using specific enzymes for detection of specific lesions, also oxidized purines and pyrimidines. The extent of DNA damage as determined by the intensity of the tail of the

comet was quantified using LUCIA Comet Assay (Laboratory Imaging, Czech Republic) software for image analysis. The results were used for the comparison of DNA damage before and after the anaesthesia of the patients. Statistical evaluation was performed in SigmaStat 3.5 (Systat Software, USA).

Results showed a statistically significant increase of DNA damage caused by anaesthesia during minor surgery. These findings imply further investigations, namely evaluation of the changes in the capacity of the affected lymphocytes to repair DNA damage.

The study was supported by MH CZ – DRO (UHHK, 00179906) and Department of Research and Development, University Hospital Hradec Králové.

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SECTION OF CHEMICAL SCIENCES

DEVELOPMENT OF UHPSFC-PDA METHOD FOR IMPURITY PROFILING IN ACTIVE PRINCIPLE INGREDIENT ATOMOXETINE

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The aim of this project was an optimization of UHPSFC method for determination of atomoxetine and its impurities mandelic acid, o-cresol, phenol, phenoxyatomoxetine, benzyl atomoxetine and atomoxetine carbamate. Atomoxetine is used as centrally acting sympathomimetic agent for the treatment of hyperkinetic disorders such as Attention Deficit Hyperactivity Disorder (ADHD).

Measurements were carried out on the UHPSFC system Aquity UPC2 with PDA detector and column Torus Diol 1.7 µm (3.0 × 100 mm). ABPR pressure was optimized at 2000 psi and the column temperature at 40 °C. Flow rate of mobile phase was 1.5 ml/min. Additional optimization parameters were the mobile phase composition, gradient elution conditions (initial composition of mobile phase, gradient slope, gradient time, gradient curves) and the effect of analysis time on the resolution of critical peak pairs. PDA detector parameters were examined, including comparison of data acquisition in 3 D and 2 D mode, selection of detection wavelength, resolution, sampling rate, filter time constant and mode selected for data acquisition, in order to obtain the maximum sensitivity of the method.

Optimal conditions for the impurity profiling in the drug substance atomoxetine were chosen as folows: CO_2/MeOH + 0.1% NH_4OH as mobile phase with a gradient from 1% to

40% in 14 minutes. Detection was made in the 3 D of compensated mode at 215 nm with a resolution of 4.8, with a sample rate of 20 points/sec, the filtering time constant in the normal mode during a 15 minute analysis. The method was validated properly including SST (retention time, peak area, resolution, peak symmetry, peak width at half height) and determination of parameters of precision, accuracy, linearity, selectivity and robustness of the method.

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DETERMINATION OF PHENOLIC COMPOUNDS IN APPLES

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This work deals with a development and optimization of an HPLC method for determination of selected phenolic compounds including gallic acid, chlorogenic acid, caffeic acid, catechin, epicatechin, rutin, quercetin, quercitrin, phloretin and phloridzin.

In the optimization step several types of stationary phase (C18, phenyl-hexyl, biphenyl, amino, cyano and monolithic column), gradients of the mobile phase and other separation and extractionconditions (temperature, extraction, solvent) were tested and a partial validation of the method was carried out.

Separation of the selected compounds was obtained using a precolumn Ascentis Express C18 (5 × 4.6 mm × 5 µm) and the chromatographic column Kinetex C18 column (150 × 4.6 mm × 5 µm). The detection was performed by a DAD spectrophotometric detector at wavelengths of 255, 280, 320 and 365 nm. Column temperature of 30 °C and gradient elution with mobile phase composed of acetonitrile and water (pH adjusted to 2.8 with acetic acid) was used. Injection volume was 10 µl and the flow rate 1 ml/min. During the optimization real apple extracts of the pulp and peel were also tested. The evalueted validation parameters included the chromatographic system suitability test (peak resolution, symmetry factor, capacity factor and repeatability), linearity and robustness.

The developed method is intended for determination of the selected phenolic compounds in different varieties of apples during their storage under various conditions (low temperature, controlled atmosphere).

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MODIFICATION OF THE CAPILLARY WALL FOR THE SEPARATION PURPOSE

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Capillary electrophoresis is a separation method for the analysis of charged molecules. Interest on this method in recent years is growing along with it develops further electromigration methods, such as capillary electrochromatography. In terms of improvement separation efficiency offers modification of these methods. One option is the modification of the inner wall of the capillary in order to influence the electroosmotic flow, which contributes to the mechanism of separation largely.

This work deals with modification of the inner capillary wall and subsequently testing the prepared capillaries. One possibility is the chemically coating. The second method of coating is Layer-by-layer and the layered different modifiers. On the surface of the capillary is linked polyelectrolyte poly(diallyldimethylammoniumchlorid)e and graphene dispersion together in several layers. The choice of graphene is very advantageous because it has excellent adsorption properties due to its morphological configuration. For the analysis and subsequent optimization of separation conditions were selected mixture of parabens. They were tested for various optimization parameters such as the influence of the nature of the electrolyte (pH, concentration) and temperature on separation efficiency and speed of analysis. During measurement sacrificing efficiency, in particular capillary coated by chemical means. This phenomenon has been attributed to the gradual washout layers, which were accompanied by changes in electroosmotic mobility.

Method of coating Layer-by-layer was selected to be more appropriate procedure modifications as provided stable conditions for the reproducible analysis of mixtures of parabens.

SPECTROPHOTOMETRIC DETERMINATION OF CHOLINESTERASE ACTIVITY USING CARBAMATE INHIBITORS

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Nowadays, two types of cholinesterases are known. The first one is acetylcholinesterase (AChE, EC 3.1.1.7), whose function is the cleavage of acetylcholine in the central and peripheral nervous system. The second type is butyrylcholinesterase (BChE, EC 3.1.1.8), sometimes called plasmatic cholinesterase. Whole significance of BChE is yet not fully

understood. Carbamates dominate among reversible cholinesterase inhibitors. Their effect can be decreased from the reason of (possible) hydrolyzation to inactive compounds.[1]

The goal of this experimental work was retrieval of kinetic parameters of AChE and BChE, and optimization of the method for determination of selected carbamate inhibitors – carbofuran (a representative of pesticides) and physostigmine/eserine (a pattern natural compound for obtain the effect of carbamates).[2] Determination of cholinesterase activity was carried with Ellman method. The mentioned enzymes were used for cleavage of thioesters (acetylthiocholin for AChE, butyrylthiocholine for BChE) to acetic, resp. butyric acid, and thiocholine. Thiocholine reacts with a 5,5′-disulfanediylbis(2-nitrobenzoic acid) – DTNB. Thus releasing 5-sulfanyl-2-nitrobenzen (TNB) can be detected spectrophotometrically at 412 nm.[3]

It turned out, firstly, that both inhibitors act very similarly, secondly, that BChE is more resistant to named inhibitors. Reversibility of inhibition will be even further investigated.

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NMR SPECTROSCOPY: A POWERFUL TOOL EMPLOYED IN ELUCIDATION OF UNKNOWN CHEMICAL STRUCTURES

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Nuclear magnetic resonance spectroscopy is a powerful analytical tool used to elucidate unknown chemical structures. Employing the behaviour of nuclei with odd nucleon number under appropriate conditions in a magnetic field with radio frequency ranged pulses applied, various useful data about the individual nuclei and their surroundings can be obtained.

