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SECTION OF CHEMICAL SCIENCES

DESIGNING A METHOD FOR SALT-ASSISTED DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION IN A "LAB-IN-SYRINGE" SYSTEM

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The sequential injection analysis (SIA) is a technique derived from flow injection analysis technique. The system generally consists of a computer controlled syringe pump, a selection valve and a detector, all connected by inert plastic tubing. It is used to automate laboratory procedures. The "Lab-In-Syringe" is a modified SIA used to carry out parts of the experiment inside the used syringe pump. Using a PTFE-coated magnetic-propelled stirring bar inside the syringe[1] allows, for example, to homogenously mix the syringe contents and perform dispersive liquid-liquid micro-extraction (DLLME)[2].

In this work, the approach to perform salt-assisted in-syringe DLLME was explored and evaluated for the first time. Starting with a one-phase system, the analyte was extracted from water into n-propanol. For this, a highly-concentrated solution of magnesium sulfate was used to increase the polarity of the aqueous phase. The high polarity causes the separation of the two normally miscible liquids.

Measuring the absorbance in the organic phase was studied both in-syringe and at the outlet and yields precise analysis of the sample content. Astraphloxine and riboflavin were used as model analytes and various conditions, i.e. salt concentration and water/solvent ratio were tested. The method performance and parameters were studied, evaluated and improved for the highest preconcentration factor and the fastest phase separation.

The highest achieved preconcentration factor was 6.68. The fastest phase separation took < 5 sec. The reproducibility of 3 repetitive extractions was generally < 1% RSD.

Using n-propanol, even compounds of moderate polarity can be extracted with high-efficiency. Furthermore, n-propanol is a HPLC compatible solvent, so the extract can be optionally analyzed on-line in modern HPLC systems.

In conclusion, the salt-assisted DLLME presents an interesting approach to perform a fast, precise, and automated extraction in small scale for the analyte preconcentration using an environment-friendly and HPLC compatible solvent.

The study was supported by SVV 260 184.

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DEVELOPMENT OF MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY METHOD FOR THE ANALYSIS OF ILLEGAL FAT-SOLUBLE FOODSTUFF DYES

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A microemulsion electrokinetic chromatography (MEEKC) method was developed and proposed for the determination of fat-soluble dyes (Sudan I, Sudan II, Sudan III, Sudan Red 7B, Sudan Orange G, and Methyl Red) illegally used in foodstuffs.

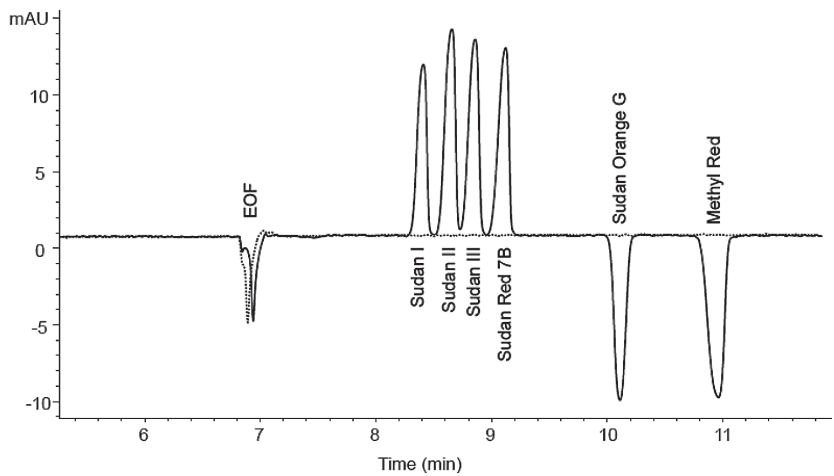

Fig.1. MEEKC separation of standard mixture of illegal foodstuff dyes under optimum conditions.

The effect of surfactant, co-surfactant, organic modifier and oil as well as the capillary length were examined in order to optimize the separation. Final background electrolyte (solution of the microemulsion) for MEEKC was composed of 30mM phosphate buffer (pH 7.5), 1.2% (w/v) sodium dodecylsulfate, 1.2% (v/v) of n-hexane, 15% (v/v) of butan-1-ol, and 20% (v/v) of acetonitrile.

A baseline separation of these six dyes was achieved within 11 min by using fused-silica capillary with 75 μm i.d. and effective length 36.5 cm. The applied voltage was 20 kV and temperature 25 °C was maintained. The VIS detection wavelengths were 500 and 400 nm.

The repeatability of the migration times and peak areas were characterized by RSD values ranging from 0.3 to 0.9% and 1.7–2.7% (n = 5), respectively. The calibration curves were linear for all analytes ($R^2 \geq 0.9990$) and the limits of detection ranged from 0.19 μg/ml (for Sudan III) to 1.27 μg/ml (for Sudan Red 7B).

The method devised is suitable for the analysis of suspected foodstuffs after appropriate sample pretreatment to eliminate matrix effects and to achieve sample pre-concentration.

The study was supported by SVV 260 184 and the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

PREPARATION AND PHOTOPHYSICAL EVALUATION OF TETRA-3,4-PYRIDOPORPHYRAZINES SUITABLE FOR THE PHOTODYNAMIC THERAPY

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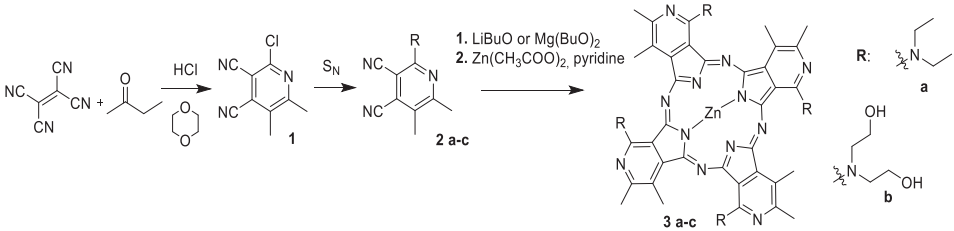
Tetra-3,4-pyridoporphyrazines (TPyPz) are aza-analogues of phthalocyanines. Their large system of conjugated bonds enables them to absorb light in the red part of the absorption spectrum. Due to their ability to produce singlet oxygen, they can be potentially used as photosensitizers in photodynamic therapy (PDT). Its mechanism is based on co-functioning of three elements – photosensitizer, light and oxygen. Photosensitizer excited by light absorption transfers its energy into tissue oxygen, thus, creating cytotoxic singlet oxygen. This method is beneficial for its high selectivity, low toxicity, minimal invasion and fast effect.

The aim of this work was to synthetize and study water-soluble TPyPz suitable for PDT. Water solubility was achieved by introduction of hydrophilic non-charged substituents (OH), quarternized amines (structure of target TPyPzs see below), forming of salts or using suitable delivery systems (hydrophilic emulsion). Firstly, appropriate precursors for TPyPz (i.e., 2-substituted-5,6-dimethylpyridine-3,4-dicarbonitriles) were prepared by nucleophilic substitution according to the scheme below. Then, cyclotetramerization of **2 a–c** with butoxide as an initiator of the reaction gave required macrocycles. Obtained TPyPz were transferred into metal free derivatives under acidic condition and zinc was then coordinated



into the center. All prepared TPyPz were characterized by physico-chemical properties and biological activity.

The study was supported by SVV 260 183.

PREPARATION AND PHOTOPHYSICAL EVALUATION OF TETRA-3,4-PYRIDOPORPHYRAZINES CARRYING CHARGED SUBSTITUENTS ON THE PERIPHERY

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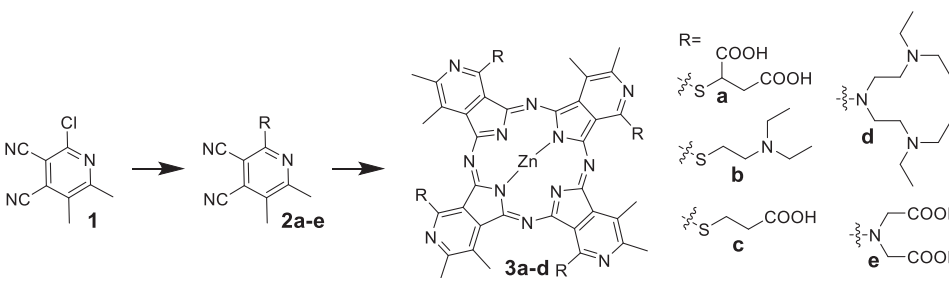
Phthalocyanines are planar organic molecules, which have a metal cation coordinated in their center. This work deals with their aza-analogues – tetra-3,4-pyridoporphyrazines (TPyPz). TPyPz can absorb light in red part spectrum and then produce singlet oxygen. Due to this ability, they may be used in photodynamic therapy (PDT) of cancer. PDT's mechanism is based on three components: photosensitizer, light and singlet oxygen. Photosensitizer transfers energy of absorbed light to oxygen making, thus, cytotoxic singlet oxygen.

The goal of this project was to synthesize water soluble TPyPz absorbing in red part of spectrum. TPyPzs bearing different charged substituents will be compared within the series.

The synthesis consisted of preparation of 2-chloro-5,6-dimethylpyridine-3,4-dicarbonitrile (**1**), which was the starting precursor for other reactions. Nucleophilic substitution of **1** was used for the introducing of hydrophilic substituents **a–e**. Prepared precursors **2a–e**



underwent cyclotetramerization leading to final TPyPz **3a–d**. Structures were characterized by physico-chemical properties and in *in vitro* testing.

The study was supported by SVV 260 183.

OXADIAZOLES AS POTENTIAL DRUGS

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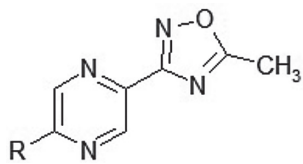
Due to the increasing resistence of bacteria and fungi against conventional drugs, it is imperative to design and develop new antibacterial or antifungal agents[1]. Some derivatives of 1,2,4-oxadiazoles exert antibacterial activity and they are known as the compounds with promising future in this direction. The 1,2,4-oxadiazole ring is located in some biologically active compounds such as muscarinic receptor agonists, tyrosine kinase inhibitors, anti-inflammatory agents, antitumor agents, selective H_3 receptor antagonists, monoamine oxidase inhibitors, anticonvulsant and anti-HIV agents[2]. The 1,2,4-oxadiazoles are bioisosteres for amides and esters with higher hydrolytic and metabolic stability[3].

In the experimental part of this study, six new oxadiazole derivates have been synthesized. First, six pyrazine-2-carbonitriles have been prepared differently alkylated in position 5. Afterwards the nitriles were converted to corresponding amidoximes using hydroxylamine hydrochloride. In the last step amidoxime reacted with acetic anhydride in xylene to form corresponding oxadiazoles.



R = *terc*-butyl, isobutyl, propyl, isopropyl, pentyl, hexyl

Six novel 3-(pyrazil-2-yl)-1,2,4-oxadiazoles were obtained characterized by melting points, IR and NMR spektra. Their purity was checked by TLC and elemental analysis. The compounds were tested *in vitro* for their antifungal and antibacterial activity.

This study was supported by project SVV 260 183.

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STUDY OF IN-SYRINGE ANALYSIS FOR THE AUTOMATION OF IMMERSED SINGLE-DROP MICROEXTRACTION AS A SAMPLE PRETREATMENT TECHNIQUE

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Lead belongs to the most toxic elements with respect to human health due to its negative effect on metabolism of human beings. Moreover, lead has proven to be a carcinogen. The recommended limit of lead in tap water by the World Health Organization[1] is 10 μg L^{-1}. In this work, an immersed single-drop microextraction for the spectrophotometric determination of lead in tap water is presented for the first time.

Dithizone was used as a reagent, which creates a rose-coloured complex with lead. Mixture of toluene and n-hexanol was used as a solvent for the pre-concentration of the complex into the single drop. Ammonium-acetate buffer was used for keeping basic pH conditions. The method took place in the void of an automated syringe pump. A magnetic stirring bar was used for mixing the syringe's content continuously. The parameters of the method were optimized including the toluene/n-hexanol ratio, drop size, pH value of buffer, volumes of the dithizone reagent, buffer, and sample, extraction time and rotation speed of the stirring bar.

The calibration curve was linear over the range of 100–700 nmol L^{-1} with a correlation coefficient of $r^2 = 0.999$. The limits of detection (LOD = 39.2 nmol L^{-1}) and quantification (LOQ = 130.6 nmol L^{-1}) were also evaluated. Repeatability for the concentration range of 100–700 nmol L^{-1} was proven and the relative standard deviation (RSD) was 2.8%. Using a pre-concentration time of 300 s, the whole analysis took about 500 s. The extraction efficiency was in the range of 25%.

Important advantages of the proposed method are a small instrumentation size and thus its portability, so it can be used for on-site analysis and a high sensitivity.

The study was supported by the specific research, No. SVV 260 184.

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SYNTHESIS OF POTENTIAL ORGANOCATALYSTS BASED ON QUINAZOLINE ALKALOIDS

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A series of substances derived from vasicine-type alkaloids was synthesized. The compounds were prepared using different α-hydroxy carboxylic acids (lactic and mandelic) (Fig. 1) and α-amino carboxylic acids (phenylglycine, alanine, proline and valine) (Fig. 2). These derivatives are currently being tested for their organocatalytic activity on a series of reactions, such as asymmetric enamine catalyzed aldolisation and conjugate addition of aldehydes to nitroalkenes.

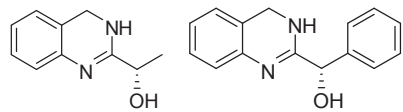

Fig. 1.

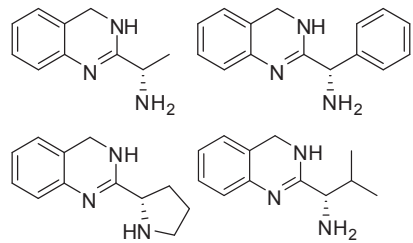

Fig. 2.

The study was supported by GA UK (No. 5671/2012), GA ČR (No. 15-07332S) and Charles University Research (SVV-260-183).

ON-LINE SPE HPLC METHOD OPTIMIZATION FOR DETERMINATION OF PATULIN MYCOTOXIN IN APPLE DRINKS

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The issue of food contamination with mycotoxins is a serious problem worldwide. These substances are highly toxic to humans and chronic effects on the human organism

in very low doses may cause long-term medical complications. Some mycotoxins are also substances stable and persistent in the environment, from where they can get through agricultural (crop and livestock) production further into the human food chain. From this standpoint it is therefore necessary to monitor whether the limits are not exceeded for individual mycotoxins and for this purpose to develop sensitive and selective analytical techniques for their detection. In our work we focus on one of the most common dietary mycotoxins – patulin, which is found in apples and related products (especially apple juices). High performance liquid chromatography (HPLC) coupled with on-line solid phase extraction (SPE) using a column switching technique for sample treatment was developed for determination of mycotoxin patulin in apple drinks and juices. A volume of 250 µl of juice sample was injected directly into the on-line SPE-HPLC system. After injection of the sample the extraction of patulin from juice matrix was carried out on SPE precolumn. SPE precolumn 25 × 3 mm was filled with Supel MIP Patulin sorbent, which is a specific "molecularly imprinted polymer" (MIP) designated for the selective extraction of patulin from an apple matrix. As the washing solution for removing ballast matrix was selected 1% solution of $NaHCO_3$, which flowed through MIP precolumn at flow rate 0.75 ml/min for 2.5 minutes. After this period a valve was switched and the residual ballastmatrix, retained on the extraction precolumn, together with patulin were further separated on an analytical column Kinetex Biphenyl 150 × 4.6 mm (particle size 5 µm). The mobile phase composition of 20% ethyl acetate in acetonitrile with water in ratio 20 : 80, flow through the column at 1 ml/min in gradient elution. Detection was performed by UV-VIS detector at a wavelength of 276 nm. The total analysis time of one juice sample, including its online pretreatment, was less than 9 minutes. The detection limit of this method was found at level 50 µg/l, which is the value corresponding to the maximum allowed levels of patulin in apple juices according to EU standards.

The study was supported by the Charles University project no. SVV 260 184.

DEVELOPMENT OF AN ABSOLUTE METHOD FOR DETERMINATION OF SINGLET OXYGEN QUANTUM YIELDS OF PHTHALOCYANINES

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Photodynamic therapy (PDT) with a singlet oxygen as an essential agent is believed to be a promising way of cancer treatment or treatment of some cutaneous diseases. Thanks to its high selectivity, harmful adverse effects are significantly decreased. The principle of PDT is based on excitation of a photosensitizer by light absorption, followed by transfer of energy to oxygen ($^3O_2$) forming cytotoxic singlet oxygen ($^1O_2$)[1]. The efficiency by which photosensitizer transforms absorbed energy to singlet oxygen production is characterized by *singlet oxygen quantum yields (Φ_Δ)*.

The aim of this study was to develop and optimize absolute method for determination of Φ_Δ. In comparison to a relative method, no reference is needed in this case, which enables accurate results with lower error. Verification of the new method was performed in *N*,*N*-dimethylformamide with zinc phthalocyanine as a model photosensitizer because of its well-known singlet oxygen quantum yield and with 1,3–diphenylisobenzofuran (DPBF) as a chemical quencher of $^1O_2$.

Different sources of light for excitation and different set-ups of the instrumentation were tried and compared. Efficient and accurate method for absolute determination of Φ_Δ was successfully developed. This method will be used for Φ_Δ measurements of the new compounds prepared in our research group.

The study was supported by SVV 260 183.

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SEPARATION OF TOCOPHEROLS USING HPLC TECHNIQUE

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Vitamin E represents eight related compounds α-, β-, γ-, δ-tocopherols (saturated phytyl side chain) and α-, β-, γ-, δ-tocotrienols (unsaturated phytyl side chain). α-Tocopherol was the most studied isomer in the past and its anti-inflammation and proliferative effect was described. Therefore most of cancer prevention trials have been focused on α-tocopherol. Present research studies have described the important role of γ- and δ-tocopherols in cell proliferation, anti-inflammation and tumor burden[1]. Also it has been demonstrated that γ-tocopherol inhibits colon, prostate, mammary and lung tumorigenesis in animal models, suggesting to have a high potential in the prevention of human cancer[2].

