SEKCE
CHEMICKÝCH VĚD

SEKCE CHEMICKÝCH VĚD

Středa 22. 4. 2015
(Posluchárna B)

9:00 Název přednášky: **Study of in-syringe analysis for the automation of immersed single-drop microextraction as a sample pretreatment and pre-concentration technique**

Autor přednášky: Kateřina Fikarová
Katedra: Katedra analytické chemie
Školitel: Burkhard Horstkotte, Ph.D., M.Sc

9:15 Název přednášky: **Designing a method for salt-assisted dispersive liquid-liquid micro-extraction in a "Lab-In-Syringe" system**

Autor přednášky: Ondřej Bešťák
Katedra: Katedra analytické chemie
Školitel: Burkhard Horstkotte, Ph.D., M.Sc

9:30 Název přednášky: **Preparation and photophysical evaluation of tetra-3,4-pyridoporphyrazines suitable for the photodynamic therapy**

Autor přednášky: Pavel Čermák
Katedra: Katedra biofyziky a fyzikální chemie
Školitel: doc. PharmDr. Veronika Nováková, Ph.D.

9:45 Název přednášky: **High throughput method for determination of caffeine in coffee drinks**

Autor přednášky: Lýdia Mihalčíková
Katedra: Katedra analytické chemie
Školitel: Warunya Boonjob, Ph.D.

10:00 Název přednášky: **Synthesis and skin permeation-enhancing effects of 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanoic acid derivatives**

Autor přednášky: Ondřej Kučera
Katedra: Katedra anorganické a organické chemie
Školitel: doc. PharmDr. Kateřina Vávrová, Ph.D.

10:15 Název přednášky: **Synthesis of potential organocatalysts based on quinazoline alkaloids**

Autor přednášky: Lukáš Górecki
Katedra: Katedra anorganické a organické chemie
Školitel: Mgr. Jiří Mikušek

10:30 Přestávka

10:45 Název přednášky: **Synthesis of Sulfonamide Analog of Cardioprotective Drug Dexrazoxane**

Autor přednášky: Josef Škoda
Katedra: Katedra anorganické a organické chemie
Školitel: PharmDr. Jaroslav Roh, Ph.D.

11:00 Název přednášky: **Substituted tetra(3,4-pyrido)porphyrazines as potential photosensitizers**

Autor přednášky: Magda Vavrečková
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: doc. PharmDr. Petr Zimčík, Ph.D.

11:15 Název přednášky: **Tacrine-Benzothiazole Hybrids-Novel Multitarget Agents To Combat Alzheimer's Disease**

Autor přednášky: Lucie Svobodová
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Marta Kučerová, Ph.D., PharmDr. Jan Korábečný, Ph.D, Mgr. Evžénie Nepovímová

11:30 Název přednášky: **Synthesis of substituted pyridines using tris(2-furyl)phosphine gold(I) catalyst**

Autor přednášky: Martin Janoušek
Katedra: Katedra anorganické a organické chemie
Školitel: Mgr. Pavel Horký

11:45 Název přednášky: **Phthalocyanines and their aza-analogues with bulky diphenylphenylsulfanyl substituents**

Autor přednášky: Anna Málková
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: doc. PharmDr. Petr Zimčík, Ph.D.

12:00 Název přednášky: **The utilization of the near infrared spectroscopy in the evaluation of the homogeneity of the powder blend**

Autor přednášky: Jaroslav Plot, Klára Morávková
Katedra: Katedra anorganické a organické chemie, Katedra farmaceutické technologie
Školitel: doc. Zdeňka Šklubalová, Ph.D.; PharmDr. Karel Palát, CSc.

12:15 Přestávka na oběd

13:15 Název přednášky: **The fast HPLC method for determination of arginine and its metabolites in monitoring of wound healing process**

Autor přednášky: Hana Piskáčková
Katedra: Katedra analytické chemie
Školitel: PharmDr. Jana Aufartová, Ph.D.

13:30 Název přednášky: **Tacrine – HYNIC Heterodimers –Anticholinesterase and Antioxidant Ligands with Good Toxicological Profile**

Autor přednášky: Ngoc Lam Pham
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: doc. RNDr. Veronika Opletalová Ph.D.

13:45 Název přednášky: **Design and Synthesis of Novel Centrally Acting Cholinesterase Reactivators**

Autor přednášky: Thuy Duong Nguyen
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Marta Kučerová, Ph.D.