Our task was to determine the structures of samples MC-SN-1 and MC-27, both of which are disubstituted tetrazoles. We ran standard 1H, 13C and 15N 1D experiments, followed by advanced 2D homocorrelated and heterocorrelated experiments: gCOSY, zCOSY, NOESY, gHSQC, gHMBC, 15N gHMBC, which provided us with conclusive proof of their structural arrangement.

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SYNTHESIS OF NEW ORGANIC COMPOUNDS CONTAINING CHALCOGENS

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The focus of this thesis is on the synthesis of new organic compounds containing chalcogens, particularly sulfur and selenium.

New compound containing selenium was prepared by Huisgen 1,3-dipolar cycloaddition of alkyne and azide, known as the click reaction. Another three compounds contain both selenium and sulphur and had been prepared by substituting the SeCN fragment. These compounds are scheduled to be tested for biological activity in the near future. Due to the presence of chalcogen, they are expected to show antioxidant, anticancer, antifungal or antibacterial effects or their combination.

Additionally, two more compounds had been synthetised by Huisgen 1,3-dipolar cycloaddition of allyl thiocyanate and allyl selenocyanate with sodium azide. The final products are, however, highly unstable and prone to rapid degradation and are therefore unsuitable for further testing.

SYNTHESIS OF CHIRAL IONIC LIQUIDS

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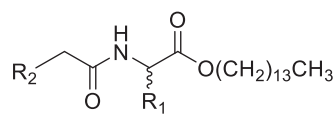
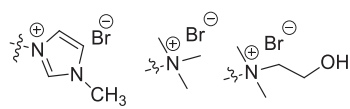
The aim of this project was to synthesize series of chiral ionic liquids, which would differ at the chiral centre, leading to three possible libraries,[1] either derived from L-alanine, D-phenylglycine or L-serine. The polar edge of the final products resulted from the reaction of α-bromoacetyl intermediates with their nucleophilic counterparts (Scheme 1).



R_1 = Me, Ph, CH_2OH

R_2 =



Scheme 1.

The products underwent a screening for the capability as additives to background electrolytes in capillary electrophoresis leading to a possible chiral recognition ability.[2]

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STUDY OF TRANSALKYLATION REACTION OF ALKYL ARYL SULFIDES

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Recently, an atypical transalkylation reaction was observed during synthesis of unsymmetrical quaternized phthalocyanines (Pc) containing quaternizable imidazole moieties and tert-butylsulfanyl susbtituents. The tert-butylsulfanyl substituents that were present on the Pc ring were replaced by methylsulfanyl groups that most likely originated from the alkylating agent – methyl iodide. In a preliminary study, similar transalkylation occurred also in tert-butylsulfanylphthalonitrile, a starting material to Pc. This reaction is unprecedented in literature, so we decided to study this reaction in more detail.

The main objective of this experiment is to present how different parameters (temperature, time, type and excess of the alkylating agent) influence the conversion and the yield of reaction. The subjects of research was primarily simple tert-butylphenylsulfide which underwent transalkylation reactions with different alkylating reagents (methyl iodide, ethyl iodide, dimethyl sulfate, dimethyl carbonate, butyl iodide). The reaction temperature was varied from room temperature to 160 °C with time ranging from 30 minutes to 72 hours. It was observed that the percent of conversion is strictly connected with the time of reaction and the reaction temperature (Graph A). The experiments indicated that methyl iodide is the most efficient alkylating agent for this reaction (Graph B), and that dimethylcarbonate did not induce any change even at 160 °C. Decomposition of the material was observed upon longer reaction times with dimethylsulfate.

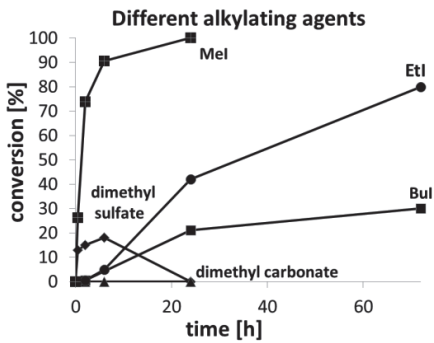

Fig. 1.

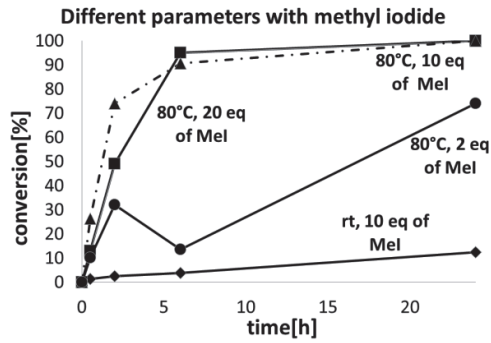

Fig. 2.

SYNTHESIS AND STUDY OF PHOTODYNAMIC PROPERTIES OF SULFONATED AZAPHTHALOCYANINES

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Photodynamic therapy is one of the methods used for destruction of undesirable cells. It combines three essentially nontoxic components: light, oxygen and a photosensitizer. Azaphthalocyanines (AzaPc) are promising compounds with photosensitizing properties. Their major disadvantage is their low water solubility and significant aggregation that decrease their photodynamic activity.

The aim of this work was to synthesise an anionic derivative of AzaPc substituted with sulfonic groups on periphery characterised by good solubility in water and to evaluate its photodynamic properties. The first step in synthesis was condensation of diaminomaleonitrile and benzil giving 5,6-diphenylpyrazine-2,3-dicarbonitrile. Subsequently the cyclotetramerisation with zinc acetate using 2-dimethylaminoethanol as a solvent was performed. The final product was obtained by sulfonation with chlorosulfonic acid followed by hydrolysis with sodium hydroxide (Fig. 1). The green coloured product was then purified by gel chromatography using Superdex® as stationary phase and by short preparative reverse phase column. Synthesised compound is soluble in water but according to absorption spectra it is partially aggregated. The tests for photodynamic activity were performed on HeLa cells using serum-free medium (phototoxicity $EC_{50} = 0.833 \pm 0.312$ μM, dark toxicity $TC_{50} = 534 \pm 27$ μM). It was practically inactive in serum-containing medium due to its binding to plasma proteins.

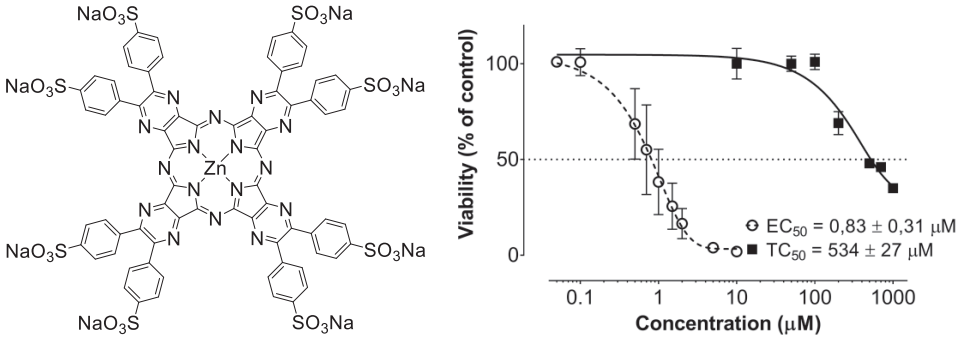

Fig. 1.

The study was supported by Charles University, project SVV 260 401.