In this study the novel sensitive method for analysis of α-, β-, γ-, δ-tocopherols was developed using the High Performance Liquid Chromatography (HPLC) technique. All the measurements were carried out on the chromatographic system HPLC Prominence LC 20, Shimadzu (Kyoto, Japan).

During the method development core-shell columns (Kinetex) with different stationary phases (HILIC, Biphenyl, Pentafluorophenyl) were tested under various conditions such as temperature, injection volume, flow rate and composition of mobile phase. The best results were achieved using Kinetex Pentafluorophenyl column (4.6 × 100 mm, 2.6 μm) and mixture of methanol and water as a mobile phase.

The newly developed analytical method will be used for analysis of breast and gastrointestinal cancer patients. Results of this research can provide closer knowledge about the tocopherols metabolic pathway and their role in human body.

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DEVELOPMENT OF SPE AND UHPLC-MS/MS METHOD FOR THE DETERMINATION OF QUERCETIN AND ITS 9 METABOLITES

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Quercetin (Fig. 1) is one of the major flavonoids belonging to subgroup of flavonols. It is one of the most potent plant antioxidants which is distributed in edible plants (tea, red wine, fruits and vegetables). Consumption of quercetin may be associated with a decreased risk of coronary, bacterial and viral diseases.[1] However, in some newly published works the correlation of plasma levels of quercetin in adults and antioxidant capacity has not been confirmed.[2]

The aim of this study was to develop and validate a new extraction method for the preparation of biological samples for the determination of quercetin and its nine potential

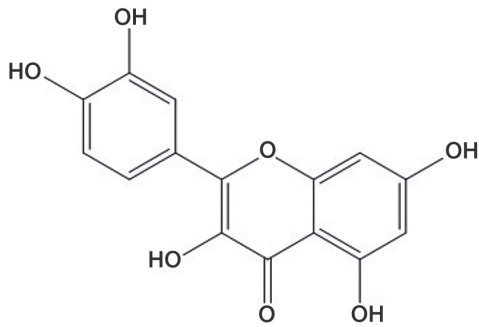

Fig. 1. The structure of quercetin.

metabolites: phloroglucinol, 3,4-dihydroxyphenylacetic acid, homovanilic acid, 3-hydroxyphenylacetic acid, 3-(3-hydroxyphenyl)propionic acid, rutin, quercetin-3-glucuronide, tamarixetin and isorhamnetin. These metabolites differ substantially in physicochemical properties (pKa, molecular weight, log P, chemical structures etc).

For the first screening in extraction procedure, the silica-based cartridges with C8, C18 ligand and polymer-based MAX (Mixed- mode Anion-eXchange) sorbents were used. MAX is mixed-mode polymeric sorbent that has been optimized to achieve higher selectivity and sensitivity for extracting acidic compounds with anion-exchange groups, which is the case of small acid metabolites of quercetin.

Due to the best retention and subsequent elution of all tested analytes the MAX cartridge was chosen. Other sorbents demonstrated poor retention of small polar acidic molecules. The optimization of elution solvent included optimization of methanol concentration (60–95%) and the choice of organic acid (formic acid 0.5–10% and trifluoracetic acid 0.1–1%). The mixture of 95% methanol and 0.5% trifluoracetic acid demonstrated the best recovery (60.9–105.7% with RSD 0.4–9.4%). Therefore it was chosen as optimal solvent for elution. The acidic pH was defined as a critical factor for the retention of analytes. Subsequently the wash solvent was optimized. The wash procedure consisted of two steps – the washing with water component (0.1–5% ammonium hydroxide, water, 0.01 M ammonium formate buffer with pH 5.0) and the washing with organic solvent (1–10% methanol). Due to good selectivity, the lowest losses of analytes, the best clean-up and removing of interfering compounds the combination of 0.01 M ammonium formate buffer with pH 5.0 and 1% MeOH was chosen.

The optimized extraction method was used for the determination of quercetin and metabolites in biological samples. The determination of quercetin and its 9 metabolites was performed by developed chromatographic method using ultra high performance liquid chromatography (Acquity UPLC) with tandem mass spectrometry (Quattro Micro triple quadrupole mass spectometer). The best selectivity between the critical pair of analytes (tamarixetin and isorhamnetin) was achieved using column BEH Shield RP C18 (2.1 × 100 mm; 1.7 μm) and gradient elution with methanol and 0.1% formic acid. The ionization was performed in electrospray polarity switching mode and the quantification by selected reaction monitoring (SRM). The method will be validated in terms of linearity, limit of detection and quantification, accuracy, precision, selectivity and matrix effects.

The advantages of newly developed SPE method for the preparation of biological samples prior to UHPLC-MS/MS analysis are simultaneous analysis and extraction of the compounds with different polarity, good recovery and repeatability.

The study was supported by SVV/2015/260184.

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SYNTHESIS OF SUBSTITUTED PYRIDINES USING TRIS(2-FURYL)PHOSPHINE GOLD(I) CATALYST

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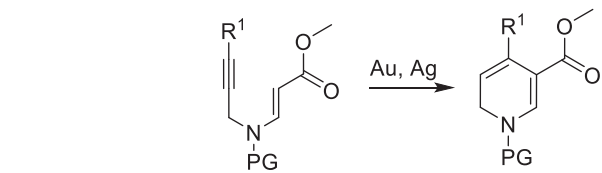
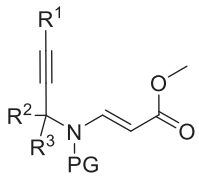
Synthesis of various types of heterocycles is possible from 1,5-enyne precursors using cationic gold(I) species as a catalyst. In order to extend previous research[1] we focused on cyclisation of 1,5-enynes with various aryls and silyls in the position R^1. The influence of various protecting groups and substituents in positions R^2 and R^3 was also investigated.





R^1 = phenyl, 3-methoxyphenyl, thienyl, 4-chlorophenyl
R^2,R^3 = H, alkyl
PG = *tert*-butylcarbamate, 4-methoxybenzenesulfonamide

The study was supported by SVV 260 183, GAČR P207-15-073325.

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5-ALKYLAMINO-*N*-PHENYLPYRAZINE-2-CARBOXAMIDES AS POTENTIAL ANTITUBERCULARS

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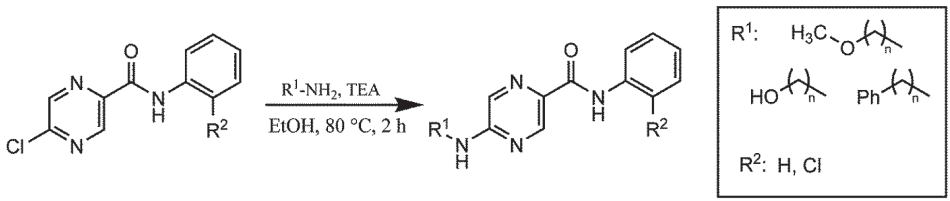
This study is focused on new derivatives of pyrazinamide (PZA) prepared as potential antituberculars. PZA itself is a well-established first-line antitubercular agent and a constituent of all basic tuberculosis treatment regimens. The design of final compounds was based on the previously synthesized 5-alkylamino-*N*-phenylpyrazine-2-carboxamides[1], which possessed promising *in vitro* antimycobacterial activity with MIC ranging from

0.78 to 3.13 μg/mL. The object of this study was to test the activity of derivatives with alkylamino chain modified with terminal phenyl, hydroxyl or methoxy group.

Final compounds were prepared by nucleophilic substitution of chlorine with respective amines in refluxing EtOH (Scheme 1). Reaction yields, after all purification steps, were 5887%. Compounds were characterized by ^{1}H and ^{13}C NMR, IR, elementary analysis and melting point.



Scheme 1. Synthesis of the final compounds.

Final compounds were tested for *in vitro* antimycobacterial, antibacterial and antifungal activity. Only six substances, out of total of 16 newly prepared, showed moderate activity against *M. tuberculosis* H37Rv and *M. kansasii* (MIC =12.5–50 μg/mL, $MIC_{(PZA)}$ = 6.25 μg/mL). All compounds were ineffective against *M. avium* and other tested pathogens. All compounds with R^2 = Cl were inactive. Detailed structure-activity relationships will be discussed.

This publication is a result of the project implementation: 'Support of establishment, development, and mobility of quality research teams at the Charles University', project number CZ.1.07/2.3.00/30.0022, supported by The Education for Competitiveness Operational Programme (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic. Additional support was provided by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 183), and Ministry of Health of the Czech Republic IGA NT 13346 (2012).

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SYNTHESIS OF ON THE RING SUBSTITUTED PHENYLGUANIDINES WITH BIOLOGICAL ACTIVITY

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The increasing frequency of systematic mycoses caused by opportunistic fungi resistant to antifungal drugs is a great health problem in the last years. It leads to high mortality especially of immunocompromised patients. Therefore it is necessary to find new substances with antifungal activity.

Series of four on the ring substituted phenylguanidines (I) were sythesized for their potential antibacterial, antifungal, and antimycobacterial activities in this study.

$R^1 = -C_{15}H_{31}, -C_{14}H_{29}$

(I) $R^2 = -H, -CH_3$, R3 = $-H, -CH_3, -CH_2CH_3$

Products were synthetized in the four-step synthesis[1,2]. Alkylarylsulfides were prepared by the reaction between alkylthiols and 4-chloro-3-nitrotoluene or *p*-chloronitrobenzene with active copper as a catalyst in the first step. The nitro group on the ring was reduced to amino group by the reaction with stannous chloride under nitrogen atmosphere in the second step. Sulfanylphenylamines were then transferred by the reaction with gaseous hydrogen chloride to ammonium chlorides. Phenylguanidines were prepared by the reaction of these salts with cyanamide or dialkylcyanamides in the last step.

All compounds were evaluated *in vitro* for antimicrobial activity by the broth microdilution method against representative human pathogenic fungi: *Candida albicans*, *Candida tropicalis, Candida krusei, Candida glabrata, Trichosporon asahii, Aspergillus fumigatus*, *Absidia corymbifera, Trichophyton mentagrophytes*; and bacteria: *Staphylococcus aureus*, *Staphylococcus aureus* Methicilin resistant, *Staphylococcus epidermidis, Enterococcus sp., Escherichia coli, Klebsiella pneumoniae, Klebsiella pneumoniae ESBL positive, Pseudomonas aeruginosa*.

The substance 1,1-dimethyl-3-[5-methyl-2-(tetradecylsulfanyl)fenyl]guanidine has significant antifungal activity. Its activity against some strains of fungi was higher than activity of ketoconazole.

This study was financially supported by the grant GA14-08423S.

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SYNTHESIS OF POTENTIAL CHOLINESTERASE INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE – TACRINE DERIVATES

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Alzheimer's disease (AD) affects currently about 6.1 million people in Europe. The threefold increase of this number can be expected due to population aging.

AD manifests as memory loss, social and spatial disorientation and gradual deterioration of intellectual capacity.

Although AD is described since 1907, the effective treatment has not been found yet. The reason is the most likely a multifactorial origin of AD.

To date, the pharmacotherapy of AD is relies on acetylcholinesterase inhibitors (AChEIs) – tacrine, rivastigmine, donepezil and galanthamine. More recently memantine, an antagonist of *N*-methyl-D-aspartate receptor, has been approved for moderate to severe stages of AD.

New approaches for AD therapy have emerged in recent years. One of them, multi-targed-directed ligands (MTDLs) capable of interaction with multiple target are currently being extensively investigated.

In our contribution we would like to introduce series of newly designed molecules based on MTDLs. These MTDLs combine tacrine, 6-chlortacrine or 7-methoxytacrine (7-MEOTA) as AChEIs with some amino acids using different linkers. It can be assumed that the molecules are capable to simultaneously bind peripheral anionic site (PAS) as well as catalytic anionic site (CAS) of AChE. The coumpounds exhibited very good inhibitory profile with IC_{50} values ranging from micromolar to sub-nanomolar concentration scale.

Supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), Long Term Development Plan – 1011 and by the Grant Agency of the Czech Republic (No. P303/11/1907). No. SV/FVZ 201409, No. SVV 260 183.

DESIGN AND SYNTHESIS OF HYBRID COMPOUNDS BASED ON TACRIN/RESVERATROL DERIVATIVES

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder, in which a progressive dementia appears. The cause of AD is currently unknown, however, scientific research has revealed several pathological hallmarks – β-amyloid plaques and neurofibrillary tangles. These changes cause gradual disintegration of nerve cells and they change the metabolism in the brain. The current drugs are not able to treat the cause of the disease, being able only to delay the onset of severe symptoms. The basic drugs for AD treatment are acetylcholinesterase (AChE, E.C. 3.1.1.7) inhibitors and, more recently approved, *N*-methyl-*D*-aspartate (NMDA) receptor antagonist memantine. These drugs are able to increase cholinergic activity or preventing glutamate excitotoxicity in the patient's brain, thus improving cognitive functions and delaying severe stages of the disease. One of the emerging approaches in drug synthesis represents multi-target-directed ligands (MTDLs). Apart from the ability to inhibit AChE, they can also target more pathological processes at once. As such, they are able to bring an added value in a single molecule. In this work, we turned our attention to the preparation of hybrid compounds based on tacrine and resveratrol moieties. Tacrine scaffold act as cholinesterase inhibitor, whereas resveratrol is a strong antioxidant, naturally occurring in the vine. We assumed that coupling of these moieties could lead to the derivatives affecting multiple pathological targets of the disease and consequently represent new leads for AD therapy.

Supported by SVV 260 183 and by Erasmus program.

PREPARATION OF BENZODIAZINES WITH BRONCHODILATORY ACTIVITY

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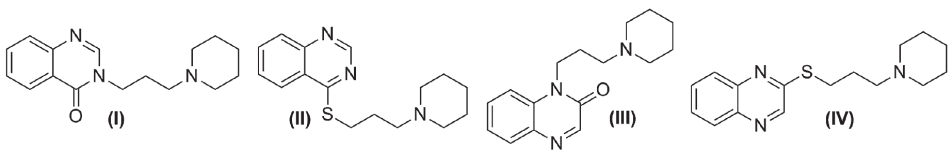
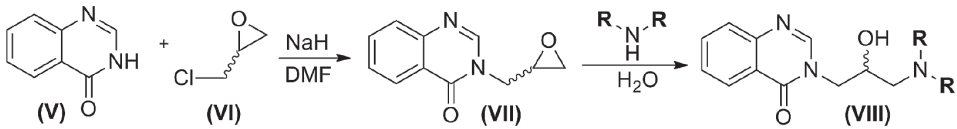
The most active compounds from previous screening contained (piperidine-1-yl)propyl moiety attached to quinazoline (**I**, **II**) or quinoxaline rings (**III**, **IV**).[1,2] The goal of this project was to introduce hydroxyl into the three-membered carbon linker with a possibility of further modification.



Bronchodilatory activity: ED_{50} = 3 – 25 μmol/L

The synthesis was carried out employing the reaction of commercially available quinazolinone (**V**) and racemic epichlorohydrine (**VI**) leading to epoxide (**VII**) which undergoes nucleophilic attack resulting in hydroxyquinazolinones (**VIII**). Similarly, this reaction sequence was applied on quinoxalinones.



However, the attempts with sulphur analogues of quinazolines and quinoxalines failed.

The relationship between the bronchodilatory effect and the prepared compounds will be discussed.

This work was supported by Charles University (SVV-260-183).

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SYNTHESIS AND SKIN PERMEATION-ENHANCING EFFECTS OF OF 6-((7-NITROBENZO[C][1,2,5]OXADIAZOL-4-YL)AMINO)HEXANOIC ACID DERIVATIVES

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Transdermal permeation enhancers are used to increase absorption of drugs through skin or, more importantly, through the stratum corneum, which is the uppermost layer of the skin. Mechanism of action of enhancers is not fully understood. In general the most active enhancers consist of hydrophilic and hydrophobic parts. Fluorescent dye 7-nitrobenzo[c][1,2,5] oxadiazol (later only NBD) is fairly hydrophilic, so we thought that it could act as a hydrophilic head of the potential enhancer. Such fluorescent enhancers could help us understand more about the mechanism of action, since it enables imaging of its penetration pathways in the skin.

We synthesised compounds containing the NBD as the hydrophilic head and ester-linked C8–C12 alkyls as hydrophobic tails. Aminocaproic acid reacted with 4-chloro-NBD and then with a series of alcohols to give us different lengths of alkyl chains. Then we applied these enhancers to human skin in Franz diffusion cells using two model drugs theophylline and hydrocortisone in two different media. We measured the concentrations of the drugs and also the enhancers beneath the skin in time to yield the flux values as well as concentrations in the skin after the test.

We found that the drug permeation was two to three times higher in the presence of 1% ester enhancers (NBD-acid was inactive) in comparison with control (only drug without the NBD-enhancer). Significant enhancer concentrations were found in epidermis and dermis and we also observed significant ester hydrolysis in the skin. Since these esters show strong fluorescence, they can provide interesting visual data, where in the skin are these enhancers located, and what structures they possibly interact with. Thus, the next step would be imaging the skin after the application of a selected enhancer using fluorescent microscopy.

The study was supported by The Charles University, No. SVV 260 183.

IN SILICO SCREENING OF SIRT6 INHIBITORS

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SIRT6 is called NAD-dependent protein deacetylase sirtuin-6 and it is a member of sirtuin protein family. Its deacetylase activity is targered on histone H3K9Ac and H3K56Ac and it modulates acetylation of H3 histone during the S phase. The SIRT6 enzyme is an interesting drug target because of its role in DNA replication, glycolysis and inflammation – that is why, the design of SIRT6 inhibitors is relevant in context of diabetes melitus, arthritis and cancer.[1,2]

There are about 9 known SIRT6 inhibitors published by Kokkonen 2014 and Parenti 2014. The aim of the work was to find new possible ligands of this enzyme using methods of computational chemistry and molecular modeling. We tried to find specially some new lead structures with possibility to be optimized in next phases of the drug discovery process.

The 9 known inhibitors and crystal structure of SIRT6 (PDB code 3K35) were used as input data during the modeling. The screening was done on the databases Enamine and Chembridge. Pharmacophoric and molecular similarity search were selected from the group of ligand-based methods. The pharmacophore was defined after structural alignment of four known ligands and tested on set of ligands and non-ligands. As pattern molecules for molecular similarity search (BIT_MACCS fingerprint), known ligands and their fragments were used.

