STUDENT SCIENTIFIC CONFERENCE MUNI PHARM 2021

The Book of Abstracts

MASARYK UNIVERSITY PRESS

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Inhibitors of casein kinase 1 for treatment of leukemia

Pavlína Janovská [1], Prashant Khirsariya [2], Petra Lesáková [1], Václav Němec [2], Pavlína Mrhálková [1], Kamil Paruch [2] and Vítězslav Bryja [1]

[1] Department of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic
[2] Department of Chemistry, Faculty of Science, Masaryk University, Czech Republic

The presentation will discuss the recent discovery of highly potent and very selective inhibitors of casein kinases 1 (CK1) for the treatment of leukemias, lymphomas, and solid tumors. Our novel CK1 inhibitors have high activity against primary targets (single-digit to sub-nanomolar IC_{50} values in the primary biochemical assays) and exceptional selectivity profile *in vitro* (virtually no off-targets in the panel of 200 human kinases). Our compounds are orally bioavailable, show on-target effects *in vivo*, and are well-tolerated at therapeutic doses (mouse model).

This pharmacologically-oriented research builds on our research of chronic lymphocytic leukemia (CLL) and lymphomas; the role of CK1 in CLL pathogenesis has been shown recently by our research group (Janovska et al., Blood, 2018). However, we believe that our inhibitors could be also used in the treatment of other CK1-driven malignancies such as solid tumors (e.g. breast cancer, melanoma, prostate cancer, pancreatic cancer, ovarian cancer, hepatocellular carcinoma), or other types of leukemia (AML).

Janovska, P.; Verner, J.; Kohoutek, J.; Bryjova, L.; Gregorova, M.; Dzimkova, M.; Skabrahova, H.; Radaszkiewicz, T.; Ovesna, P.; Vondalova Blanarova, O.; Nemcova, T.; Hoferova, Z.; Vasickova, K.; Smyckova, L.; Egle, A.; Pavlova, S.; Poppova, L.; Plevova, K.; Pospisilova, S.; Bryja, V. Casein Kinase 1 is a Therapeutic Target in Chronic Lymphocytic Leukemia. *Blood* **2018**, 131(11), 1206-1218.

MASTER SECTION A

Scientific Committee Members

prof. RNDr. Jozef Csöllei, CSc.

PharmDr. Margita Dvorská, Ph.D.

Mgr. Hana Pížová, Ph.D.

Mgr. Ing. Jiří Václavík, Ph.D.

Morus alba - isolation and identification of prenylated phenolic substances

Martina Poláková [1], Karel Šmejkal [1]

[1] Department of Natural Drugs, Faculty of Pharmacy, Msaryk University, Czech Republic

Key words: Morus alba, separation, isolation, identification, skin, skin disorders, cosmetics

Introduction
Morus alba plant, the dominant species of the genus Morus, is known primarily as a food source for the silkworm (Bombyx mori L.), used for silk production. However, it is also valued for its healing properties and has long played an important role in traditional medicine mainly in Asian countries. Its therapeutic potential has already been confirmed by multi-member research, which has revealed the presence of various substances with bioactive properties, while it also finds a certain application in the field of cosmetics.

Aims
Experimental part focuses on the analysis, separation and isolation of potentially bioactive phenolic substances from chloroform fraction of ethanolic extract of the root bark of M. alba, which is their rich source, using selected chromatographic methods.

Methods
For experimental part TLC, analytical and preparative HPLC as well as column chromatography were used. In addition, a database of already obtained substances was used to identify isolates.

Results
A search was carried out on the topic of M. alba, their constituents, where in addition to the general focus on their general effectiveness, the search was related to the properties applicable in the field of cosmetics. The obtained isolates were compared with the database of already obtained substances and following identification was performed via the HPLC measurement of the co-injection of the sample with the standard. Cudraflavon B, kuwanon H, albanol B and moracin M were identified. In one case of isolated substances was obtained a pure, yet unidentified substance (a new substance) which needs to be examined by identification methods. Substances and fractions suitable for further experiments were selected.

Conclusions
Obtained compounds cudraflavone B, albanol B and moracin M are known to have specific effects that have the ability to intervene in processes of pathological skin changes, and these substances have the potential to be useful in the field of cosmetics. Further assays will be carried out, especially evaluating anti-inflammatory and antibacterial activity.

Author‘s CV: Martina Poláková is a fifth-year student of the master‘s degree program at the Faculty of Pharmacy of Masaryk University.

Supervisor: prof. PharmDr. Karel Šmejkal, PhD.

Morus alba – potential in therapy of Alzheimer‘s disease and isolation of content compounds

Lucie Robošová [1], Karel Šmejkal [1]

[1] Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Morus alba, root bark, phenolic compounds, prenylated flavonoids, Alzheimer‘s disease, neuroprotective activity, isolation, identification

Introduction
The root bark of Morus alba L. contains prenylated phenols, biologically active substances with a specific hydrophilic-lipophilic structure. These compounds could offer new possibilities in the treatment of Alzheimer‘s disease (AD), a neurodegenerative disease affecting a significant part of the aging population.

Aim
The aim of the theoretical part was to conduct research and present M. alba, Alzheimer‘s disease, and the potential of prenylated M. alba phenols in the therapy of AD. The purpose of the experimental part was to isolate content compounds of the root bark of M. alba, to identify the substances by comparison with the database and to select fractions for further experiments.

Methods
The research for the theoretical part was performed mainly using Web of Science database. In the experimental part, 10 fractions from M. alba root bark extract were selected for processing. Substances were analysed, isolated, and identified using chromatographic methods (TLC, column chromatography, analytical and preparative HPLC) and by comparison with a database of M. alba root bark compounds and with standards of substances.

Results
In the theoretical part, M. alba, Alzheimer‘s disease and prenylated phenols of M. alba with possible neuroprotective activity were described. A scheme of AD pathogenesis and a summary table of activities of selected prenylated phenols were created. Moracin S, mulberrofuran D2, kuwanon C, mulberrofuran G and kuwanon G were identified as prenylated M. alba phenols that could have significant potential in the treatment of AD. In the experimental part, 40 fractions were isolated from the material obtained in previous experiments. Sanggenon H, kuwanon T, morusin, cudraflavone B, mulberrofuran A and mulberrofuran B were identified within 9 fractions. 5 pure substances were selected for subsequent testing of biological activity and 10 pure fractions for identification by other methods.

Conclusion
Some prenylated phenols, isolated from the root bark of M. alba, show strong, multitarget activity, possibly interfering with the pathogenetic processes of Alzheimer‘s disease. Therefore, they could potentially be used in the future to increase the effectiveness of AD prevention and treatment.

Author‘s CV: Lucie Robošová is currently studying the fifth year of the Pharmacy master‘s degree programme at the Faculty of Pharmacy of Masaryk University.

Supervisor: prof. PharmDr. Karel Šmejkal, Ph.D.

Isolation of constituents from fractions obtained from Paulownia tomentosa fruit

Miriama Dupľáková [1]

[1] Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Paulownia tomentosa, flavonoids, multidrug resistance, ABC drug efflux transporters, chromatographic methods

This work deals with flavonoids that could reverse multidrug resistance and increase the effectiveness of chemotherapy by inhibiting efflux pumps. P-gp, MRP1 and BCRP are among the most important efflux pumps that are involved in the development of resistance of tumor cells to cytostatic treatment, therefore this thesis is mainly focused on these efflux transporters. Part of work is focused on fast growing tree Paulownia tomentosa (Paulowniaceae) rich in flavonoids, especially that geranylated. The experiment

desribes the isolation and identification of substances from PT3K fraction that was obtained from chloroform portion of the ethanolic extract of P. tomentosa fruits. The fractions were separated by various chromatographic methods. The identification of the obtained compounds was performed by UV/VIS spectrophotometry, IR, HRMS, NMR spectrometry and circular dichroism, which determined the absolute configuration of the substance. Using preparative HPLC we isolated and identified one pure substance – tomentone B.

Author's CV: Miriama Dupľáková is student in the fifth year at the Faculty of Pharmacy of Masaryk University.

Supervisor: prof. PharmDr. Karel Šmejkal, Ph.D.

Active constituents of Amorpha fruticosa L., Fabaceae

Nikol Jurčová [1], Emil Švajdlenka [1], Milan Malaník [1], Renata Kubínová [1], Dagmar Jankovská [1]

[1] Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Amorpha fruticosa, anticholinesterase activity, extraction, isolation, chromatography

This thesis is focused on the isolation of active constituents from *Amorpha fruticosa L.* by using chromatographic methods and their further identification. *Amorpha fruticosa* L. is a shrub from the Fabaceae family, which is native to North America.

The methanolic extract of flowers was tested for inhibition of acetylcholinesterase and butyrylcholinesterase and demonstrated significant inhibitory activity. Cholinesterase inhibitors are used in the treatment of neurodegenerative disorders such as Alzheimer's disease. The aim of this thesis was to isolate and identify substances that could be potentially responsible for this activity. The ethyl acetate fraction of the methanolic extract of flowers was prepared and was further separated by column chromatography and preparative liquid chromatography.

Separation techniques succeeded in the isolation of dominant substances from the ethyl acetate extract. These substances were identified by using spectral methods (UV, MS, NMR) as derivatives of putrescine and spermidine, namely N^1-(E)-N^6-(Z)-di-*p*-coumaroylputrescine (mongolicine A), N^1,N^6-(E)-di-*p*-coumaroylputrescine, N^1-(E)-N^5,N^{10}-(Z)-tri-*p*-coumaroylspermidine (safflospermidine B), N^1,N^5-(Z)-N^{10}-(E)-tri-*p*--coumaroylspermidine and a mixture of tri-*p*-coumaroylspermidine isomers. Compounds of this type have been described in *Amorpha fruticosa L.* for the first time. For isolated substances, anticholinesterase activity or cytotoxicity will be verified.

Author's CV: Nikol Jurčová is currently studying her last year at the Faculty of Pharmacy, Masaryk University. This research used for Student's Scientific Conference is also her diploma thesis.

Supervisor: PharmDr. Dagmar Jankovská, Ph.D.

Determination of isomaltulose in food supplements by HPLC with evaporative light-scattering detector (ELSD)

Tomas Crha [1], Jiri Pazourek [1]

[1] **Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University**, Czech Republic

Key words: isomaltulose; Palatinose; maltodextrins; dietary supplements; HILIC; ELSD

Isomaltulose (Palatinose) is a disaccharide, an isomer of sucrose, which differs in the position of the bond between the glucose unit and fructose. Human hydrolases cannot break down isomaltulose very well, so it is a long-term source of energy for the human body. It has a relatively low glycaemic index, so it could be used as an auxiliary antidiabetic in the treatment of type 2 diabetes mellitus. This disaccharide occurs exceptionally in nature, it is produced by a biotechnological process using bacterial enzymes. The aim of this paper was to develop and apply a method for the determination and quantification of isomaltulose in addition to sucrose in commercially available samples with isomaltulose content.

HPLC (high performance liquid chromatography) is one of the most popular instrumental methods used in pharmacy. With a suitable combination of polar and non-polar components of the mobile phase, we can quickly separate carbohydrates. However, since carbohydrates do not contain any chromophore in their structure, a conventional UV-detector is unusable, and thus an evaporative light-scattering detector (ELSD) can be advantageously used. HILIC (hydrophilic interaction liquid chromatography) is a HPLC technique, which is nowadays used for polar analytes. In most cases stacionary phase is modified silica with polar groups (hydroxy, amine, amide,...). Mobile phase is usually mixture of water (polar) and acetonitrile (nonpolar).

The method for resolute these two isomers was developed and tested on a Dionex UltiMate 3000 UHPLC kit (ThermoFisher Scientific) with a HALO Penta-HILIC 4.6 x 150 mm column with a stationary phase of particles with a maximum size of 2.7 µm and an ELSD detector Varian 380-LC (Varian). Chromeleon and MS Excel software were used to evaluate the obtained data. After optimizing the conditions of the method, the temperature was determined to be 10 °C, the flow rate of the mobile phase 2 ml per minute and the use of a mixture of acetonitrile and 30 mmol/l ammonium formate buffer as mobile phase. Gradient elution was used. Detection conditions were determined at a nitrogen flow of 1 slm, a temperature of nebulizer and an evaporator of 40 °C.

Under our optimized conditions, the method can determinate isomaltulose in 5.57 min, isomaltulose--sucrose resolution 1.70; repeatability (peak area) 1.1%; repeatability (isomaltulose retention time) 0.2%; LOD 20 mg/ml and LOQ 66 mg/ml.

Acknowledgement: IGA project 317/2019/FaF of Veterinary and Pharmaceutical University Brno, Czech Republic

Author's CV: Tomas Crha tries to improve his knowledge in the field of liquid chromatography, especially liquid chromatography with hydrophilic interaction (HILIC). This area has fascinated me for a long time and I hope that after obtaining a master's degree in Muni Pharm I will continue to study analytical chemistry.

