49th Conference
Synthesis and Analysis of Drugs 2021
Hradec Králové

BOOK OF ABSTRACTS

Compiled and edited by Jan Zitko

Hradec Králové, 2021

Dear colleagues and friends,

It is my great pleasure to invite you to the 49th conference "Synthesis and Analysis of Drugs", which is held on September 16th – 17th, 2021. Our international meeting was postponed from the September 2020 because of covid-19 pandemic. The virtual conference will take place at the Faculty of Pharmacy, Charles University, Czech Republic.

I believe, the conference will be very pleasant and fruitful. There will be many opportunities to make new contacts and discuss current challenges in the field of medicinal chemistry, pharmaceutical analysis and other disciplines. Especially young scientist and PhD students are encouraged to present online the results of their work.

Dear colleagues, I look forward to meeting you virtually at this traditional annual conference for experts coming not only from the Czech and Slovak Republic but also from many other countries.

Prof. PharmDr. Martin Doležal, Ph.D.

Organising committee chairman

Organising committee:

Prof. RNDr. Jarmila Vinšová, CSc.,

vice-chairman of Section of Synthetic Drugs

Assoc. Prof. PharmDr. Radim Kučera, Ph.D.,

chairman of Section of Pharmaceutical Analysis and Bioanalytics

PharmDr. Marta Kučerová, Ph.D.

PharmDr. Petr Kastner, Ph.D.

Assoc. Prof. PharmDr. Martin Krátký, Ph.D.

Assoc. Prof. PharmDr. Jan Zitko, Ph.D.

The conference was sponsored by:

PLENARY LECTURES

PL-1

ADVANCES IN THE USE OF PHTHALOCYANINES AND THEIR ANALOGUES IN PHOTODYNAMIC THERAPY

ZIMČÍK P.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: zimcik@faf.cuni.cz

Phthalocyanines (Pcs) are planar macrocyclic dyes with rich photophysical properties, including strong production of singlet oxygen or fluorescence upon excitation by light. Both these properties are highly emphasized in photodynamic therapy (PDT) that is based on production of highly cytotoxic singlet oxygen upon excitation of photosensitizers (like Pcs) by light. The planar Pc core is, on the other hand, also a source of undesirable aggregation that eliminates any photodynamic activity. During our research, we focused on the reduction of the aggregation mostly by strategy of introduction of charged peripheral substituents (anionic or cationic) that efficiently decreased the aggregation by electrostatic repulsive forces and lead to the highly active Pcs.[1-3] In several cases, compounds with EC_{50} in nanomolar range upon activation and TC_{50} in hundreds of micromoles (no activation) where obtained. Their fate on the subcellular level was deeply investigated leading mostly to the conclusion that the primary targets are lysosomes and that prevalent mode of cell death is necrosis. By series of detailed experiments, also a statistically significant difference in activities between cationic and anionic species was explained.[1] The discovered structure-activity relationships are helpful in future rational development of new photosensitizers.

The study was supported by Czech Science Foundation (grant. No. 20-09212S).

References

1. KOLLAR, J., MACHACEK, M., HALASKOVA, M., *et al.*: J. Med. Chem., 63, 2020, 7616-7632.
2. GHAZAL, B., MACHACEK, M., SHALABY, M.A., *et al.*: J. Med. Chem., 60, 2017, 6060–6076.
3. MACHACEK, M., DEMUTH, J., CERMAK, P., *et al.*: J. Med. Chem., 59, 2016, 9443–9456.

PL-2

TARGETING TUMOR-RELEVANT PROTEINS – INHIBITORS OF THE SUBUNIT INTERACTION OF PROTEIN KINASE CK2

PIETSCH, M.,[1] VIHT, K.,[2] GÖTZ, C.,[3] HELMS, V.,[4] JOSE, J.,[5] URI, A.,[2] NEUNDORF, I.,[6] NIEFIND, K.[6]

[1] Center of Pharmacology, Medical Faculty and University Hospital Cologne, University of Cologne, Germany

[2] Institute of Chemistry, University of Tartu, Estonia

[3] Medical Biochemistry and Molecular Biology, Saarland University, Germany

[4] Center for Bioinformatics, Saarland Informatics Campus, Saarland University, Germany

[5] Institute of Pharmaceutical and Medical Chemistry, Westfälische Wilhelms-Universität Münster, Germany

[6] Institute of Biochemistry, Department of Chemistry, University of Cologne, Germany

e-mail: markus.pietsch@uk-koeln.de

The serine/threonine protein kinase CK2 is over-expressed in various types of human tumors, with increased transcript levels being correlated to poor prognosis outcome and survival rate. Both downregulation of CK2 and inhibition of CK2 activity reduce growth and proliferation of tumor cells and increase their apoptotic activity. The CK2 holoenzyme is characterized by a heterotetrameric architecture containing two catalytic subunits (CK2α) attached to a central dimer of noncatalytic subunits (CK2β). A recently described strategy to inhibit CK2 focuses on the interference with the CK2 subunit interaction to prevent holoenzyme formation.[1]