All molecules from ligand-based screening were docked into SIRT6 crystal structure with several algorithms in software MOE, Glide and InducedFit and scored with different methods (London dG, GBVI/WSA dG and Glide score).

After final selection, 32 molecules were recommended for *in vitro* testing.

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DEVELOPMENT OF HPLC METHOD FOR DETERMINATION OF VANCOMYCIN IN CLINICAL RESEARCH – PART I

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Vancomycin, a glycopeptide antibiotic, provides bactericidal effect against gram-positive bacteria such as methicillin resistant *Staphylococcus spp.*, *Streptococcus spp.* and *Enterococcus spp.* It is useful mainly in serious bacterial infectious disease when resistances or allergies to penicillin or oxacillin in patients were indicated. Optimizing of vancomycin therapy is beneficial because of narrow therapeutic index and potential toxicity in high serum level.

Aim of this study was development of a clinical routine practice suitable method for determination of vancomycin levels in biological fluids (serum, urine and body effusion fluid).

In the first part of the method development separation of vancomycin standard solution and cefuroxime (internal standard) was done. The best chromatographic conditions were carried out by Kinetex™ C18 column, 2.6 μm particle size, 100 A, 50 × 4.6 mm (Phenomenex, Torrance, USA) in combination with potassium phosphate buffer (pH 4.5) and acetonitrile (90:10, v/v) as the mobile phase. For analytes determination UV detection at 220 nm wavelength was applied. Real patient serum samples, after simple protein precipitation with zinc sulphate (4%) and methanol, were analyzed.

This new HPLC-UV method will be applied in clinical study interested in optimizing antibiotic dosing in surgical patients with Systemic Inflammatory Response Syndrome (SIRS) caused by multi-trauma or serious bacterial infection and accompanied with fluid sequestration.

The study was supported by the project SVV 260 184; the European Social Fund and the state budget of the Czech Republic, TEAB project no. CZ.1.07/2.3.00/20.0235. and IGA Ministry of Health project NT14089-3/2013.

PHTHALOCYANINES AND THEIR AZA-ANALOGUES WITH BULKY DIPHENYLPHENYLSULFANYL SUBSTITUENTS

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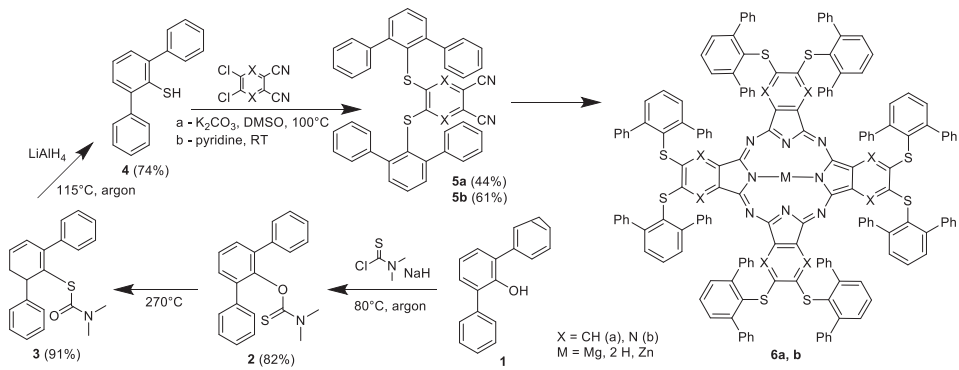
Phthalocyanines (Pcs) and their aza-analogues (AzaPc), chemical substances used in photodynamic therapy, are characterized by interesting photophysical properties which may substantially vary in dependence on the character of peripheral substituents. For example, sum of singlet oxygen and fluorescence quantum yields reaches typically a value of one for Mg and Zn complexes while is significantly decreased for metal-free derivatives. It has been suggested from several previous experiments that this effect can be influenced by bulkiness of the peripheral substituents. The aim of this work is therefore the synthesis of bulky 2,6-diphenylphenylsulfanyl substituted Pcs and AzaPcs and afterwards their photophysical characterisation.

The synthesis started from 2,6-diphenylphenol (**1**), a commercially available substance, which was converted to *O*-carbamothioate **2** with dimethylcarbamoyl chloride. Isomeric *S*-carbamothioate **3** was prepared from *O*-carbamothioate using Newman-Kwart rearrangement at high temperatures and then reduced to thiol **4** with $LiAlH_4$. The thiol was used for the nucleophilic substitution of two dicarbonitrile precursors with pyrazine (**5b**) and a benzene ring (**5a**). Subsequent cyclotetramerisation and following exchange of the central cations led to the Pc and AzaPc macrocycles **6a,b** bearing different central cations (Mg, 2H, Zn) that were subject of the following photophysical study.



The study was supported by SVV 260 183.

NMR SPECTROSCOPY – THE IDENTIFICATION OF THE ISOLATED SUBSTANCE FROM *NERINE BOWDENII*

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The sample was obtained from the Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Hradec Králové and was isolated from plant *Nerine bowdenii* (Amaryllidaceae). The family of Amaryllidaceae is well known for the presence of alkaloids, especially large group of isoquinoline-derived alkaloids.

The unknown substance was characterized employing basic ^{1}H and ^{13}C NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC and gCOSY. After identifying the substructural fragments, the final skeleton (Fig. 1) of molecule was constructed.

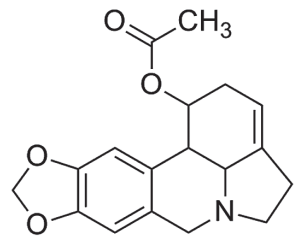

Fig. 1.

The resultant isoquinoline alkaloid has not been yet fully characterized in the literature.

This work was supported by the Charles University (SVV-260-183).

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HIGH THROUGHPUT METHOD FOR DETERMINATION OF CAFFEINE IN COFFEE DRINKS

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Caffeine is a xanthine alkaloid acting like a stimulant of heart and central nervous system. Quantification of caffeine in coffee drinks is significant to show how much of caf-

feine was in each cup which has been taken per day prior to prevent a caffeine overdose. The development of high-throughput sequential injection analysis (SIA) spectrophotometric assay for the determination of caffeine in coffee drinks was performed. Sample was treated with carrez reagent for matrix suppression followed by filtration thereafter analyte was isolated from organic acids by a short monolithic column. The flow rate was 10 μL s^{-1} with 10% *v/v* of methanol as the elution solvent. Caffeine was detected directly at 274 nm. The influence of main parameters affecting the quantification of caffeine were optimized. Under optimal conditions, the method was successfully applied to determine caffeine in different real samples including the soluble coffee, coffee from espresso machine and brewed-coffee drinks. The limit of detection (LOD) and limit of quantification (LOQ) were 0.01 and 0.03 mg L^{-1}, respectively. Linear range was 0.03–15 mg L^{-1} and determination coefficient (r^2) was 0.9969. The relative standard deviation (RSD) was 4.5% (n = 3).

The study was supported by the specific research, No. SVV 260 184.

DESIGN AND SYNTHESIS OF NOVEL CENTRALLY ACTING CHOLINESTERASE REACTIVATORS

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Exposure to the organophosphates (OP), which are used in the form of pesticides (e.g. paraoxon, malaoxon) or as warfare nerve agents (OPNAs, e.g. tabun, sarin, soman) can have fatal consequences. The toxic effect involves irreversible inhibition of acetylcholinesterase (AChE) that causes an accumulation of acetylcholine in central and peripheral synapses leading to overstimulation of the cholinergic receptors, seizures and ultimately respiratory arrest and death. Current treatment for OPNAs intoxication combines an antimuscarinic drug, anticonvulsant drug and AChE reactivator based on pyridinium aldoxime scaffold.

The design strategy, that we introduce, combines tacrine moiety (itself or its structural modifications: 7-MEOTA, 6-chlorotacrine, 7-phenoxytacrine) and pyridine-4-aldoxime *via* various linkers. The major advantage of such reactivators is that the binding of tacrine moiety to the peripheral anionic site allows the oxime better access to the catalytic anionic site of AChE. We assume that despite the presence of permanently charged pyridinium moiety, the molecule will still be lipophilic enough to cross the blood-brain barrier and be able to reactivate OP-inhibited brain AChE. Combinations of structures like tacrine with pyridine-4-aldoxime represent a promising approach for further drug development in this field.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011), specific research (No. SV/FVZ201409) and SVV 260 183.

EVALUATION OF STABILITY OF NOVEL AROYLHYDRAZONES IN PLASMA USING HPLC

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Aroylhydrazone iron chelators are potential drug candidates which both *in vitro* and *in vivo* pharmacodynamics studies demonstrated promising antioxidant and cardioprotective properties. Salicylaldehyde isonicotinoyl hydrazone (SIH) is the compound of the aroylhydrazone class that was the most thoroughly studied. The results that were obtained showed that its hydrazone bond makes SIH susceptible to hydrolysis in biological materials, decreasing its biological half-life. Various novel derivatives were developed in the direction of improving the stability of SIH in biological materials. The main aim of the research was firstly, to develop suitable chromatographic methods for the analysis of 8 novel iron chelators of the aroylhydrazone class (H21, H22, H23, H24, H25, H26,H32, H54) and secondly, utilize those methods to evaluate their stability in plasma *in vitro*.

The appropriate separation of all compounds, their precursors and the different internal standards was achieved on reversed stationary phase (Ascentis C18, 100 × 3 mm, 3 μm, Sigma-Aldrich) protected by the same type of guard column. The mobile phase was composed of a mixture of 10 mM of phosphate buffer (with the addition of 2mM EDTA) with either methanol or methanol/acetonitrile in various ratios. Flow rate of 0.3 ml/min, a column temperature of 25 °C and an injection volume of 20 μl were utilized. The UV detection was performed at a maximum absorbance for each compound.

The stability of the eight different compounds in rabbit plasma *in vitro* (100 μM, 37 ± 0.5 °C, 10 hours) displayed different results. Most of the tested chelators demonstrated clearly superior stability comparing to SIH, whose concentration decreased to 10% of its initial concentration after 3 hours of incubation. A drop in concentration down to 66.9% in 4 hours followed by further decrease to 40.6% after 10 hours was observed for H26 chelator which belongs to the compounds that decomposed quickly. For H23 chelator a decrease to 77.9% after 2 hours of incubation and a further decomposition of 33.7% was observed after 10 hours. The rest of the compounds demonstrated a smaller degree of decomposition.

The work was supported by SVV 260 183.

TACRINE-HYNIC HETERODIMERS – ANTICHOLINESTERASE AND ANTIOXIDANT LIGANDS WITH GOOD TOXICOLOGICAL PROFILE

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by general cognitive impairment such as memory loss, disorientation and behavioral issues that gradually lead to dementia. Multifactorial nature of AD includes loss of cholinergic function, protein misfolding and aggregation, oxidative stress and free radical formation, mitochondrial abnormalities, and neuroinflammation. Also the etiology of the disease has not been fully understood yet. All these facts make the therapy very difficult. In addition, the therapeutic options on the market are severely narrow: acetylcholinesterase (AChE) inhibitors – tacrine, donepezil, galantamine, rivastigmine; *N*-methyl-*D*-aspartate (NMDA) receptor antagonists – memantine. These drugs are only able to hit a single target in organism and that is one of the reasons why pharmacotherapy is not sufficient. Therefore, the drugs which are able to affect multiple targets have a great potential in the treatment of neurodegenerative diseases; these are so called multi-target-directed ligands (MTDLs).

In this work, we focused on the design of new tacrine (THA) heterodimers with antioxidant activity, specifically tacrine-6-hydrazinylnicotinamide (THA-HYNIC) compounds. THA was the first AChE inhibitor launched to the market against AD. Additionally, due to its synthetic accessibility it remains the cornerstone in AD drug discovery. Involvement of HYNIC moiety as a derivative of vitamin B_3 was expected to provide antioxidant properties. *In vitro* assays performed on the whole series have shown that these compounds inhibit cholinesterases in micro-nanomolar concentration scale. Moreover, DPPH assay revealed that these heterodimers exhibit even better antioxidant properties compared to standard antioxidants (trolox, *N*-acetylcystein). Finally, the acute toxicity of three selected candidates demonstrated better toxicological profile than tacrine. Therefore, pursuant above-mentioned results we may assume that THA-HYNIC heterodimers could be interesting candidates for further studies as potential AD drugs.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by the University of Defence (Long Term Development Plan – 1011), specific research (No. SV/FVZ201409) and Charles University SVV 260 183.

OPTIMIZATION, VALIDATION AND COMPARISON OF UHPSFC AND UHPLC METHODS FOR THE DETERMINATION OF AGOMELATINE AND ITS IMPURITIES

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Agomelatine is a synthetic compound with chemical structure N-(2-(7-methoxynapht-1-yl)ethyl)acetamide (Fig. 1). It is an analogue of epiphysis hormone melatonin and the first antidepressant from a new group of melatonin agonists and selective serotonin antagonists (MASSA). By influencing MT_1, MT_2 and $5\text{-}HT_{2C}$ receptors agomelatine regulates circadian rhythms and the release of noradrenaline and dopamine. This effect allows its indication for treatment of depression disorders in adults.

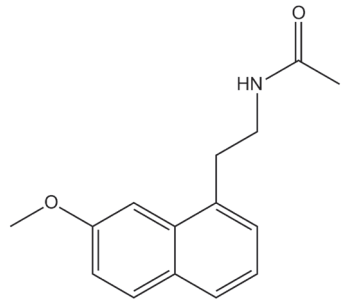

Fig. 1. The structure of agomelatine.

The aim of this study was to develop and validate UHPSFC and UHPLC methods with UV detection for the separation and the determination of the group of structurally similar substances, agomelatine and its six impurities: (7-methoxynapht-1-yl)ethylamine hydrochloride, (7-methoxynapht-1-yl)acetonitrile, bis[2-(7-methoxynaphtalen-1-yl)-ethyl] amine, (7-methoxynapht-1-yl)acetamide, (7-methoxynapht-1-yl)acetonitrile, (7-methoxynapht-1-yl)acetic acid.

Although these substances are structurally close to agomelatine, their physicochemical properties differ substantially. Therefore, one of the main goals was to compare the retention and selectivity not only in supercritical fluid chromatography system and liquid chromatography system but also under different separation conditions. The UHPSFC separations were accomplished using four different stationary phases (Acquity UPC^2 BEH, Acquity UPC^2 BEH 2-EP, Acquity UPC^2 CSH PFP and Acquity UPC^2 HSS C18), all of them with 100 × 3.0 mm dimension and with particles sizes 1.7 μm. Gradient elution was performed using modified CO_2 with gradient program started at 5% of modifier and ended at 30% in 3 minutes. Methanol with different additives including 20mM ammonium acetate, 20mM ammonium formate and 20mM ammonium formate with addition of 5%

of water was used. Flow rate was set at 2 ml/min, the temperature at 40°C and BPR at 2000 psi. The UV detection was performed by Acquity UPC^2 PDA detector at 275 nm. The column BEH 2-EP and gradient elution with 20mM ammonium formate with the addition of 5% of water were chosen due to the best selectivity and resolution results.

The stationary phases for UHPLC system included Acquity UPLC CSH C18, Acquity CSH Fluoro-Phenyl, Acquity UPLC BEH Shield RP18, Acquity UPLC BEH Phenyl and Acquity UPLC BEH C18 with column dimension 2.1 × 50 mm × 1.7 µm or 2.1 × 100 mm × 1.7 µm. Methanol, acetonitrile and a mixture of acetonitrile and methanol in the ratio 1:1 were tested as a organic component of mobile phase using gradient elution with different gradient slopes, gradient curves and buffers (pH 2.0, 3.0, 9.0, 9.5 and 10.0). The column temperature was 30°C and the UV detection was performed at 275 nm. The final conditions were chosen as follows: column BEH Shield RP18, gradient elution with a mixture of methanol and acetonitrile (ratio 1 : 1) and buffer with pH 9.5 started at 5% and increased up-to 70% within 5 minutes under gradient curve number 4.

Both developed methods were properly validated according to ICH guidelines. The methods were validated in terms of linearity, sensitivity (LOD, LOQ), accuracy and precision. The UHPSFC method was linear in the range 0.7–70 µg/ml for all analytes with accuracy and precision ≥ 95.5% and RSD ≤ 2.4 for impurities and ≥ 97.6% and RSD ≤ 0.9 for API. The UHPLC method was linear in the range 0.1–10 µg/ml with accuracy ≥ 95.7% and RSD ≤ 2.6 for impurities and ≥ 95.2% and RSD ≤ 1.5 for API.

The measurement of real samples of agomelatine tablets was performed and the methods were compared in the selected parameters as shown in Fig. 2.

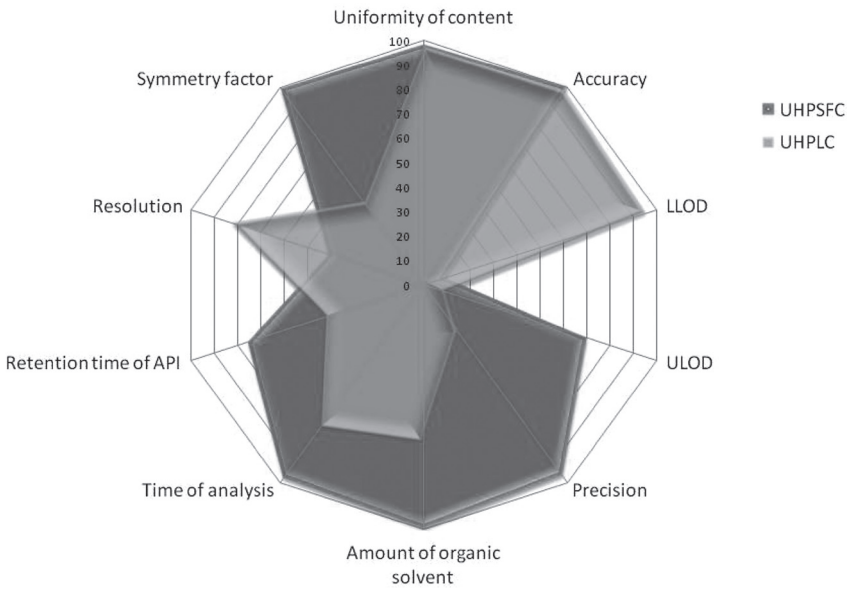

Fig. 2. The comparison of UHPSFC and UHPLC methods in selected parameters.

The study was supported by SVV/2015/260184.