Supervisor: doc. RNDr. Jiří Pazourek, Ph.D

Determination of aminopeptidase N inhibitory activity of newly synthesised nitrogenous compounds

Radka Zackova [1], Veronika Ballayova [1], Peter Zubac [1], Oldrich Farsa [1]

[1] Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: aminopeptidase N, IC50, enzyme assay

Aminopeptidase N (AP-N) is a zinc metalloprotease with broad specifity releasing an N-terminal amino acid from unsubstituted oligopeptides, amides and arylamides. It is widespread in the human body and it has been linked to processes such as tumorigenesis, regulation of blood pressure, antigen presentation and host-virus interaction. The aim of this research was to determine the AP-N inhibitory effect of sub-

stances falling into 3 categories: basic thiosemicarbazone and semicarbazone derivatives of acetophenone, basic diacylhydrazine derivatives and their quaternary salts; by estimating their half maximal inhibitory concentration (IC_{50}). The initial set of data has been obtained by performing UV–vis spectrophotometric enzyme assays at the wavelength of 405 nm using the multi-mode microplate reader Cytation 3 and Gen5 Data Analysis Software. These values have then been used for estimating the relative (the response halfway between the estimates of the lower and upper plateaus) and/or the absolute (the mean of the 0% and 100% assay controls) IC_{50} using the 4-parameter logistic model (4PL) and exponential function. The results have been divided into sets based on the method used to get the estimate and the subsequent aim is to find correlations between lipophilic, electronic and steric parameters and these sets of data in quantitative structure–activity relationship models.

Acknowledgement: The study was supported by the project MUNI/A/1682/2020

Author's CV: Radka Zackova is a fourth-year student of the Pharmacy Master's degree program at Masaryk University. She is currently working on her Diploma Thesis, which is focused on determination of activity of aminopeptidase N potential inhibitors, at the Department of Chemical Drugs.

Supervisor: doc. PharmDr. Oldřich Farsa, Ph.D.

Synthesis and evaluation of natural 2-arylbenzofuran analogues as anti-inflammatory agents

Michal Greguš [1], Pavel Bobál' [1]

[1] Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: mulberrofuran Y, benzofuran, Stille reaction, geranylation, palladium catalyzed cross-coupling

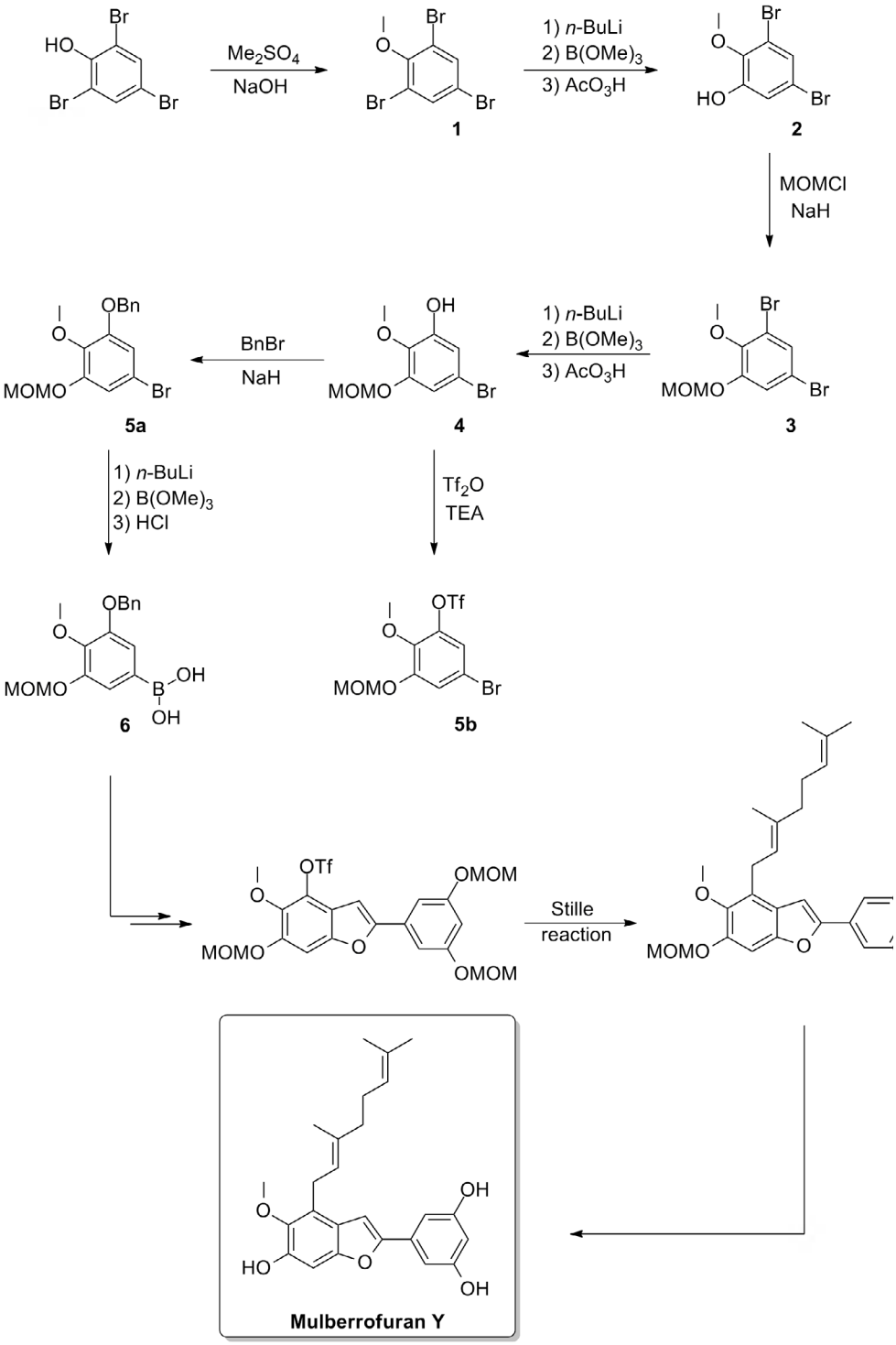
Introduction
Mulberrofurans are a family of biologically active compounds known for over 35 years which have been studied as novel anti-inflammatory agents. From various mulberrofurans, which were isolated from natural sources and synthesized, mulberrofuran Y represents a challenge to be laboratory prepared despite its relatively simple structure. Thus, this work's main aim was a total synthesis and spectral evaluation of the mulberrofuran Y.

Methods
2,4,6-tribromophenol has been chosen as a starting material and was subsequently modified. The purity and structural properties of prepared intermediates were analyzed by TLC, MS, NMR, and IR spectrometry.

Results
The work mainly provides optimized synthetic procedures of compounds 1 – 6 (Fig. 1). Furthermore there was discovered inability to prepare geranylated intermediate in this stage of synthesis either by conventional way employing butyllithium or by Stille cross coupling reaction. The latter method showed to be ineffective despite the effort to differentiate triflate leaving group from bromide in compound 5b (Fig. 1). Geranylation step was therefore put on hold and the main focus was to obtain stemofuran skelet through boronic acid 6 (Fig. 1). Obtaining this intermediate proved to be unexpectedly difficult and time-consuming as well as inefficient as the most successive method provided very poor yields only around 16%.

Conclusion
The synthesis of mulberrofuran Y could not have been finished due to an unexpected obstacle in synthesizing key intermediate 6. Thus, this work brings the basis for the future development of this particular synthetic pathway.

Figure 1: Brief review of the synthesis pathway



Acknowledgement: This work was supported by the project GACR no. 16-07193S.

Author's CV: Michal Greguš is currently finishing the pregraduate study program at the Faculty of Pharmacy, Masaryk University.

Supervisor: doc. Ing. Pavel Bobáľ, CSc.

Synthesis of new 1,3,4-thiadiazol derivates with potential antibacterial and antitubercular activity

Petr John [1]

[1] Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

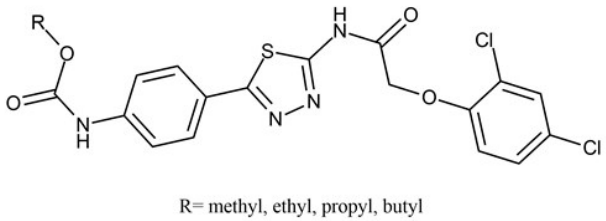
Key words: antibacterial drugs, 1,3,4-thiadiazole, antitubercular drugs, carbamate, multidrug-resistant microorganisms

Jim O'Neill states in his publication that in 2050, the number of deaths caused by multi-resistant microorganisms will be up to 10 million per year [1]. A synthesis of new antimicrobial drugs of a different structure may be a possible solution of this antimicrobial resistance crisis. Examples of these drugs might be variously substituted derivatives of 1,3,4-thiadiazol-2-amine that represent an attractive potential pharmacophore due to their multispectral biological activity incl. antibacterial, antifungal and antitubercular activities [2]. The aim of this study was therefore to prepare new compounds of this structure that could contribute to the fight against resistant strains of various pathogens.

The method of a five-step synthesis of 1,3,4-thidiazol-2-amine derivatives was used in this work, with a 4-aminobenzoic acid as a starting molecule. The initial step of the synthesis was the formation of a carbamate group. Subsequently, the thiosemicarbazide fragment was attached and cyclized in the next step. In the last part, the 2,4-dichlorophenol molecule was added by two methods, a one-step and a two-step method. The progress of the reactions and the purity of the synthesized compounds were monitored by a thin layer chromatography. The structure of all newly prepared substances was verified by NMR and IR spectroscopy.

Sixteen 1,3,4-thidiazol-2-amine derivatives were synthesized. These derivatives have not yet been published in any accessible literature. The final products were prepared only by a two-step method. When using a one-step method, a mixture of unknown products was prepared which could not have been identified.

All 16 newly prepared derivatives were provided to screening of microbial activity (the Department of Molecular Pharmacy FaF MU). The study demonstrated a mild to moderate growth-inhibitory effect of tested compounds on the reference microorganisms (Escherichia coli, Staphylococcus aureus, Candida albicans).



R= methyl, ethyl, propyl, butyl

Newly synthesized 1,3,4-thiadiazol derivates

[1] TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS. (2016, May). Jim O'Neill. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf
[2] Jain, A. K., Sharma, S., Vaidya, A., Ravichandran, V., & Agrawal, R. K. (2013). 1,3,4-Thiadiazole and its Derivatives: A Review on Recent Progress in Biological Activities. Chemical Biology & Drug Design, 81(5), 557–576. https://doi.org/10.1111/cbdd.12125

Author's CV: Petr John is a master's degree student in pharmacy. He is interested in organic chemistry and for this reason he has chosen the Department of Chemical Drugs for his diploma thesis. The results presented by him will be part of his master's thesis.

Supervisor: Mgr. Petr Mokrý, Ph.D.

MASTER SECTION B

Scientific Committee Members

doc. PharmDr. Peter Kollár, Ph.D.

Mgr. Marie Brázdová, Ph.D.

PharmDr. Milan Malaník, Ph.D.

PharmDr. Dominik Rotrekl

PharmDr. Veronika Orendášová

Optimization of the methodology for determining the inhibitory activity of natural substances on bacterial biofilm formation

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[1] Department of Molecular Pharmacy, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Staphylococcus epidermidis, antibacterial activity, antibiofilm activity, MIC, MBIC, cannabinoids

Introduction
With the spread of antimicrobial resistance and with increasing number of chronic biofilm-associated infections, the natural products may become new sources of antibacterial agents. The main aim of this study was to experimentally evaluate the antibacterial and antibiofilm activity of selected cannabinoids as potential natural compounds facing an approaching antibiotics crisis.

Methods
Cannabinoids were isolated from Cannabis sativa subsp. indica, specifically cannabinol (CBN), Δ-9-tetrahydrocannbinol (THC), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabidiol (CBD) and tetrahydrocannabinolic acid (THCA). Antibacterial and antibiofilm activity of these cannabinoids were evaluated on gram-positive bacteria Staphylococcus epidermidis, whose main virulence factor is biofilm formation. The antibacterial effects were evaluated by the microdilution method, in which the minimum inhibitory concentration (MIC) of every compound was determined. According to MIC value, concentration used for inhibitory effect on bacterial biofilm was chosen. Determination of antibiofilm activity was performed using the crystal violet staining assay, in which the minimum biofilm inhibitory concentration (MBIC) of every compound was determined.

Results
All tested cannabinoids showed an antibacterial effect against S. epidermidis, which in most of cases also correlated with an inhibitory effect on bacterial biofilm formation. The most effective antibacterial and antibiofilm compounds were CBN, CBD and CBG with MIC and MBIC 2 µg/ml. Antibacterial and antibiofilm activity did not correlate only with compound CBGA, which even at 1/8 MIC (8 µg/ml) inhibited 50% of biofilm.