PHENOL-SUBSTITUTED AZAPHTALOCYANINES: pH SENSORS WITH TUNABLE PKa

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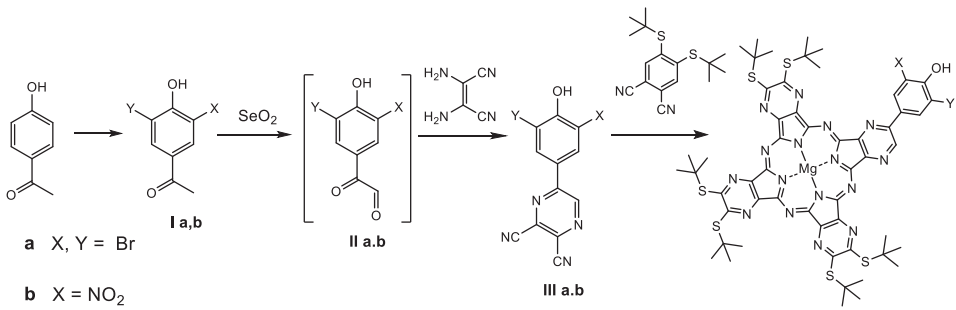
Azaphthalocyanines (AzaPc) are macrocyclic compounds containing a large system of conjugated double bonds which enables them to absorb light in the red part of the spectrum that is promising in biological applications. They are characterized by intense red fluorescence as one of the relaxation pathways of the excited state after absorbing photon. The fluorescence of AzaPc substituted with a phenol on the periphery can be switched ON/OFF depending on the pH of the environment and the pKa of the phenolic group. In basic medium the molecule occurs as phenolate and undergoes so-called intramolecular charge transfer (ICT) between the phenolate group serving as a donor and the electron-deficient nitrogen-rich macrocyclic core serving as an acceptor. As a consequence of this process, the fluorescence is quenched. Switching between ON/OFF states in phenol-substituted AzaPc is dependent on the proton concentration and thus can be utilized in sensing pH.[1]

The aim of this work was to synthesize phenol-substituted AzaPc and to study the effect of different substituents on the pKa of the phenolic group. The synthesis (Fig.1) started with preparation of appropriate precursors (i.e. substituted 5-(4′-hydroxyphenyl) pyrazine-2,3-dicarbonitriles). Electrophilic substitution of commercially available 4-hydroxyacetophenone (bromination in substance Ia and nitration in Ib) was performed. The products were treated with selenium dioxide affording corresponding ketoaldehydes that were not isolated but directly reacted in a condensation reaction with diaminomaleonitrile. To obtain unsymmetrical AzaPc, a mixed cyclotetramerization (statistical condensation) of these precursors (A) with 5,6-bis(tert-butylsulfanyl)pyrazine-2,3-dicarbonitrile (B) was performed using magnesium butoxide as an initiator. Resulting mixture contained six different congeners (i.e. AAAA, AAAB, AABB, ABAB, ABBB, BBBB) from which the required congener (ABBB) was separated using column chromatography. Substance IVa was incorporated to lipophilic particles (microemulsion) in water and the fluorescence changes were investigated as a function of pH of the buffer. Dependence of fluorescence on pH allowed determination of pKa value (see the picture below).



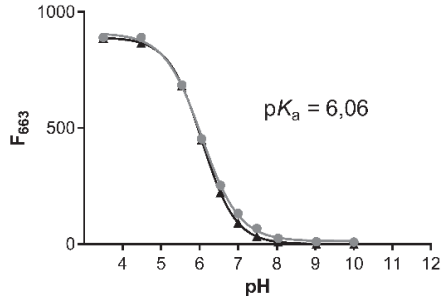


Fig. 1.

The study was supported by SVV 260 401.

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DESIGN AND SYNTHESIS OF NOVEL HYBRID MOLECULES MODULATING ACTIVITY OF M1 ACETYLCHOLINE RECEPTORS WITH ACETYLCHOLINESTERASE INHIBITION PROPERTIES

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Alzheimer's disease (AD) is a neurodegenerative disorder of complex etiology, with insidious progression and fatal consequences. The worldwide incidence of AD is very high. AD affects primarily aging population manifesting as progressive decline of cognitive and intellectual functions. Histopathological hallmarks include the presence of β-amyloid neuritic plaques, neurofibrillary tangles composed of hyperphosphorylated τ protein and atrophy of brain tissue. Neurotransmitter levels are decreased in case of acetylcholine (ACh) while glutamate levels are elevated. Nowadays, there are two pharmacological groups employed in the treatment of AD; acetylcholinesterase inhibitors (AChEIs) and antagonist of N-methyl-D-aspartate receptors (NMDARs) – memantine. Both groups act only symptomatically, lacking disease-modifying effect.

The aim of our study was preparation of a series of novel hybrid molecules combining AChEIs, namely tacrine, 7-methoxytacrine and 6-chlorotacrine with molecule BQCA – positive allosteric modulator of M1 subtype of muscarinic ACh receptors (mAChRs). Inhibitory effectiveness of the newly synthetized compounds against cholinesterases (ChEs) was determined *in vitro* by the Ellman's colorimetric method and expressed as IC50. Effect on mAChRs was determined by measurement of intracellular calcium concentration using fluorescent indicator. The results demonstrated the ability of newly

synthesized molecules to inhibit ChEs in the micromolar and sub-micromolar IC50 values, with antagonist activity towards mAChRs.

The study was supported by SVV 260 401 (Faculty of Pharmacy in Hradec Králové, Charles University) and Long-term development plan (Faculty of Military Health Sciences).

SYNTHESIS OF PURPUREALIDIN-INSPIRED BROMOTYRAMINES

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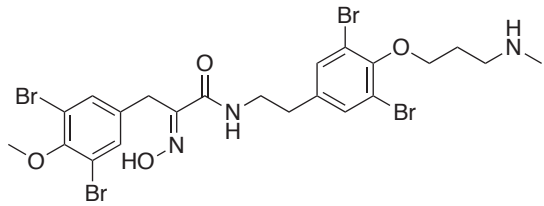
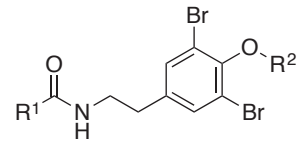
Biodiversity in the sea is a valuable source of lead structures and potential drug candidates.[1] Two bromotyrosine alkaloids purpurealidin I and J were isolated from the marine sponge *Pseudoceratina (Psammaplysilla) purpurea* by the National Institute of Oceanography, Goa, India in 2012.[2]

In this study, simplified dispyrin-like[3] bromotyramine analogs **1** of purpurealidin I were synthesised *via* purpurealidin E. Five of the novel compounds were tested against a hepatitis C virus (HCV) replicon cell model showing activity but also high cytotoxicity. Further research focus has therefore shifted onto studying prospective anticancer effects. A well-established synthetic route allowed the synthesis of variously modified structures. The previous together with improved purification methods fostered a creation of a library of derivatives studied for their activity towards hEAG K^+ channels and melanoma cell lines. Both synthetic approaches and selected biological results will be discussed.