THE FAST HPLC METHOD FOR DETERMINATION OF ARGININE AND ITS METABOLITES IN MONITORING OF WOUND HEALING PROCESS

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Wound healing is characterized by three phases – inflammatory, proliferative, and maturation. Nevertheless, the relationship among these phases is not always linear since this process can progress forward and backward. The healing process depends on intrinsic and extrinsic factors. Long-acting negative influences may disrupt this process and lead to chronic condition. Each phase is characterized by certain events that require specific components[1,2,3]

Wound healing is multi-factorial process, however, the nutritional factor have a basic role in their development. One of these factors is the level of arginine. Arginine is the sole precursor of nitric oxide, a signal molecule, among others, involved in immune responses, angiogenesis, epithelization and formation of granulation tissue, all essential aspects accompanying wound healing[4]. According to previous studies, the ratio of arginine and its metabolites, ornithin and citrulline, is expected to help in the treatment of chronic wounds as an indicator of the healing process[1,5].

The aim of this study was to develop fast and sensitive chromatographic method for analysis of arginine, citruline, and ornithin in wound exudates. Analytical determination was performed using HPLC system Prominence LC 20 Shimadzu (Koyto, Japan) with fluorescence detector, since low concentrations in complex matrix required the use of derivatization reagent. The mobile phase of sodium acetate buffer (pH 7.3) and mixture of acetonitril and methanol (9 : 2, v/v), respectively and a monolithic column Chromolith HighResolution, RP-18e, 100 × 4.6 mm (Merck, Darmstadt, Germany) were used. Sample preparation was performed by ultrafiltration using Microcon Centrifugal Filters (Merck, Millipore, Darmstadt, Germany).

The new HPLC-FD method for determination of arginine and its metabolites in wound exudates was developed. After full validation will be used for monitoring of chronic wounds treatment of patients at Internal Gerontometabolic Clinic in University Hospital, Hradec Králové.

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THE UTILIZATION OF THE NEAR INFRARED SPECTROSCOPY IN THE EVALUATION OF THE HOMOGENEITY OF THE POWDER BLEND

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Working with the powder blends, particularly their mixing, is very commonly used method in pharmaceutical technology. In this area, the biggest problem is reaching for the homogeneity of the mixtures and its measurement. Mixing of active substances and excipients is one of the key factors in the preparating.

The main aim of the study was to introduce a suitable method for the evaluation the homogeneity of the powder blends by using near infrared spectroscopy (NIR). After that, it was monitored the appropriateness of this method with respect to its application during the routine mixing in Pharmaceutical Technology.

To study the homogeneity, the mixture of acetylsalicylic acid (ACS) and microcrystalline cellulose (MCC) with a concentration of active substance of 20% was prepared at different experimental conditions. The total amount of mixture 40 and/or 200 g was homogenized at speed 17 and 34 rpm in the mixing cube Erweka KB 15S. Five samples of mixture were taken at time of 0, 5, 10, 20, 40, 80 and 160 seconds. To prepare tablets, each sample was compressed on the material tester Zwick/Roell Z050. Infrared spectra were measured on a spectrometer Nicolet 6700 at wavenumbers in the range 10000 to 4000 cm^{-1}. In order to evaluate the concentration of ACS, the calibration tablets containing 0–20% of ACS were prepared and measured the same way. The area under the curve (AUC) was used in evaluation of the NIR spectra.

Testing the calibration samples, the best strip for a range of wavenumbers 9020–8750 cm^{-1} by using a horizontal baseline 9032 cm^{-1} was selected and used in evaluation of the homogeneity of powder blends. It was detected that the best homogenization was achieved with a larger amount of the powder blend at the faster speed; the homogeneity was completed after 10 seconds.

The study was supported by the student grant SVV 260 183.

HPLC METHOD DEVELOPMENT FOR ARTIFICIAL COLORANTS DETERMINATION IN GREEN BEER

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Food colorants are an important class of food additives. They are widely used in drinks, juices, meat products and sweets to preserve or restore the natural color of food products and enhance appeal. Natural food dyes have been used more and more for consumer preference, however, synthetic food dyes are still widely used in food and feed industry because of their low cost and high stability. Most of the synthetic dyes show good resistance against degradation and pose little threat to human and animal health. But some of these substances and their metabolites pose potential health risk to human beings and may even be carcinogenic, especially when they are consumed in excessive amounts. Therefore, the use of synthetic dyes in foodstuff is strictly controlled by legislation throughout the world[1].

HPLC method was used and validated for the simultaneous determination of synthetic water-soluble dyes: E 102 – tartrazine, E 104 – Quinoline Yellow, E 110 – Sunset Yellow, E 131 – E 132 Patent blue – Indigo carmine, E 142 – Green S, E 133 – Brilliant Blue FCF and E 143 – Fast Green FCF. The method was applied for direct determination of these dyes in samples of green beers Jarní pivo 11° (Primátor, Náchod), Krasličák 14° (Ježek, Jihlava), Zelený král Vratislav 12° (Konrád, Vratislavice), Velikonoční speciál 14° (Starobrno, Brno), Velikonoční ležák 12° (Radniční pivovar, Jihlava).

Analytical Chromolith Performance CN 100 × 4.6 mm and guard column Chromolith CN 5 × 4.6 mm Merck were used and mobile phase contained 40% (v/v) methanol / 2% (v/v) acetic acid buffer with addition of ammonia for pH adjustment to value 7.0. Successful separation was obtained for all the compounds using an optimized gradient elution within 12 minutes. Analysis was carried out at temperature of 30 °C and the flow rate 2 ml/min, the injection volume was set at 10 µl. The diode-array detector (DAD) was used for monitor the dyes at the 3 wavelenghts 420 nm ((Tartrazine a Quinoline yellow), 482 nm (Sunset yellow) a 625 nm (Indigo carmine, Green S, Brilliant blue FCF, Patent blue a Fast green FCF).

The study was supported by: SVV 260 184.

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PHYSICO-CHEMICAL PROPERTIES OF DRUGS – MEASUREMENT OF DISSOCIATION CONSTANT AND USAGE IN PRACTICE

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To test physico-chemical properties of new molecules is necessary during drug development. It could be helpful to understand or predict the pharmacocinetic parametres of a new drug *in vivo*/*in vitro* experiments.

One of this parameters is a dissociation constant (pK). Dissociation constant is defined as "Number on pH scale, wherein is just fifty percent of molecule in a ionization condition". In real case this number can help us to know where in the gastro-intestinal tract (GIT) the drug will be absorbed. In GIT only molecules exhibiting pK from 3 to 11 could be absorbed. Out of this range it is not possible.

In this work I would like to introduce the ways of experimental measurement of pK values. I am working with two methods to measure the pK values of water-soluble compounds. The spectrophotometric method and the potentiometric one. Water-insoluble compounds can be experimentally measured, too. The dissociation constant is affected by functional groups in the molecule. So I also tried to compare a variety of functional groups on pyrazine heterocyclic to show how a pK value is changed by introduction of various functional groups. I also studied if using of both method to measure every compounds is possible, or not.

The study was supported by Ing. Vladimir Kubiček, CSc. SVV – 260 183.

TACRINE-BENZOTHIAZOLE HYBRIDS NOVEL MULTITARGET AGENTS TO COMBAT ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive fatal neurodegenerative disorder and the most common type of dementia. It is manifested by a variety of neuropsychiatric symptoms such as memory loss, visuospatial deficits etc. Ethiology of the disease has a multifactorial character and is not well known. Among the major pathological features belong: presence of extracellular amyloid plaques, mainly represented by amyloid-beta peptide, intracellular aggregates of hyperphosphorylated tau protein and neuronal loss, especially of cholinergic

neurons. Also the oxidative stress of the neuronal cells contributes to the patophysiology of the disease. Because AD is affected by the multiple factors, the main strategy of the treatment is to affect the multiple targets in the brain as well. Such drugs are denoted as multitarget-directed ligands (MTDLs) and they target the different molecular abnormalities of AD.

In our contribution we would like to introduce tacrine-benzothiazole hybrids combining tacrine with the benzothiazole moiety. Linkers of different lenghts were used to connect these two scaffolds. Tacrine was the first drug approved for AD treatment by FDA. Its mechanism of action is based on inhibition of cholinesterases and thereby increasing the levels of synaptic acetylcholine. On the other hand, benzothiazole, as a planar molecule, could prevent the protein–protein interactions and thus could have anti-amyloid effect. Moreover, benzothiazole moiety represents the core of inhibitors of amyloid-binding alcohol dehydrogenase (ABAD). ABAD is a mitochondrial enzyme that contributes to oxidative stress in AD progression. Therefore its inhibition could avoid ROS production and act neuroprotectively. Pursuant above-mentioned facts, molecules bearing tacrine and benzothiazole motif could become promising drug candidates in AD therapy. Nevertheless, just *in vitro* and *in vivo* determination of biological activity will reveal their real value in the field of Alzheimer's disease.

The work was supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), by University of Defence (Long Term Development Plan – 1011) specific research (No. SV/FVZ201409) and by Charles University, Faculty of Pharmacy, specific research (No. SVV 260 183).

SYNTHESIS OF SULFONAMIDE ANALOG OF CARDIOPROTECTIVE DRUG DEXRAZOXANE

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Anthracyclines such as daunorubicin or doxorubicin are widely used anticancer drugs. However, the administration of anthracyclines is connected with cardiotoxicity leading to irreversible damage and congestive heart failure. The reason of their toxicity is unknown yet, there are two main theories. It is assumed that the complexation of anthracyclines with intracellular iron ions catalysis the formation of reactive oxygen species. The second theory involves inhibition of topoisomerase II. The only known drug effective against the anthracycline cardiotoxicity is dexrazoxane (DEX). The mechanism of cardioprotection is also unknown yet. One theory involves chelation of iron ions, the second involves interaction with topoisomerase II in heart. In this study we deal with the synthesis of a new analog of DEX and with the study of its cardioprotective effect (Fig. 1).

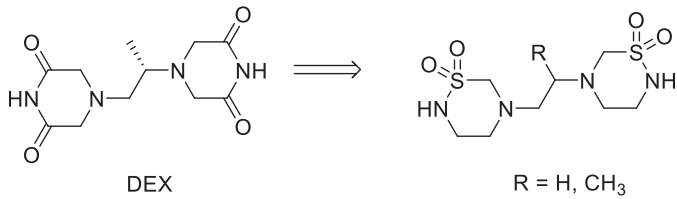

Fig. 1. Structure of dexrazoxane (DEX) and its sulfonamide analog.

The new analog was designed to have sulfonamide group, which mimics the original imide group in DEX and importantly has similar acidity. In the case when R = H synthesis started from triethylenetetramine. In the case when R = CH_3, 1,2,5-thiadiazinane-1,1-dioxide was prepared and subsequent reaction with 1,2-dibromopropane would provide target compound.

The study was supported by the Charles University (Charles University Research Centre UNCE 204019/304019/2012 and project SVV 260 183).

WHEN –CONH– BECOMES –NHCO–: PYRAZINYL BENZAMIDES AS POTENTIAL ANTITUBERCULARS

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Rising incidence of mycobacteria resistant to known antitubercular drugs opens new space for the search for new antitubercular compounds. Our work was aimed at new derivatives of pyrazinamide, more specifically on derivatives of 5-chloro-*N*-phenylpyrazine-2-carboxamides (**I**) with various substituents on the phenyl ring, which were previously shown to possess significant *in vitro* antimycobacterial activity (MIC = 0.78–3.13 μg/mL *M. tuberculosis* H37Rv).[1] Chemical modifications or model compound (**I**) were focused on the carboxamide moiety, which was inverted from CO–NH to NH–CO.

Final compounds **II** were prepared by aminolysis (Fig. 1) of commercially available benzoyl chlorides by 5-chloro-2-aminopyrazine in dichloromethane as a solvent, maximiz-

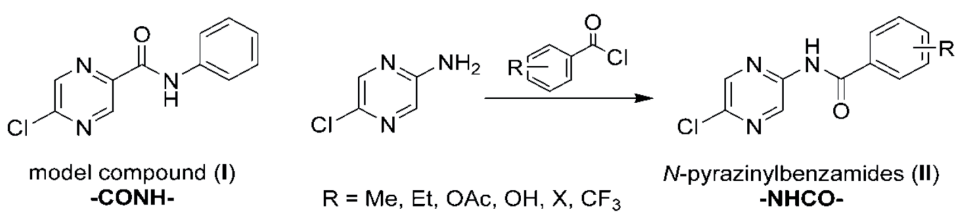

Fig. 1. Model compound I and synthesis of final compounds II.

ing the yields by working in non-aqueous environment. Compounds with R = OH were obtained using the acetyl protected chlorides of hydroxybenzoic acids. Final compounds were characterized by ^{1}H and ^{13}C NMR, IR, elementary analysis and melting point.

Final compounds will be tested for *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv, *M. kansasii, M. avium* and *M. smegmatis*. Additionally, compounds will be tested for activity against selected bacterial and fungal strains of clinical importance. The results and structure-activity relationships will be discussed.

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SUBSTITUTED TETRA(3,4-PYRIDO)PORPHYRAZINES AS POTENTIAL PHOTOSENSITIZERS

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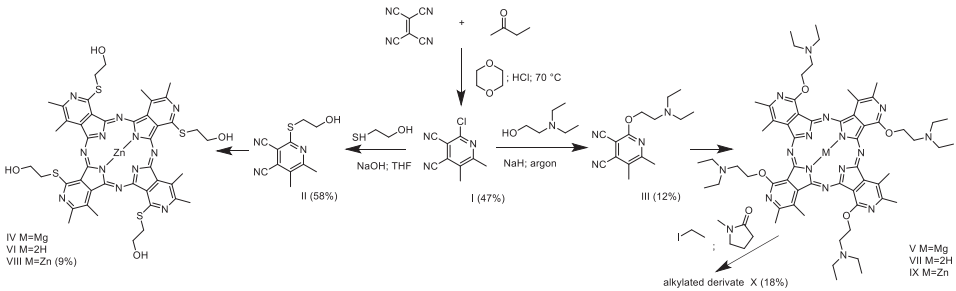
Substituted tetra(3,4-pyrido)porphyrazines represent new structural type of potential photosensitizers with interesting properties in the area of photodynamic therapy (PDT). The aim of this work was to synthesize two types of tetra(3,4-pyrido)porphyrazines with hydrophilic substituents as potential photosensitizers. Photosensitizers are substances with an ability to produce singlet oxygen, the key toxic species in PDT, after irradiation.

2-Chloro-5,6-dimethylpyridine-3,4-dicarbonitrile (I) was prepared in the first step by condensation of tetracyanoethylene and butan-2-one. In the next step, an hydrophilic substituent was attached by nucleophilic substitution. Compound II was prepared by reaction of I with 2-mercaptoethanol in aq. NaOH. Similarly, compound III was prepared by reaction with *N*,*N*-diethylaminoethanol in the presence of NaH. The third step involved cyclotetramerization with magnesium butoxide as initiator that gave Mg complexes (IV, V). Mg complexes were converted to metal-free derivatives (VI, VII) and then to Zn complexes (VIII, IX). Complex IX was subsequently quaternized by ethyl iodide to the final



compound (X). Zn complexes VIII and X were tested for photodynamic activity and toxicity on tumor HeLa cells.

The study was supported by SVV 260 183 and Czech Science Foundation 13-27761S.

SYNTHESIS OF COMBRETASTATIN ANALOGUES AS POTENTIAL ANTITUMOR AND ANTIMICROBIAL AGENTS

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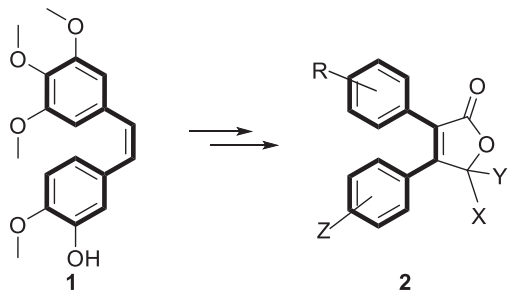
Combretastatins are naturally occurring molecules possessing remarkable *in vitro* cytotoxicity against human cancer cell lines. These compounds, such as combretastatin A-4 **(1)**, are known to disrupt mitosis through the inhibition of tubulin assembly. Furthermore, some of them also exhibit antivascular and antiangiogenic effects.[1] The compounds, however, are highly lipophilic and insufficient in terms of chemical stability. Our aim was, therefore,



to synthesize a library of α,β-diphenyl furanones analogous to combretastatins with improved pharmacological properties and subject it to biological activity screening.

Variously functionalized α,β-diphenyl furanones are possible to be obtained from commercially available acetophenones and phenylacetic acids in good to excellent yields. Derivatizations of aromatic cores as well as of γ-position are subsequently performed in order to improve the solubility in aqueous media.

To date, a series of compounds (**2**) was prepared and screened for cytostatic and antimicrobial activity. Our molecules display interesting both antineoplastic and antibacterial activities in micromolar concentrations. Structural modifications responsible for boosting antimicrobial effects were observed.

The study was supported by Charles University (SVV-260-062 and GA UK 1906214) and Czech Science Foundation (P207/10/2048).

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SECTION OF OTHER PHARMACEUTICAL SCIENCES

CONSISTENCY OF THE SEMISOLID PREPARATIONS

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Semi-solid preparations are intended for local or transdermal delivery of active substances, or for their emollient or protective action. They consist of a simple or compound base in which, usually, one or more active substances are dissolved or dispersed. The base is not inert carrier of an API but influences the effect of the preparation. Commonly used hydrophobic bases are yellow and white soft paraffin, purified mixture of semisolid saturated hydrocarbons obtained from petroleum. Only for these bases the measurement of consistency by penetrometry as control of quality is required in the pharmacopoeia. The question is why among all hydrophobic viscosifiers just only soft paraffin must be tested and how its consistency should be changed not to comply with pharmacopoeial requirement.

In our study, white soft paraffin was mixed either with liquid paraffin or with solid paraffin to change its consistency. The samples for penetrometry measurement were prepared either by methods A or method C according to Ph.Eur., and measured at 25 ± 0.5 °C. The consistency of the samples was significantly affected by the chosen method of preparation. The value of consistency of samples prepared in semisolid state (method A) is 215, the consistency of the samples prepared by melting (method C) is 143 i.e. by 44% less. The addition of 10% of liquid paraffin increased the value of consistency in 290, near to the

upper pharmacopoeial limit in case of method A, while in case of method C the value of consistency was not influenced. The value of consistency of white soft paraffin with 10% of solid paraffin was significantly decreased, samples with 20% of solid paraffin prepared by melting even did not comply with pharmacopoeial requirement.