Conclusion
Fytocannabinoids, specifically CBN, CBD and CBG showed potential antimicrobial and antibiofilm activity. We have identified CBD and CBG as the most suitable candidates for future experiments, mostly because of they are not psychoactive.

Acknowledgement: The study was supported by the project Internal Grant Agency VFU Brno 310/2019/FaF.

Author's CV: Curious pharmacy student interested in innovations in medicine, currently finishing master study program Pharmacy at Masaryk University. The topic of my diploma thesis was to evaluate antimicrobial and antibiofilm activity of different cannabinoids.

Supervisor: PharmDr. Jakub Treml, Ph.D.

Optimalization of Methodology and Testing of Antibacterial and Synergistic Potential of Kuwanon E, Isolated from Morus alba Root Bark

Veronika Porostlá [1], Zdenka Florková [1], Gabriela Škovranová [1], Alice Sychrová [1]

[1] Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Antimicrobial Susceptibility Testing; Checkerboard Method; Kuwanon E; Microdilution Broth Method; *Morus alba*; MRSA; Optimalization of Methodology

Introduction

There are approved guidelines for microdilution broth method for standard antibiotics (SA). Unfortunately, in case of natural compounds, these standards as EUCAST and CLSI protocols are slightly modified according to individual scientist. These modifications can be responsible for incorrect interpretation of results obtained and impossibility to compare them with other studies. Thus, the aim of the diploma thesis was to optimize the procedure of antimicrobial susceptibility testing (AST) of natural compounds. The next goal was to define the synergistic potential of natural substance Kuwanon E with conventional antibiotics.

Methods

At the first step, comparison of EUCAST and CLSI protocols for microdilution broth method for SA was performed and adapted to gram-positive and gram-negative microorganisms used in our laboratory. The resulting minimum inhibitory concentrations were confronted with the breakpoints determined by EUCAST and CLSI. Then, the influence of various conditions as inoculum size, incubation time, type of solvent and method of MIC detection was tested. Synergistic potential of Kuwanon E was determined using checkerboard titration technique. Antibiotics with detected resistance in *Staphylococcus aureus* strains were used for combinations.

Results

Bacterial growth and the evaluation of the results were significantly affected by the inoculum size. A standard concentration in the well of $5 * 10^5$ CFU/ml was introduced experimentally. Duration of incubation was less important for MIC reading and it was standardized to 18–20 hours. It is well known, that the solvent dimethyl sulfoxide (DMSO) inhibits the growth of *Staphylococcus aureus* strains at a concentration of 16 % [1]. In our experiment, a concentration of less than 8 % DMSO was introduced in order not to alter the antibacterial effect of the natural substance. Kuwanon E alone inhibited microorganisms at a concentration of 4–8 µg/ml. The synergistic potential of Kuwanon E was tested in total 12 combinations with oxacillin, tetracycline, and erythromycin. The additive effect was established in 6 combinations.

Conclusions

The diploma thesis presented the need to optimize the microdilution broth method for natural, mostly lipophilic, substances. We optimized variable conditions such as the size of inoculum in the well, the duration of incubation, or the effect of DMSO solvent on bacterial growth. MIC endpoint determinations with the best reproducibility gave visual or spectrophotometric methods. In the future, it will be necessary to continue with this standardization, but results obtained for SA combating gram positive or negative bacteria will be used as the template for optimized testing of natural compounds. The experiment revealed the additive effect of Kuwanon E with oxacillin and tetracycline. It is proposed to test the synergistic potential of this natural substance with other conventional antibiotics.

[1] CAMP, Jason E., Simbarashe B NYAMINI a Fraser J. SCOTT. Cyrene™ is a green alternative to DMSO as a solvent for antibacterial drug discovery against ESKAPE pathogens. RSC Medicinal Chemistry [online]. 2020, 11(1), 111-117 [cit. 2021-03-25]. ISSN 2632-8682. Doi: 10.1039/c9md00341j

Author's CV: Veronika Porostlá is engaged in research of bacteria and antibacterial natural compounds. She discovered her passion for microorganisms during her one-month internship in summer 2018 in a microbiological laboratory at the Institut Teknologi Bandung in Indonesia.

Supervisor: PharmDr. Alice Sychrová, Ph.D.

Synergistic interactions between selected essential oils and antifungal drugs against Candida for local application

Veronika Hulková [1]

[1] Department of Molecular Pharmacy, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Candida, resistance, synergy, essential oil, clotrimazole, yeast infections, volatile substances

The declining susceptibility of yeasts to antifungals is becoming an alarming problem. The solution requires synthesis and isolation of new antifungals, as well as new treatment methods. Essential oils are promising substances with a lower risk of developing resistance, diverse mechanisms of action, and additional biological activities. This thesis is focused on the antifungal potential of Cymbopogon flexuosus, Mentha arvensis and Syzygium aromaticum essential oils used in combination with imidazole antifungal agent clotrimazole, intending to prove their effectiveness in the local treatment of Candida albicans, Candida krusei and Candida parapsilosis infections.

After the initial screening of antifungal activity, which determined the substances used further in the study and their MICs, the pharmacodynamic interactions of essential oils and clotrimazole were examined in a microtiter plate. Every well contained a different combination of concentrations. After the cultivation, the absorbance was measured, the FIC index was calculated, and the absence of living Candida cells was verified with the TTC test. The synergy was confirmed by the disk diffusion method. The thesis contains a comparison of the effectiveness of liquid and gaseous phases of essential oils.

In a microtiter plate, S. aromaticum essential oil showed mostly weak synergy with clotrimazole against all three yeast species. The combination of M. arvensis essential oil and clotrimazole showed negative interactions against non-albicans species. C. flexuosus had synergy with clotrimazole against C. krusei. Negative interactions were not confirmed by the disc diffusion method. However, most synergies were not confirmed either. The difference can be due to the variability of yeast growth, the difference in the starting density of the yeast culture, difficult diffusion of antifungal and essential oil through agar in the case of the disk diffusion method, and finally, different evaluation of the synergy by the microdilution and disk diffusion method. The synergy of clotrimazole and S. aromaticum essential oil against C. albicans was proved. Small concentrations of clotrimazole potentiated the effect of essential oil. The antifungal effect of the gas phase of essential oils was confirmed. Essential oils as volatiles act even without direct contact of the liquid with the tissue and microorganism. The gaseous phase of C. flexuosus had the same or higher efficiency compared to the liquid phase. It may contain a different ratio of individual components of the essential oil. However, it cannot be said, that the effect of all essential oils is greater in the gaseous phase as the antifungal effect of eugenol, the main ingredient of S. aromaticum essential oil was lower.

Despite the unconvincing results of the synergy, we have shown that the use of essential oils in the treatment of minor local candidiasis could be beneficial. Essential oils did not reduce the effectiveness of antifungal therapy and possess proven antifungal and additional biological activities. Especially C. flexuosus and S. aromaticum essential oils have the potential for separate use, but also for the use together with clotrimazole.

Author's CV: Veronika Hulková is in her fifth year of studying at the Faculty of Pharmacy at Masaryk University, currently working on her master's thesis concerning resistance to antifungals. She has been interested in microbiology since her internship at the Department of Clinical Microbiology and Erasmus+ program in Helsinki.

Supervisor: Ing. Marcela Nejezchlebová

Synergistic effect of selected essential oils and antibiotics for external use against Staphylococcus aureus

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Key words: Staphylococcus aureus, synergistictic effect, essential oils, antibiotic resistance

Staphylococcus aureus is a frequent cause of skin diseases with a complicated treatment due to frequent resistance to antibiotics. In connection with increasing bacterial resistance, it is necessary to look for new treatment alternatives. One option is to combine an already used antibiotic with a natural substance that shows antimicrobial potential. The aim of this study was to demonstrate the synergistic effect between antibiotic clindamycin and essential oils of Syzygium aromaticum and Cymbopogon flexuosus against different strains of Staphylococcus aureus. Microdilution and disk diffusion methods were used to determine synergy. The Microdilution method allows direct contact of substances at different concentrations with the microorganism in one well of a microtiter plate. The disk diffusion method enables us to determine the synergy using two disks impregnated with the tested substances on an inoculate culture plate. Syzigium aromaticum essential oil showed a synergistic effect with antibiotic in resistant and also sensitive strains of Staphylococcus aureus, while Cymbopogon flexuosus essential oil in combination with the antibiotic was effective only in sensitive strains of Staphylococcus aureus. Topical application of a combination of clindamycin and essential oil in cutaneous staphylococcal infections could potentially reduce the dose of antibiotics applied due to a synergistic effect between the substances.

Author's CV: Hana Janečková tried to determine the synergistic effect between essential oils and the antibiotic in order to find a new solution to antibiotic resistance. This is her first scientific publication as a diploma thesis at Masaryk University at Faculty of Pharmacy.

Supervisor: Ing. Marcela Nejezchlebová

Mechanism of antitumor action of Pt(IV) complexes with double and triple effect containing estramustine

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Key words: antiproliferative activity, platinum complexes, estramustine, complexes with double and triple effect

Successful chemotherapeutic treatment is usually combination of several drugs acting by different mechanisms against cellular targets in the cancer cell. We can combine more drugs in one complex and formulate multi-target drugs.

The aim of this study was to experimentally verify the effectiveness of new synthetized Pt(IV) complexes containing in their molecule axially bound estramustine and another self-acting ligand.

The experiment was performed in vitro on multiple cell lines. We evaluated the antiproliferative activity of the Pt(IV) complexes, cisplatin and estramustine itself. Furthermore, we investigated the lipophilicity, cellular accumulation, and DNA binding of the new compounds. We examined whether the Pt(IV) complexes containing estramustine act as androgen antagonists and the effect of dichloracetate in complexes.

Pt(IV) complexes with estramustine showed a higher antiproliferative activity than estramustine and cisplatin alone. Prodrugs containing another self-acting ligand showed even more promising results. The most active drugs were complexes carrying HDAC inhibitors - valproate and phenylbutyrate as ligands. Compared to cisplatin they were 145-fold and 92-fold more efficient. Cellular uptake and DNA platination is the initial step in the mechanism of antitumor activity of platinum complexes. Platinum from the examined complexes was taken up in the cell and bind to DNA more efficiently than platinum from cisplatin (cellular accumulation 37-fold to 64-fold more). A clear correlation between the complex lipophilicity, cellular accumulation, and DNA platination was found. The Pt-estramustine complexes downregulated the androgen receptor and prostate-specific antigen expression, which affect the development of prostate cancer. Complexes containing estramustine can act as androgen antagonists as well as estramustine itself. Dichloracetate induced a significant decrease in the glucose uptake more than molecule without it.

Our experiment showed that the new synthesized Pt(IV) complexes show higher activity against tumor cells than cisplatin or estramustine alone. To conclude the investigated Pt-estramustine compounds represent promising multiaction chemotherapeutics for treatment of prostate cancer because of their multimodal mechanism of activity.

Acknowledgement: The study was supported by the Czech Science Foundation (grant 18-09502S) and the Israel Science Foundation (grant 1002/18) and the Alex Grass Center for Drug Design and Synthesis. The study was supported in part by the Israel Cancer Association (ICA) with the help of donation from Bruce Youngman.

Author‘s CV: Tereza Ctvrtlikova‘s highest obtained acquirements is a high school with a graduation (Slovanské gymnázium Olomouc 2008 - 2016). She is currently studying at the Faculty of Pharmacy MUNI (expected graduation in 2021). Cooperation with the Institute of Biophysics of the ASCR has been ongoing since 2019.

Supervisor: PharmDr. Mgr. Alžběta Kružicová

Comparison of Thrombolysis Induced by Tenecteplase and Alteplase in a Rat Model of Systemic Arterial Embolism

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Key words: arterial embolism, thrombolysis, alteplase, tenekteplase, in vivo model, stroke

Background
The aim of the experimental work was to compare the efficacy of clinically available thrombolytics alteplase (rtPA) and tenecteplase (TNK-tPA) in an in vivo model of rat systemic arterial embolism.

Methods
Male Wistar outbreed rats (120-180 g) were used for the work. A total of 32 rats were included in the experiment. All animals received 3 $BaSO_4$ labelled fibrin-based artificial clots 10 mm long and 0.76 mm in diameter into the aorta via the left common carotid artery. The rats were randomly divided into three groups. In group A, rtPA (n = 12) was administered intravenously at a dose of 0.9 mg/kg BW/hr with an

initial bolus of 10 % of the calculated dose. The intravenous infusion was given for 60 minutes. In group T, TNK-tPA (n = 12) was intravenously administered as a bolus dose of 0.25 mg/kg BW. The control group (n = 8) received saline in an equivalent regimen as in group A. Thrombolysis was detected directly by X-ray microfluoroscopy. A radiograph was taken every 5 minutes for 60 minutes. The relative change in the area of the artificial clot shadow was evaluated and the rate of fibrinolysis was calculated.