Purpurealidin I



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NUCLEOPHILE ASSISTED GOLD(I) CATALYZED CYCLIZATIONS

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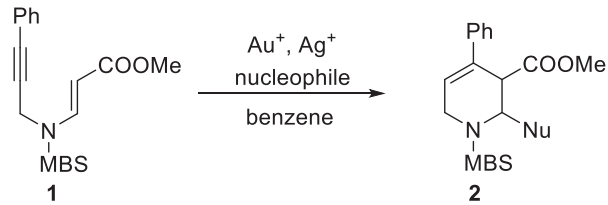
We have developed cyclizations of substitued enynes using gold(I) catalyst turnishing substituted dihydropyridines.[1] In the next step, we focused on gold(I)-catalyzed cyclizations of 1 in the presence of a nucleophile (e.g. methanol) which give substituted dihydropyridines.[2] The formal addition of nucleophile generates a new stereogenic center, whose relative configuration was determined by advanced NMR experiments. Screening of various gold catalysts was performed and the cyclization step optimized.



Scheme 1. Nucleophile-assisted cyclizations.

This work was supported by Charles University (SVV 260 401 and GAUK 262416) and Czech Science Foundation (Project No. 15-07332S).

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SYNTHESIS OF DEXRAZOXANE ANALOGUES AS POTENTIAL CARDIOPROTECTIVE AGENTS

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Anthracycline antibiotics (ANTs) such as doxorubicin or daunorubicin belong to anticancer drugs widely used to treat diverse forms of cancer. A high risk of the cardiotoxity represents the most serious side effect connected with the administration of ANTs. Dexra-

zoxane (DEX) has been the only compound capable of the protection against the ANT cardiotoxicity so far. However, the mechanism of its cardioprotective effect is still unknown. The aim of this study was to prepare, evaluate and optimize the preparation of four DEX analogues, in particular *meso*-dimethyl analogue ICRF-193 (Fig. 1).

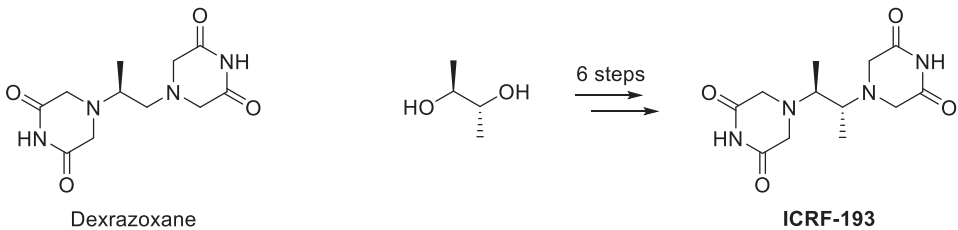

Fig. 1. Structure of DEX and its *meso*-dimethyl analogue ICRF-193.

The synthesis of ICRF-193 was accomplished *via* six-step procedure starting with the mesylation of *meso*-2,3-butanediol and subsequent substitution with sodium azide. The obtained *meso*-2,3-diazidobutane was reduced in order to prepare *meso*-2,3-diaminobutane which was subsequently converted to the corresponding diminobutane tetraacetic acid in two-steps. Its cyclization in formamide provided ICRF-193, which was studied for *in vitro* protection against ANT cardiotoxicy. In the pilot experiment ICRF-193 showed higher *in vitro* cardioprotective action than DEX.

The study was supported by the Czech Science Foundation (13-15008S) and Charles University (SVV 260 401).

SYNTHESIS AND *IN VITRO* EVALUATION OF NOVEL IRON CHELATORS BASED ON SALICYLALDEHYDE ISONICOTINOYL HYDRAZONE

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Iron (Fe) is an element essential to all living cells. However, this transition metal may also catalyze the Fenton reaction which results in the formation of toxic reactive oxygen species (ROS), such as hydroxyl radicals.

Salicylaldehyde isonicotinoyl hydrazone (SIH) is a tridental chelator selectively forming complexes with Fe ions. As a result of its low molecular weight and good lipophilicity, SIH can be administered orally. It readily enters the cells, effectively chelates the intracellular Fe ions, and is therefore able to very efficiently inhibit the Fe-dependent processes,

such as production of ROS, but also the synthesis of some proteins and enzymes and the processes they regulate (e.g., cellular growth and proliferation).

In this work we focused on the design, synthesis and *in vitro* evaluation of novel SIH analogues with modified ligands, in particular the thio-analogue of SIH, analogues derived from (di)hydroxybenzophenone (**1**) and 2,6-dihydroxybenzaldehyde (**2**, Fig. 1).

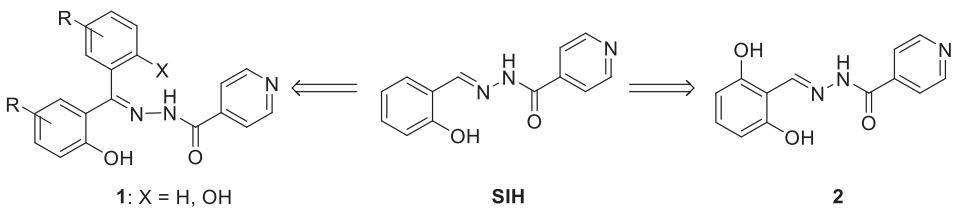

Fig. 1. Structures of SIH and its analogues studied in this work.

We prepared 17 analogues of SIH so far and assessed their ability to protect H9c2 cardiomyoblast cells against hydrogen peroxide-induced injury, studied their toxicity in the same cell line and their antiproliferative effects in HeLa and MCF-7 cell lines. Among the studied compounds, 2,6-dihydroxybenzaldehyde 4-chlorobenzohydrazone showed the most promising results.

The study was supported by the Czech Science Foundation (1315008S) and Charles University (SVV 260 401).

TOTAL SYNTHESIS OF HUMAN 6-HYDROXYSPHINGOSINE

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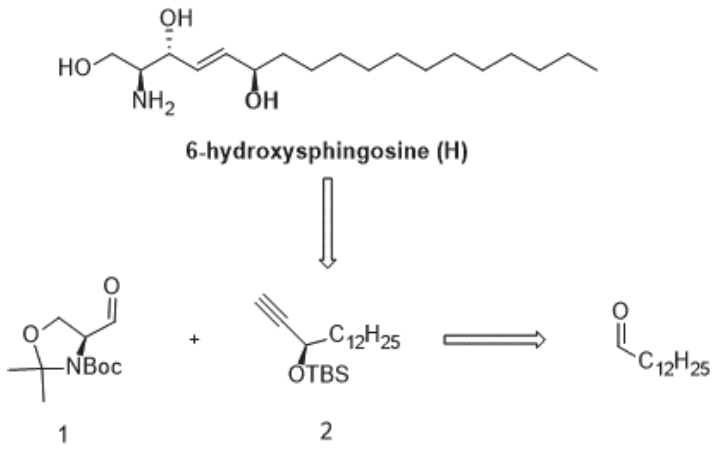
Ceramides (Cer) as members of sphingolipid family, play an important role in cell signalling. On the other hand, Cer occur in the human skin, where play another role in barrier function. Cer together with free fatty acids and cholesterol (in equimolar ratio) form intercellular multi-lamellar lipid matrix of the uppermost skin layer (stratum corneum). The primary function of this lipid matrix is to ensure a permeability barrier, thus, to provide water and electrolyte homeostasis, and event entry of harmful substances.[1]

Cer are composed of amino alcohol, e.g., sphingosine, and fatty acid acyl part. Cer derived from 6-hydroxysphingosine (H),3 i.e., (2S,3R,4E,6R)-2-aminooctadec-4-ene-1,3,6-triol (4E-t18:1), are the most unusual sphingolipids. 6-Hydroxyceramides (H-Cer) are not typical for all mammals, i.e., none were detected in pig skin but were found in some dog breeds.[2] Moreover, their function and biosynthesis are still unclear. However, various studies showed a relationship between lower concentrations H-Cer classes (relative to the

healthy skin) and atopic dermatitis.[3] The major limitation of understanding the nature of H-Cer is that these species are not commercially available.