The result of the test of consistency by penetrometry i) can be influenced with method of preparation of the samples, ii) is required only for yellow and white soft paraffin, and iii) limit is in very wide range. Rheological characteristics as consistency coefficient and flow index, or yield stress and viscosity on yield stress can be suggested as more suitable and more useful.

The study was supported by SVV 260 183.

ONLY ONE THIRD OF PATIENTS WITH COPD IS FULLY ADHERENT TO INHALATION THERAPY

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Patient adherence to treatment in chronic obstructive pulmonary disease (COPD) is essential to optimise disease management. Poor adherence is common and results in increased rates of morbidity, healthcare expenditures, hospitalisations and possibly mortality, as well as unnecessary escalation of therapy and reduced quality of life. The major problem is failure to adhere to inhaler technique. The aims of this project were to assess inhaler adherence for different types of devices and to analyze various aspects of adherence in a cohort of patients with severe COPD in the Czech Republic.

An observational multicentre study with the participation of 12 centres in the Czech Republic was conducted in cooperation with the Czech Multicentre Research Database of COPD (http://chopn.registry.cz). The inclusion critera are: severe COPD without fibrosis and FEV1 < 60%. The assessment was structured into five steps to be followed while using an inhaler. Adherence to each step was assessed in a dichotomous manner (performed properly/improperly). Each respondent was asked to report how often he/she rinses his/her mouth after using the corticosteroid inhaler (always: 75–100%, sometimes: 25–75%, never: 0–25%).

Three hundred and forty-three patients were enrolled in the study (mean age of 67 years). They used various types of inhalers, sometimes in combination. The most often used devices were pressurized metered-dose inhaler (pMDI) (N = 171) and dry powder inhalers (DPIs) – Handihaler (N = 151), Aerolizer (N = 146) and Diskus (N = 68). The assessment of the adherence to inhalation technique revealed that less than 35% of the study cohort adhered properly to each of the five steps. Correct adherence to each inhaler was following: pMDI (31.9%), Handihaler (28.2%), Aerolizer (27.2%) and Diskus (12.7%).

For all types of inhalers, the highest rate of failure to was observed for step 3 (failure to breathe out completely in one breath before taking the medicine with the next breath). After using an inhaler containing corticosteroid, 62% of respondents always rinse out their mouth, 29% sometimes, and 9% never. Patients who fully adhere to inhaler use technique rinse out their mouth every time after inhalation of corticosteroids in 71% of cases while those who fail to use the inhaler properly only in 57% of cases ($p = 0.036$).

Patients failed to adhere to inhaler technique more often while using aerosol inhalers. Powder inhalers are used improperly by more than half of the respondents. The most common error was complete exhalation before inhaling the drug. Patients who adhere to inhaler technique rinse out their mouth after inhalation of corticosteroids more often than those who failed to adhere to one or more steps.

The study was supported by project SVV 260 187. The COPD project is registered in Clinical Trials.gov with the identifier NCT01923051.

PREPARATION OF MICROPARTICLES BY MICROFLUIDIC METHOD

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The aim of this work was to prepare hydrogel microparticles based on silated-hydroxypropyl methylcellulose (HPMC-Si). The microparticles are expected to be obtained by self-hardening of HPMC-Si from the microdroplets formed by the emulsification in the continuous phase.

The microparticles were prepared by microfluidic method. This method is based on phase separation of a droplet in a non-miscible continuous phase by using microchannels. A 3% w/w solution of HPMC-Si in the sodium hydroxide solution 0.2M (pH 13.2), the HEPES buffer (pH 3.5) and a fluorescent dye FITC-Si were utilized to form microparticles. Sesame oil was tested as a continuous phase.

In order to form microparticles of controlled size and to improve their stability, various experimental conditions were tested. Parameters like temperature, speed rate of the continuous and dispersed phase, length of the microchannel and the use of the surfactant Plurol in a concentration of 1% to 3% were investigated.

The results showed that the particles were well spherical and more uniform by choosing suitable values of speed rate. By influencing the temperature and the length of the outlet microchannel, the stability of particles was ameliorated minimizing the fusion of particles. Although the results are preliminary, this research proved that it is possible to prepare microparticles and encapsulate FITC-Si using microfluidic method.

The study was supported by student grant SVV 260 183 and Erasmus+ programme.

PHARMACEUTICAL CARE AND CONTINUAL PROFESSIONAL DEVELOPMENT IN GREECE

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The political line of the health systems in EU is patients oriented and demands increased rates of training and well understanding of pharmaceutical services and pharmaceutical care. Either in hospital pharmacies or in community pharmacies. Almost half of the annual pharmaceutical expenses accrued by the so called expensive drugs special category. Almost half of the annual turnover of community pharmacies is of the OTCs and the negative list of medicines. So examine the aspects of Continuing Professional Development (CPD) within deepened economic crisis in Greece in order to find professional solutions for the near future as well as by means of survival.

We deal with European surrounding and the present situation in basic education and continual training of community pharmacies in Greece taking into account the assumption that community pharmacies are part of the primary health care. We trace the real needs of well-trained community pharmacists to improve value for both the benefit of patients and the Health Care System.

It is the outcome of collaboration with the Institute for the Continual Training and CPD (IDEEAF) of PanHellenic Pharmaceutical Association which merely performs the unique thorough effort on the matter in Greece under the auspices and financing by pharmaceutical companies. The method of study includes a disseminated simple questionnaire to all those freely participating to IDEEAFs lessons.

The anonymous responds give a first impression and we can conclude in the following points.

a) The urgent dramatic changes – as main proposal – of the basic educational program within pharmacy faculties in Greece.
b) The direct collaboration of the PanHellenic Pharmaceutical Association (through IDEEAF) with international institutions specialized on social pharmacy.
c) The evaluation of pharmaceutical services by ETESTA the company of the PanHellenic Pharmaceutical Association for the research statistics and analysis of pharmaceuticals as basic pharmacoeconomic support.
d) The ongoing procedure of questionnaires in a second phase proposed by PGEU at a more centralized way.
e) The immediate establishment of an accreditation system certified by the PanHellenic Pharmaceutical Association.
f) A separate part of therapeutic categories of services for the emergency and/or the "heroic" drugs.

THE ANALYSIS OF THE CARE OF PHARMACY CLIENTS WITH THE RISK OF ARTERIAL HYPERTENSION

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Arterial hypertension (AH) is still very serious worldwide health problem. AH belongs among the most important risk factors for coronary heart disease, stroke, or peripheral arterial disease. Since, the detection and treatment of AH received considerable attention, in the clinical practice, satisfactory results have not been achieved yet. This problem can be improved by the involvement of pharmacists in the care of pharmacy clients as shown by published data. The aim of this work is to present the results of the analysis focused on the involvement of pharmacists in the care of pharmacy clients with the risk of AH.

Data were collected in one community pharmacy in the town with 3,500 inhabitants in Pilsen region from September 2014 to January 2015. Blood pressure measurement was included in each interview with the selected pharmacy client. The following data were recorded: socio-demographic characteristics; opinion on blood pressure measurement in a pharmacy; risk factors of AH or atherosclerosis; illness in anamnesis; using drugs including dietary supplements; the result of blood pressure measurement; interventions of pharmacist or identified drug-related problems. The measurement of blood pressure was carried out in accordance with valid current recommendations. The obtained data were evaluated using frequency analysis. Drug problems were classified according to the modified Pharmaceutical Care Network Europe classification V5.01.

Analysed data were obtained from 199 pharmacy clients (55.3% women; mean age 49.7 years). 30 clients, of which 12 clients were without diagnosed AH, had blood pressure above 140/90 mm Hg. Further, there were identified 43 drug-related problems related to pharmacotherapy of AH. Each client received the recommendation to promote the adherence to healthy life style or pharmacotherapy. If necessary, the recommendations, how to use medications, were provided. Some clients were recommended to visit their general practitioners.

The blood pressure measurement as a part of counselling in a pharmacy can be regarded as the suitable method for identification of persons with undiagnosed AH. Particular attention should be given to smokers or persons with a higher BMI. More detailed knowledge of anamnesis can contribute to optimization of treatment plan, e. g. by the identification and solution of drug-related problems.

The study was supported by SVV 260 187.

INFLUENCE OF EXCIPIENTS ON THE MECHANICAL PROPERTIES OF ORODISPERSIBLE TABLETS

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Orodispersible tablets are modern solution to the administration of drugs, mainly characterized by a short disintegration time and rapid onset of drug effect. Due to the required disintegration in the oral cavity, a necessary step in the formulation is also a choice of suitable sweeteners and flavours to influence palatability.

This work studies the mechanical properties of orodispersible tablets containing an active ingredient (VF) in confrontation with the used pre-compression/compression force and selected excipients. Tablets were produced by a direct compression method using two combinations of pre-compression/compression forces. The effect of the addition of hypromellose 5–10% (Methocel E5) and/or crospovidone 5–15% (Polyplazdone XL) on tablet friability, disintegration time and strength was studied.

Experimental results shown that mechanical strength and disintegration time of tablets are directly influenced with compression force, however, individual formulations were also influenced by the composition of the tablet. At the higher concentration of hypromellose decrease in the strength of tablets was detected while the disintegration time was prolonged. A similar effect on the tablet strength was observed when crospovidone was used. Extension of disintegration time was observed only for the combination of lower pre-compression/compression forces. At higher forces, however, relationship was not completely linear. The strength and disintegration time of tablets was significantly affected by the addition of sweeteners sucralose and/or sodium saccharin in both formulations investigated.

The results of this work allow to suggest a suitable formula for orodispersible tablets for administration of the active ingredient (VF) with the optimum strength and required disintegration time.

The study was supported by student grant SVV 260 183 and Zentiva, k.s.

ETHICS IN PHARMACEUTICAL COMPANIES

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According to Study on Corruption in the Healthcare Sector one third of people think that taking of bribes and abuse of positions of power for personal gain are widespread among people working in the public health sector. The most serious situation is in the certification and procurement of medical equipment and in the autorisation and procure-

ment of pharmaceuticals.[1] Problems of ethics are connected with pharmaceutical industry inherently. Therefore number of pharmaceutical companies try to enhance their reputation by various of ethical tools and activities. Nevertheless having the working business ethics program is very complex, difficult and neverending process. Ethics are part of all activities of business and if the ethics program is efficient it helps company increase its profit and meet its goals. Sophisticated ethics program includes ethical tools such as a code of ethics, an ethical audit, a corporate social responsibility, an education in ethics, an organisation structures, a stakeholder analysis, a whistleblowing and many more. The aim of this work is to describe these tools, a process of creating an ethics program and find out the advance of ethics program in pharmaceutical companies operating in the Czech Republic. For this purpose we used the questionnaire sent by post to the pharmaceutical companies.

According to the answers collected for our study, all of them consider ethics to be an important part of the business. Most of them think the level of business ethics in pharmacy has developed, even though a lot of these companies do not use full range of tools of business ethics.

The study was supported by the Charles University (Project SVV 260 187).

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PRESENCE OF DRUGS INTERACTIONS IN CZECH POPULATION

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Interaction between drugs is an important problem of our nowadays´ society. The risk of new interaction between drugs is raised with every one added medication. It is not only a matter of the amount of drugs people use. The number of doctors that cure a person and write prescriptions for them is also very important factor in this case. In these cases when there are lot of doctors prescribing medications for a person we could avoid interactions between drugs by using a system where doctors curing one person know about each other and know who prescribes what kind of medication. There are a lot of studies interested in these problems abroad. Unfortunately there are very few studies taking care of this theme so far in our country. My work is aimed to describe prevalence of these interactions and their importance within the population of Czech Republic and point out the need to be aware of its danger to the public.

The study was supported by Association of Innovative Pharmaceutical Industries.

THE PERMEABILITY AND MICROSTRUCTURE OF MODEL STRATUM CORNEUM LIPID MEMBRANES CONTAINING NON-HYDROXYLATED AND (R)- AND (S)-A-HYDROXYLATED CERAMIDES

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Ceramides (Cer) are essential components in the uppermost layer of the skin, called *stratum corneum* (SC). In the SC, Cer with cholesterol (Chol) and free fatty acids (FFA) are in equimolar ratio. Cer molecules are amphiphilic structures with a small polar head and two hydrophobic chains (Fig. 1). Cer contain sphingoid bases, which are amino alcohols sphingosine (S), phytosphingosine (P), dihydrosphingosine (dS) or 6-hydroxysphingosine (H). These sphingoid bases are *N*-acylated by non-hydroxylated (N), α-hydroxylated (A) or ω-hydroxylated fatty acid, mostly by lignoceric (C24) acid.[1,2]

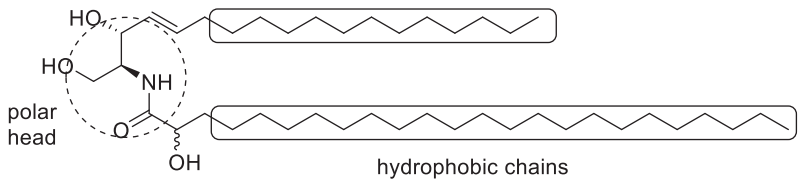

Fig. 1. Structure of Cer AS: the dashed circle shows small polar head and the rectangles show hydrophobic chains.

The goal of this work was to study the permeability and microstructure of the model membranes containing non-hydroxylated Cer including the commercially unavailable Cer NH, and further to study the effects of additional α-hydroxyl group in Cer including their stereochemistry at Cer α-C. Therefore, we prepared model membranes containing Cer/FFA ($C_{16–24}$)/Chol and small amount of CholS (5%wt) where Cer were either non-hydroxylated (NS, NdS, NP, and NH) or α-hydroxylated Cer (2′*R*) and (2′*S*)-diastereomers (AS, AdS, and AP). We investigated four permeability markers: electrical impedance, water loss through the membrane and flux of the theophylline and indomethacin. The microstructure and miscibility of Cer with other lipids were analyzed by infrared spectroscopy and X-ray powder diffraction.

The results confirmed that every type of Cer has unique properties and every change in their structure (type of sphingoid base, α-hydroxylation and stereochemistry) leads to differences in barrier function of model lipid membranes.

The study was supported by Charles University (SVV: 260 183 and GAUK 1868214).

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YIELD STRESS OF SEMISOLID PHARMACEUTICAL PREPARATIONS

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Semisolid preparations (gels, ointments, creams) do not flow until the applied stress exceeds a certain critical stress, known as the yield stress. When the semisolid preparation is sheared at very low shear rates, in the range between 0.01–0.1 s^{-1} and below a critical strain the system is subjected to work hardening. This is characteristic of solid-like behaviour. At a shear rate in the range of 0.8 of the critical shear rate the reinforcing structure will start to break down. This is the point where the instantaneous viscosity reaches a maximum. This maximum corresponds to a critical value of shear stress – the yield stress. The chart shows the dependence of viscosity on the shear stress. The yield stress of this sample is determined from the viscosity maximum (Fig. 1).

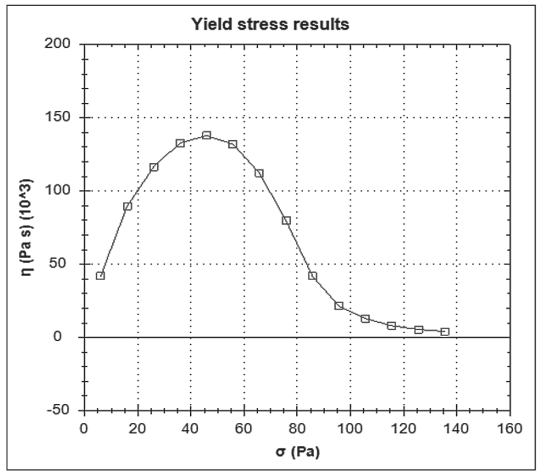

Fig. 1. Viscosity data for Paraffin soft white.

The aim of this work was to use yield stress analysis as simple but more standard method of characterisation of consistency of ointments and gels in comparison to penetrometry. Yield stress by stress ramp of Paraffin soft white, Carbomer gel, Cellulose derivatives gels etc. were measured on Malvern Kinexus rheometer using a CP4°/40 cone geometry, at

25.0 °C, and shear stress range from 0.1 to 300 Pa. Values of yield stress and viscosity on yield stress were expressed and compared with previously determined parameters of Power Law Model and values of consistency measured by penetrometry.

The study is supported by SVV 260183.

ANALYSIS OF SELF-MEDICATION WITH ANTIBIOTICS IN KOSOVO

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Evidently, the irrational and overuse of antibiotics continues to be a significant problem in Kosovo despite the increased risks of antibiotic resistance and adverse drug reactions. The main aim of this study was to analyze the knowledge, attitude and practice towards self-medication with antibiotics among the population in Kosovo.

The study was conducted in a community pharmacy in Prishtina where a total of 300 patients participated. Data was collected through using a validated, self-administered questionnaire which was developed in English at the department of Social and Clinical Pharmacy at Charles University, Faculty of Pharmacy in Hradec Králové. This questionnaire was spread to two groups of patients: To every patient that visited the pharmacy and to patients who specifically wanted to purchase antibiotics.

The prevalence of non-prescription use was high. In the first group 76.8% of the patients reported using non-prescribed antibiotics while in the second group 47.6% of the patients did not present a prescription at the time of the purchase. Utilization of an old prescription was the most common source of non-prescribed antibiotic use. In both groups the most common reasons for antibiotic consumption were urinary inflammations, cough and influenza which were followed by gastrointestinal and gynecological inflammations. In the first group 73% of the patients stored antibiotics at home while in the second group 60.3% of the patients did the same. On the other hand the majority of the patients were not aware of antibiotic resistant bacteria and the fact that antibiotics can kill off normal flora as well.

Results showed that unfortunately self-medication with antibiotics is common in Kosovo, indicating that there is a need for educational campaigns which will help the public understand the proper antibiotic use and diminish the inappropriate consumption of antibiotics.

The study was supported by SVV 260 187.