Results
Out of a total of 96 artificial clots (ACs), 69 ACs were visualized and analysed in the abdominal cavity of the animals. When comparing the percentage loss of the shadow area of ACs, slightly better efficiency of TNK-tPA compared to rtPA was observed after only 5 minutes. However, the difference was not statistically significant. These results were calculated as the average of the values within the whole groups from all arteries. Analysis of the loss of the shadow area by blood vessels separately showed comparable efficacy of both thrombolytics in the renal artery and the ileocaecocolic artery. In the mesenteric artery, artificial clots were better dissolved by TNK-tPA, but again statistically insignificant. When evaluating the rate of fibrinolysis (%/min) between alteplase and tenecteplase, the differences in the results were statistically insignificant at 15 minutes (1,05 %/min vs. 1,33 %/min, p = 0.19) and 60 minutes (0,94 %/min vs. 1,17 %/min, p = 0.07). There was a statistically significant difference at 30 minutes (0,97 %/min vs. 1,56 %/min, p = 0.03), when TNK-tPA dissolved artificial clots faster than rtPA.

Conclusions
At the end of this study, there is not much difference in the thrombolytic activity of rtPA and TNK-tPA. However, when evaluating the rate of fibrinolysis at 30 minutes, TNK-tPA was 50 % more effective than rtPA. This fact may be clinically important and the effect of tenecteplase needs to be further studied, especially with regard to the treatment of stroke.

Acknowledgment This work has been supported by the European Regional Development Fund - Project INBIO (No. CZ.02.1.01/0.0/0.0/16_026/0008451).

Author's CV: Currently, Michaela Heglasová studies in her fifth year at the Faculty of Pharmaxcy, Masaryk University. She has been part of the INBIO project research team, where she studied the pharmaceutical activity at *in vivo* model. During her studies, she gained valuable experience within the Erasmus+ Programme, both for studies and practical training.

Supervisor: MVDr. Peter Scheer, Ph.D.

MASTER SECTION C

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Price and non-price competition tools applied in pharmacy system

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Key words: The marketing mix, pharmacy specifics, STP concept, questionnaire survey, pharmacies, competition tools

Background and Aims
Although pharmacy belongs to medical institutions, they must use appropriate price and non-price competition tools and analyze their possibilities using a marketing mix. It is including analysis of the product, price, promotion, and place. This work aimed to compare tools of price and non-price competition used in a pharmacy.

Methods
On the website, Click4Survey has created a survey. A questionnaire was distributed to all pharmacy types in the Czech Republic, including basic type pharmacy, pharmacy with the professional workplace, and separate drug dispensing department of a pharmacy. There were 32 questions related to characterization and location, pharmacy assortment, promotion, presence of online pharmacy, and drug price. Price and non-price competition tools were evaluated according to pharmacy type, number of other pharmacies nearby, or number of doctor's offices nearby.

Results
The final evaluation included 402 questionnaires. The most used competition tools are reservation E-recipe (95,4%), individual drug preparation (IDP) (87,0%), flyer with action account (85,9%), client card (85,8%) and payment by voucher (84,5%). On the other hand, least used competition tools are advice guaranteed by the Czech Chamber of Pharmacists (36,9%), consultation place (19.9%), less than 20 % providing pharmaceutical service (the exception is the measurement of blood pressure which provides 62,5% of pharmacies), delivering over-the-counter drugs (OTC) to the home (15,9%) and propagation of pharmacy on the internet (57,2 %). In a chain-pharmacies propagation of pharmacy on the internet, flyer with action account, client card, and online pharmacy prevail. Non-chain pharmacies prefer to do more IDP; they offer advice guaranteed by the Czech Chamber of Pharmacists, they have consultation places, and sponsor events around the pharmacy. If a pharmacy has low competition around, they can do more IDP, distribute OTC to the home, and sponsor events. They could extend advice guaranteed by the Czech Chamber of Pharmacists, a self-service choice of drugs in a pharmacy, and propagation of pharmacy on the internet. On the contrary, pharmacies with high competition around offer a self-service choice of drugs in a pharmacy, online pharmacy, and a discrete zone. They could extend delivering OTC to the home and sponsoring events. Pharmacies with a small number of doctor's offices nearby provide a self-service choice of drugs in a pharmacy, publish their own magazine, distribute OTC to the home, and sponsor events. Due to the small number of doctor's offices nearby, IPL is not so popular; they could increase the offer of pharmaceutical service and consultation place for the patient.

Conclusions
The results show that price competition tools are used more in chain-pharmacies and in pharmacies with high competition around. Tools of non-price competition which have educational character are more applied in non-chain pharmacies. Tools included various pharmaceutical services for patients are used in non-chain pharmacies and pharmacies with low competition around. All types of pharmacies apply some competition tools out of the pharmacy; they use either distribution OTC to the home, event sponsoring, or online pharmacy.

Author's CV: Kristýna Šudomová is in the 5. year of the Pharmacy study. She writes diploma thesis at department of Applied Pharmacy.

Supervisor: PharmDr. Bc. Dana Mazánková, Ph.D.

Quality of life assessment in patients with tinnitus

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Key words: Tinnitus, quality of life, questionnaire survey, Mini-tinnitus questionnaire (MTQ), Five Well--Being Index (WHO-5), Brief-Illness Perception Questionnaire (B-IPQ)

Background and aims
Tinnitus, auditory perception with no source in the external environment, is a disease whose prevalence is constantly increasing. The aim of this thesis was to investigate the effect of tinnitus and its severity on quality of life and investigate the areas of life that are affected by the disease.

Methods
A complex questionnaire was developed, including a Mini-tinnitus questionnaire (MTQ) examining the severity of tinnitus, a Five Well-Being Index (WHO-5) concerning the patient's mental state, and a Brief--Illness Perception Questionnaire (B-IPQ) reflecting the self-regulation model of the behavior of patient with tinnitus. Six hypotheses were established, where it was possible, a verity was decided based on the statistical $\chi 2$ test of independence (α=0.05). The questionnaire was created in electronic form using Google Forms and published on Facebook in groups associating people suffering from tinnitus.

Results
A total of 190 questionnaires was accepted for evaluation. Scores from MTQ and WHO-5 were calculated for each of the respondents. According to the MTQ results, an even distribution of tinnitus severity can be observed among respondents - 24% low tinnitus severity, 28% moderate tinnitus severity, 22% severe tinnitus, and 26% very severe tinnitus. The premise was that respondents suffering from more severe tinnitus (higher MTQ score) have lower well-being and therefore, lower quality of life (lower WHO-5 score). However, the interdependence of these scores has not been proven. The incidence of depression in respondents was 15 %, the incidence of anxiety was 30 %, and the incidence of sleep disorders was 37 %. The hypothesis that people with tinnitus suffer from these disorders more often has not been proven; however, the incidence of these diseases in respondents is at the upper limit of the generally reported prevalence. The quality of sleep turned out to be the most affected area of life for the respondents. 44% of respondents reported that they woke up unrested in more than half of the days in the two previous weeks, 71% of respondents claimed the need for a longer time to fall asleep caused by tinnitus. Further, 82% of respondents stated that it was at least partially valid for them that their tinnitus are too strong to be ignored, and 89 % of respondents claimed that it was at least partially true for them that they perceive tinnitus from the time they wake up to the time they go to sleep. In answer to the question concerning the disease's expected duration, 74% of respondents stated the assumption that they would suffer from tinnitus until death. The fact that the average score from WHO-5 was 58%, and three-quarters of respondents consider more than half of their lives to be filled with things of their interests shows that many respondents live a happy life.

Conclusions
The questionnaire results showed that tinnitus has a negative effect on the quality of life, mainly in questions of sleep and rest. However, clear dependence between tinnitus severity and mental well-being was not confirmed.

Author's CV: Jana Janyová, a fifth-year student at the Faculty of Pharmacy of Masaryk University.

Supervisor: PharmDr. Bc. Dana Mazánková, Ph.D.

Waiver of value added tax on medicinal products imported from the third countries

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[1] Faculty of Law, Masaryk University, Czech Republic

Key words: VAT, law, import, DPH, zákon, dovoz, VAT waiver, directive

In the context of import of medicinal product ZOLGENSMA from the United States of America, the discussion about obligation to pay several millions of Czech korunas as a value added tax (VAT) on import was opened. This case was closely followed by the media, when in 2020 multiple Czech families were fundraising money for purchase of medicinal product ZOLGENSMA and facing possible VAT assessment. This medicinal product was not registered nor sold in the EU at that time. In the end ZOLGENSMA was paid form the public health insurance.

Through analysis of mentioned cases and legislation the author will evaluate the impossibility of individual VAT waiver on importation of medicinal products from the third countries. Author will present the results of the analysis in the context of VAT waiver at the time of COVID-19 pandemic. The possible solutions of this situation will be shown, including changes of the national and European legislation de lege ferenda.

Author's CV: Damir Solak is the fifth year student of the Master study programme at the Faculty of Law, Masaryk University. Currently, he is writing his diploma thesis at the Department of Financial Law and Economics under the supervision of doc. JUDr. Petr Mrkývka, Ph.D. The thesis is focused on the income tax in Croatia.

Preparation and evaluation of mannitol microparticles for lung application

Martin Veselý [1], *Jakub Karas* [1], *Hana Hořavová* [1], *Jan Gajdziok* [1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: spray drying, microparticles, mannitol

Dry powder formulations are used for lung application to provide a local or systemic effect. The effect of an incorporated active substance depends on the degree and location of deposition in airways and the time of persistence. The current trend is the preparation of large porous particles with low density. Their deposition degree is similar to common microparticles, and their benefits lie in better aerosolization, processability, and evasion of lung cleansing mechanisms. Spray drying is one of the promising ways of such particles preparation.

The study aimed to find convenient formulation and process parameters that would ensure optimal aerodynamic parameters of particles designed for lung application.

Correlations between process parameters and properties of particles were evaluated. Followed process variables were: drying temperature, drying airflow, and pump speed. According to the Box-Behnken plan, each parameter was assigned three combined values (13 samples). 10 % (w/w) D-mannitol solution was used. Afterward, experiments with different mannitol solution concentrations and under middle process parameters (t = 120°C; pump speed v = 20 rpm; airflow s = 35 units – device settings) were conducted to evaluate formulation parameters. Spray drying was carried out on the LabPlant PD-06 device. The disper-

sion was atomized through a two-liquid nozzle with 2 mm diameter using pressure of 3 bar. Bulk and tapped densities were measured to evaluate produced particles. Hausner ratio was calculated afterward. The geometric diameter was measured by laser diffraction and aerodynamic diameter on APS (aerodynamic particle sizer) device. The morphologic appearance was assessed by SEM.

Geometric size, aerodynamic diameter, and particles´ morphology were affected by different process parameters. The value of aerodynamic diameter was increased along with the temperature in all samples. Geometric diameter and Hausner ratio increased as well in the majority of cases. The same trend of the Hausner ratio and geometric diameter increase was observed by increasing the pump speed. Finally, higher aerodynamic diameter and lower tap density of samples were linked to an increase in airflow.

Lower drying temperature and airflow lead to the production of particles with lower aerodynamic diameters. Optimal product morphology was achieved when middle and low drying temperature was used. Particles with the lowest geometric diameter were produced with all variables set to low or medium values. Optimal morphology, lowest aerodynamic diameter, and medium geometric diameter values particles were obtained using mid-temperature, pump speed, and airflow values (t = 120 °C; pump speed v = 20 rpm; airflow s = 35 units). Most convenient aerodynamic and geometric diameter values (AD = 6.20 µm; S = 4.95 µm) were achieved using 10 % D-mannitol solution with medium process parameters.

Acknowledgement: The study was supported by the project MUNI/A/1213/2020.

Author's CV: Martin Veselý is a student of 5th year of pharmacy faculty, Masaryks university in Brno. Currently he is working on his master thesis which is dealing with preparation and evaluation of microparticles produced by spray drying.

Supervisor: doc. PharmDr. Jan Gajdziok, Ph.D.

Innovative highly porous matrix pellets for the detection of chemical warfare agents

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Key words: ammonium bicarbonate, camphor, carriers, detection tubes, extrusion, menthol, Neusilin, pellets, phosgene, spheronisation

Introduction

Chemical warfare agents are still a threat in some parts of the world. Detection tubes (DT) are used by armies to quickly assess their presence. The current development of second-generation DT requires, in addition to new detection reagents, a new type of carrier with a high specific surface area (SSA), which would allow better adsorption of toxic substances and detection reagents on the carrier surface. The aim of this study was to prepare porous carriers with high SSA.