Therefore, the aim of this work was to explore of synthetic route towards H as a precursor of all known H-Cer subclasses.



Scheme 1. Structure and retrosynthesis of 6-hydroxysphingosine.

The total syntheis of H was based on the reaction of commercially available tridecanal with trimethylsilyl acetylene. Strategy for synthesis of H involved an alkynylation of (S)-Garner's aldehyde (protected L-serinal) (1) with protected (R)-pentadec-1-yn-3-ol (derived from tridecanal), followed by selective two-step trans-reduction of triple bond(2). The reduction was performed by mild and selective [Cp*Ru(CH3CN)3]PF6-catalyzed Trost's hydrosilylation followed by protodesilylation.

In conclusion, physiological sphingoid base H was prepared in 7 reaction steps. Moreover, Cer NH (N-lignoceroyl-6-hydroxysphingosine) was also prepared by the acylation of free H with lignoceric acid. In the future, the free sphingoid base (H) will serve as precursor for the synthesis of all known H-Cer subclasses, i.e., alfa-hydroxylated Cer AH, omega-hydroxylated Cer OH and Cer EOH (with ester-linked linolenic acid).

This work was supported by the Czech Science Foundation (16-25687J) and Charles University (SVV 260 401).

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PYRAZINE DERIVATIVES AS POTENTIAL ANTITUBERCULOTICS, SYNTHESIS AND BIOLOGICAL EVALUATION

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Over the past few years, the treatment of tuberculosis proved to be more and more challenging due to appearance of many resistant strains of *Mycobacterium spp*. Therefore, once considered not so important systematic research of new potential antituberculotics is becoming to be one of the priorities in research among developed countries.[1]

The importance of pyrazinamide in treatment of tuberculosis is undeniable. However, because of commonly and rapidly growing resistance to this potent antituberculotic drug, modern research is trying to find other new drugs or at least other derivatives of known ones as potent as pyrazinamide to overcome the resistance problem. In 1978, Foks and Janowiec[2] described antimycobacterial activity of 1-phenyl-3-(pyrazin-2-yl)urea but did not continue with the research in this area.

Our research group decided to further investigate this finding in hope to find another potent antituberculotic by preparing and testing derivatives of this promising molecule.

We synthetized a series of 1-phenyl-3-(pyrazin-2-yl)urea derivatives by substituting phenyl group with other aromatic compounds, and had them tested so far on 3 strains of Mycobacteria: *M. aurum, M. phlegmatis and M. tuberculosis*. None of the synthetized proved to be sufficiently active against *M. aurum* and *M. phlegmatis*, but some showed activity against *M. tuberculosis*. With more time, our research will continue and we hope to find other potential new antituberculotic drugs.

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COMPOUNDS COMBINING PYRAZINAMIDE AND *PARA*-AMINOBENZOIC ACID AS POTENTIAL ANTITUBERCULARS

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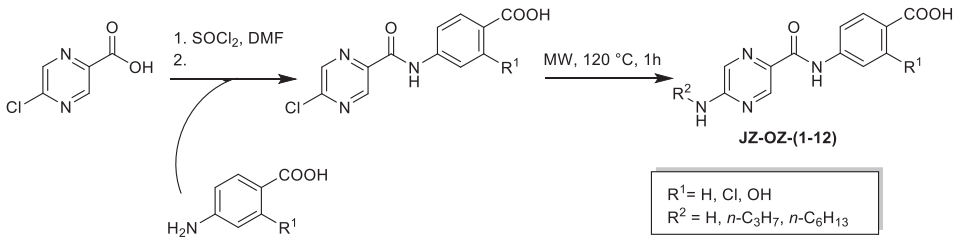
A series of new compounds combining pyrazinamide and *p*-aminobenzoic acid was prepared and *in vitro* tested for antimycobacterial activity against *M. tuberculosis* H37Rv, *M. avium*, *M. kansasii*, *M. aurum* and *M. smegmatis*. Previously prepared 4-(5-chloro-

pyrazine-2-carboxamido)-2-hydroxybenzoic acid (R^1 = OH) exerted micromolar activity against *M. tbc* H37Rv and low *in vitro* cytotoxicity in HepG2 cells.[1] *Para*-Aminosalicylic acid (PAS) has significant antitubercular properties based on its resemblance to *p*-aminobenzoic acid and interference with the folate pathway in mycobacteria.[2] To assess the role of the PAS fragment, we designed and prepared derivatives with modified substitution on the phenyl ring (R^1). Further modification was the exchange of 5-Cl on the pyrazine core with alkylamino substituent (JZ-OJ-1 to 12), which was a successful modification in our previous series.[3]

Some of the 5-propylamino compounds (incomplete results) proved micromolar activity against *M. tbc* H37Rv. Structure-activity relationships will be discussed.



The study was supported by Czech Science Foundation project No. 17-27514Y and by SVV 260 401.

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DERIVATIVES OF 5-ALKYLPYRAZINE-2-CARBOXYLIC ACID AS POTENTIAL ANTI-INFECTIVES

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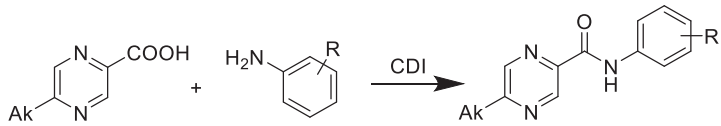
In our previous study, we have demonstrated that 5-alkylamino-*N*-phenylpyarazine-2-carboxamides with longer alkyl chain (C_5–C_8) exerted micromolar growth inhibition activity against *M. tuberculosis* H37Rv.[1] We speculated that the long alkylamino chain could facilitate the penetration of lipophilic mycobacterial cell envelope. To test this hypothesis, we performed the amino to methylene isosteric exchange and designed a series of 5-alkylamino-*N*-phenylpyarazine-2-carboxamides. 5-Alkylpyrazine-2-carboxylic acids (5-Ak-POA) were prepared by homolytic alkylation of commercially available pyrazine-2-carbonitrile by respective alkanoic acid, followed by hydrolysis of the carbonitrile group.

Final derivatives were prepared by CDI mediated coupling of 5-Ak-POA with corresponding aniline at RT.

Final compounds were described by melting point, elementary analysis, IR spectroscopy and ^{1}H, ^{13}C NMR. Then they were tested *in vitro* for antimycobacterial activity against *M. tuberculosis* H37Rv and several non-tuberculous mycobacterial strains. Several compounds exerted MIC of 3.13–6.25 μg mL^{-1}. Compounds with R = 3-CF_3 had a broad spectrum of activity covering the non-tuberculous mycobacteria. Detailed structure-activity relationships will be discussed.



The study was supported by Czech Science Foundation project No. 17-27514Y and by SVV 260 401.