We have developed a fluorescence anisotropy (FA) assay to quantify binding of ligands to the CK2α-CK2β site of $CK2\alpha^{1-335}$ and two Microscale Thermophoresis (MST)-based approaches to study effects of such ligands on the CK2 subunit interaction.[2,3] Rational ligand design based on the crystal structure of the cyclic peptide Pc bound to the CK2α-CK2β site of $CK2\alpha^{1-335}$ (PDB: 4IB5)[4] and docking experiments[2] in combination with fusion of the ligands to the cell-penetrating peptide sC18 resulted in the cell-permeable Pc derivative sC18-I-Pc, which is able to bind to $CK2\alpha^{1-335}$ with a K_D value in the high nanomolar range, to inhibit the CK2-catalyzed phosphorylation of a substrate that requires the intact holoenzyme, and to exhibit selective cytotoxicity towards cancerous HeLa cells over non-cancerous HEK-293 cells.[5]

Characterization of the CK2 ligand ARC-3140, a hybrid molecule with a heteroaromatic part linked to an acidic peptide, also started from the crystal structure of the complex with

CK2α$^{1-335}$, which revealed interaction of the ligand with the ATP and substrate binding sites as well as with the CK2α-CK2β site of CK2α$^{1-335}$ (PDB: 6SPW).[3] The structural data were confirmed by FA binding assays and a kinase activity assay providing K_D values in the picomolar and micromolar range for binding of ARC-3140 to these two sites, respectively. Furthermore, inhibition of CK2 subunit interaction by ARC-3140 (K_i = 5 μM) was quantified by two separate MST methods.[3]

This work was supported by the Deutsche Forschungsgemeinschaft (grants NI 643/4-1, NI 643/4-2, and PI 806/2-2), the Estonian Research Council (grants IUT20-17 and PRG454), the EU project ISOLATE, and the Graduate Program in Pharmacology and Experimental Therapeutics of the University of Cologne and the Bayer AG, Leverkusen (Germany).

References

1. IEGRE, J., ATKINSON, E. L., BREAR, P. D. *et al.*: Org. Biomol. Chem., 19, 2021, 4380–4396.
2. HOCHSCHERF, J., LINDENBLATT, D., STEINKRÜGER, M. *et al.*: Anal. Biochem., 468, 2015, 4–14.
3. PIETSCH, M., VIHT, K., SCHNITZLER, A. *et al.*: Bioorg. Chem, 96, 2020, 103608.
4. RAAF, J., GUERRA, B., NEUNDORF, I. *et al.*: ACS Chem. Biol., 8, 2013, 901–907.
5. LINDENBLATT, D., HORN, M., GÖTZ, C. *et al.*: ChemMedChem, 14, 2019, 833–841.

PL-3

DEVELOPMENT OF RADIOTRACERS AS ACTIVITY-BASED PROBES FOR THE DETECTION AND IMAGING OF TRANSGLUTAMINASE 2

LÖSER, R.,[1] PIETZSCH, J.,[1] WODTKE, R.,[1]

[1] Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Germany

e-mail: r.loeser@hzdr.de

The general impact and utility of radiotracers and molecular imaging on drug discovery and pharmacology will be briefly outlined (see for example Ref.[1] for a short overview).

The main part of the talk will focus on the development of molecular probes derived from irreversible inhibitors labelled with fluorine-18 and iodine-123 for activity-based detection of transglutaminase 2. This enzyme is increasingly recognised as key player in neoplastic and fibrotic diseases.[2,3] The identification of radiotracer candidates based on structure-activity relationships will be highlighted and methods for radiolabelling including the synthesis of precursor compounds will be briefly mentioned.[4,5] The pharmacological investigation of the resulting radiolabelled probes with regards to the application as tool compound for the characterisation of binding of other ligands and the quantitative detection of transglutaminase 2 in biological specimens as well as their in vivo behaviour including the metabolic stability will be reported.

The study was supported by the Saxon ministry for science and arts.

References

1. AUBERSON, Y. P.: Chimia, 70, 2016, 868-873.
2. ECKERT, R. L.: Mol. Carcinog., 58, 2019, 837-853.
3. BENN, M. C., WEBER, W., KLOTZSCH, E., *et al.*: Curr. Opin. Biomed. Eng., 10, 2019, 156-164.
4. WODTKE, R., HAUSER, C., RUIZ-GÓMEZ, G., *et al.*: J. Med. Chem., 61, 2018, 4528-4560.
5. WODTKE, R., WODTKE, J., HAUSER, S., *et al.*,: J. Med. Chem., 64, 2021, 3462-3478.

PL-4

BORON CLUSTERS AS NEWLY EMERGING 3D PLATFORM FOR DESIGN OF SPECIFIC INHIBITORS OF PHARMACEUTICALLY RELEVANT ENZYMES

GRŰNER B.,[1] HOLUB J.,[1] NEKVINDA J.,[1] EL ANWAR S.,[1] KUGLER M.,[2] HAJDŮCH M.,[2] POKORNÁ J.,[3] KONVALINKA J.,[3] BRYNDA J.,[3] ŘEZÁČOVÁ P. [3]

[1] Institute of Inorganic Chemistry of the Czech Academy of Sciences, Řež, Czech Republic