14:00 Název přednášky: **Development of an absolute method for determination of singlet oxygen quantum yields of phthalocyanines**

Autor přednášky: Lenka Hrubá
Katedra: Katedra biofyziky a fyzikální chemie
Školitel: doc. PharmDr. Veronika Nováková, Ph.D.

14:15 Název přednášky: **Preparation and photophysical evaluation of tetra-3,4-pyridoporphyrazines carrying charged substituents on the periphery**

Autor přednášky: Jiří Demuth
Katedra: Katedra biofyziky a fyzikální chemie
Školitel: doc. PharmDr. Veronika Nováková, Ph.D.

14:30 Název přednášky: **Synthesis of combretastatin analogues as potential antitumor and antimicrobial agents**

Autor přednášky: Manuela Voráčová
Katedra: Katedra anorganické a organické chemie
Školitel: prof. RNDr. Milan Pour, Ph.D.

14:45 Přestávka

15:00 Název přednášky: **Evaluation of stability of novel aroylhydrazones in plasma using HPLC**
Autor přednášky: Panagiotis Mingas
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: doc. PharmDr. Petra Kovaříková, Ph.D.

15:15 Název přednášky: **HPLC method development for artificial colorants determination in green beer samples**

Autor přednášky: Ivana Stachová
Katedra: Katedra analytické chemie
Školitel: doc. RNDr. Dalibor Šatínský, Ph.D.

15:30 Název přednášky: **Separation of tocopherols using HPLC technique**

Autor přednášky: Michaela Hutníková
Katedra: Katedra analytické chemie
Školitel: RNDr. Lenka Kujovská Krčmová, Ph.D

15:45 Název přednášky: **In silico Screening of SIRT6 Inhibitors**

Autor přednášky: Tomáš Kučera
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: Maija Lahtela-Kakkonen, Ph.D., prof. PharmDr. Martin Doležal, Ph.D.

16:00 Název přednášky: **Physico-Chemical properties of Drugs – Measurement of dissociation constant and usage in practice**

Autor přednášky: Miroslav Suchý
Katedra: Katedra biofyziky a fyzikální chemie
Školitel: Ing. Vladimír Kubíček, CSc.

16:15 Název přednášky: **Development of HPLC method for determination of vancomycin in clinical research – part** I

Autor přednášky: Kateřina Kučerová
Katedra: Katedra analytické chemie
Školitel: RNDr. Lenka Kujovská Krčmová, Ph.D.

16:30 Přestávka

16:45 Název přednášky: **NMR Spectroscopy – the identification of the isolated substance from Nerine bowdenii**

Autor přednášky: Jana Maříková
Katedra: Katedra anorganické a organické chemie
Školitel: doc. PharmDr. Jiří Kuneš, CSc.

17:00 Název přednášky: **Oxadiazoles as Potential Drugs**

Autor přednášky: Pavlína Dzámová
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Marta Kučerová, Ph.D.

17:15 Název přednášky: **Synthesis of potential cholinesterase inhibitors for the treatment of Alzheimer´s disease – tacrine derivates**

Autor přednášky: Michaela Jarošová
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: doc. RNDr. Veronika Opletalová, Ph.D., PharmDr. Jan Korábečný, Ph.D.

Čtvrtek 23. 4. 2015

(Nová posluchárna)

9:00 Název přednášky: **Synthesis of on the ring substituted phenylguanidines with biological activity**

Autor přednášky: Dominika Jarešová
Katedra: Katedra anorganické a organické chemie
Školitel: PharmDr. Karel Palát, CSc.

9:15 Název přednášky: **5-Alkylamino-N-phenylpyrazine-2-carboxamides as potential antituberculars**

Autor přednášky: Alena Janoutová
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Jan Zitko, Ph.D.

9:30 Název přednášky: **Design and Synthesis of Hybrid Compounds Based on Tacrin/Resveratrol Derivatives**

Autor přednášky: Jakub Jeřábek
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Jan Korábečný, Ph.D.

9:45 Název přednášky: **Development of microemulsion electrokinetic chromatography method for the analysis of illegal fat-soluble foodstuff dyes**

Autor přednášky: Jana Bradová
Katedra: Katedra analytické chemie
Školitel: PharmDr. Klára Petrů, Ph.D.

10:00 Název přednášky: **Development of SPE and UHPLC-MS/MS method for the determination of quercetin and its 9 metabolites**

Autor přednášky: Lucia Chrenková
Katedra: Katedra analytické chemie
Školitel: doc. PharmDr. Lucie Nováková, Ph.D.

10:15 Název přednášky: **Pyrazinyl benzamides as potential antituberculars**

Autor přednášky: Ondřej Valášek
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Jan Zitko, Ph.D.