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PYRAZINECARBOXYLIC ACID DERIVATIVES AS POTENTIAL ANTIMYCOBACTERIAL COMPOUNDS

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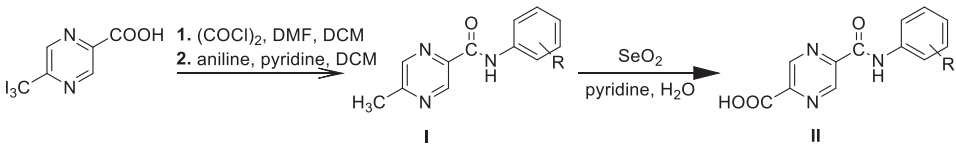
A series of new compounds related to pyrazinoic acid (POA) was synthesized, characterized by analytical data and *in vitro* tested for antimycobacterial activity against *M. tuberculosis* H37Rv and several non-tuberculous mycobacteria.

Anilides of 5-methylpyrazine-2-carboxylic acid (**I**) were prepared to broaden the previously published series of POA anilides with different substitution on the pyrazine core.[1] One of the proposed mechanisms of action of POA is the inhibition of mycobacterial trans-translation by binding to ribosomal protein RpsA. Lipophilic substituents in C-5 or C-6 of POA should be compatible with the binding mode of POA to RpsA[2] and could enhance the permeation of mycobacterial cell wall due to the increased lipophilicity. With this intention, we designed and prepared lipophilic derivatives of POA of general structure **II** by oxidation of **I** by selene dioxide. Among the tested compounds (incomplete results), **I** with R = 4-Cl proved to be the most potent compound against *M. tbc* H37Rv (MIC = 1.65 μg mL^{-1}). Generally, oxidation of the methyl to carboxylic moiety decreased the activity. Structure-activity relationships will be discussed.



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STRUCTURAL STUDIES OF SELECTED PROTEIN-LIGAND INTERACTIONS

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Molecular docking is a computational method that predicts interaction between two molecules (receptor and ligand). This method studies the binding mode and the position of the ligand in a protein's binding site.

The goal of the work was to predict interactions of novel compounds designed on Department Toxicology and Military Pharmacy before their synthesis. Based on the predicted data, compounds for chemical synthesis and biochemical testing were chosen.

Our targets were human BACE-1 (beta-secretase 1) that plays a role in Alzheimer's disease, GluN2B that is a part of glutamate (*N*-methyl D-aspartate) receptor and its antagonists have neuroprotective and anti-Parkinsonian effects, and COX-2 (cyclooxygenase-2) as a target for non-steroidal anti-inflammatory drugs.

We chose appropriate complexes from RCSB Protein Data Bank and we prepared proteins and ligands for molecular docking using software Avogadro, Chimera and AutoDock Tools. The computational part was realized through AutoDock. Suitability of the approach was verified by re-docking of ligands co-crystalized with the targets.

EVALUATION OF CLINICAL PHARMACIST´S INTERVENTIONS IN THE HOSPITAL

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The Drug-Related Problem (DRP) is an event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes.[1] DRP may disrupt patient's safety. Pharmaceutical care allows solution of DRPs and contributes to optimization of pharmacotherapy.

The aim of the research was to describe and evaluate the most common DRPs occurring in the selected department in the hospital.

The research was provided in the hospital in Šumperk, at the department of the internal medicine, the nursing section. Data was collected from medical records of hospitalized patients. Subjects for analysis were patient's characteristics, diagnosis, using drugs, selected laboratory markers and drug-related problems. DRPs have been identified during reviews of the clinical pharmacist. Description and evaluation of DRPs include management, acceptation of the pharmacist's intervention and economic aspect of intervention. The classification of DRPs was according to the Pharmaceutical Care Network Europe classification version 5.01. Results were evaluated by statistical method. 53 patients were involved in the research. DRPs were detected at 85 percent of analyzed patients. On average one patient suffered from 1.9 DRPs. The most frequent DRPs were "Drug choice problem" (58%), followed by "Dosing problem" (26%), "Others" (9%), "Drug use problem" (4%), "Interaction" (3%). "Adverse reaction" were not identified even once.

The most related drugs to DRPs were omeprazole, cholecalciferol and atorvastatin. Recommended interventions of the clinical pharmacist were discussed with doctor and mostly accepted. Interventions did not mean cost reduction on drugs. Occurrence of DRPs in hospital is high. Clinical pharmacist helps to prevent and identify DRPs and minimize their occurrence among hospitalized patients.

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ANALYSIS OF DRUG-RELATED PROBLEMS IN HEALTHCARE FACILITY

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Drug-Related Problems (DRPs) are defined as pharmacotherapy related events, which actually or prospectively interfere with a therapeutic purpose.[1]

The goal of the work is focused on obtaining and evaluating of DRPs in *Hamzova odborná léčebna pro děti a dospělé* (Luže-Košumberk), which is the important center of rehabilitation in the Czech Republic. Patient´s data were collected from their medical documentation, uploaded to special web database, and statistically evaluated by descriptive statistic. The data of 94 patients from the three departments of the rehabilitation center were analyzed. Together patients used 672 drugs (i.e. 7.15 drugs per patient). Overall, 272 DRPs were registered and 84 (91%) patients had at least one DRP. Most of them were related to drugs from the anatomical group C – Cardiovascular system in accordance with ATC classification of drugs. According to PCNE (Pharmaceutical Care Network Europe) classification the most problems were recorded in groups P3 (Dosing problem, 47%) and P2 (Drug choice problem, 33%).

The most frequent DRPs were regarding to unclear drug indication, drug absence despite the clear indication, bad dosing scheme, too high drug dose and the unclear drug signature. DRPs had a prospective character. Because of the methodology, this study contains no information about undesirable side effects. Most of the DRPs were considered of little or medium importance.

The conclusion of the work is that influence of pharmacotherapeutic audits is very important because they help to detect and solve DRPs frequently occurring in healthcare facilities. The most occurred DRPs were discussed with physicians.

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PRESCRIBING OF POTENTIALLY INAPPROPRIATE MEDICATIONS TO ELDERLY PEOPLE NEGATIVE OUTCOMES

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Older persons represent a quickly growing segment of the population worldwide and more vulnerable population to various adverse drug reactions and adverse outcomes. Many explicit criteria of potentially inappropriate medications (PIMs) have been developed in different countries in order to improve the quality and safety of geriatric prescribing and to reduce the risk of drugs in older adults.

The aim of this diploma thesis was to summarize (using narrative literature review) the outcomes of potentially inappropriate medications documented in pharmacoepidemological studies published by 2016 year.

Based on the systematic literature review using PubMed, Web of Science datasets during the period 10/2015–1/2017, literature review was conducted. 421 studies were identified during the primary literature search and after thorough consideration of abstracts 69 (16%) of studies were selected for works on summary tables. In the literature review only outcomes studies published during the period 2003–2016 were included. 36 (52%) of prospective, 27 (39%) of retrospective and 6 (9%) of cross-sectional studies were identified. The majority of studies included patients aged ≥ 65 years living in community, nursing homes or hospitalized patients. 57 (83%) of studies were made on representative sample of population (> 300 patients). The most of studies found positive association with hospitalization, impairments in physical functioning, higher health care cost and higher health care utilization. No negative impact on mortality, HRQOL, occurrence of ADE/ADR or drug-drug interactions was observed.

Further outcome studies using improved methodology (particularly in the part of the number of older people, length of data collection period, study design) are needed to better understand relevant outcomes of PIM use in older patients in Europe.