[2] Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

[3]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic

e-mail: gruner@iic.cas.cz

This contribution gives an overview over the boron cluster compounds and their emerging potential as unconventional 3D pharmacophores in drug design. In particular, our original results are presented that cover successful synthesis of specific inhibitors designed for two different types of medicinal targets. We have previously identified metallacarboranes as a promising class of specific inhibitors of HIV protease (HIV-PR) enzyme.[1] Recently, advances in the molecular design of carborane and metallacarborane inhibitors have been made targeting CA IX isoenzyme. This is associated with solid hypoxic tumors and belongs currently to validated targets for cancer therapy and diagnostics. Indeed, the optimized structures led to significantly enhanced *in vitro* activities of CA IX, from initial values in low micromolar range, to picomolar inhibition constants.[2,3] The scope of currently available exo-skeletal modifications on various boron cages is critically overviewed that enabled synthesis of both types of inhibitors. These results are complemented by synchrotron structures of enzyme-inhibitor complexes and by a brief report on pharmacologically relevant factors presented on a panel of selected active compounds.

The study was supported by Czech Science Foundation, Project No. 21144095S.

References

1. ŘEZÁČOVÁ P, GRÜNER B, KONVALINKA J., *et al.*: Chapter 1.3., in: Boron Science- New Technologies and Applications Hosmane N.S., Ed., CRC Press, Boca Raton, 2012. p 45-63.
2. BRYNDA J., MADER P., ŠÍCHA V., *et al.*: Angew. Chem., Intl. Ed. Eng., 52, 2013, 13760-13763.
3. GRÜNER B., KUGLER M., EL ANWAR S., *et al.*: Chempluschem, 86, 2021, 352-363.

PL-5

ASPH INHIBITORS FOR THE TREATMENT OF METASTATIC CANCER

OLSEN, M.J.,[1,2]

[1] Department of Pharmaceutical Sciences, College of Pharmacy - Glendale, Midwestern University, USA

[2] Crenae Therapeutics, USA

e-mail: molsen@midwestern.edu

Aspartyl(asparaginyl)-beta-hydroxylase (ASPH) is an iron-dependent dioxygenase family enzyme that hydroxylates specific aspartic acid and asparagine residues in certain calcium binding Epidermal Growth Factor (cbEGF) domains.[1] ASPH plays an important role in embryological development, including uterine implantation by tropoblasts.[2] However, ASPH is inappropriately overexpressed by a variety of aggressive metastatic cancers, including hepatocellular carcinoma and cholangiocarcinoma.[3] Tetronimide-type ASPH inhibitors have demonstrated potent, orally bioavailable suppression of tumor growth and metastasis in a wide range of tumor models, including a highly metastatic cholangiocarcinoma model.[4] These compounds can be deuterated, yielding surprisingly chemically stable enantiomers that display radically different stabilities in human and mouse liver microsomes and resulting in novel compounds for the treatment of ASPH expressing cancers.

The study was supported by Midwestern University.

References

1. KANWAL, M., SMAHEL, M., OLSEN, M., *et al.*: J. Exp. Clin. Cancer Res., 39(1), 2020, 163.
2. GUNDOGAN, F., ELWOOD, G., GRECO, D., *et al.*: Human Pathol., 38(1), 2007, 50–59.
3. LAVAISSIERE, L., JIA, S., NISHIYAMA, M., *et al.*: J. Clin. Invest., 98, 1996, 1313–1323.
4. NAGAOKA, K., OGAWA, K., JI, C., *et al.*: Dig. Dis. Sci., 66(4), 2021, 1080–1089.

PL-6

SYNTHESIS OF MODIFIED NUCLEOSIDES, DERIVED FROM PYRIMIDINE, AND THEIR BIOLOGICAL PROPERTIES

KACZMAREK, R.,[1] DEMBINSKI, R.[1,2]

[1] Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

[2] Department of Chemistry, Oakland University, 146 Library Drive, Rochester, Michigan 48309-4479, USA

e-mail: dembinsk@oakland.edu

Modified nucleosides represent potential tools for fluorescent tagging, studying nucleoside metabolism, 2′-deoxyribonucleoside kinase activity, and biological activities. "*Consanguineous*" methodologies to access pyrimidine-based alkynyl nucleoside analogs are discussed. The focus is placed on highly fluorescent 5-alkynylfuropyrimidines, designed in the form of ribose acetyl esters, which antiviral properties investigated *in vitro* for acetyl derivatives. Regiochemistry of the introduction of the alkynyl substituent was achieved via 5-*endo*-*dig* electrophilic halocyclization of acetyl 5-*p*-alkylphenyl-2'-deoxyuridines. Diverse alkynyl substituents were introduced at the heterobicyclic base C-5 position via Sonogashira coupling of 5-iodo-2'-deoxyribofuranosyl-furo[2,3-*d*]pyrimidin-2-ones. The resulting compounds have fluorescence emissions of 452-481 nm. High quantum yields of 0.53-0.60 were observed for 9-ethynyl-9-fluorenol and propargyl alcohol/methyl ether-modified furopyrimidines. Antiviral assays against a broad spectrum of DNA and RNA viruses showed that in human embryonic lung (HEL) cell cultures some of the compounds showed antiviral activity (EC_{50} 1.3-13.2 μM) against varicella-zoster virus (VZV). The alkynyl furopyrimidine with two *p*-pentylphenyl substituents emerged as the best compound with reasonable and selective anti-VZV activity. Further functionalization include conversion of alkynyl function into their dicobalt hexacarbonyl complexes, which were investigated for anticancer properties.