Methods

A mesoporous magnesium aluminometasilicate (Neusilin® US2) with high SSA was utilized along with increasing concentration (7.5–20.0 %) of volatile substances (VS) such as menthol, camphor and ammonium bicarbonate. Spheronization–extrusion was used to prepare pellets. To further increase carrier SSA, sublimation of VS from carriers at elevated temperature was performed. Prepared samples were tested in

terms of physicochemical parameters. SSA was measured by Brunauer–Emmett–Teller method and morphology of carrier surface was assessed by electron microscopy. In cooperation with company Oritest s.r.o., samples were impregnated with detection reagent o-phenylenediamine-pyronine for the detection of phosgene/diphosgene.

Results

Samples containing 20 % of menthol or camphor in powder mixtures were most porous (Figure 1) and had highest SSA (Figure 2), which was double that of the blank. Physicochemical parameters of all batches met the requirements for their application in DT. After impregnation, samples prepared using menthol or camphor were found to provide a red fluorescence under the UV light in addition to the eye-visible red-violet color (Figure 3).

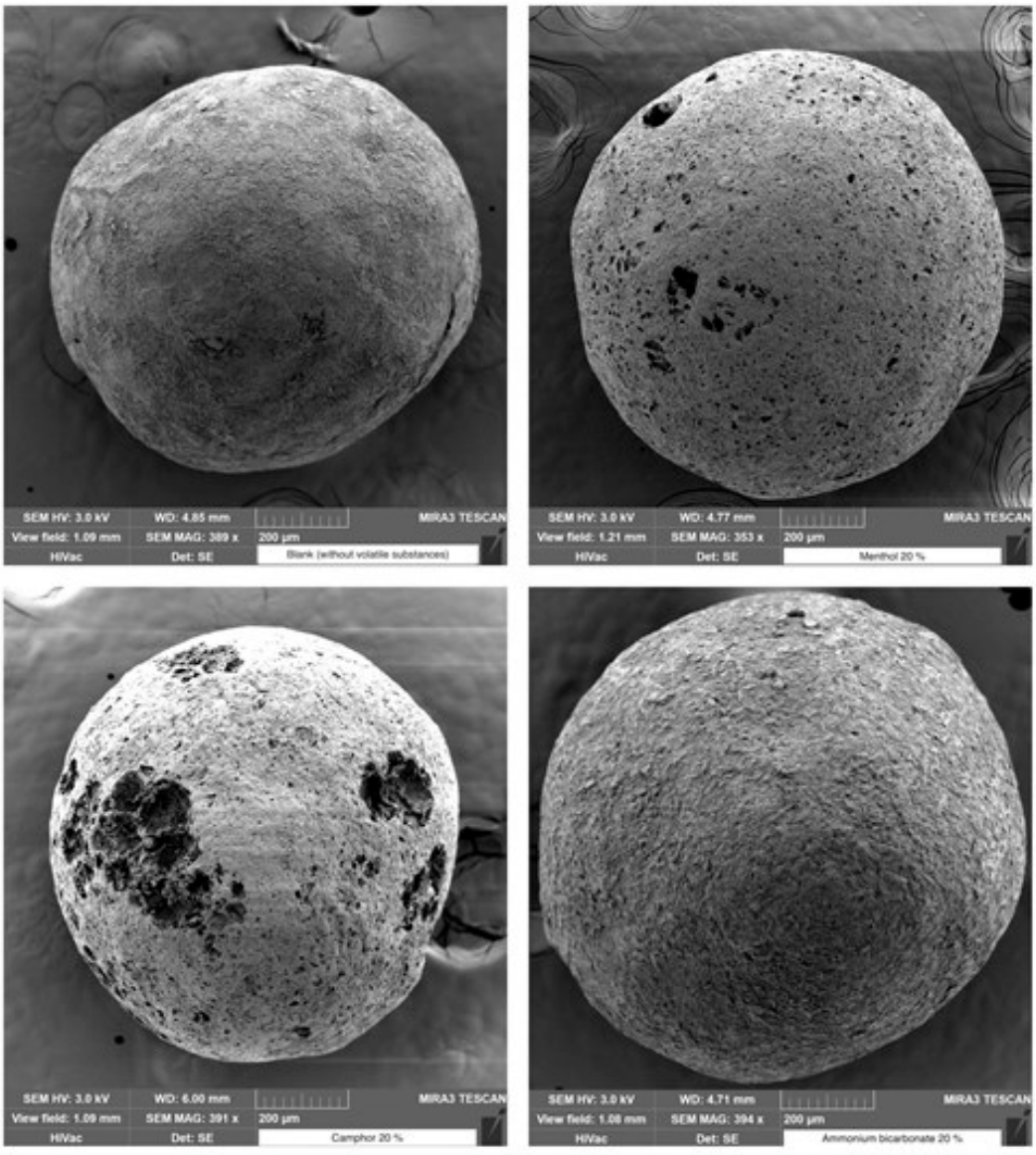

Figure 1.

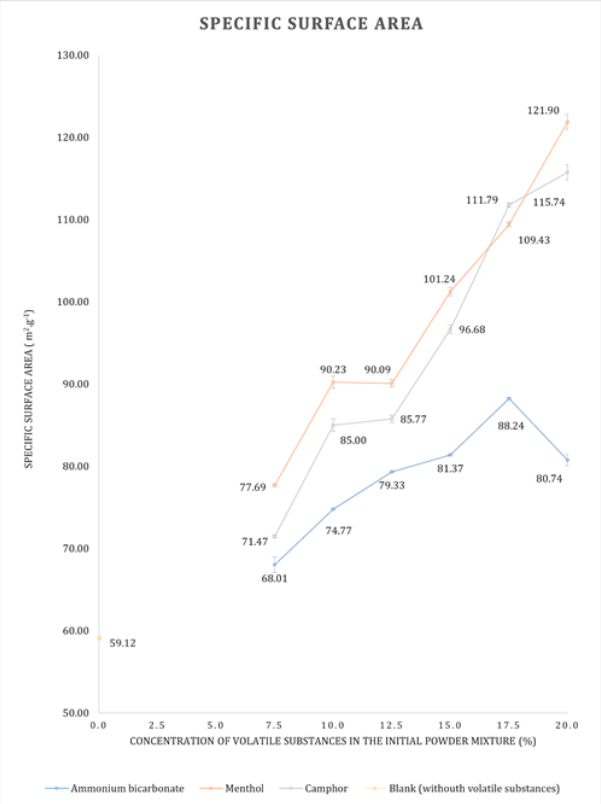

Figure 2.

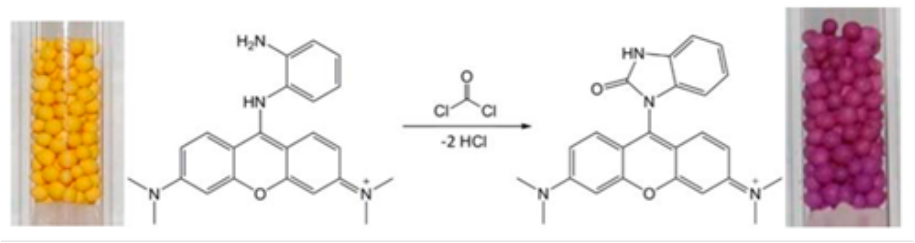

Figure 3.

Conclusions
Samples prepared using menthol or camphor provided highest SSA and suitable physicochemical properties. Unlike blank or ammonium bicarbonate samples, they give red fluorescence under UV light which further improve their sensitivity to phosgene. In addition, these carriers can also be used for other applications, such as the preparation of rapid release dosage forms.

Acknowledgement: The study was supported by the Security Research Program of the Ministry of the Interior of the Czech Republic, no. VI20192022172.

Author'sCV: Adam Staňo is pregradual student of Faculty of Pharmacy MU. His research is focused on preparation and evaluation of porous carriers used for detection of chemical warfare agents. He is currently working in pharmacovigilance company in Global PV Department and SÚKL (national drug authority) in the Department of Reimbursement and Pricing.

Supervisor: PharmDr. Jiří Zeman, Ph.D.

Preparation and evaluation of matrix dosage forms with sustained drug release for dynamic biorelevant dissolution study.

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Key words: Sustained drug release, dynamic biorelevant dissolution, Golem v2 apparatus, USP apparatus, similarity factor f2

The dissolution method determines the amount of active ingredient released from a solid oral dosage form in the specific medium at the specific time. This method is crucial for development of new dosage forms, quality control and bioequivalence studies. Standard dissolution testing cannot provide absolute simulation of GIT. For this reason, many new apparatuses, which enable to measure bioequivalent dynamic dissolution and to simulate behavior of solid drug forms, are being developed in these days. One of these apparatuses is Golem v2. It works as multi-compartment system allowing passage of dissolution medium. Usually this kind of apparatus is used for testing of immediate release dosage forms, although controlled release dosage forms are becoming more and more popular. The aim of this diploma thesis was a pilot study of dissolution profiles for different types of matrix tablets with prolonged drug release in a single-compartment setup, and to evaluate suitability of Golem v2 for this type of measurement. Hydrophilic hypromellose matrix tablets, lipophilic matrix and dual hydrophilic-lipophilic matrix tablets were prepared. Caffeine as a model API and pH 6.8 phosphate buffer as a dissolution medium were used. For each sample three tablets were evaluated at given time points by HPLC apparatus to obtain dissolution profiles. Each batch underwent also a standard USP dissolution testing. Obtained results were compared using similarity factor f2, that measures the similarity between two profiles. This can be used to judge the influence of formulation or process parameters. Within a given batch, the results showed a discrepancy between the dissolution profiles obtained using the standard USP method and the profiles measured in a Golem v2 instrument, indicating the potential of developing dynamic dissolution for controlled release formulations. In lipophilic tablets of two different batches prepared with different compression strengths, the similarity of the profiles was demonstrated. The obtained data will be further used to optimize the Golem v2 device.

Author's CV: Gabriela Koutná is a student of 6th year at Pharmaceutical Faculty. Currently preparing herself for final state exams. During her studies she tried to gain experience for her further education abroad e.g. an internship in Baxter, Germany, one year of Erasmus studies at University Complutense Madrid, an internship in pharmacy, Spain.

Supervisor: PharmDr. Jakub Vysloužil Ph.D.

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Collagen-carboxymethylcellulose blend films as a novel wound dressing

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Key words: collagen, carboxymethylcellulose, film wound dressing, technology

Background
Collagen has a very good film forming property and films from this natural polymer are extensively used in tissue engineering and food industry. However, its application as in the way of a potential wound dressing is limited because of its weak mechanical properties. For that reason, modifications or combinations with different materials are investigated. Carboxymethylcellulose (CMC) offers suitable properties and also a good film forming capacity, so it may be a promising material for combination with collagen. The aim of this study was to prepare and evaluate a novel composite collagen-CMC film wound dressing.

Methods
The collagen-CMC films with three collagen types (porcine, bovine, equine), two plasticizers (glycerine, macrogol 300) and two collagen:plasticizer ratios (1:2, 1:3) were prepared using the solvent casting method. Organoleptic, microscopic and physicochemical properties (degree of swelling, mechanical properties, surface pH) of all the samples were evaluated especially in terms of the practical application on the wound.

Results
All the samples confirmed its potential application as a film wound dressing due to its suitable organoleptic properties. All the films exhibited a mild degree of swelling, equine films were less absorbent than porcine and bovine ones. Microscopic observation of the prepared films confirmed a presence of microfibrillar structure of the CMC, which led to satisfactory mechanical properties of all the films even after wetting. The surface pH of all the samples was acidic, which is favourable for the process of wound healing.

Conclusion
The films were successfully prepared by the solvent casting method. Based on the statistical testing, it can be concluded that film properties were primarily influenced by the collagen type and to a lesser extent by collagen-plasticizer ratio.

Acknowledgement: This work was supported by finances of the Technology Agency of the Czech Republic (Research Project no. TH04020540).

Author's CV: Kateřina Tenorová is a PhD student at the Department of Pharmaceutical Technology, Faculty of Pharmacy, Masaryk University. The topic of her dissertation is Collagen in combination with materials of natural origin in the technology of medical devices and cosmetic preparations.

Supervisor: doc. PharmDr. Ruta Masteiková, CSc.