POLYPHARMACY IN THE ELDERLY – NEGATIVE OUTCOMES

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Older persons usually suffer from multiple chronic disorders and consequently use more medications than younger adults and often polypharmacy. The aim of this diploma thesis was to summarize by narrative literature review the negative outcomes of polypharmacy in older adults documented in pharmacoepidemological studies published between 2005 and 2015 years. The outputs of this diploma thesis create part of results of the research subgroup "Aging and Changes in the Therapeutic Value of Medications in the Aged" and the EU COST Action initiative IS1402 (2015–2018).

Using narrative literature review of Web of science, Medline, PubMed, EMBASE datasets during the period (2014–2015), we summarized outcome studies on polypharmacy in older patients published between 2005–2015 years. 563 studies were identified during the primary literature search and after reading of studies' abstracts, 70 (13%) of studies were

selected for summary tables. 496 (87%) of studies were excluded because they did not focus on outcomes of polypharmacy.

We identified 23 (33%) prospective, 22 (32% retrospective and 25 (35%) cross-sectional studies. Seniors mainly aged 65 years from ambulatory care, nursing homes, acute care or living in their homes were included. The main outcomes positively associated with polypharmacy were mortality, falls, hospitalizations, non-adherence, poorer nutritional status and GIT symptoms and poor quality of life. Cognitive decline was not significantly associated in most of the studies.

We confirmed that polypharmacy in older adults have mostly negative impact on health status in the elderly, especially on mortality, falls, higher rate of hospitalizations, adherence and other factors. Further outcome studies are needed to better understand relevant negative outcomes of polypharmacy in older patients in Europe.

PARALLEL RE-EXPORT OF MEDICINES FROM CZECH REPUBLIC

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Re-export of medicines is one of the most discussed issues, both in professional and public scene of pharmacy and therefore, we decided to focus on this topic and try to help keeping balance between shortages of particular drugs and income of distributors.

There are still severe shortages of vital human medicines on the Czech market as a result of their mass parallel re-export to other countries. This is clearly a long-term problem that has not been dealt with effectively. The parallel re-export of medicines as a result of low prices on the Czech market has led to national shortages.

Under EC law, Member States can impose limits on the percentage volume of medicines that can be exported from the country if those medicines cannot be substituted for another substance with the same medicinal properties. Under EC law, Member States can take action within the framework of their own laws to limit exports of medicinal products (maximum re-export limits) for the purpose of protecting human health and human lives and on the basis of the proportionality of such action.

Aim of the study was to get complex knowledge from distributors, pharmacies with approval for distribution and producers on recent situation including legislative, common praxis with essential data and also their own experiences and opinions how to solve the problem, in order to maximize results of the research. Personalised questionnaires were made and the answers were analyzed, amplified with further up-to-date information from verified sources.

Conclusions are greatly interesting and promising, so continuing long-term research is going on already.

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ANALYSIS OF COMMUNITY PHARMACY MARKETING

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Community pharmacy works as any other economical subject so it needs to generate profit from its customers. Therefore, it is important to analyze customers' needs and decide for the right marketing communication.[1] According to growing number of patients that use internet and social media for health related reasons it is good to use these technologies as the part of marketing communication also in the community pharmacy.[2]

This research focuses on the issues of patients' choice of pharmacy and investigates their opinion on pharmacy marketing practice and importance of internet communication. Data were collected via the questionnaire survey among the users of internet and customers in one independent community pharmacy in Beroun. Link on the electronic version of questionnaire was placed on Facebook sites dealing with the medicines and healthy life style and on the internet forums for mothers and pregnant women. Customers of community pharmacy in Beroun were asked to complete the questionnaire right in the store.

More than one-half of respondents prefer the nearest pharmacy. The third of them (33.7%) appreciate the quality of provided services. Almost one-half of addressed people assess marketing activities of pharmacies as positive and 30.9% of respondents admitted that these activities influence them. For twenty-four percent of respondents aged between 25 and 64 years is important communication of pharmacy on the internet.

According to outcomes of this research, there is significant part of patients seeking for high-quality marketing communication and internet presentation of the community pharmacy.

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MEDICATION ADHERENCE AND SELF-MANAGEMENT IN KIDNEY TRANSPLANT RECIPIENTS

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Kidney transplantation (KT) requires a lifelong medical regimen of immunosuppressive treatment (IS). Our study is focused on medication adherence (MA) in KT outpatients, as the medical non-adherence is one of the leading (yet preventable) cause of graft rejection.

Furthermore, we focus on analysis of the main self-management tasks. The prospective cross-sectional study was undertaken in one year period from March 2016 to March 2017 at the Haemodialysis Centre in the Teaching Hospital Hradec Králové. Patients ≥ 18 years old and at least 3 weeks after KT were addressed within their regularly scheduled visit to the nephrologist. Structured interview was performed by pharmacist to determine patients' self-reported MA to IS using validated Czech version of Medication Adherence Report Scale (MARS-CZ). In addition, patients were interviewed about other self-management issues. Simultaneously, medication records were reviewed. Data analysis was performed by means of descriptive statistics. A total of 211 patients with the mean age of 55.8 ± 12.41 years completed the interview. Patients were 7.4 ± 5.75 years after KT and used in average 11.3 ± 2.96 drugs. The mean score of MARS-CZ was 24.7 ± 0.74 (MIN 16; MAX 25). Using the cutpoint of < 23, signs of non-adherence to IS were observed in 6 patients. In terms of medication taking, tacrolimus on empty stomach was taken by 44 (35.2%) patients and prednisone after the breakfast by 160 (79.6%) patients. A number of 174 (82.5%) patients followed their dietary recommendations. Out of 211, 153 (72.5%) patients used some kind of sun protection, 172 (81.5%) measured blood pressure at home and 24 (11.4%) were regular smokers.

According to current findings, the level of self-reported MA seems to be satisfying. Nevertheless, lower acceptance of other self-management tasks may also cause serious problems. Interventions on multiple levels including education and psychosocial support should be implemented to daily routine to minimize the risks of therapy failure. The engagement of the pharmacists should be beneficial in this point. This is the first complex study in patients after KT conducted in the Czech Republic.

This study was supported by Charles University (Project SVV 260 417).

ATTITUDES TOWARDS TREATMENT AND KNOWLEDGE OF HORMONAL CONTRACEPTIVES AMONG FINAL YEAR PHARMACY STUDENTS

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Hormonal contraception (HC) belong among worldwide birth control methods used in a broad age groups of women. Based on the health status, lifestyle, user's preferences or non-contraceptive benefits it is possible to choose from the wide spectrum of HC which differs in dosage and composition. The aim of this thesis was to determine the attitudes toward the treatment and knowledge about the HC among the final-year pharmacy students upon the completion of mandatory 6-months long practical training in pharmacy and knowledge acquired during the studies. The data were collected using online questionnaire. The questionnaire consisted of 33 questions, which were divided into several sections focused on obtaining for instance social-demographic information, information about dispensation of HC during practical training, knowledge about HC risks, information about correct and safe dosage of HC etc. The questionnaire was sent during one month to the students' school e-mail addresses at 2 faculties of pharmacy (Faculty of pharmacy in Hradec Králové (HK) and Faculty of Pharmacy in Bratislava (BA)). A total of 382 students were requested. The collected data were processed using the descriptive statistics. In the questionnaire, 109 students from HK (57.1%) and 74 students from BA (38.7%) have responded. Most of the respondents were women, in HK it was 95 (87.2%) and in BA 64 (84.2%). The mean age of the respondents was 23.6 years (SD = 0.9) in HK and 23.6 years (SD = 1.1) in BA. If students provided advice on the supply of HC (HK 43.1%, BA 32.8%), they focused mainly on the new users and counseling was mostly in the range of dispensing minimum (HK 61.5%, BA 47.3%). As absolute contraindications of combined HC students from both faculties reported correctly mostly venous thromboembolism history, breast carcinoma and smoking more than 15 cigarettes by women over 35 years, however in case of progestin-only HC, majority of students did not know the correct answers. Only 17.6% of students in BA and 21.1% students in HK would properly advise the patient how to solve the problem with unstuck contraceptive patch.