References

1. KACZMAREK, R., TWARDY, D.J, OLSON, T.L., *et al.*: Eur. J. Med. Chem., 209, 2021, 112884.

PL-7

PROTACS FOR THE DEGRADATION OF CYCLIN-DEPENDENT KINASES AND BEYOND

GÜTSCHOW, M.

Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, University of Bonn, Germany

guetschow@uni-bonn.de

Proteolysis-targeting chimeras (PROTACs) have received tremendous attention as a new and exciting class of therapeutic agents that promise to significantly impact drug discovery. These bifunctional molecules consist of a target binding unit, a linker, and an E3 ligase binding moiety. The chemically-induced formation of ternary complexes leads to ubiquitination and proteasomal degradation of target proteins. Among the plethora of E3 ligases, only few have been utilized for the novel PROTAC technology, mainly cereblon (CRBN) and von-Hippel-Lindau (VHL).[1] Cyclin-dependent kinases (CDKs) 4 and 6 are important regulators of the cell cycle. CDK4/6 inhibitors such as palbociclib possess high activity in breast cancer and other malignancies. We developed palbociclib-based PROTACs which addressed several ligases and showed potent and longlasting degrading activity in human and mouse cells.[2] When studying fluorination, CRBN binding and antiangiogenic effects of thalidomide-derived immunomodulatory drugs (IMiDs), a correlation between the latter two phenomena was not found.[3] Furthermore, we assembled PROTACs from two cereblon ligands as well as from a cereblon and a VHL ligand and demonstrated a PROTAC-induced homo- or heterodimerization of the E3 ligases leading to an efficient degradation of CRBN.[4,5]

References

1. BRICELJ, A., STEINEBACH, C., KUCHTA, R. *et al.*: Front. Chem., 9, 2021, 642273.
2. STEINEBACH, C., NG, Y.L.D., SOSIČ, I. *et al.*: Chem. Sci., 11, 2020, 3474–3486.
3. HEIM, C., MAIWALD, S., STEINEBACH, C. *et al.*: Biochem. Biophys. Res. Commun. 534, 2021, 67–72.
4. STEINEBACH, C., LINDNER, S., UDESHI, N.D. *et al.*: ACS Chem. Biol., 13, 2018, 2771–2782.
5. STEINEBACH, C., KEHM, H., LINDNER, S. *et al.*: Chem. Commun., 55, 2019, 1821–1824.

PL-8

CHIRAL SEPARATION OF BORON CLUSTER COMPOUNDS

KUČERA, R.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: radim.kucera@faf.cuni.cz

The boron atom is located next to the carbon atom in the periodic table of elements. Unlike carbon, boron can create attractive abiotic three-dimensional clusters with unique properties, i.e. 3-D aromaticity, high lipophilicity and stability to natural enzymatic systems. These properties together with low chemical reactivity allow applications of boron cluster compounds in medicinal chemistry. The research of carboranes reflects their similarity to a rotating phenyl ring. The exoskeletal substitution can modify their physical and chemical properties and often leads to chiral compounds without a discrete centrum of chirality. It is of utmost importance to investigate the conditions for chiral separation of these compounds concerning their potential clinical use. Moreover, chiral analytical methods are required to evaluate the optical purity of chiral boron clusters.

HPLC was successful in chiral separations of numerous zwitterionic carborane derivatives but failed in separating anionic species on cyclodextrins[1] and polysaccharides.[2] Nevertheless, electrophoretic experiments proved that cyclodextrins could, in principle, discriminate almost any type of substituted carboranes, regardless it is ionic or not. This presentation summarises the experimental prerequisites for chiral discrimination of anionic carboranes by HPLC on β-cyclodextrins.[3]

The study was supported by the Charles University projects GAUK 168 120 and PROGRES.

References

1. HORÁKOVÁ, H., GRÜNER, B., VESPALEC, R.: Chirality, 23, 2011, 307–319.
2. MENGELINGS, D., VANDER HEYDEN, Y., VESPALEC, R.: Biomed. Chromatogr. 28, 2014, 694–707.
3. HORÁČEK O., PAPAJOVÁ-JANETKOVÁ M., GRÜNER B., *et al.*: *Talanta.* 222, 2021, 121652.

PL-9

NEW PSYCHOACTIVE SUBSTANCES: GLOBAL HEALTH THREAT OR A POOL OF PHARMACEUTICALS?