10:30 Přestávka

10:45 Název přednášky: **Preparation of benzodiazines with bronchodilatory activity**

Autor přednášky: Věra Ježková
Katedra: Katedra anorganické a organické chemie
Školitel: PharmDr. Marcel Špulák, Ph.D.

11:00 Název přednášky: **Optimization, validation and comparison of UHPSFC and UHPLC methods for the determination of agomelatine and its impurities**

Autor přednášky: Kateřina Plachká
Katedra: Katedra analytické chemie
Školitel: doc. PharmDr. Lucie Nováková, Ph.D.

11:15 Název přednášky: **Design and synthesis of rutaecarpine analogs as potential cytotoxic agents for cancer chemotherapy treatment**

Autor přednášky: Tadeáš Pešek
Katedra: Katedra farmaceutické chemie a kontroly léčiv
Školitel: PharmDr. Jan Korábečný, Ph.D., Guozheng Huang, Ph.D., PharmDr. Marta Kučerová, Ph.D.

11:30 Název přednášky: **On-line SPE HPLC method optimization for determination of patulin mycotoxin in apple drinks**

Autor přednášky: Anežka Holznerová
Katedra: Katedra analytické chemie
Školitel: doc. RNDr. Dalibor Šatínský, Ph.D.

Seznam abstraktů:

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

Fikarová K., Horstkotte B., Šrámková I., Sklenářová H.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University in Prague, Czech Republic

Lead belongs to the most toxic elements with respect to human health with 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.

References

1. World Healt Organization: Guidelines for drinking-water quality, 4th ed., Geneva, WHO, 2011, 383-384.

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

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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.

References from journals:

1. Horstkotte, B., Suarez, R., Solich, P., Cerda V.: In-syringe-stirring: A novel approach for magnetic stirring-assisted dispersive liquid–liquid microextraction. Anal. Chim. Acta 788, 2013, 52-60.
2. Maya, F., Horstkotte, B., Estela, J. M., Cerdà V.: Automated in-syringe dispersive liquid-liquid microextraction. TrAC 59, 2014, 1-8.

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

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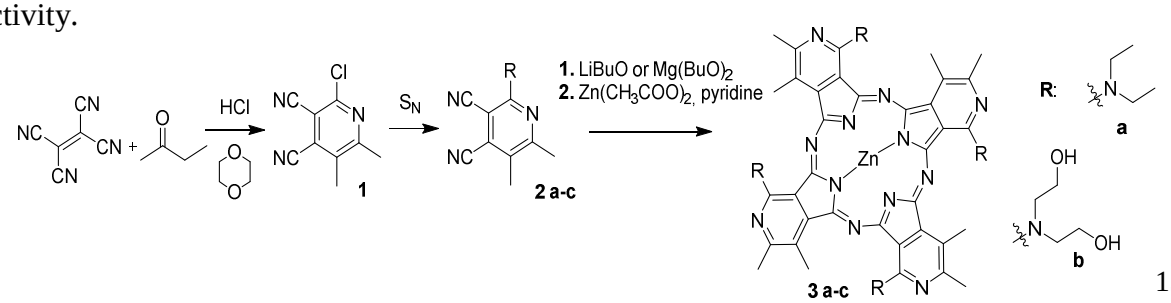
Tetra-3,4-pyridoporphyrazines (TPyPz) are aza-analogues of phthalocyanines. Their large system of conjugated bonds enables them to absorb light in the red part of the absorption spectrum. Due to their ability to produce singlet oxygen, they can be potentially used as photosensitizers in photodynamic 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.

HIGH THROUGHPUT METHOD FOR DETERMINATION OF CAFFEINE IN COFFEE DRINKS

Lýdia Mihalčíková, Warunya Boonjob, Hana Sklenářová, Petr Solich

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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 caffeine 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).

Acknowledgement

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

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

Kučera O.[1], Kopečná M.[1], Kováčik A.[1], Vávrová K.[1]

[1] Department of Organic and inorganic chemistry; Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic

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 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.