SECTION OF BIOLOGICAL SCIENCES

ALKALOIDS OF SELECTED *GALANTHUS, LEUCOJUM* AND *NARCISSUS* SPECIES AND THEIR BIOLOGICAL ACTIVITY

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More than 50% cases of dementia are nowadays caused byAlzheimer's disease (AD). AD is a progressive neurodegenerative disease and it causes gradual memory loss, disorientation and behavioral disorders which affect patient's social and occupational life. AD is characteristic by loss of neurons in some regions of brain – for example hippocampus and cortex. Ethiopathogenesis of this disease is not completely known – that is why the treatment is still just symptomatic. Formation of β-amyloid deposits in brain tissue plays an important role – it is a protein which creates extracellular plagues around neurites and causes their degeneration and death. Intracellular tangles are made up of the changed τ-protein. These tangles also cause death of the neuronal cell. The degeneration of neurons is supported by reactive oxygen radicals too. The another problem is a glutamatergic system disorder. This set of excitatory amino acids is important for correct long-term memory formation. Patients with AD suffer from glutamatergic system overactivation which leads to the formation of neuronal perturbation, excitotoxicity and apoptosis of neuronal cells. In patients with AD the acetylcholine (ACh) production is damaged. ACh is a neurotransmitter and its lack participates in the development of AD. ACh is physiologically decomposed by enzyme acetylcholinesterase (AChE). The second enzyme taking part in ACh degradation is a butyrylcholinesterase (BuChE). In severe forms of AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with normal condition, while the concentration of BuChE increases. That's why the new inhibitors with dual enzymatic activity against AChE and also BuChE are sought.

Galanthus, Leucojum and *Narcissus* species belong to *Amaryllidaceae* family. Plants of this family produce wide range of specific chemical substances called *Amaryllidaceae* alkaloids. These alkaloids have various biological effects like anti-inflammatory, antivirotic, antineoplastic, antiparasitic, antimycotic and they are also able to inhibit erythrocytic AChE (HuAChE) and serum BuChE (HuBuChE).

Alkaloidal extracts of seven selected species and cultivars were analysed by GC/MS and alkaloids were identified from their mass spectra, retention times and retention indexes. Summary extracts were tested *in vitro* for their ability to inhibit HuAChE and HuBuChE using Ellman's method. Interesting inhibitory activities were shown by alkaloidal extracts

of *Galanthus woronowii* ($IC_{50,HuAChE}$ = 8.65 ± 1.20 μg/mL), *Galanthus elwesii* ($IC_{50,HuAChE}$ = 10.29 ± 1.00 μg/mL), *Narcissus* cv. QUIRINUS ($IC_{50,HuAChE}$ = 17.72 ± 2.41 μg/mL) and *Narcissus* cv. VIRGINIA SUNRISE ($IC_{50,HuAChE}$ = 10.72 ± 0.83 μg/mL; $IC_{50,HuBuChE}$ = 29.62 ± 3.47 μg/mL) when galanthamine was used as a standard ($IC_{50,HuAChE}$ = 1.71 ± 0.007 μM; $IC_{50,HuBuChE}$ = 42.30 ± 1.30 μM).

Using preparative TLC (To : Et_2NH, 95 : 5) one alkaloid was isolated from alkaloidal extract of *Narcissus* cv. PROFESSOR EINSTEIN. The isolated compound was identified as a homolycorine and tested for its inhibitory activity against HuAChE (IC_{50} = 63.7 ± 4.3 μM), HuBuChE (IC_{50} = 151.0 ± 15.2 μM) and prolyloligopeptidase ($IC_{50,POP}$ = 173 ± 40.6 μM). Galanthamine and Z-Pro-prolinal ($IC_{50,POP}$ = 3.27 · 10^{-3} ± 0.02 · 10^{-3} nM) were used as positive standards.

The study was supported by SVV 260 184.

IN VITRO CULTURES OF MEDICINAL PLANTS XVII.

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Milk thistle*, Silybum marianum* L. Gaertn., is a source of flavonoid taxifolin and flavonolignans – silymarin complex (silybin, silydianin, silycristin and isosilybin). Due to the the main active component of silymarin – silybin, milk thistle is used as hepatoprotectivum and antioxidant, in skin-health, and in the therapy of some kinds of cancer. New therapeutic potentials of *Silybum marianum* are still discovered.

Milk thistle is usually obtained by field cultivation. Alternative way for getting the active components, is the use of *in vitro* cultures. But the production of secondary metabolites by the *in vitro* cultures is low in comparison with plant. One of the possibilites how to increase this produciton is the method of elicitation.

In this study, ethephon as the elicitor, in the concentrations of 500 μmol/l, 400 μmol/l, 200 μmol/l, 100 μmol/l and 50 μmol/l was used with the aim to increase secondary metabolite production in suspension and callus cultures. The effect of ethephon was compared to its inhibitor (120μM $AgNO_3$). The levels of flavonolignans and taxifolin were measured by the method of HPLC. The samples were taken 24, 48, 72, 96 and 168 hours after the ethephon application and inhibitor treatment. The nutrient medium of suspension culture was also tested for the possibity of secondary metabolites releasing into medium.

The highest content of flavonoid taxifolin was found in the suspension culture medium after 48 h treatment with ethephon in conc. of 400 μmol/l. The level of taxifolin was increased by 197-fold to 1,97 mg/100 ml, compared to control sample.

The statistically significant production of taxifolin in the callus culture was reached after 96 hours of treatment with ethephon in conc. of 50 μmol/l (0.11 mg/g DW).

The statistically significant production of silybin A was reached in the nutrient medium 72 h after application of 400μM ethephon (0.51 mg/100 ml).

The statistically significant positive effect of $AgNO_3$ as inhibitor was found in the case of taxifolin in the medium, 168 hours after application of 400μM ethephon. Inhibitor increased taxifolin content by 58-fold to 0.58 mg/100 ml.

The statistically significant negative effect of inhibitor $AgNO_3$ was on silybin A content in medium, 168 hours after application of 400μM ethephon. Inhibitor completely decreased the effect of ethephon.

The study was supported by the Charles University, SVV 260 186.

DEVELOPMENT OF NEMATODES RESISTANCE TO ALBENDAZOLE

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Nematodoses, including haemonchosis, disease caused by *Haemonchus contortus,* are responsible for substantial losses in livestock farming. *Haemonchus contortus* inhabits the abomasum of small ruminants and causes anemia and gastritis. Currently available anthelmintics used to treat haemonchosis are ineffective in many breeds because the increasing incidence of multiresistant strains of *Haemonchus* worldwide. Therefore, the research of the mechanism of drug resistance of these parasites is very actual and important. The aim of my work was to study whether short-term contact of eggs or adults of *Haemonchus* with anthelmintics can affect the expression of certain enzymes and can lead to development of resistant individuals. The study had two parts.

In the first part, the influence of anhelmintic drug albendazol and its main metabolite albendazol sulfoxide on *Haemonchus* eggs isolated from the feces of infected sheep was tested. In the second part, expression changes of the reference and selected genes in adult males and females of *Haemonchus* from two strains with different sensitivity to anthelmintics were studied. One group of adults of both genders and strains were exposed to culture medium with 10μM albendazole for 12 and 24 hours. ABZ-untreated group (controls) were exposed to culture medium without drugs for 12 and 24 hours. The genes for UGTs (UDP-glucosyl trasferases) were monitored, because in a previous study, higher UGTs activity and increased ability to deactivate albendazole via conjugation with glucose was found in the drug-resistant strain than in sensitive strain.

In my work, I tested the expression of UGT7, 12 and 13, but I didn't find statistically significant differences in their expression between strains. The contact of *Haemonchus* with albendazole also did not affect expression of these enzymes. For that reason, tested enzymes probably don't contribute to increased metabolism of albendazol in a resistant strain. Due to a large amount of UGTs (more than 40) in *Haemonchus*, it's possible that deactivation of albendazole is catalyzed by other UGTs. Study of UGTs is a part of an on-

going project which deals with mechanisms of helminths resistance and which promises other more interesting results.

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EFFECT OF SULFORAPHANE ON BIOTRANSFORMATION ENZYMES IN RAT

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Sulforaphane (SF) is a diet-based isothiocyanate, which is occurring in *Brassicaceae* (cruciferous vegetables) for example in broccoli or cabbage. In vegetables it is in the form of glucoside – glucoraphanin. By the following cutting or chewing, it is hydrolysed into the corresponding isothiocyanate SF either by the plant thioglucosidase myrosinase or by bacterial thioglucosidases in the colon. Because of its lipophilicity and molecular size, SF is likely to passively diffuse into the enterocytes. Myrosinase is inactivated by higher temperature. So when we want SF to be absorbed from GIT, the best way to do so is eating raw vegetables or making juice from it. SF has several beneficial effects on human health, e.g. anticancer, antioxidant or neuroprotective effects.

The aim of our study was to evaluate the effect of SF on the activity of selected biotransformation enzymes. The effect of SF was studied on rat liver subcellular fractions and primary culture of rat hepatocytes. Hepatocytes were incubated with SF, with β-naphthoflavone (β-NF) and with SF and β-NF together, all for 24 hours. The activities of conjugating enzymes, reduction enzymes and cytochromes P450 were studied. In subcellular fractions was studied only an inhibitory effect of SF, but in hepatocytes the induction of abovementioned enzymes was studied as well.

Our results show, that SF in concentrations 10μM, 20μM, 50μM and 100μM weakly inhibits glutathione S-transferase (GST) in cytosol. But in contrast, in hepatocytes was observed induction of GST by 10μM SF itself or in combination with β-NF. Sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) were not detected in subcellular fractions**.** No effect of SF on UGT activity was proven in hepatocytes too.

In hepatocytes, SF induces aldo-keto reductase (AKR1C) alone or with β-NF. The same results were obtained with carbonyl reductase (CBR) and on top of that, there is a great synergism of SF and β-NF. They induce CBR together more than alone SF. We proved that SF induces NAD(P)H:quinone oxidoreductase 1 (NQO1) in rat hepatocytes and the synergism of SF and β-NF combination was also observed. In rat liver cytosol SF slightly inhibits NQO1.

The effect of SF on cytochromes P450 (CYPs) was studied as well. In subcellular fractions no effect of SF on CYP1A1 and CYP1A2 activities was proven. In hepatocytes we confirmed the inhibitory effect of SF on CYP1A1, but only when CYP1A1 was induced by β-NF at first. The activity of CYP3A was not found in hepatocytes, in subcellular fractions SF inhibits CYP3A.

The obtained results show that SF affects several biotransformation enzymes in rat. Therefore, sulforaphane appears to be a promising compound with induction effect on detoxifying enzymes.

This study was supported by the Charles University, SVV 260 186.

THE ENTRANCE OF INTRACELLULAR PATHOGEN *FRANCISELLA TULARENSIS* INTO B CELLS

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The main aim of the study was the analysis of entrance of intracellular pathogen *Francisella tularensis* into B lymphocyte.

Francisella tulerensis is intracellular gram-negative bacterium and it was chosen for its high virulence. It can be easily abused as a biological weapon.

The interaction with B cells is important, because a studies shown *F. tularensis* is able to induce an apopthosis in them. In the first part of the work we wanted to find out the influence of blocking receptors for entrance into cell on infection caused by *F. tularensis*.

It was detected by transmission electron microscopy and flow cytometry. We observed blocation on receptors CR1, CR2, CR3, CR4 a BCR. Next part of this was investigate effect of opsonization by antibody and complement system on the same infection. Their inhibitors were used for detection signal pathaways.

In the second part of this research we observed the cell fate – colocalization of bactery with endo-lysozomal markers. For the first part of work we used murine B lymphocytic cells line A20 and peritoneal B cells BALB/c. The colocalization was observed on the A20 cells only.

The study was supported by SVV 260 185.

EFFECT OF AMYGDALIN ACTIVATED WITH B-D-GLUCOSIDASE ON HELA, MCF-7 AND PC-3 CANCER CELLS PROLIFERATION.

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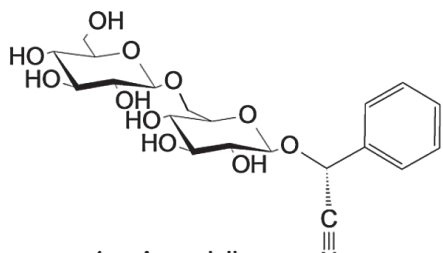
Stone fruits from tribe Amygdaleae of Rosaceae family are known for their antioxidant activity and amount of nutrients and vitamins. Their seeds are connected with content of cyanogenic glycoside amygdalin and its possible effect on inhibition of cancer cells growing.

The anti-proliferative activity brought by stand-alone amygdalin (**1**) and amygdalin activated with β-D-glucosidase from almonds was evaluated in HeLa (cervical), MCF-7 (breast) and PC-3 (prostatic) human cancer cell lines. The MTT viability assay showed that all samples inhibited growth of all three cell lines in dose and time dependent manners. IC_{50} values on the proliferation of the three cell lines for 24 h were more than 15 mg/mL for stand-alone amygdalin and less than 7 mg/mL for amygdalin combined with β-D-glucosidase.

In vitro degradation study of amygdalin with β-D-glucosidase was examined by rp-HPLC to characterize enzymatic hydrolysis rate. Experiments showed that amygdalin could be decomposed to benzaldehyde during the first 1.5 h. Optimum reaction conditions were determined as follows: 37 °C, phosphate buffer system (pH 7.4), the ratio of amygdalin/enzyme 1 : 0.12. The results indicate that amygdalin in combination with β-D-glucosidase significantly inhibit *in vitro* growth of the carcinoma cells.



1 Amygdalin

The study was supported by the Charles University, SVV 260 184.

SYNTHESIS OF TETRAZOLE DERIVATIVES WITH HIGH ANTIMYCOBACTERIAL ACTIVITY AND THEIR INITIAL *IN VITRO* TOXICITY ASSESSMENT

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Tuberculosis is persistently considered to be one of the most dangerous and widespread infectious disease with estimated 1.5 million annual deaths worldwide (one third of entire population is infected). It is very specific illness with unique pathogenesis, progression and

complicated treatment. The most frequent etiological agents of this disease are strains of *Mycobacterium tuberculosis*. Nowadays we can diagnose and cure this disease with success. On the other hand, it is still causing fatal issues in developing countries and is often found in immunosuppressed patients and people co-infected with HIV virus. Resistance to antituberculosis drugs has been rising constantly – the emergence of extensively drug resistance tuberculosis (XDR) has worsen the situation even more. From this reason a pharmaceutical laboratories all over the world are trying to develop new effective drugs that would provide faster and more effective treatment of this illness and prevent the forming and spreading of resistant strains.

The aim of this study was to synthetize three novel derivatives and subsequently to provide basic *in vitro* evaluation of their cytotoxicity (together with other substances from this series).

Cytotoxicity experiments were performed on 3T3 mouse nonmalignant fibroblast cell line. Cellular viability was assessed using Neutral Red uptake assay in 96-well plates. Epifluorescence microscopy was used for obtaining information of cellular and sub-cellular morphology changes using CellMask Green (cytoplasmic membrane), Hoechst 33342 (nucleus) and MitoTracker Red FM (mitochondria) fluorescent probes. Additionally changes in mitochondrial inner membrane potential were monitored using JC-1 fluorescent probe.

Results of this study showed absence of inherent toxicity of the most of studied compounds up to their solubility limit in waterbased media (up to 100 μM). Antimicrobial activities (MICs) of these substances are in ≈ 0.03–1 μM concentrations – this in combination with the low cytotoxicity render these substances as very promising antituberculosis agents.

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SCREENING FOR CYTOTOXIC ACTIVITY OF *AMARYLLIDACEAE* ALKALOIDS

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Oncological diseases are one of the leading causes of death in the developed countries and the increase of its prevalence seems to be inevitable. According to World Health Organization's International Agency for Research on Cancer (IARC) in Loyn, France, the incidence of cancer is expected to increase by more than 75% by the year 2030 in

developed countries. In most cases oncological patients die due to resistance of cancer to therapy, metastasis and dissemination of cancer cells into vital organs. The standard treatment covers surgical intervention, radiotherapy and/or chemotherapy.

Additionally convential anticancer treatments damage healthy tissue, resulting in a variety of side effects. Therefore, substantial efforts are being invested into identifying and developing compounds that would be able selectively target tumor cells while not damage healthy cells.

The search for new lead anticancer compounds is a crucial element of modern natural products research. Among various natural sources that have been investigated for constituents with potential use in cancer treatment, plants of the *Amaryllidaceae* family have been particularly promising and fruitful.

To date, about 50 of these alkaloids were tested against different cell lines. From these pilot studies we can conclude that the most active substances fall into free structural types: namely lycorin, crinine and pancratistatin. Most of these active compounds were studied for IC_{50} values on diverse mammalian cells howewer mechanism of action remains to be determined.

In curent study we screened and determined IC_{50} *in vitro* growth inhibitory activity (using the MTT colorimetric assay) of 15 *Amaryllidaceae* alkaloids at concentrations up to 100μM in to cancer cell lines Caco-2 (human epithelial colorectal adenocarcinoma cells) and HT-29 (human colon adenocarcinoma cells) using the MTT colorimetric assay. A human normal intestine cell line (FHS-74int) was used as a control fot the overall toxicity. All tested alkaloids have been previously isolated in our laboratory from three plant species *Zephyrantes robusta*, *Chlidantus fragrans* and *Nerine bowdenii*.

Among the tested compounds lycorine, haemanthamine and haemanthidine exhibited the most potent cytotoxic potential against both tested cell lines, with IC_{50} values of 0.99–3.28 μM for Caco-2 and IC_{50} 0.59–1.72 μM for HT-29. Lycorine and haemanthamine showed only moderate toxicity against normal cells (15 μM < IC_{50} < 30 μM) in comparison to used standard vinorelbine, which is significant toxic to used normal cells (IC_{50} 3.98 ± 0.26 μM). Other tested alkaloids showed moderate (10 μM < IC_{50} < 25 μM), weak (25 μM < IC_{50} < 100 μM) or no (IC_{50} > 100 μM) cytotoxic potential against all tested cell lines.

Further step of the current study is the preparation of semisynthetic analogues by changing different parts of the structure of the most active compound haemanthamine. This compound is isolated in our laboratory in sufficient amount (10 g) allowing such structure-activity relationship (SAR) study. The first series of haemanthamine analogues have been already synthetized. The prepared analogues are assayed for their cytotoxic activity.