PAŠKAN, M.,[1] SPÁLOVSKÁ, D.,[2] JURÁSEK, B.,[3] KUCHAŘ, M.,[3] RIMPELOVÁ, S.,[4] KOHOUT, M.,[1]

[1] Department of Organic Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Czech Republic

[2] Department of Analytical Chemistry, Faculty of Chemical Engineering, University of Chemistry and Technology Prague, Czech Republic

[3]Forensic Laboratory of Biologically Active Substances, Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Czech Republic

[4] Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

e-mail: michal.kohout@vscht.cz

The prevalence of new psychoactive substances (NPS) that are not controlled under existing legislation is a complex phenomenon affecting the health and safety of citizens on the global level. The most important groups of recreational drugs represent cathinones, synthetic cannabinoids, phenethylamines, tryptamines, piperazines and arylcyclohexylamines. Among these substances, there are many pharmaceuticals previously approved for clinical use and subsequently discontinued or even pharmaceuticals that are still being used in human or veterinary medicine.[1] Many NPS are chiral, however, they are typically available in the racemic form. Since enantiomers may interact differently with chiral receptors of living organisms, their effect can significantly differ, *i.e.*, one enantiomer may be psychoactive or toxic while the other may have plausible medicinal effect. Hereby, we present and discuss several recent examples of NPS, which can be potentially used in medicine to treat various diseases, and the workflow towards individual enantiomers and their characterization.

The study was supported by Czech Science Foundation, grant number 21-31139J and Ministry of the Interior of the Czech Republic, grant number MV0/VI20172020056.

References

1. TOBIAS, J. D., MARTIN, D. L., WETZEL, R. C.: Crit. Care Med. 18, 1990, 819–821.

SHORT COMMUNICATIONS

SC-1

MODIFICATIONS OF HDL COMPONENTS´ GENE EXPRESSION IN LIVER OF RAT WITH ADJUVANT ARTHRITIS

VYLETELOVÁ, V.[1]; NOVÁKOVÁ, M.[1]; BAUEROVÁ K.[2]; PONIŠT S.[2]; OBLOŽINSKÝ, M.[1]; PAŠKOVÁ, Ľ.[1]

[1] Department of Cellular and Molecular Biology of Drugs, Pharmaceutical Faculty of Comenius University, SK-83232 Bratislava, Slovakia

[2] Centre of Experimental Medicine, Slovak Academy of Sciences, SK-84104 Bratislava, Slovakia

e-mail: vyletelova6@uniba.sk

In rheumatoid arthritis (RA), the remodelling of protein cargo of HDL particles, thus switching from anti-inflammatory to pro-inflammatory particle, accelerating atherosclerosis, occurs[1]. Since the data from animal models of arthritis are limited, we studied alterations of HDL subunits´ gene expression in hepatocytes of Lewis rats with adjuvant arthritis (AA) induced by intradermal administration of *Mycobacterium butyricum*. We observed increased expression of pro-inflammatory cytokines, like tumour-necrosis factor α (TNFα), C-reactive protein (CRP) or interleukin 1β (IL-1β). Expressions of for anti-inflammatory HDL typical components like apolipoproteins apoA-I and apoE, antioxidant enzymes glutathione seleno-peroxidase 3 (GspX3) or paraoxonase 1 (PON1) and enzyme influencing the HDL metabolism lecithin-cholesterol acyltransferase (LCAT) were decreased, whilst Lp-PLA2, enzyme with antioxidant and PAF-hydrolysing activity, was over-expressed. We observed enhanced expression of some acute phase reactant or other HDL components such as haptoglobin, ceruloplasmin, C3 complement component or fibrinogen. Comparing to data from literature, the changes in hepatic expression of HDL components in rats with AA are in accordance with clinically observed changes in protein cargo of HDL during inflammation, thus may represent suitable model for studying and development of HDL-modifying drugs[2].

The study was supported by VEGA 2/0115/19, VEGA 1/0429/21 and FaF UK/10/2021.

References

1. CHOY, E., GANESHALINGAM, K., SEMB, A.G, *et al.*: Rheumatology, 53, 2014, 2143–2154
2. FEINGOLD, K.R., BRINTON, E.A., GRUNFELD, C.: The Effect of Inflammation and Infection on Lipids and Lipoproteins, Endotext *[Internet]*, 2019. PMID: 26561701

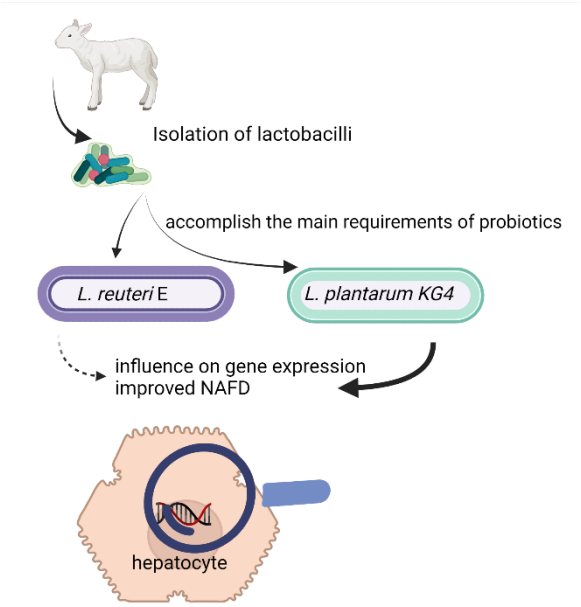
SC-2

EFFECT OF HEAT-KILLED LACTOBACILLI ON THE EXPRESSION OF GENES RELATED TO LIPID METABOLISM AND INFLAMMATION

NOVÁKOVÁ M.[1], BILKOVÁ A.[1], KIŇOVÁ-SEPOVÁ H.[1], DUDÍK B.[1], VYLETELOVÁ, V.[1], OBLOŽINSKÝ M.[1], PAŠKOVÁ Ľ.[1]