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

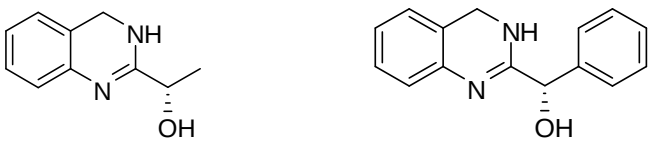
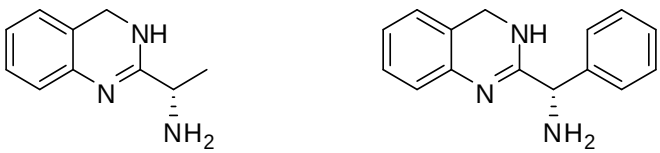
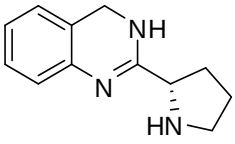
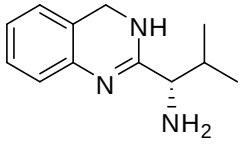


Fig. 2







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

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 (Figure 1).

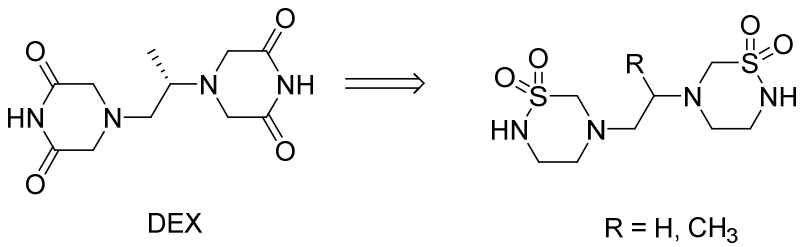

Figure 1. Structure of dexrazoxane (DEX) and its sulfonamide analog

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 in Prague (Charles University Research Centre UNCE 204019/304019/2012 and project SVV 260 183)

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

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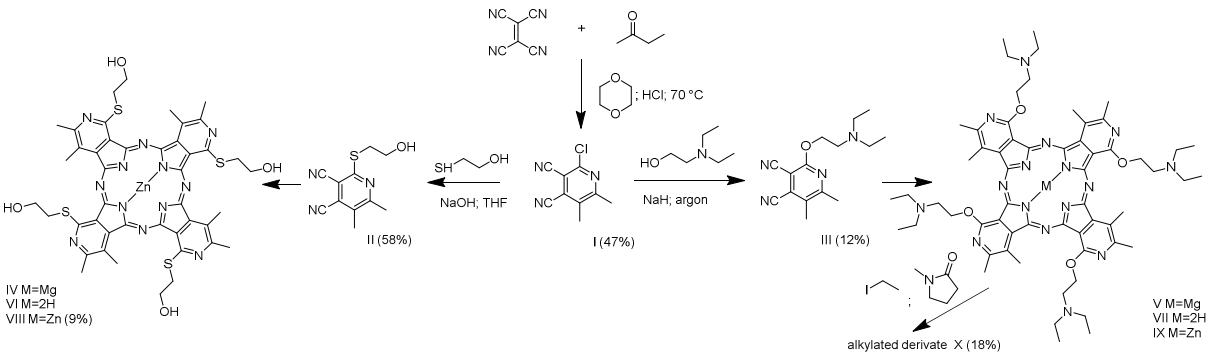
Substituted tetra(3,4-pyrido)porphyrazines represent new structural type of potential photosensitizers with interesting properties in the area of photodynamic therapy (PDT). The aim of this work was to synthesize two types of tetra(3,4-pyrido)porphyrazines with hydrophilic substituents as potential photosensitizers. 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**.*



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 Charle's University in Prague, Faculty of Pharmacy, specific research (No. SVV 260 183).

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

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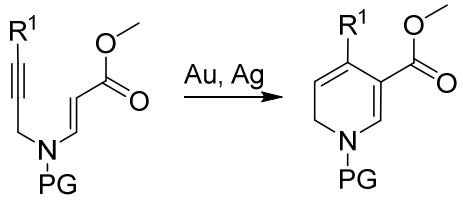
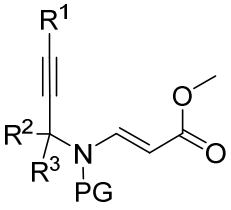
Synthesis of various types of heterocycles is possible from 1,5-enyne precursors using cationic gold(I) species as a catalyst. In order to extend previous research[1] we focused on cyclisation of 1,5-enynes with various aryls and silyls in the position R^1. The influence of various protecting groups 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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PHTHALOCYANINES AND THEIR AZA-ANALOGUES WITH BULKY DIPHENYLPHENYLSULFANYL SUBSTITUENTS