Ink-jet not just for printing docs

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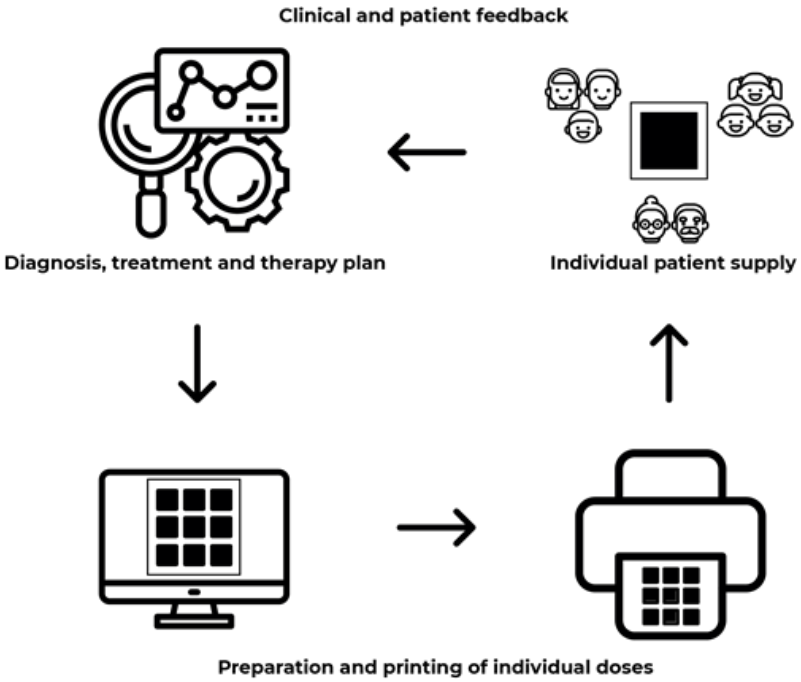
Key words: inkjet, pharmaceutical printing, personalized medicine, orodispersible forms

The research aimed to verify the utilization of Canon iP7250 inkjet printer for the preparation of orodispersible dosage forms in individualized therapy of patients. Printing data was prepared in the Adobe Illustrator® program in colors of the CMYK color model. The reference substrate for the evaluation of ink deposition was edible starch paper. Quantitative determination of ink deposition was performed by UV--VIS spectrophotometry. The highest concentration of printed ink was in the range of 0.42 µl/cm^2 (cyan) to 1.22 µl/cm^2 (magenta). At the required concentration of 10 to 300 mg of API in the ink, 16.8 µg to 1.5 mg of the drug could be printed on a 4 cm^2 area. The increase of ink content in the sample was performed by repeated printing cycle. Due to the limited absorption capacity of the substrate, the application of the third layer led to a decal of ink on the printer feed mechanism.

Edible water-based ink was subsequently chosen to verify the accuracy of dosing and the highest possible deposition of ink on the substrate. Due to the limited solubility of some APIs in water, PEG and ethanol-based inks were prepared. The PEG ink was not sufficiently absorbed into the substrate structure. Smearing and decal have occurred in contact of the film with another material. The ethanol-containing ink dried up inside nozzles, resulting in a reduction of the amount of ink in printed patterns. The Canon iP7250 printer needs construction modifications for the use in the individualized preparation of drugs. Printhead technology does not allow the utilization of ink with a lower boiling point than water due to drying up in the nozzles. Due to the high dosing accuracy and printing speed, there is a potential to use inkjet printers for the preparation of personalized medicine and tailored dosage forms.



Author's CV: Dagmar Blaháčková is currently working on the optimization of ink-jet printing technology for the preparation of orodispersible dosage forms. She has started with optimization of ink-jet technology for preparing of pharmaceuticals at the University of Veterinary and Pharmaceutical Sciences Brno, where she also obtained her master degree.

Supervisor: doc. PharmDr. Jan Gajdziok, Ph.D.

3D printing of multilayered orodispersible films: A comparison of drying methods

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Key words: 3D printing, orodispersible films, various drying time

Background
Orodispersible films manufactured by 3D printing are an innovative dosage form whose main advantage is their application comfort and the possibility of personalization. An innovative approach was developed where a thin layer of the film-forming dispersion was printed and dried before applying the next layer.

Aims
The work aimed to formulate orodispersible films using modified semisolid extrusion 3D printing method and to compare the influence of different drying times on mechanical and physical properties of the films.

Methods
The study compared the impact of drying time in two experiments. In both were formed five batches of films with different thicknesses (45-205 µm). In the first experiment, drying took place during the printing itself. In the second experiment, the drying continued after printing (overall drying time was uniform for all batches). The evaluated parameters were films' weight uniformity, thickness, moisture content, surface pH, disintegration time, hardness, and tensile strength. The results were statistically processed.

Results
In the mutual experiment comparison, statistically significant differences in the moisture content, which subsequently affected the disintegration time, were found. Moisture content in films of experiment I. was 1.925 ± 0.419 % and in films of experiment II. was 1,466 ±0.505 %, respectively. The difference in the average moisture content between experiments was 23.85 %. The residual moisture content affects film plasticity, closely connected to other film properties. Statistically significant differences in mechanical properties (hardness, tensile strength) between films from different experiments were also proved. The results from the second experiment showed higher hardness and tensile strength. The average disintegration time in experiment I. was 17.4 ±0.85 s and in experiment II. it was 18.2 ±0.65 s.

Conclusion
Mutual comparison of the manufactured orodispersible films showed that the drying time affects their physical and mechanical properties. The films from the second experiment showed higher mechanical resistance and longer disintegration time. It was proved that the in-process drying time of films from the first experiment was sufficient, and this approach speeds up the manufacturing process.

Acknowledgement: The study was supported by VFU and Masaryk University.

Author's CV: Natália Janigová works on the development of dosage forms using a 3D printer. He is currently preparing multi-layer films. Her diploma thesis was focused on the use of 3D printing as a drug dispenser and the preparation of solid filaments for a 3D printer.

Supervisor: doc. PharmDr. Jan Gajdziok, Ph.D.

Preparation of spray-dried microparticles containing N-acetylcysteine for lung application.

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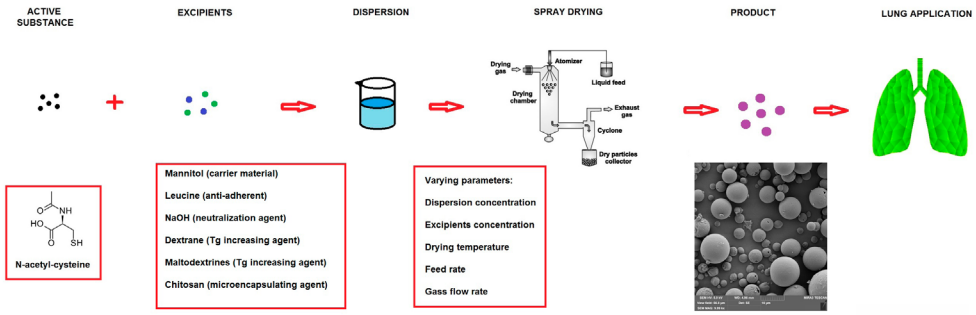
Key words: N-acetylcysteine; dry powder for inhalation; spray drying

Introduction

This work aimed to prepare dry microparticles containing N-acetylcysteine (mucolytic agent) by spray drying. The influence of formulation parameters and different excipients addition on particles' morphology was evaluated.

Methods

Sixteen formulations containing mannitol as a carrier, N-acetylcysteine (NAC) as an active substance, and leucine as an anti-adherent were spray dried. The effect of drying temperature and concentration of the feed dispersion was evaluated. Subsequently, a neutralization additive (sodium hydroxide) was added to regulate hygroscopicity by salt formation. The impact of maltodextrin with a different dextrose equivalent (DE) and different concentrations of dextran addition (glass transition temperature increasing agents) or different concentrations of chitosan addition (microencapsulation of NAC) on particles' morphology visualized by electron microscope was evaluated.



Process of preparation of spray-dried microparticles

Results

For batches without any additive spray drying at higher temperature and lower solid content led to the formation of particles with a smoother surface. For neutralized batches prepared at higher temperatures, more porous particles and small agglomerates were formed. The required spherical shape of particles was achieved with a higher concentration of dextran and maltodextrin with lower DE. With a higher concentration of chitosan, despite a superior macroscopic appearance, crystalline unencapsulated structures appeared.

Conclusion

In general, added excipients lead to a better particles' morphology compared with additive-free batches. However, under the given conditions, sticky products with agglomerates of primary particles were formed. This limitation could be solved using a larger drying chamber, increasing the proportion of leucine in the formulation, or adding another excipient reducing the hygroscopicity of microparticles.

Acknowledgement: This work was supported by the project MUNI/A/1213/2020.

Author's CV: The author is currently in the 3rd year of postgraduate doctoral studies at the Department

of Pharmaceutical Technology, FAF, MUNI. She deals with preparing spray-dried microparticles and incorporating liposomes into them in collaboration with Veterinary Research Institute Brno.

Supervisor: Doc. PharmDr. Jan Gajdziok, Ph.D.

Modified compartments for biorelevant dissolution apparatus Golem v2

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Key words: dissolution, oral dosage forms, hydrodynamics, dissolution apparatus, Golem

Dissolution testing is a key tool for quality control and research & development in pharmaceutical industry, providing information about the behaviour of oral dosage forms under controlled in vitro conditions which ideally substitute the GI environment. A multi-compartment dynamic dissolution apparatus Golem was formerly developed to simulate chyme transit and biorelevant conditions in the human stomach and three parts of small intestine, each compartment represented by a modified plastic infusion bag. The current apparatus version v2 has been recently upgraded with an electrically driven mixing mechanism and a new design of dissolution compartments. In this work, we tested the effect of inner modification of the compartments on dissolution and hydrodynamics.

We performed two consecutive test rounds, with 7 and 8 compartments, respectively. All experiments were performed with caffeine immediate release tablets – commercial tablets (1st round) and in-house tablets with reduced intravariability (2nd round). The tested compartment modifications represented mainly a narrowing of the rectangular bags in the middle to increase hydrodynamics of the media flow through the constricted section. The compartments were tested at medium and at the highest agitation speed (3 and 7 “squeezes” per minute – SPM), and with two different dissolution volumes (250 mL representing fasted state administration in stomach, and 100 mL in small intestine). Results were additionally subjected to analysis of variance (ANOVA) and principal component analysis (PCA).
Based on robustness, represented by the median of relative standard deviation, and dissolution efficacy, represented by the % of drug dissolved at 60 min and the time to reach 60% eventually 30% of dissolved drug, we selected two best performing compartments. Version “B” provided higher robustness, and version “D” higher discrimination to dissolution changes, including agitation speed and dissolution volume.

Overall, the increase in dissolution volume led to higher dissolution rates and measurement reproducibility (ANOVA; $p < 0.05$). The agitation speed played a role in the homogenization of dissolution content, although not reaching statistical significance. Additionally, we standardized basic experimental settings, such as tablet administration and positioning of sampling tubes to minimize variability. As a result, with 250 mL of dissolution volume and the highest agitation speed (7 SPM), the dissolution performance in the Golem v2 was comparable to a standard pharmacopoeial apparatus (USP 2). The working range of Golem v2, however, allows to better simulate biorelevant conditions, with lower dissolution volumes and agitation. These findings provide a crucial step for further utilization of the Golem v2.

Author‘s CV: Ivan is currently in his last year of doctoral studies at the Faculty of Pharmacy, Department of Medicinal Chemistry. His research is focused on biorelevant dissolution studies and utilization of the novel dissolution apparatus Golem v2.

Supervisor: doc. Ing. Jiří Dohnal, CSc., MBA

Synthesis of new carbamate compounds as cholinesterase inhibitors

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Key words: acetylcholinesterase inhibitors, new aryloxyaminopropanol derivatives, carbamate

Alzheimer's disease, the most common cause of adult-onset dementia is characterized by a progressive decline of cognitive functions accompanied by behavioral manifestations. Long-term clinical use shows that some drugs used in therapy of Alzheimer disease can only produce partial effective in early stages of disease, and with severe adverse phenomenon, such as bradyarrhythmia, nausea and vomiting, even hepatotoxicity, like tacrine. Compounds that contain a carbamate functional group in their structure, have proven to be suitable candidates which are better tolerated and safer. Rivastigmine and the experimental drug fenserin are examples of such drugs.

The work deals with the synthesis of new aryloxyaminopropanol derivatives, in which eight new derivatives were prepared by modification in the basic part and the carbamate fragment. We can expected, that new carbamate derivatives can inhibit acetyl and/or butyrylcholinesterase, respectively they can shown other interesting biological effects. The final aryloxyaminoalcohols were prepared by a three-step synthesis. meta-Aminophenol, respectively para-aminophenol derivatives were used as starting materials. Aminophenol isomers reacts with alkyl chloroformates and provided carbamate derivatives, in which the ether functional group was introduced by reaction with epichlorohydrin. Reaction of the epoxy derivatives with 4-benzylpiperidine opened the epoxy ring to give the aryloxyaminopropanol compounds from which the corresponding salts were prepared. One dimensional 1D NMR and two dimensional 2D NMR spectroscopy was used for determination of chemical structure of new carbamate compounds. Antimicrobial tests on the strain of Escherichia coli and Staphylococcus aureus by disk diffusion method showed, that all meta-substituted carbamate derivatives inhibit the growth of bacteria of Staphylococcus aureus (Figure 1).