[1] Department of Cellular and Molecular Biology of Drugs, Pharmaceutical Faculty of Comenius University, SK-83232 Bratislava, Slovakia

e-mail: novakova92@uniba.sk



Alteration in microbiome could improve symptoms of non-alcoholic fatty liver disease (NAFLD). A close relationship between inflammation and dysregulation of lipid metabolism was established. Recent findings suggest, that the anti-inflammatory effect of some bacterial strains is mediated by ligand of TLR2/TLR10 signalling cascade.[1] At the Department of Cell and Molecular Biology of Drugs, the Faculty of Pharmacy, Comenius University in Bratislava, several strains of lactobacilli were isolated from animal sources. For the study of NAFLD prevention therapy, we chose two strains of lactobacilli, *L. reuteri* E and *L. plantarum* KG4, which show high resistance to bile acids and low resistance to antibiotics.[2,3] NAFLD was simulated *in vitro* in the HepG2 cell line by using conjugate of 0.5 mM oleic acid with bovine serum albumin (OA-BSA). We observed differences in gene expression of lipid metabolism in cells pre-treated for 24 hours with heat-killed (30 minutes/95°C) lactobacilli before exposition to the pathological concentration of OA-BSA. The changes in gene expression mediated by *L. plantarum* KG4 showed a higher potential in the prevention of NAFLD than *L. reuteri* E.

The study was supported by VEGA 1/0429/21 and FaF UK/8/2021

References

1. PAVELJŠEK D., IVIČAK-KOCJAN K., *et al.*:. Cellular Microbiology, 23, 2020, e13264.
2. KIŇOVÁ SEPOVÁ H., BILKOVÁ A.: Folia Microbiol., 58, 2013, 33–38.
3. MÁJEKOVÁ H., KIŇOVÁ SEPOVÁ H., *et al.*: Folia Microbiol., 60, 2015, 253–257.

SC-3

SYNTHESIS, PHOTOPHYSICAL, PHOTOCHEMICAL AND PHOTOBIOLOGICAL PROPERTIES OF AMPHIPHILIC ZN(II) AND FREE BASE TRIPYRIDYLPORPHYRINS

MUŠKOVIĆ, M.,[1] DŽEBA I.,[2] BASARIĆ, N.,[3] MIHALJEVIĆ, B.,[2] RATKAJ, I.,[1] MALATESTI, N. [1]

[1] Department of Biotechnology, University of Rijeka, Rijeka, Croatia

[2] Radiation Chemistry and Dosimetry Laboratory, Ruđer Bošković Institute, Zagreb, Croatia

[3] Laboratory of Synthetic Organic Chemistry, Ruđer Bošković Institute, Zagreb, Croatia

e-mail: martina.muskovic@biotech.uniri.hr (presenting author); nela.malatesti@biotech.uniri.hr (corresponding author)

Photodynamic therapy (PDT) is a cancer treatment that has been increasingly investigated. Photosensitizer (PS), light and oxygen are main components of PDT, and in their combination reactive oxygen species (ROS) are produced, which can lead to cytotoxic effect within the cell and consequent destruction of cancerous tissue [1]. The desired characteristics of the PS include stability of the molecule, high production of singlet oxygen and other ROS, good absorption in red or infrared region, negligible toxicity without irradiation and relative fast excretion from the body [2]. Porphyrins are often used as PSs because their structure can be relatively easily modified to achieve adequate lipophilicity of the molecule, or chelated by different cations, such as Zn(II) in our work. Amphiphilic molecules can facilitate cellular uptake while maintaining good water solubility [3], and Zn(II) is expected to increase the lifetime of the excited triplet state (^{3}PS*), and thus the production of singlet oxygen [4]. In addition to the PS, light also plays an important role in PDT, so the optimal dose of light and the appropriate irradiation wavelenght should be selected [5]. Here we will present two groups of tripyridylporphyrins, Zn(II) and free-based, both conjugated with chains of different length. Spectroscopic properties of the ground state, as well as the excited state, of the obtained compounds were studied by using laser flash photolysis (LFP) and time-resolved single photon counting (TC-SPC). Lipophilicity and singlet oxygen production, which was investigated by using modified relative methods, will also be presented. *In vitro* studies of the PSs' biological properties included evaluation of cytotoxicity on melanoma cell line (MeWo) and human foreskin fibroblasts (HFF), with two light sources used for photoactivation, with irradiation wavelength at 605 nm (orange light) and 645 nm (red light).

The study was supported by the University of Rijeka grant (uniri-prirod-18-173).

References

1. NICULESCU, A. G., GRUMEZESCU, A.M.: Applied Sciences, 11(8), 2021, 3626.
2. JOSEFSEN, L. B., BOYLE, R.W.: Theranostics, 2(9), 2012, 916-966.
3. PISAREK, S., MAKSIMOVA, K., GRYKO, D.: Tetrahedron, 70(38), 6685-6715.
4. BENOV, L., CRAIK, J., BATINIC-HABRELE, I.: Anti-Cancer Agents in Medicinal Chemistry, 11(2), 2012, 233-241.
5. KIM, M., DARAFSHEH, A.: Photochemistry and Photobiology, 96, 2020, 280-294.