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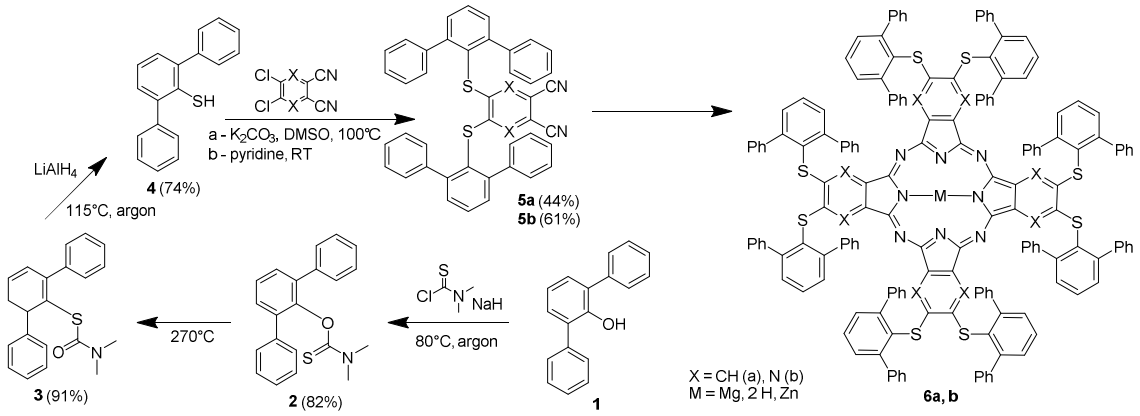
Phthalocyanines (Pcs) and their aza-analogues (AzaPc), chemical substances used in photodynamic therapy, are characterized by interesting photophysical properties which may substantially vary in dependence on the character of peripheral substituents. For example, sum of singlet oxygen and fluorescence 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.


LiAlH4
115°C, argon
4 (74%)
a - K2CO3, DMSO, 100°C
b - pyridine, RT
5a (44%)
5b (61%)
X = CH (a), N (b)
M = Mg, 2 H, Zn
6a, b
NaH
80°C, argon
1
270°C
2 (82%)
3 (91%)

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

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 x 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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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 leads to dementia. Multifactorial nature of AD that includes loss of cholinergic function, protein misfolding and aggregation, oxidative stress and free radical formation, mitochondrial abnormalities, neuroinflammation as well as the exact etiology of the disease is not fully known yet, makes 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 a treatment of neurodegenerative diseases, these are so called multi-target-directed ligands (MTDLs).

In this work, we were focusing on the design of new tacrine (THA) heterodimers with antioxidant activity, specifically tacrine-hydrazine nicotinate (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 B3 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*-acetyl-cystein). Finally, the acute toxicity of three selected candidates demonstrated better toxicological profile than tacrine. Therefore, pursuant to 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 SVV 260 183.

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.

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

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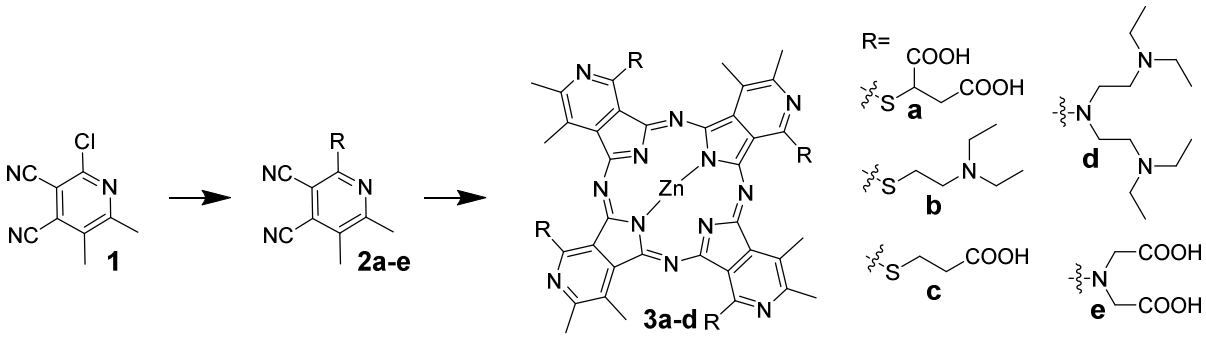
Phthalocyanines are planar organic molecules, which have a metal cation coordinated in their center. This work deals with their aza-analogues - tetra-3,4-pyridoporphyrazines (TPyPz). TPyPz can absorb light in red part spectrum and then produce singlet oxygen. Due to this ability, they may be used in 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.