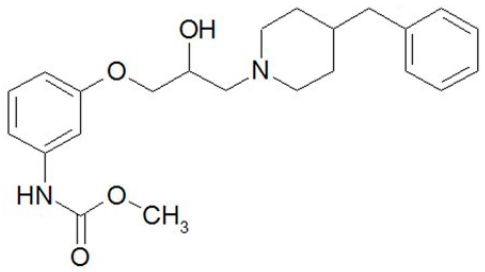

Figure 1. Methyl {3-[2-hydroxy-3-(4-benzylpiperidin-1-yl)propoxy]phenyl} carbamate

Para-Substituted carbamate derivatives did not show inhibition activity on this strain. para-Carbamate derivatives showed only a slight inhibitory effect on bacteria strain of the Escherichia coli. The highest ability to inhibit the growth of bacteria strain of the Staphylococcus aureus was found in the meta-derivatives with a methyl- substituent with a value of 128.5 % RIZD.

Author's CV: RNDr. Lucia Ungvarská Maľučká, PhD. is curently working on the synthesis of new biologically active compounds (carbamate derivatives with an aryloxyaminopropanol fragment in the molecule), the study of the relationship between the structure and biological activity of substances which are classified as cholinesterase inhibitors. She completed PhD. study at the Pavol Jozef Šafárik University in Košice, Faculty of Science, in the field of organic chemistry.

Supervisor: prof. RNDr. Jozef Csöllei, CSc.

Asymmetric Organocatalyzed Friedel–Crafts Reaction of Chloral and Phenols

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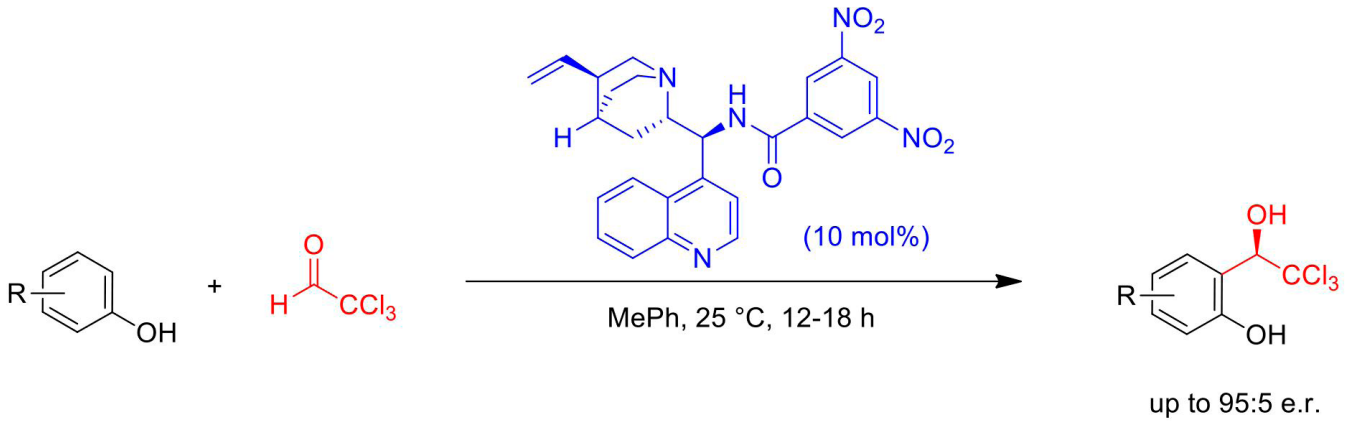
Key words: Enantioselective organocatalysis, Cinchona alkaloids, trichloromethyl carbinols.

Optically pure trihalogenmethylalcohols are direct precursors for the synthesis of biologically active compounds (e.g., efavirenz, ondacatinib, acetofenate). They could also be transformed by stereospecific reactions to α-amino-, α-hydroxy-, α-fluoroacids, or epoxides, which are important building blocks for the preparation of new chiral nonracemic molecules. One of the possibilities to obtain these aromatic trihalogenethanols is the asymmetric organocatalyzed Friedel–Crafts reaction of phenol and trihalogenacetaldehyde, investigated in this project. Accordingly, we prepared 40 organocatalysts that were tested in the model reaction of sesamol and chloral. Cinchona-based amide derivatives showed the best enantioselectivity in the preliminary catalyst screening. Additional improvements of the catalyst structure resulted in discovering 3,5-dinitrobenzamide of 9-aminoepicinchonidine as the most convenient molecule. After that, a series of optimization reactions were performed in order to establish the most suitable reaction conditions (catalyst load, solvent type, amount of chloral, temperature, and reaction time). Further, the above catalyst was utilized for the asymmetric Friedel–Crafts reaction of chloral and various electron-rich phenols. High yields and enantiomeric ratios (up to 95:5) of adducts were accomplished within 12–18 h at 25 °C. Our current work represents the first organocatalyzed method for the enantioselective synthesis of this type compounds.



up to 95:5 e.r.

Acknowledgement: This work was supported by the project MUNI/A/1510/2020.

Author's CV: David Švestka received his master's degree in 2019 at Faculty of Pharmacy UVPS Brno. He is currently a Ph.D. student at the Department of Chemical Drugs PHARM MUNI. His dissertation thesis is focused on the development of organocatalyzed asymmetric syntheses of biologically active molecules.

Supervisor: doc. Ing. Pavel Bobál', CSc.

Design, synthesis, and antiproliferative activity of newly designed derivatives of N-hydroxycinnamamide

Magdaléna Onuščáková [1], Hana Pížová [1], Tereza Kauerová [2], Peter Kollár [2], Pavel Bobál' [1]

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Key words: HDAC inhibitor, Hydroxamic acid, Antiproliferative activity

Histone acetylation is a process controlled by histone deacetylases (HDAC) and histone acetyltransferases (HAT), and it plays an essential role in the regulation of gene expression. HDACs are a family of enzymes that modulate the acetylation status of histones and non-histone proteins. Inhibitors of HDAC (HDACi), which form a complex with Zn^{2+} ion in the enzyme active site, are novel antiproliferative agents that induce tumor cell death, differentiation, and/or cell cycle arrest. We believe that they have a great potential for cancer therapy. Based on the structure, the HDACIs can be divided into several groups, namely aliphatic acids, hydroxamic acids, 2-aminoanilides, cyclic peptides, electrophilic ketones, and others.

We have designed and synthesized a series of *p*-coumaric acid-based compounds, based on the common pharmacophore, with various substituted anilides (Fig.1). These novel HDACIs comprise a common hydroxamic acid group as a zinc-binding group (ZGB). The compounds were initially evaluated for their inhibitory activity. A preliminary screening of antiproliferative activity was performed on a selected monocytic leukemia cell line THP-1, and the activity was evaluated by WST-1 analysis. Vorinostat® (SAHA), the first registered HDACi, was used as the positive control.

Derivatives bearing substituents like methoxy or chlorine on anilide function displayed a good level of antiproliferative activity. Compound with chlorine substituent on meta position of aromatic ring was the most potent from the tested series with an IC_{50} value <3 µmol/l (SAHA IC_{50} <1 µmol/l).

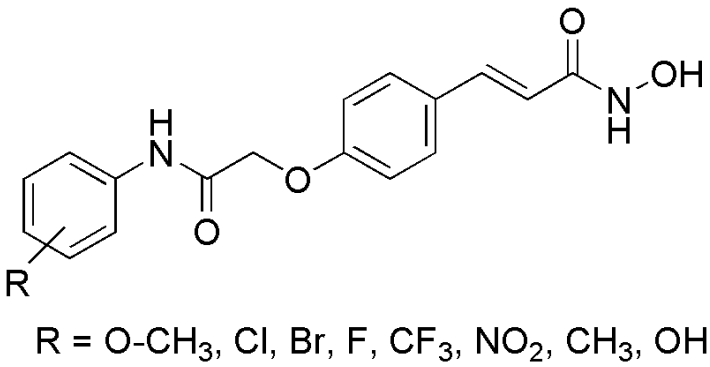

Figure 1. The structures of N-hydroxycinnamamide derivates

Acknowledgement: The study was supported by the projects MUNI/A/1598/2020 and MUNI/A/1682/2020

Author's CV: Magdalena Onuscakova graduated from the Faculty of Pharmacy at Comenius University in Bratislava in 2013. Currently, she is a third-year Ph.D. student at the Faculty of Pharmacy at Masaryk University in Brno. Her research is mainly focused on the synthesis of hydroxamic acids as potential HDAC inhibitors.

Supervisor: doc. Ing. Pavel Bobáľ, CSc.

Basic derivatives of aromatic-aliphatic ketones as potential inhibitors of aminopeptidase N

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Key words: Aminopeptidase N, thiosemicarbazone derivative of acetophenone, semicarbazone derivative of acetophenone

The aminopeptidases are a group of enzymes which hydrolyse peptide bonds at the N-terminal end of polypeptides. Aminopeptidase-N (APN) is a neutral zinc metalloenzyme. APN is a ubiquitous enzyme present in a wide variety of human organs, tissues and cell types. Inhibitors of APN may offer effective therapy for indications such as inflammatory diseases, pain management, or vasopressin release.

The main aim of this research is synthesis of unreported basic thiosemicarbazone and semicarbazone derivatives of acetophenone (Fig. 1) with inhibitory effect on the enzyme aminopeptidase-N.

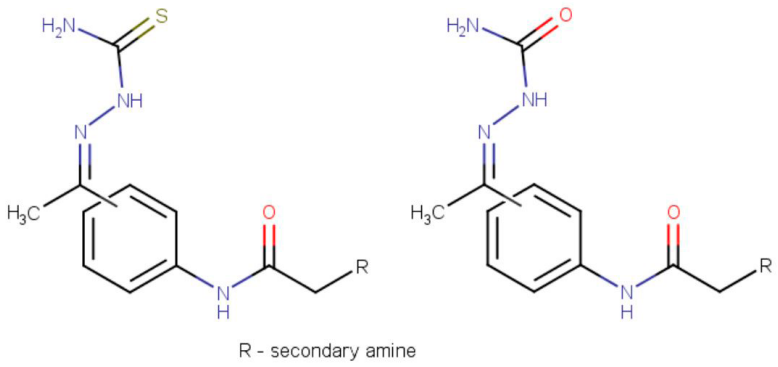

Figure 1: The general structure of basic derivatives of aromatic-aliphatic ketones

The synthesis consists of three steps. The first step is acetylation of aminoacetophenone to give N-(acetylphenyl)-2-chloroacetamide. In the second step, the chlorine atom of the intermediate obtained from the previous synthesis step is substituted for the nitrogen atom in the secondary amine. There are used symmetrical secondary amines and heterocyclic amines with saturated heterocyclic skeleton. The third step of synthesis is variable. The synthesis is depending on the structure of the target product and the reaction is focused on ketonic group of acetophenone. There are prepared thiosemicarbazone derivatives and semicarbazone derivatives. Moreover, perchlorate salts and hydrochloride salts of thiosemicarbazone derivatives of acetophenone are synthetized. The activity of these derivatives is verified by the optical photometric method of absorbance in the visible and ultraviolet region at wavelength of 405 nm. The measurement is performed by using a hybrid multi-mode microplate reader Cytation 3 and appropriate Gen5 software.

By measuring the half maximal inhibitory concentration of the mentioned substances, it was found that most of the synthesized products exert an inhibitory effect on the enzyme aminopeptidase N.

Acknowledgement: This work was supported by the project MUNI/A/1682/2020.

Author's CV: Veronika Ballayová graduated from the Faculty of Pharmacy at University of Veterinary and Pharmaceutical Sciences Brno in 2020. Nowadays, she is a first year Ph.D. student at the Faculty of Pharmacy at Masaryk University in Brno. Her research is focused on the synthesis of Schiff bases and their potential therapeutical effect.

Supervisor: doc. PharmDr. Oldřich Farsa, Ph.D.

Synergistic activity of flavonoid derivatives from Morus alba root bark against MRSA isolates

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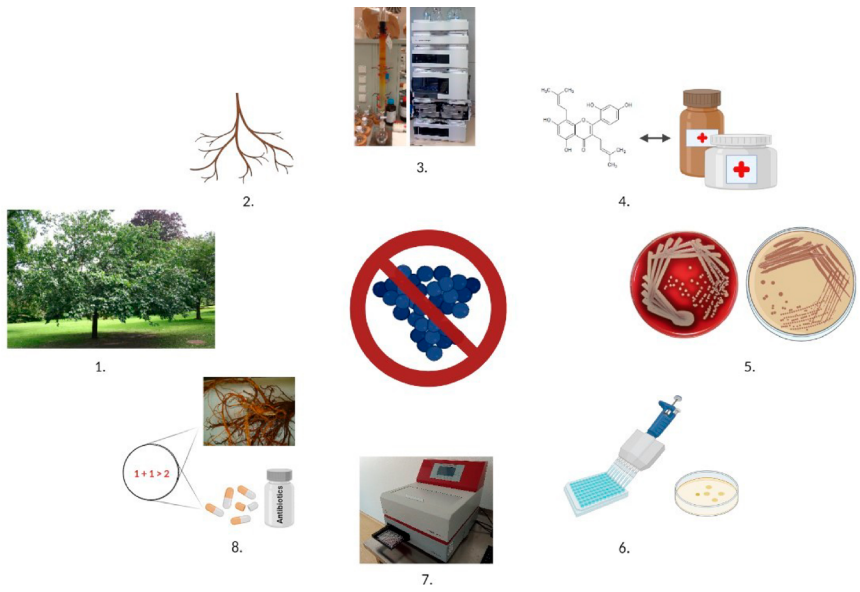
[1] Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: antimicrobial activity, Morus alba, MRSA, kanamycin, synergy

Methicillin-resistant Staphylococcus aureus (MRSA) shows antibiotic resistance not only to β-lactams but also to other commonly used antibiotics such as aminoglycosides, quinolones, macrolides, and tetracyclines. Plant-derived natural products provide a significant source of potent antimicrobial agents. The combination of conventional antibiotics and natural compounds is a promising strategy for overcoming bacterial resistance.