SC-4

TRIAZINYL-SUBSTITUTED BENZENESULFONAMIDES AS INHIBITORS OF BACTERIAL CARBONIC ANHYDRASE

HAVRANKOVA, E., [1] CSÖLLEI, J., [1]

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

e-mail: havrankovae@pharm.muni.cz

Carbonic anhydrases (CA, EC 4.2.1.1) are metalloenzymes present in pathogenic bacteria. CAs play an important role in growth and survival in bacteria. Inhibition of bacterial CAs leads to growth retardation, growth defects and makes bacterias vulnerable to host defense mechanisms. Bacterial CAs are therefore a very promising target in the search for new antibiotics. The new 1,3,5-triazinyl aminobenzenesulfonamide derivatives containing aminoalcohole, aminochalcone and other structural moieties were synthesized, and their biological activity was evaluated. Some of the tested compounds exhibit a significant inhibitory activity against vancomycin resistant *Enterococcus faecalis*, while against relevant human CAs they showed almost negligible inhibitory activity. In conclusion, newly prepared compounds have a great potential as antibacterial agents with high activity and at the same time with high selectivity for bacterial CA in comparison with metabolically important hCA isoenzymes (e.g. hCA I, hCA II) found in the human body.

The study was supported by INGA MU (grant number MUNI/A/1202/2020).

SC-5

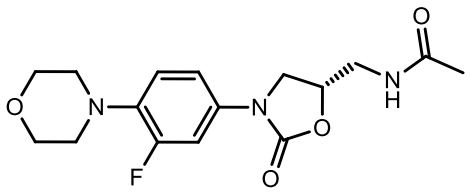
SYNTHESIS OF OXAZOLIDINONE DRUGS LINEZOLID AND RIVAROXABAN VIA ASYMMETRIC HENRY REACTION

VRBICKÝ, M., DRABINA, P.

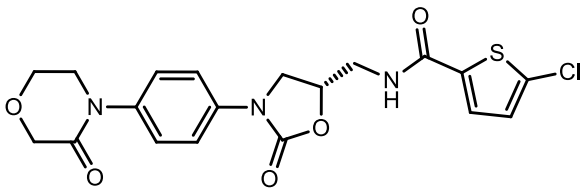
Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Czech Republic

e-mail: martin.vrbicky@student.upce.cz

Oxazolidinones are new class of antibiotics used to treat serious skin and bacterial infections. They are active against large spectrum of gram-positive bacteria, including methicillin- and vancomycin-resistant staphylococci. An oxazolidinone derivative for other purposes is Rivaroxaban, which is approved by the FDA for venous thromboembolism prophylaxis.[1] Herein, we are presenting significant improvement in synthesis of antibacterial agent Linezolid, previously reported by Piccionello *et al.*,[2] and anticoagulant drug Rivaroxaban. Both reaction sequences involve six steps overall, starting from commercially available and inexpensive materials. The stereogenic center was introduced by asymmetric Henry reaction catalyzed by the complexes of copper(II) acetate and selected ligands. The use of imidazolidin-4-one or bis(oxazoline) ligands provide high yields and enantioselectivities of the chiral precursors of both drugs (up to 94%, up to 90% ee). With regard to obtained results, the most efficient catalysts and appropriate reaction conditions were selected for subsequent research that deals with the modification of the carbamate functional group of starting aldehydes and its influence on the enantioselectivity of asymmetric Henry reaction.



Linezolid



Rivaroxaban

References

1. ROEHRIG, S., STRAUB, A., *et al.*: J. Med. Chem., 48, 2005, 5900–5948.
2. PICCIONELLO, A. P., PIERRO, P., *et al.*: RSC Adv., 3, 2013, 24946–24951.

SC-6

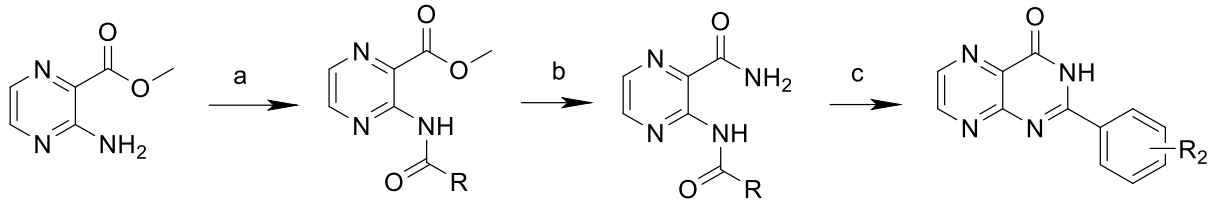
DESIGN, SYNTHESIS, SAR AND IN SILICO STUDIES OF 3-AMINOPYRAZINE-2-CARBOXAMIDES AS ANTIMICROBIALS

PALLABOTHULA, V. S. K.,[1] ZITKO, J.,[1] KERDA, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: pallabov@faf.cuni.cz