SYNTHESIS OF COMBRETASTATIN ANALOGUES AS POTENTIAL ANTITUMOR AND ANTIMICROBIAL AGENTS

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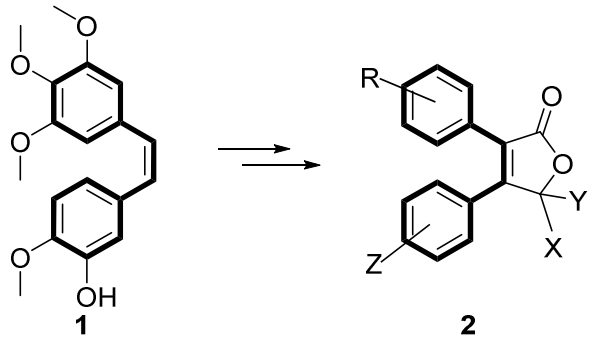
Combretastatins are naturally occurring molecules possessing remarkable *in vitro* cytotoxicity against human cancer cell lines. These compounds, such as combretastatin A-4 **(1)**, are known to disrupt mitosis through the inhibition of tubulin assembly. Furthermore, some of them also exhibit antivascular 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.



1 **2**

R = halogen, alkyl, alkoxy, hydroxy
X,Y = hydroxymethyl
Z = alkyl, alkoxy, hydroxy, methylsulfonyl

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 in Prague (SVV-260-062 and GA UK 1906214) and Czech Science Foundation (P207/10/2048).

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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 x 3 mm, 3µm, 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.

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 x 4.6 mm and guard column Chromolith CN 5 x 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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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 x 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.

Acknowledgements

The authors thank for financial support to 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 IRP UK 2015 "Využití mikrotitračních destiček pro zpracování a chromatografickou analýzu biologických vzorků v klinickém výzkumu".

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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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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 Kubicek, CSc. SVV – 260 183

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 x 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.

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.

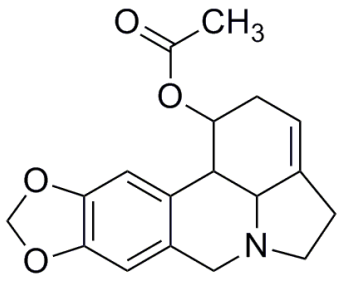

Fig. 1

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

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

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OXADIAZOLES AS POTENTIAL DRUGS

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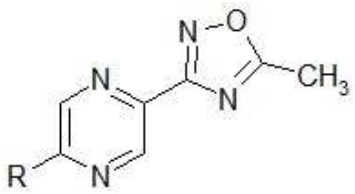
Due to the increasing resistence of bacteria and fungi against conventional drugs, it is imperative to design and develop new antibacterial or antifungal agents.[1] Some derivatives of 1,2,4-oxadiazoles exert antibacterial activity and they are known as the compounds with promising future in this direction. 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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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.

Acknowledgment*: 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

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

R^2=-H, -CH_3, R^3=-H, -CH_3, -CH_2CH_3

(I)

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

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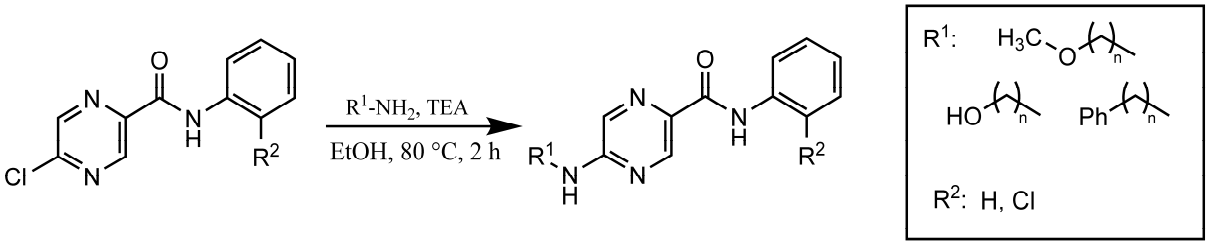
This study is focused on new derivatives of pyrazinamide (PZA) prepared as potential antituberculars. PZA itself is a well-established first-line antitubercular agent and a constituent of all basic tuberculosis treatment regimens. The design of final compounds was based on the previously synthesized 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 58-87%. 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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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.

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.

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.