In conclusion, knowledge and attitudes of pharmacy students at both faculties were comparable, but there are still some limits of knowledge, which should be improved to enhance rational pharmaceutical care.

ATTITUDES TOWARDS TREATMENT AND KNOWLEDGE OF HORMONAL CONTRACEPTIVE USERS

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Hormonal contraception (HC) is very popular and reliable birth control method, although, as any other pharmacological therapy it is not entirely risk-free. The main aim of this work was to determine the attitudes towards treatment and awareness of HC risks of its users.

The research was carried out as questionnaire survey. Anonymous questionnaire consisted of 35 questions to obtain sociodemographic data, information concerning HC use as well as form of HC, information about the extent of knowledge concerning HC risks and HC misconcepcion awareness. Last part of questionnaire was focused on smoking while using HC. Questionnaires were distributed among women older than 15 years of age who attended a small private pharmacy in the centre of Prague with prescriptions to get their HC. The respondents could fill out the written questionnaire by themselves directly in pharmacy but mostly they chose to fill it out at home and send it to the Faculty in the prepaid envelope. The survey lasted from October 2016 to January 2017 and totally 51 (respond rate 63.8%) questionnaires were gathered. Collected data were then evaluated by descriptive statistics.

The mean age of respondents was 28 ± 8.2 years while more than 50% of women reached higher education. Survey results show that respondents were mostly aware of side effects that do not pose a direct threat to their health and most of them are not even proven to be caused by HC. One quarter of respondents was also smokers but only 23.1% of them were afraid of thromboembolism while using this form of birth control method and smoking. Respondents took mostly affirmative attitude (agreed or strongly agreed) for a claim that HC users are able to enjoy sexual relationship to the greater extent.

It was discovered that women's knowledge and awareness is quite limited, especially when it comes to the most serious risks of HC. However, these outcomes cannot be globalized because of small number of respondents, yet, they can serve as a background for further research needed for obtaining more accurate results in this topic.

PHARMACY STUDENTS' QUALITY OF LIFE

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The quality of life of pharmacy students may change throughout their university life and differ from the quality of life of the general population. In the Czech Republic, quality of life of pharmacy students was not yet examined.

The aim was to determine the quality of life of second year students at the Faculty of Pharmacy at Charles University in Hradec Králové. Secondary aim was to compare the results of the Bern Subjective Well-Being Questionnaire with the short version of World Health Organization Quality of Life questionnaire and the Subjective quality of life analysis questionnaire.

The data were collected during 2011. Questionnaires were filled in by second-year students of Faculty of Pharmacy in Hradec Králové. The Bern Subjective Well-Being Questionnaire, the short version of World Health Organization Quality of Life questionnaire and the Subjective quality of life analysis questionnaire were used. The data were analyzed using the MS Excel computer program.

The results of the questionnaires showed that most of the students rated their quality of life as good or very good (82%). Fifty three percent and seventeen percent of the students were satisfied and very satisfied with their health, respectively. Students were mostly satisfied with the environment they lived in (housing, health care, safety, money). They were least happy with the lack of free time. Students lacked time for relaxation, hobbies and friends. A statistically significant difference between the quality of life of men and women was confirmed in third domain of the short version of World Health Organization Quality of Life questionnaire, at the scale of somatic disorders, at the scale of self-respect and at the scale of depressed mood of the Bern Subjective Well-Being Questionnaire ($p < 0.05$). At the other scales, a statistically significant difference was not confirmed ($p > 0.05$). Worse results in quality of life of our respondents, compared with Prague's population have not been confirmed. We found relationships between second domain of the short version of World Health Organization Quality of Life questionnaire and the scale of a positive attitude towards life of the Bern Subjective Well-Being Questionnaire, between third domain of the short version of World Health Organization Quality of Life questionnaire and the scale of joy of life of the Bern Subjective Well-Being Questionnaire and between second dimension of the Subjective quality of life analysis questionnaire and the scales of somatic disorders of the Bern Subjective Well-Being Questionnaire.

We can indicate that quality of life of students from second year was good although our students were not satisfied with their free time. The same conclusion was found in students from the Czech Technical University in Prague.[1] Further investigation into quality of life of pharmacy students across all years is needed.

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UP-SCALING AND FURTHER DEVELOPMENT OF MATRIX LIPOSOMES

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Liposomes have been investigated as promising drug delivery systems since their first discovery back in the 1960s. Up to date, scientists are facing many challenges in order to increase their bioavailability after oral administration, improve and simplify manufacturing processes and avoid microbial contamination with the aim to extend their shelf-life.[1] Matrix liposomes are small spheroid vesicles whose lipid bilayer is composed of egg-phosphatidylcholine and cholesterol. Entire particles of liposomes are embedded in a gelatin matrix while the final formulation is solid at room temperature. In addition, the presence of gelatin suggested to increase the stability of the formulation under gastric and intestinal conditions in the GI-tract. Therefore, it is considered to become a prospective formulation for oral protein drug delivery.[2]

The aim of this study is to up-scale and optimize the matrix liposomes formulation by dual asymmetric centrifugation method. This method is based on a combination of two *contra* rotating movements which generate shear forces and thus lead to efficient homogenization of lipid blend.[3]

Due to the development of a new speed mixing device, liposomes were formulated in higher batch sizes while the step of forming dried lipid film completely omitted. The new technology is not only less time-consuming but also enables to decrease consumption of organic solvents and make the entire process more environmentally friendly.

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STUDY OF SKIN DISEASES USING MONOLAYER LIPID MODELS

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Skin is an organ that protects the body against external influences and excessive water loss. The proper skin barrier is localized in the outermost layer of the epidermis, the stratum corneum (SC). The function of this barrier depends on the composition and or-

ganization of the extracellular space of SC. It consists mainly of ceramides (CERs), free fatty acids and cholesterol in approximately equimolar ratio. An imbalance in the lipid composition leads to disruption of the skin barrier function.[1]

Ceramidase is an enzyme which cleaves CERs or glucosylated ceramides (precursor of CERs) to lysosphingolipid (lysoSph) and fatty acid. Abundance or deficit of this enzyme causes dysfunction of skin barrier and the manifestation of skin diseases, e.g. Farber's disease. This systemic disease causes the development of subcutaneous nodules, which appear a few months after birth and lead to death during the first few years of life.[2,3]

The aim of this work was to study the monolayer lipid models whose compositions reflect the abundance of ceramidase in the skin. Lipid models were studied by Langmuir monolayers at the gas-liquid interface and at the solid surface, including Brewster angle microscopy and atomic force microscopy.

With increasing addition of lysoSph (0–75%) – including increasing addition of free fatty acids, the theoretical area per molecule of the lipid is decreasing. The modus of compressibility of the lipid mixtures is increasing to the 10% addition of lysoSph to the mixture containing only CERs and then it is decreasing again.

Results brought finding that the addition of lysoSph to the lipid mixture affects the lipid organization and therefore it affects the function of the skin barrier.

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