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CIRCADIAN RHYTHM SLEEP DISORDER

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Current lifestyle with more stress factors helps to impair physiological programming of circadian rhythm, because of that the number of patients with sleep disorders is growing.

Study was conducted at the Centrum of Disorders of Sleep and Biorhythm in University hospital in Hradec Králové, Czech Republic in co-operation with doc. MUDr. Petr Smolík. A total of 51 patients were enrolled in the study, each of them was controlled minimally once every 3 months. The two main groups are insomnia and circadian rhythm sleep disorder patients.

Method of study was the personal contact with patients, together filling in questionnaires and follow up work with medical history and consultation with psychiatrist from sleep laboratory.

Except standard sleep distribution, we distinguish patients with advanced sleep phase syndrome – ASPS (larks) or delayed sleep phase syndrome – DSPS (owls). Patients with ASPS aren't limited in social and economical life as much as patients with DSPS. Main problems of DSPS are demonstrated on two, to point out complexity of therapy in patients with DSPS.

The cases are focused on physiological programming and individualization of therapy, especially using right dosage of drug at the right time. Therapy is also based on complex change of patient's lifestyle, which is beneficial for enhancing patient's compliance and adherence not only to pharmacological part, but also to psychological part of therapy. Incorrectly administered dosage of drugs, usually associated with drug abuse, might not help patient, in one given case study it made patient's condition worse.

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THE EFFECT OF EPIGALLOCATECHIN GALLATE ON THE INDUCTION AND REPAIR OF THE DNA DAMAGE INDUCED BY HYDROGEN PEROXIDE IN HUMAN ADENOCARCINOMA CELLS A549

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During their life, human cells are exposed to oxidative stress. The cell damage caused by reactive oxygen species (ROS) has been recognised as a major cause of cell ageing and the subsequent mutagenesis. The aim of our work was to identify the protective effects of epigallocatechin gallate (EGCG) on DNA in human lung cells A549 under oxidative stress generated by hydrogen peroxide (H_2O_2).

The A549 cells were treated with EGCG at several concentrations for one hour and subsequently exposed to hydrogen peroxide at different concentrations. In this way, the protective effect of EGCG against H_2O_2-induced damage was studied. Analogically, the DNA repair process was followed with A549 cells first exposed to hydrogen peroxide at several concentrations and subsequently incubated with EGCG at different concentrations for two reparation periods – 15 and 30 minutes. The impaired oxidised bases were detected by enzymes, endonuclease III (Endo III) and formamidopyrimidine-DNA-glycosylase (Fpg). The oxidative damage to DNA was assessed quantitatively using the Comet Assay method.

In the first case, the protective effect of EGCG against hydrogen peroxide induced DNA damage was confirmed. The A549 cells were treated with EGCG at concentrations from 12.5 up to 50 μg/ml, for one hour and then exposed to H_2O_2 at concentrations 0, 10, 25, 100 μM for 5 minutes. EGCG at concentrations, 25 and 50μg/ml has protective effect on DNA damage caused by oxidative stress compared to the control (no EGCG pre-treatment).

In the second case, A549 cells were exposed to H_2O_2 at concentrations of 0, 50, 100, 200 μM for 5 minutes, and afterwards they were treated with EGCG at concentrations of 12.5 and 25 μg/ml during two reparation periods: 15 and 30 minutes. The oxidised bases were detected using enzymes Endo III and Fpg. EGCG was found improving the reparation, however, the mechanism underlying this phenomenon has not been identified so far.

The study was supported by the Charles University, SVV 260 186.

VERIFICATION OF THE EFFICIENCY OF THE HERBAL BLEND DEFINED PD007 IN ACCELERATION ETHANOL METABOLISM AND DECREASING ITS TOXIC EFFECTS ON HUMAN ORGANISM

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This pilot evaluation assesses effects of using specific herbal blend, expecting to relieve the symptoms of organism intoxication with ethanol and accelerate ethanol metabolism.[1,4,5]

The aim of this study is to evaluate effects of the administration of standardized herbal blend tested on selected subjective and objective parameters of alcohol intoxication and monitoring the time course of alcohol levels changes in blood with or without tested herbal blend.

The herbal blend consists of *Hovenia Dulcis*, *Pueraria Lobata*, *Panax Quinquefolius*.

There are studies confirming the efficiency of individual plants in therapy of alcohol intoxication. Testing was performed on mice.[1,2,4,5,7] Scientists found that the main substance, dihydromyricetin, blocks receptors in the brain responsible for transmission of aminobutyric acids (GABA). Ethanol isn't bound on neurons and remains in the bloodstream, where it can be transferred to livers for further degradation.[1] As referred in the study: Mice remained sober in spite of excessive level of alcohol concentration in blood.[1,2,4,5] DHM accelerates liver ability to degrade both alcohol, and the toxic metabolite acetaldehyde.[1,2,3,4]

Substances contained in *Kudzu* root extract inhibits or modify the effects of alcohol dehydrogenase. It was also confirmed that isoflavonoids, found in root of *Kudzu*, increase concentration of serotonin and dopamine in the CNS, which leads to a gradual reduction of necessity to alcohol consumption in any form.[7,8,9,10]

The tested subjects use the herbal blend, which is in the form of a dry powder. Tested subject drinks a defined dose of ethanol with given alcohol concentration. The amount of the dose is calculated referring to body weight using specific mathematical formula. The aim is to achieve peak blood levels of alcohol in the range of 0.5–1 per mille. The level measuring is carried out by breathalyser. The part of monitoring the effect of alcohol on organism is performing the response test. The test shows changes in ability to focus on performance and in changes of fine motor coordination. Alcohol breath test is carried out every thirty minutes until the alcohol level is below 0.1 per mille. In the first test scheme the subjects are administered alcohol only. The values obtained will serve as a control measurement in further examinations of ethanol degradation. In the second test scheme the subject first uses the tested herbal blend and after thirty minutes drinks a specified dose of alcohol (the same dose as in scheme one). In the third test scheme monitored subject drinks alcohol (the same dose as in tests one and two) and thirty minutes from administering of alcohol the tested herbal blend is applied. According to the results of the tests number 2 and 3 the most successful scheme is selected and it will be performed with doubled dose of the herbal blend.

Data analysis evaluated referring to the duration of decreasing alcohol level below 0.1 per mille in relation to sex, body constitution and results of the response test.

The study would be important for diagnostic or therapeutic methods for medical science.[6]

The results can't be assessed at this very moment, but it is believed that one dose of herbal blend should be taken before alcohol consumption and another dose after finishing of the consumption even before going to bed to achieve maximal efficiency of the blend. If the tests will confirm the assumptions about the positive effect of the blend on reducing the symptoms of intoxication and acceleration of ethanol metabolism stated in literature than the results will serve as initiation of a broader study. The main objective of upcoming study would be obtaining objective data for possible use of the blend as a complementary therapy.[1,6,8,10]

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CHARACTERIZATION OF *LRRK2*-MUTANT IPSC-DERIVED ASTROCYTES

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Somatic cells derived from induced pluripotent stem cells (iPSC) are becoming a model tool of future that allows to study *in vivo* processes under *in vitro* conditions. The protocol for generation of iPSC-derived astrocytes was published only recently. Thanks to this fact, it is possible to study a complicated and not fully understood role of astrocytes under physiological and pathological conditions.

In our pilot study, iPSC-derived astrocytes in the primary research of Parkinson‘s disease (PD) were used for the first time ever. We focused on comparison of the gene expression profiles of iPSC-derived astrocytes obtained from a healthy individual and a patient with genetically conditioned PD. The G2019S mutation of the *leucine rich-repeat kinase 2* (*LRRK2*) gene was purposely studied. Presence of the *LRRK2* mutation also occurs in some patients with sporadic form of PD and therefore studying this mutation seems to be also beneficial for understanding mechanisms of PD in general.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR), immunocytochemistry (ICC) and western blotting (WB) were employed for an objective analysis

of both studied astrocyte lines. These methods analyzed morphological features of iPSC-derived astrocytes, astrocyte-specific markers, disease-associated markers, neuroprotective and pro-inflammatory markers, sensors of organelle functions as well as some enzymes.

Results of our work imply significant changes in astrocyte morphology and gene expression profiles that might be critical in pathology of PD.

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INFLUENCE OF TNF-α ON HENAC SUBUNITS EXPRESSION

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The human epithelial sodium channel (hENaC) or the amiloride-sensitive channel, is a type of ion channel which has the ability to control salt and water homeostasis. Therefore it is one of the main driving forces for the reabsorption of water through the alveolar epithelium. A dysfunction of this channel, respectively of this control mechanism, leads to a very severe disease – pulmonary edema and several other pathological conditions.

Previous studies tested a drug named AP301. AP301 is a cyclic protein comprising the human tumour necrosis factor-like domain sequence. This drug was recently developed as a potential treatment of pulmonary edema. The principle is that it activates hENaC by increasing the open probability. It was also shown that AP301 transiently increases the expression of hENaC subunits in mammalian cells.

In this study we used the western blot method to test the influence of tumour necrosis factor α (TNF-α) on hENaC subunits expression and we compared these results with the results from the studies with AP301.

We found that TNF-α transiently significantly increased the expression of δ subunit and it had a potential to increase the expression of α subunit. On the other hand, the expression of β- and γ-hENaC was not significantly increased.

Taken together, these results are analogical to those which were found in the studies with AP301.

The study was supported by SVV260186.

INTERACTION OF ANTIRETROVIRAL DRUG ABACAVIR WITH DRUG EFFLUX ATP-BINDING CASSETTE (ABC) TRANSPORTERS

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Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) that is frequently used in combination antiretroviral therapy of HIV infection. Pharmacokinetics of many antiretroviral drugs is often affected by the activity of ATP-binding cassette (ABC) transporters. Drug-drug interactions on ABC transporters should be, therefore, always borne in mind as they may complicate therapy. To guarantee effective and safe abacavir-based therapy it is inevitable to have complex knowledge on abacavir interactions with ABC transporters. The aim of our work was to study interaction of abacavir with human drug efflux ABC transporters ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein), ABCC2 and ABCC5 (multidrug resistance-associated protein 2 and 5) using *in vitro* method of MDCKII cell monolayer in setup of bi-directional study and concentration equilibrium. Abacavir was tested at a low non-saturating concentration of 198 nM. Using both experimental setups we observed abacavir active transport across MDCKII-ABCB1 and MDCKII-ABCG2 reaching significantly higher transport ratio after six hours > 2 in bidirectional study and > 2 in concentration equilibrium studies when compared with transport across the parent MDCKII cells. Application of higher concentrations of abacavir (50 μM and/or 100 μM) caused partial saturation of the ABCB1- but not ABCG2-mediated transport. Additionally, it was demonstrated that dual ABCB1/ABCG2 inhibitor GF120918 completely abolished transcellular transport across both MDCKII-ABCB1 and MDCKII-ABCG2 monolayers. We can therefore conclude that abacavir is a substrate of ABCB1 and ABCG2, but not ABCC2 and ABCC5 and it can be hypothesized that ABCB1 and ABCG2 may affect its pharmacokinetics at the level of absorption, distribution and elimination. Additional studies are required to confirm relevancy of abacavir drug-drug interactions on these transporters *in vivo*.

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PHARMACOLOGICAL EVALUATION OF POTENTIAL ALZHEIMER'S DISEASE DRUGS

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Alzheimer's disease (AD) is a neurodegenerative disorder affecting primarily ageing population. It is characterized by aggregates of amyloid plaque, neuofibrillary tangles of tau proteins and by loss of cholinergic neurons in the basal forebrain and hippocampus.[1] Cause of AD is still unknown and only symptomatic treatment is available thanks to acetylcholinesterase inhibitors (AChEI) and memantine. M_1 muscarinic positive allosteric modulators (PAMs) represent another variant of treatment that can improve cholinergic transmission. Thanks to their selectivity, they are able to decrease side effects.

The aim of the study was to measure novel compounds' abilities to inhibit AChE and BChE and simultaneously act as M_1 PAM. Enzymes inhibition was measured spectrophotometrically (according to Ellman's method) using 96 microwell plates and IC_{50} values were determined. The CHO cell line stably expressing the M_1 subtype mAChR was used for fluorescent measurement of compounds interaction with mAChR. Fluo-4 NW was used as fluorescent indicator, oxotremorine as an orthosteric agonist and BQCA (benzyl quinolone carboxylic acid) as a positive allosteric modulator[2]. Statistical analysis of results was performed in GraphPad Prism6.

Unfortunately, none of the tested compounds acted as a PAM/allosteric agonist. All novel compounds acted as M_1 inhibitors. Moreover, AChE and BChE inhibition was comparable with standards.

The study was supported by grant Ministry of Defence, A long-term developing plan 1011.

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COPPER CHELATING AND REDUCING EFFECTS OF QUERCETIN METABOLITES

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Copper is an essential microelement in particular due to its ability to easily convert between both redox forms: oxidized (Cu^{2+}) and reduced (Cu^{+}).[1] Flavonoids are common components of the human diet and they may positively influence human health.[2] They are converted into smaller phenolic acids during digestion by bacteria in the colon. Although properties of flavonoids have been well studied, the same is not true for their metabolites – phenolic acids.[3]

In this *in vitro* study, 10 phenolic acids, which are known metabolites of commonly tested flavonoid quercetin, were analyzed for their copper chelating activity and copper reducing activity at 4 pathophysiologically relevant pHs. Simple spectrophotometric methods based on hematoxylin and bathocuproinedisulfonic acid disodium salt were used for the assessment chelation and reduction of copper ions.[4]

As expected, metabolites possessing a dihydroxygroup in an *ortho* position were able to chelate cupric ions, however their chelation activity disappeared when challenged with a more powerful copper chelator. The degree of cupric reduction differed among tested compounds. All *o*-dihydroxycompounds were the most active and achieved 100% of cupric ion reduction in low compound to copper ratio.

In conclusion, based on this study, it appears that metabolites of quercetin can influence the kinetics of copper in human.

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GENERATION OF DNA CONSTRUCTS FOR THE STUDY OF GENE REGULATION *VIA* NUCLEAR RECEPTORS

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Nuclear receptors PXR, HNF4α and CAR are transcription factors that control expression of major xenobiotic-metabolizing enzymes and transporters.

The gene reporter assay is widely used method in the study of gene regulation through nuclear receptors. The method is based on a gene reporter DNA construct with target gene promoter sequence upstream of a firefly luciferase or green fluorescence protein (GFP).

In my project I introduced GENEART^{TM}Site-Directed Mutagenesis and DNA plasmid ligation techniques to generate the following DNA constructs:

(i) the organic cation transporter-1 (OCT1) luciferase reporter constructs with mutated HNF4α response elements for the study of OCT1 regulation;

(ii) gene reporter (both luciferase and GFP) constructs for cytochrome P450 CYP3A4 gene, a target gene regulated by PXR and CAR;
(iii) generation of Constitutive androstane receptor (CAR) chimera expression construct with extra alanine residue in CAR ligand binding domain and enhancer green fluorescence protein (EGFP) for the study of CAR-mediated regulation of CYP3A4 gene and CAR activation

I generated three OCT1 luciferase gene reporter constructs with mutated HNF4α and USF1 binding sites in OCT1 promoter. Further gene reporter experiments with the constructs in HepG2 cells helped to reveal the significant roles of the factors in OCT1 regulation.

Next, we generated two CYP3A4 gene reporter constructs with XREM and proximal regulatory regions of CYP34 gene and with both firefly and GFR reporter genes. Functional cellular experiments confirmed correct construction of the plasmids.

Finally, we generated chimera CAR expression construct with inserted alanine residue and EGFP, which confers low constitutive activity and no false translocation to nucleus. In fluorescent microscopy experiments, we confirm nuclear localization of CAR+A/EGFP protein after treatment with CAR ligand in HepG2 cells and activation of CYP3A4 luciferase gene reporter construct.

I can conclude that generated DNA constructs are functional and are valuable tools for further study of gene regulation.

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CYTOMEGALOVIRUS INFECTION WITH HCMV STRAIN AND ITS RELATIONSHIP TO THE IMMUNOSUPPRESSION

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The main goal of our study is a contribution to the study of *in vitro* interaction of human cytomegalovirus, belonging to the family *Herpes viridae* with selected immunosuppressed host cells.

During the academic year 2014/2015, we were focused on the infection of human lung fibroblasts MRC-5 with human cytomegalovirus strain VR-1590. During the study, basic laboratory techniques have been used. Among these methods, we were employed to work with cell cultures and viral isolates where the concentration of cytomegalovirus has been quantified using plaque-based assay. Moreover, ionizing radiation was applied to ensure the condition of immunosuppressed host. Subsequently, selected signalling pathways of host cells have been examined in relation to the radiation and/or infection using PathScan antibody technology.

In this study, the cytomegalovirus infection model using immunosuppressed host cells has been introduced and quite well optimized. We have also investigated the target signaling pathways of host cells using specific antibody-determined technology. The relationship between immunosuppressed host cells and viral infection has been studied on the basis of changes of selected transduction pathways signals of these cells. PathScan technology will be further optimized in the context of the study of other selected signaling pathway signals in the future.

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

UHPLC-MS/MS ABSOLUTE QUANTIFICATION OF CYTOCHROME P450 ENZYMES IN C3A, CACO2 MODIFIED CELL LINES AND IN HUMAN LIVER MICROSOMES

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Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism. They can be responsible for the failure of treatment, adverse and toxic effects or drug–drug interactions. Knowledge of expression levels and their susceptibility to be either induced or inhibited would be the basic tool for personalized therapy. Therefore, *in vitro* and *in vivo* experiments of CYP mediated metabolism is an essential part of the drug development and clinical research.

In vitro studies can be done with primary cells or cell lines. Cell lines are phenotypic stable and immortal but their CYPs levels are low. From this point of view, modified C3A and CACO2 cell lines with constitutive androstane receptor (CAR) and pregnane X receptor (PXR) might be used for these experiments. CYP enzymes should be expressed continuously in these modified cell lines.

With regard to pharmacokinetic and pharmacological importance, the expression levels of metabolizing enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A5 and CYP3A4 were studied in my diploma thesis.

Absolute quantifications of CYP enzymes were carried out by UHPLC in line coupled with tandem mass spectrometry working in scheduled MRM mode. Data assessment was conducted by Skyline 2.6 software.