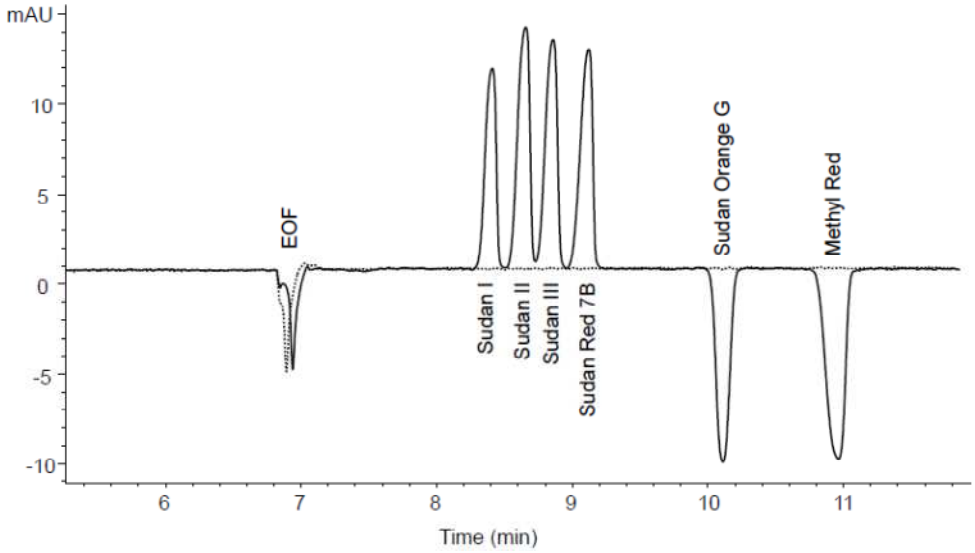

Fig.1: MEEKC separation of standard mixture of illegal foodstuff dyes under optimum conditions

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.

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]

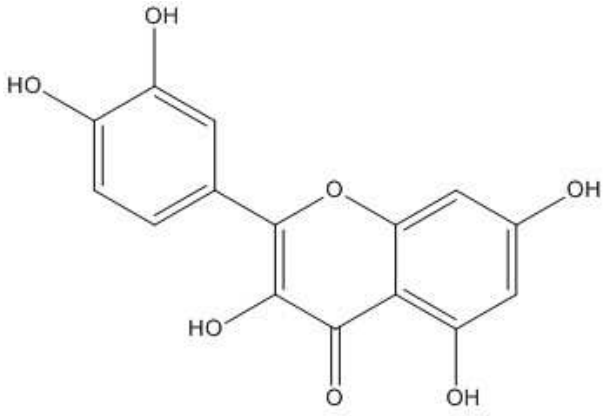

Fig. 1: The structure of quercetin

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 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 x 100 mm; 1.7μm) 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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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, maximizing 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.

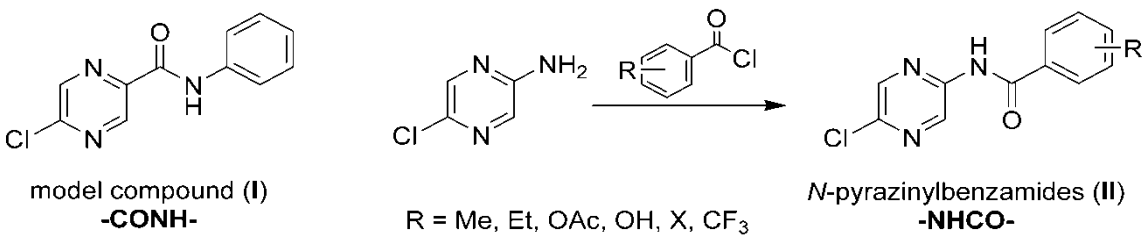

Fig. 1. Model compound I and synthesis of final compounds II.

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.

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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PREPARATION OF BENZODIAZINES WITH BRONCHODILATORY ACTIVITY

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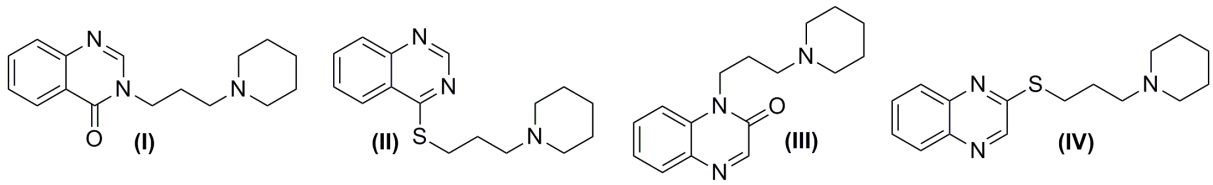
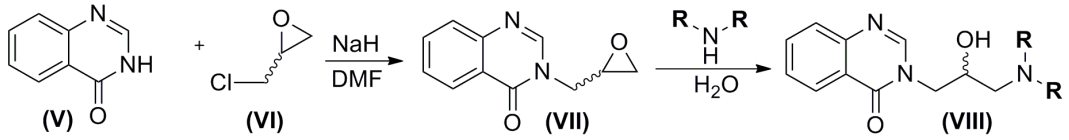
The most active compounds from previous screening contained (piperidine-1-yl)propyl moiety attached to quinazoline (**I**, **II**) or quinoxaline rings (**III**, **IV**).[1,2] The goal of this project was to introduce hydroxyl into the three-membered carbon linker with a possibility of further modification.