The aim of the study was to evaluate the antimicrobial activity of twelve compounds (six flavonoids, two Diels-Alder adducts, three 2-arylbenzofurans, and one stilbene) isolated from mulberry root bark extract against five clinical MRSA strains and three methicillin-susceptible S. aureus (MSSA) strains. Active compounds were investigated for the ability to reverse antibiotic resistance. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were assessed by the broth microdilution and agar method according to CLSI guidelines. Synergistic interaction was evaluated by the checkerboard technique. The results' interpretation is based on the FIC calculation that assesses the combination-derived MIC compared with the MIC of each agent alone. The synergism is defined as the 4-fold reduction in the MIC. Eight compounds demonstrated the activity with MICs of 2–8 µg/mL and MBCs of 4–16 µg/mL against three strains of MSSA and five strains of MRSA with MICs of 2–8 µg/mL and MBCs of 4–32 µg/mL. These compounds inhibited the growth of MRSA strains in lower concentrations than standard antibiotics such as oxacillin, erythromycin, kanamycin, ciprofloxacin, clindamycin, or tetracycline. Five compounds showed synergistic activity with kanamycin against at least one MRSA strain. The combination of all active compounds with kanamycin demonstrated the additive effect against five tested MRSA strains. Kuwanon C was the most effective structure. In comparison, none of the tested compounds showed synergistic interaction with ciprofloxacin, but the additive effect was evaluated for eight compounds.

The experimental results demonstrate the antibacterial activity of flavonoid derivatives and Diels-Alder adducts against S. aureus. The synergistic potential of selected plant compounds with kanamycin provides promising candidates for developing antibacterial combination therapy of MRSA infections.



Antimicrobial activity of flavonoid derivatives from Morus alba

Author's CV: Gabriela Škovranová evaluates the antibacterial activity of plant-derived compounds following the subject of her Master's thesis developed in Košice. Considering the Internship at Czech University of Life Sciences Prague, she is testing the synergism to reverse the antibiotic resistance of methicillin-resistant Staphylococcus aureus.

Supervisor: PharmDr. Alice Sychrová, Ph.D.

Prenylated phenolic compounds – isolation, structural analysis and biological activity

Lenka Molčanová [1], Jakub Treml [2], Veronika Leláková [2], Tereza Kauerová [3], Stefano Dall'Acqua [4], Petr Maršík [5], Karel Šmejkal [1]

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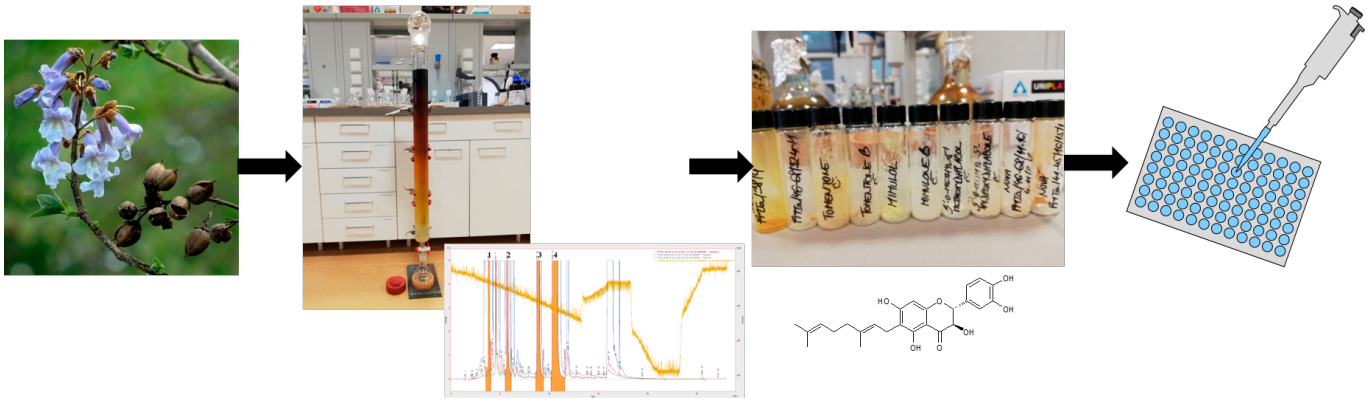
Key words: anti-inflammatory activity, cytotoxic activity, chromatography, flavonoid, geranyl, isolation, *Paulownia tomentosa*, prenyl, structural analysis

Paulownia tomentosa Steud. (Paulowniaceae), traditional Chinese medicine plant, is a rich source of multifarious secondary metabolites with potent biological activities. My work is focused on the isolation of geranylated flavonoids from fractions obtained from the fruit of *P. tomentosa* aiming to obtain compounds in sufficient amounts and purity for identification and studying their biological activities. Different chromatographic methods, such as column chromatography for basic separations, preparative RP-HPLC for precise separations, and TLC and HPLC for analytical purposes, were utilized. The structures of isolated substances were elucidated using combination of identification methods. The subtype of flavonoid skeleton was determined using UV spectroscopy, while IR spectroscopy provided information about the presence of functional groups typical for prenylated flavonoids. Exact mass and molecular formula were determined using high-resolution mass spectrometry and structures of isolated compounds were evaluated using 1D and 2D NMR spectroscopy. The absolute configurations were determined using circular dichroism. Chromatographic separation led to the isolation of 28 different geranylated flavonoid derivatives, one non-geranylated flavanone, and two other phenolic compounds. Eighteen of these compounds were isolated from a natural source for the first time. Almost all these compounds have the geranyl chain attached to the flavonoid skeleton at position C-6 and many of them have this side chain modified by further oxidation or cyclization. New subtypes of geranylated flavonoids were found, flavone and pyranoflavone, which were not described for *P. tomentosa* before. Part of my work was to create a library of geranylated flavonoids previously isolated at the Department of Natural Drugs and to add newly isolated compounds to this library. Isolated compounds are currently being tested for their ability to inhibit the NF-κB signaling pathway and some of them were more active than the standard anti-inflammatory drug prednisone, therefore, they may have the potential for treating inflammation. We believe that traditional medicine and natural compounds can still influence the modern healthcare and can be interesting source of inspiration for finding new drugs.



Geranylated flavonoids from Paulownia tomentosa – isolation, structural analysis and biological activity

Acknowledgement: The study was supported by the project IGA VFU Brno 311/2019/FaF

Author's CV: Lenka Molčanová is currently working on the isolation, structural analysis and biological activity of geranylated flavonoids from Paulownia tomentosa for her dissertation thesis at Masaryk University. She is also working as pharmacist in Brno. In the past, she studied pharmacy at University of Veterinary Medicine and Pharmacy in Košice.

Supervisor: prof. PharmDr. Karel Šmejkal, Ph.D.

Natural anti-inflammatory products and glucan particles as their drug delivery system – usability in an ex vivo and in vivo models

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Key words: Glucan particles, β-glucan, Curcumin, Diplacone, Inflammation, Drug delivery system

Traditional symptomatic treatments of chronic inflammatory diseases, such as nowadays widespread inflammatory bowel diseases, possess numerous side effects. Natural products offer a broad spectrum of potential active pharmaceuticals, often with fewer side effects compared to traditional drugs, such as corticosteroids. On the other hand, they also often have poor bioavailability or stability. To increase the bioavailability and bioactivity of the natural anti-inflammatory molecules curcumin and diplacone, we used glucan particles (GPs) – hollow shells from Saccharomyces cerevisiae composed mainly of β-1,-3-D-glucan – as their drug delivery system. GPs indigestibility and relative stability in the gastrointestinal system, combined with their immunomodulatory effects, make them promising carriers for such compounds. In this study, we at first aimed to determine how curcumin and diplacone, either alone, mixed with GPs, or incorporated in GPs affect the activity of primary porcine innate immune cells. Following ex vivo results, the next goal was to assess the beneficial effect of incorporating curcumin in GPs compared to individual pure substances or their mixtures and their impact on dextran sulfate sodium-induced colitis

in rats.

The ex vivo experiment's assessment was carried out by assessing the respiratory burst response and the secretion of pro-inflammatory cytokines in PBMCs and neutrophiles isolated from porcine blood. The anti-inflammatory effect of particular substances in vivo was evaluated based on the calculated disease activity index and by assessment of cytokines and enzymes production from the gut tissue – tumor necrosis factor α (TNF-α), transforming growth factor β1, interleukin (IL)-1β, IL-6, IL-10, IL-17, catalase, superoxide dismutase 2, myeloperoxidase (MPO), and matrix metalloproteinase 9.

Incorporating curcumin and diplacone into GPs by controlled evaporation of the organic solvent substantially reduced the respiratory burst response mediated by GPs. Incorporated curcumin in GPs also reduced GPs mediated secretion of IL-1β and TNF-α by innate immune cells. Composites of GPs with incorporated curcumin then also showed promising results with the capability to lower colitis symptoms and significantly ($p < 0.05$) decrease the production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and the activity of MPO, as well. The anti-inflammatory effect of the composites was more remarkable than those of pure GPs or curcumin.
The obtained results indicate a potentially beneficial effect of incorporating curcumin or diplacone into GPs against inflammation.

Acknowledgement: This work was supported by grant no. 16-27522A from the Ministry of Health of the Czech Republic (http://www.azvcr.cz) and by internal grants of VFU: IMA-2017-FaF-16 and IGA-312/2018/FaF

Author's CV: Dominik Rotrekl a graduate pharmacist, currently finishing his doctoral studies at the Faculty of Pharmacy of Masaryk University. His research is focused on intestinal inflammation and its treatment with natural products and glucan particles. He is a motivated young scientist who is not afraid of further challenges.

Supervisor: doc. RNDr. Jan Hošek, Ph.D.

Harmane reduces the activity of CYP2D1/2, CYP2B1, CYP2C11 and CYP3A1 after subchronic administration in rats

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[1] Department of Pharmacology, Faculty of Medicine, Masaryk University, Czech Republic
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[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Masaryk University, Czech Republic

Key words: Cytochrome P450, harmane, metabolic activity, drug-drug interactions

Aims
Harmane is a secondary metabolite of Passiflora incarnata with both in vitro and in vivo inhibitory effect on MAO A. The antidepressant-like effect of harmane in the preclinical model was proved by several investigators. Our study focused on the impact of harmane on the metabolic activity of selected rat liver cytochrome P450 (CYP) enzymes in vivo after subchronic administration.

Methods
Harmane was administered to Wistar Albino rats intragastrically at the doses of 25, 40 and 64 mg/kg/day for eight consecutive days. A vehicle (66% propylene glycol) was administered to the control group. Liver samples were drawn, and rat liver microsomes (RLM) were isolated by ultracentrifugation. In vitro incubations of RLM with CYP-specific substrates: diclofenac (CYP2C6), dextromethorphan (CYP2D1/2), phenacetin (CYP1A2), testosterone (CYP2A, CYP3A, CYP2C) were undertaken. The concentration of metaboli-

tes in samples was measured with HPLC coupled with a DAD detector. In accordance with the results of incubations, the expression of CYP2D1, CYP3A1, CYP3A2, CYP2C11 and CYP2B was further investigated with a semiquantitative western blot method.

Results
The dose-dependent inhibitory effect of harmane on the metabolic activity of four CYP isoforms was proved. The metabolic activity of CYP2D2 was significantly reduced after the administration of all three doses of harmane (25, 40 and 64 mg/kg). CYP2B1, CYP2C11 and CYP3A1 were significantly inhibited at the doses of 40 and 64 mg/kg. Neither inhibitory nor inducing effect of harmane on the metabolic activity was observed in CYP1A2, CYP2C6 and CYP2A1 enzymes.

Conclusion
Passiflora incarnata is a widely used over-the-counter herbal anxiolytic and tranquillizer supplement. Our data show the potential of harmane to influence metabolic activity in vivo. These results suggest that interaction of harmane and CYP enzymes may be of clinical importance. However, further clinical studies are needed.

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Supervisor: doc. PharmDr. Ondřej Zendulka, Ph.D.

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