CYP enzymes were not detected in CACO2 and C3A modified cell lines. However, these enzymes were found in human liver microsomes. Average values were ranging from 0.6 pmol/mg to 21.5 pmol/mg of microsomal protein. The lowest detected amounts of CYP protein were 0.006–0.210 pmol/mg of microsomal protein in a hundred times diluted human liver sample. These findings point out that CYPs protein levels in modified C3A and CACO2 cell lines were apparently below the limit of detection.

Results show that up-regulation of CYP enzymes in modified cell lines CACO2 and C3A does not reach CYPs levels in human liver microsomes. Further studies have to be conducted in order to optimize cultivation conditions, presence of co-regulators and ligands to get modified cell lines with measurable CYP levels.

The study was supported by the Charles University, SVV 260 186.

RELIABLE REFERENCE GENE SELECTION FOR QUANTITATIVE REAL TIME PCR IN *HAEMONCHUS CONTORTUS*

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Parasite anthelmintic resistance is a great problem of these days. Prophylaxis, treatment and consequences of parasitic infections represents an important economic burden on livestock production worldwide. Mechanisms of drug resistance are still not fully understood. Molecular biology methods, *e.g.* gene expression studies, could contribute to understanding of these mechanisms and thus help in resistance management. Use of suitable reference genes is essential for an accurate normalisation of gene expression levels.

Haemonchus contortus is a parasitic nematode of small ruminants, whose multi-resistance to anthelmintics means global problem. The genome and transcriptome have been published recently, allowing extensive gene expression research to be conducted. Suitable reference genes for different strains of *H. contortus* have not been validated yet.

The aim of this work was to identify and validate reliable reference genes for gene expression studies in adult *H. contortus*. 11 candidate genes were chosen for further assessment of their expression stability in males and females of two genetically divergent *H. contortus* strains: drug-susceptible (ISE) and multi-drug-resistant (WR). The candidate genes were selected based on their common use as endogenous controls, supplemented by genes identified bioinformatically based on stable expression in RNA-seq data.

Total RNA was extracted from ten adult *H. contortus* males or females and reverse transcribed to cDNA. An identical reaction without reverse transcriptase was carried out simultaneously. The resulting cDNA was diluted 1 to 50 and used for quantitative real-time PCR assay (qPCR). iQ5 Real Time PCR Detection System (Bio-Rad, USA) with SYBR green I detection was used for qPCR analyses. Specificity and efficiency of designed primer sets were checked using standard dilutions, efficiency for all primers was between 91–104%.

Expression stability was evaluated by computer programs BestKeeper, geNorm, NormFinder and the comparative ΔΔCt method. Different calculation methods caused slightly different ranking order of genes obtained from each program. However, all methods found ama, farb, gapdh, ncbp and sodc to be the five most stable genes.

By this study we demonstrated, that the combination of commonly used gapdh gene and at least one of the other best ranked genes would be appropriate for gene expression studies in *H. contortus* adults.

The study was supported by the Charles University (Research project SVV 260 186).

CHARACTERIZATION OF HUMAN WARFARIN REDUCTASE

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Warfarin is widely used anticoagulant drug. Considering the narrow therapeutic window of warfarin, it is important to fully understand its metabolism in human body. Oxidative, reductive and conjugation reactions are involved in warfarin metabolism. However, the reductive metabolism of warfarin has not been studied in details until now.

The reduced metabolite of warfarin, i.e. warfarinalcohol, is produced by the conversion of the carbonyl group of the side chain. It is known that human liver cytosolic and microsomal fractions exhibit warfarin reductase activity but the specific enzymes catalysing the reduction of warfarin are not known yet.

The aim of this study was to identify the enzyme(s) participating in reduction of warfarin and to describe enzyme kinetics. Human liver cytosolic and microsomal fractions and recombinant enzymes AKR1A1, AKR1B1, AKR1B10, AKR1C1, AKR1C2, AKR1C3, AKR1C4, CBR1 and CBR3 were incubated with warfarin at various concentrations. The produced warfarinalcohol was quantified by UHPLC and the specific activities of enzymes and subcellular fractions were determined.

The warfarin reductase activity was confirmed in cytosolic and microsomal fractions. The reduction of warfarin was higher in the liver cytosol than liver microsomes. From the enzymes tested, AKR1C3 and CBR1 were found as the main enzymes participated in the production of warfarinalcohol. Other enzymes showed only low or no activity.

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HUMAN MEMBRANE-BOUND ENZYMES AS TARGETS OF ORACIN-IMMOBILIZED AFFINITY CARRIER

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Carbonyl reducing enzymes play important role in the metabolism of various eobiotic (e.g. steroids, prostaglandins) and xenobiotic (e.g. doxorubicin, daunorubicin, oracin, NNK, haloperidol) compounds. Due to their substrate specificity, they also play a role in development of some diseases like hormone-dependent cancers and metabolic syndrome. While cytosolic carbonyl reducing enzymes are well characterised the knowledge about membrane-bound types is quite poor because their study is demanding.

Actually, until today there are only three described microsomal carbonyl reducing enzymes participating in the metabolism of xenobiotic compounds (11β-HSD1, DHRS7 and DHRS3)[1,2] of which only the 11β-HSD1 is well characterized. However, based on the research of anticancer drug oracin reduction stereospecificity, there were predicted other microsomal carbonyl reducing enzymes involved in its metabolism and inactivation[3].

In order to isolate these "unknown" enzymes the in-house developed affinity carrier with immobilized ligand oracin[4] was introduced into the purification protocol of human liver membrane-bound enzymes[3]. Proteins having affinity towards oracin were successfully captured by our affinity carrier and subsequently gently eluted by 100 mM glycine buffer, pH 10.5. Using mass spectrometry enzyme 17β-HSD6 was identified. Despite its metabolism of eobiotics (e.g. retinol, testosterone and estradiol) was already described there are still no published information about its role in the metabolism of xenobiotics. Thus its isolation and identification as a potential target of drug oracin could significantly extend our knowledge about its role in biotransformation.

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EXPRESSION OF DHRS8 AND DHRS12 ENZYMES IN HUMAN TISSUES

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Dehydrogenase/reductase (SDR family) member 8 (DHRS8, SDR16C2) and dehydrogenase/reductase (SDR family) member 12 (DHRS12, SDR40C1) are human microsomal enzymes belonging to the superfamily of short-chain dehydrogenases/reductases (SDR). This superfamily represents one of the largest protein groups. SDR enzymes participate in the metabolism of various xenobiotic and endogenous compounds and are involved in physiological and pathological processes[1,2]. However, there are still many enzymes which are only poorly characterised.

To this date, the expression on mRNA level and catalytic activity toward 5α-androstane-3α,17β-diol are the only available information about DHRS8[3,4]. Moreover, there is still no published information (apart from the prediction) regarding DHRS12.

The aim of this study was to examine the protein expression of DHRS8 and DHRS12 enzymes in various human tissues. The tissue samples were collected from five middle aged male subjects after the sudden death without apparent disease. Proteins of interest were detected using western blotting and specific antibodies. Recombinant form of searched proteins (DHRS12, DHRS8) expressed in sf9 insect cells was used as a control.

According to our results, DHRS8 is widely expressed in many tissues with the highest level in the liver and adrenal glands. On the other hand, the expression of DHRS12 was detected only in the brain. Our data could help to estimate the role of these enzymes in human body.

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STUDY OF EFFECTS OF COMMONLY USED ANTIRETROVIRALS ON ABCG2-MEDIATED TRANSPORT OF ABACAVIR ACROSS CELL MONOLAYER

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Nucleoside reverse transcriptase inhibitor (NRTI) abacavir is a drug commonly used in combination antiretroviral therapy of HIV infection. It has been previously observed in our laboratory that drug efflux ATP-binding cassette (ABC) transporter, breast cancer

resistance protein (ABCG2), recognizes abacavir as a substrate. ABCG2 thus can be an important factor affecting its absorption, distribution, and elimination. As many antiretrovirals are substrates or inhibitors of ABCG2 the aim of our study was to investigate their drug–drug interactions with abacavir on the ABCG2 transporter. For this purpose we used well established *in vitro* model of monolayer formed by MDCKII cells stably expressing ABCG2 in setup of concentration equilibrium. First we analyzed effect of Ko134 (2 μM), model inhibitor of ABCG2, that completely abolished ABCG2-mediated active transport of abacavir across MDCKII monolayer and abacavir was demonstrated to have saturation transcellular kinetics. Subsequently, we tested several concentrations (ranging from 0.1 μM to 100 μM) of eight antiretrovirals (zidovudine, didanosine, lopinavir, atazanavir, ritonavir, stavudine, nevirapine, and rilpivirine) originating from three distinct drug groups (NRTI, non-NRTI, and protease inhibitors). We observed significant inhibition in all cases tested. Protease inhibitors lopinavir (20 μM), ritonavir and atazanavir (both at concentration of 100 μM) and non-NRTI rilpivirine (0.1 μM – 20 μM) showed the highest potency to inhibit ABCG2-mediated transport reaching abacavir was translocated across the MDCKII-ABCG2 monolayer by passive mechanism only. It can be concluded that co-administration of the tested antiretrovirals with abacavir might change pharmacokinetics of abacavir. Our data thus broaden knowledge on abacavir drug-drug interaction, however, it will need to be verified *in vivo*.

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CHANGES OF MPV DURING END-STAGE RENAL FAILURE: A LINK BETWEEN PLATELET SIZE, INFLAMMATION AND MAIN CAUSES OF CHRONIC KIDNEY DISEASE

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Mean platelet volume, a simple indicator of platelet size, is automatically calculated by blood analysers. Higher MPV refers to larger platelets, which are more reactive. These thrombocytes are assumed to have the greatest role in development of haemostatic disorders and the most of other cardiovascular diseases (CVD), which are the main cause of death in patients with end-stage renal failure[1]. The chronic renal failure (CRF) also supports a small permanent inflammation in the body[2] which can be measured by C-reactive protein. The aim of the study was to find out if there is a relation between MPV and CRP in patients undergoing a continuous renal replacement therapy (CRRP). We also focused on etiopathology of renal failure. The main causes of CRF all over the world are chronic glomerulonephritis, diabetic nephropathy, hypertension, polycystosis of kidneys and chronic

interstitial nephritis[3]. We compared MPV values from three of these groups and tried to establish the correlation between pathogenesis of the disease and platelet activation.

A total of 102 patients who received the CRRP between November 2014 and February 2015 were taken into this retrospective study. The collected data included basic information such as gender, age, fundamental cause of renal failure and the length of dialysis program, if the transplantation of kidney has been realized and if the patient suffered from any cardiovascular disorders or the diabetes mellitus. Then MPV, platelet count and CRP taken from every first week in these 4 months and finally some technical information such as the method of dialysis (hemofiltration, hemodialysis or hemodiafiltration and the regular regime of dialysis.

The sex ratio (M/F) of all patients was 11 : 6, the average age was 66.2 (23–91) years. 45.1% of patients had already suffered from diabetes mellitus, 39.2% had some cardiovascular disease (ischemic heart disease, heart insufficiency, apoplexy etc.).

The mean MPV was 10.44 ± 0.96 which is closed to the upper limit of physiologic values (10.5 fl). The mean platelet count was 219×10^9/l and the mean CRP was 9.56 ± 10.70 mg/l. As predicted, the MPV values slightly diminished with increasing CRP (so with the higher level of inflammation), but the value of reliability of linear regression was very low ($R^2 = 0.0013$) that is why we could not consider it as a valuable confirmation of our theory.

The main causes of chronic renal failure were diabetic glomerulosclerosis (in 30 patients, 1st group), chronic interstitial nephritis (11, 2nd group), chronic glomerulonephritis (9, 3rd group) and other (62). The average value of MPV was the highest in the 1st group (10.79 fl) and the lowest in the 2nd group (10.57 fl).

We did not detect a significant relation between MPV and CRP, even if the trendline showed a negative correlation. The problem could be in interactions with other diseases the patients suffered, as CVD, hypertension and DM, which could influenced our values. The MPV was higher in patients with diabetic glomerulosclerosis. The other two groups could have lower MPV values due to an inflammatory etiology of the ailment.

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SOLUBILISATION, PURIFICATION AND RECONSTITUTION OF HUMAN 17-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 3 (HSD17B3)

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Short-chain dehydrogenases/reductases (SDR) superfamily is a large group of NADP(H)/NAD(H)-dependent oxidoreductases. Human SDR enzymes are classified into 47 families, including cytosolic and membrane-bound ones. The objective of this study is a human membrane-bound enzyme 17-beta-hydroxysteroid dehydrogenase type 3 (HSD17B3), which participates in the biosynthesis of steroidal hormones and mainly catalysis the conversion of androstenedione to testosterone. The main goals of this study are to find a suitable detergent for successful solubilisation, purify the enzyme and prepare a reconstitution system for studying of the pure HSD17B3 behavior in the membrane.

Microsomes containing overexpressed HSD17B3 were isolated from Sf9 insect cells (*Spodoptera frugiperda)*. The first step is the solubilisation process, which involves detergent screening. Six detergents were tested, each in final concentration of 0.1%, 0.5% and 1.0% (w/v): ASB 14-4, C12E8, DDM, CHAPS, Igepal CA-630, Triton X-100. The detergent ASB 14-4 in concentration 0.5% (w/v) has been indentified to be the best one for the HSD17B3 solubilisation.

The next step is enzyme purification, using the His10-tag located on C-terminus of the HSD17B3 and Ni-metal affinity chromatography (Ni-IMAC). This method enabled to obtain the pure protein in the concentration 248.92 μg/ml and specific activity 5.16 nmol/mg/60 min based on the reduction of androstendione to testosterone.

The last step was successful incorporation of the *HSD17B3* into custom prepared liposomes, whose phospholipid composition was based on the membrane of the human liver endoplasmic reticulum.

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EFFECT OF SESQUITERPENES ON BIOTRANSFORMATION ENZYMES IN TISSUE SLICES

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High-precision tissue slicers (e.g. Krumdieck tissue slicer[1]) allow the rapid production of equally sized tissue slices of less than 250 μm thickness. Tissue slices are viable explants of tissue, cultivated *ex vivo*, with a reproducible and defined thickness and they serve as a multipurpose *in vitro* model. It contains all cell types of the tissue in their natural environment. The thin slices realistically and reliably represent the *in vivo* situation and have been used to study the metabolism, transport and biotransformation of xenobiotics, as well as for toxicological studies and others.

The essential factors, for the viability and function of cells inside the slices, are incubation conditions and slice thickness. These limit sufficient oxygen supply to the inner cell layers and exchange of nutrients and metabolites.

The aim of the study was to evaluate influence of several sesquiterpenes, secondary metabolites mainly produced by higher plants, as possible modifiers of biotransformation enzymes. Sesquiterpenes α-humulene, β-caryophyllene and β-caryophyllene oxide were chosen for this purpose. Above mentioned sesquiterpenes showed a potential as inducers of the detoxifying enzyme glutathione S-transferase in forestomach, liver and small bowel mucosa of A/J mice[2].

In our project we used rat liver slices that were 8 mm in diameter and 200 μm thin. They were cut on the Krumdieck tissue slicer in ice-cold and oxygenated Krebs-Heseleit buffer set up to pH 7.4. After cutting, the tissue slices were incubated for 3, 6 and 24 hours in medium containing sesquiterpene and in carbogen atmosphere (95% O_2/5% CO_2). After, the tissue was homogenized. The results were compared to control homogenates from slices incubated in clear medium. Advantage of the method lies in producing multiple tissue samples that can be incubated with variable compounds in different concentrations and reducing the number of animals necessary for experiment.

So far, we managed to optimize the method and raise the viability of the tissue slices, especially the viability after 24 hours. The first sesquiterpene studied was α-humulene. However, the results did not show any induction of glutathione S-transferase in comparison to control. We also tried to assess the effect of α-humulene on sulfotransferase and quinone oxidoreductase 1, but the measured activities were at detection limit. Further studies have to be conducted in order to evaluate effect of β-caryophyllene and β-caryophyllene oxide on biotransformation enzymes.

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INTERACTION OF ANTIRETROVIRAL DRUGS ETRAVIRINE AND RILPIVIRINE WITH ABC DRUG EFFLUX TRANSPORTERS *IN VITRO*

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Etravirine and rilpivirine, are relatively new antiretroviral drugs that belong to the second-generation non-nucleoside reverse transcriptase inhibitors used in combination therapy (cART) of HIV positive patients. ABC (ATP-binding cassette) transporters are extensively expressed in normal tissues (e.g. liver, kidney, intestine, blood-brain barrier, placenta), where they are able to affect pharmacokinetic behavior of various drugs. ABCB1 (P-glycoprotein), ABCG2 (BCRP) and ABCC2 (MRP2) represent the most com-

mon drug transporters, on which drug-drug interactions (DDI) can occur. Nevertheless, the current knowledge on interactions of etravirine and rilpivirine with the transporters and their potential to create transporter-mediated DDI is only limited so far. In this study we therefore aimed to investigate inhibitory potency of etravirine and rilpivirine towards ABCB1, ABCG2 and ABCC2 drug efflux transporters employing *in vitro* accumulation/efflux studies with relevant fluorescent substrates.

The accumulation assays with Hoechst 33342 and Rhodamine 123 on MCDKII-ABCB1 cells have demonstrated that rilpivirine is a potent ABCB1 inhibitor able to bind H- as well as R-site of the transporter, while etravirine does not inhibit ABCB1 at all. In MDCKII-ABCG2 cells, both antiretrovirals revealed significant inhibitory potency to ABCG2. Nevertheless, using the efflux experiments with calcein-AM in MDCKII-ABCC2 cells neither etravirine nor rilpivirine caused inhibition of MRP2.

Since our data clearly showed that rilpivirine and etravirine are ABCB1 and/or ABCG2 inhibitors, we have additionally employed transport assays to evaluate possible DDI of these drugs with tenofovir disoproxil fumarate (TDF), another antiretroviral drug used in cART and being confirmed as ABCB1 and ABCG2 substrate. Our transport experiments on MDCKII-ABCB1 and MDCKII-ABCG2 cell monolayers demonstrate that both antiretrovirals significantly affect TDF permeability across the cellular monolayer due to inhibition of the ABC drug efflux transporters. These results suggest that etravirine and rilpivirine possess a great potential for drug efflux transporters-mediated DDI, which could have an impact on antiretroviral dosage scheme during cART in clinical practice.

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