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 in Prague (SVV-260-183).

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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-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.

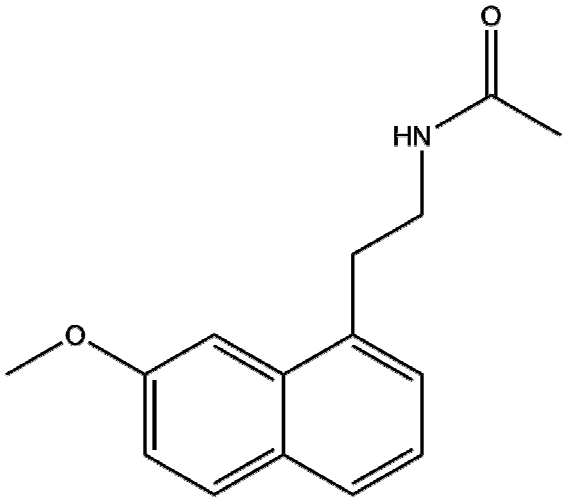

Fig.1: The structure of agomelatine

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 x 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 x 50 mm X 1.7 μm or 2.1 x 100 mm x 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.

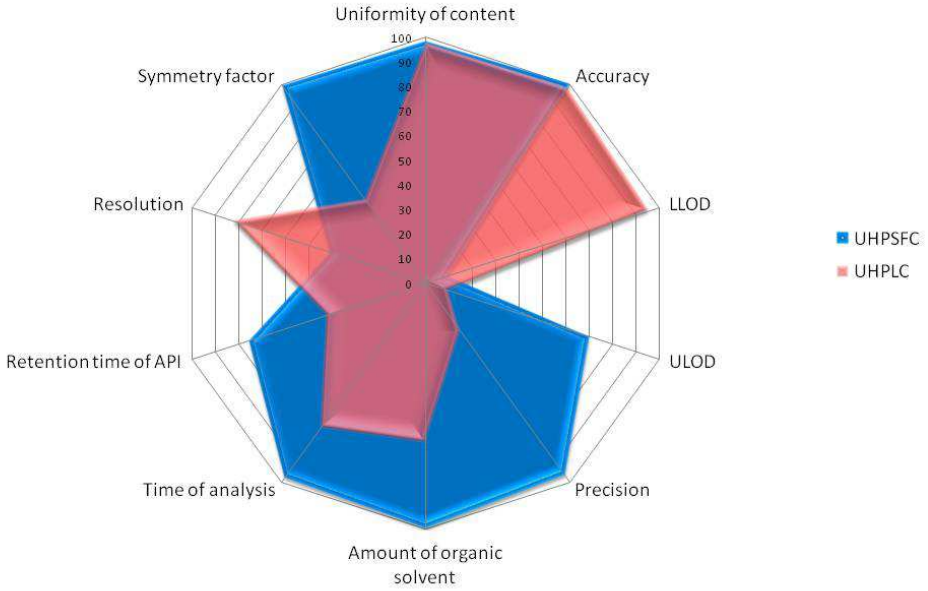

Fig.2: The comparison of UHPSFC and UHPLC methods in selected parameters

The study was supported by SVV/2015/260184.

DESIGN AND SYNTHESIS OF RUTAECARPINE ANALOGS AS POTENTIAL CYTOTOXIC AGENTS FOR CANCER CHEMOTHERAPY TREATMENT

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Rutaecarpine is a natural alkaloid isolated from plant *Evodia Rutaecarpa*. It is a well-known compound which has long been used in traditional Chinese medicine. It has been studied for its many potential pharmacological effects. This alkaloid has been proved to possess cytotoxic activity that can be exploited for treatment of unregulated cell growth leading to tumour formation. A series of rutaecarpine ethers have been developed using a simple fusion approach forming the basic heterocycle to which three different polar moieties were attached via etheric bond in position 3. The rational for choice of these moieties was to increase the solubility profile of rutaecarpine.

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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 x 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 x 4.6 mm (particle size 5µm). 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.

Keywords: HPLC, MIP, on-line SPE, patulin, mycotoxins

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