As part of our current ongoing research on pyrazinamide (a first-line antitubercular) derivatives, we report the design and synthesis of novel 3-aminopyrazine-2-carboxamides and their respective cyclic pteridine derivatives along with their biological evaluation. The synthesized compounds were prepared according to Scheme 1 and biologically evaluated for their *in vitro* activity against various mycobacterial strains and other strains of pathogenic bacteria and fungi. The active compounds are pyrazine-2,3-dicarboxamides (where R is a substituted phenyl). The most active compounds exerted MIC (Minimum Inhibitory Concentration) ranging from 1.98 to 7.81 $\mu g.mL^{-1}$ and are highly selective towards *Mtb* H37Ra and *Mtb* H37Rv inhibition (over other mycobacterial and bacterial strains). The final compounds were also studied for cytotoxicity on HepG2 cell line followed by SAR. Title compounds were also studied as potential inhibitors of (human) prolyl-tRNA synthetase based on their structural resemblance to confirmed inhibitors reported in the literature.[1]



Scheme 1. Synthesis of final compounds
a) Acyl chlorides/Pyridine/Ar Medium ; b) 2M Ammonia in EtOH ; c) KOH, H_2O, DMSO

The study was supported by the Charles University, project GA UK No. 349721, project Grant Schemes at CU (reg. no. CZ.02.2.69/0.0/0.0/19_073/00169359) and project SVV 260 547.

References

1. ADACHI, R., OKADA, K., SKENE, R., *et al.*: Biochem. Biophys. Res. Commun., 488, 2017, 393–399.

SC-7

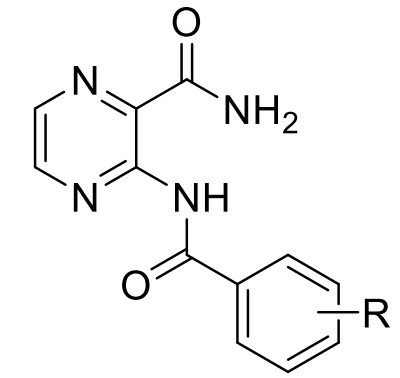
SYNTHESIS OF HYDROPHILIC SUBSTITUTED 3-BENZAMIDOPYRAZINE-2-CARBOXAMIDES AND DOCKING INTO HOMOLOGY MODELS OF MYCOBACTERIAL PROLYL-TRNA SYNTHETASE

KERDA, M.,[1] ZITKO, J.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

kerdam@faf.cuni.cz

This work relates to the effort to synthetize novel inhibitors of prolyl-tRNA synthetase (PRS). Inhibition of such an enzyme could have large scale of use including antimicrobial therapy. The scaffold is based on previously reported inhibitor of human prolyl-tRNA synthetase (hPRS).[1] Compounds with intended structure (R= 4-Cl, 4-Br) showed good antimycobacterial activity (MIC = 1.95 μg/ml *M. tuberculosis* H37Rv) and no significant *in vitro* cytotoxicity (HepG2). Based on the recent confirmation that such compounds bind to ATP site of hPRS[2], we suggest that title compounds could have better affinity to mycobacterial PRS. We used online homology modelling tools to determine any possible differences between mycobacterial and human version of the enzyme. We used SWISS-MODEL Interactive Workspace (expasy.org) and PHYRE2 Protein Fold Recognition Server (ic.ac.uk) for creating of the homology models. Final models were achieved by various refinement tools and compared with model created by AlphaFold Protein Structure Database (ebi.ac.uk). Molecular docking was performed to conclude achieved results.



R = 4-OH, 4-NH_2, 2-OH

The study was supported by the Charles University, project GA UK No. 349721 and by Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

References:

1. ADACHI, R., OKADA, K., SKENE, R., *et al.*: Biochem. Biophys. Res. Commun. 488(2), 2017, 393–399.
2. PANG, L., WEEKS, S.D, *et al.*.: Int. J. Mol. Sci., 22, 2021, Article Number 7793.

SC-8

STRUCTURAL CHARACTERIZATION AND STABILITY OF PROTEINS IN SOLID FORMS

BOLJE, A.,[1] GOBEC, S.[1]

[1] The Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Slovenia

e-mail: aljosa.bolje@ffa.uni-lj.si

Protein therapeutics are becoming increasingly important as an alternative treatment for a variety of diseases. For better stability, proteins are often formulated as solid dosage forms. Their stability depends on the preservation of the native structure of the proteins. Proteins are exposed to various stress factors that, in combination with the excipients, can affect the protein structure in the final solid form. If the native structure of the protein is not maintained, this may be reflected in an unstable final pharmaceutical product and consequently in its quality, safety and efficiency. Characterization of proteins in solid form is less established, as most analytical methods evaluate critical properties in solution, which is not necessarily indicative of adequate stabilization of the protein in the solid phase and thus long-term stability of the pharmaceutical form. In addition to structural characterization, monitoring protein aggregation is also very important. Together with denaturation and surface adsorption, aggregation can affect the activity and stability of proteins.[1] In this work, the study of protein structure and stability in solid dosage forms using analytical methods such as FTIR, NIR, Raman, solid-state fluorescence, solid-state UV-Vis and solid-state NMR spectroscopy, as well as circular dichrosim, DSC and X-ray powder diffraction is presented. Aggregation phenomena were also studied by size exclusion chromatography and dynamic light scattering.[2]

The study was supported by the Ministry of Education, Science, and Sport, Republic of Slovenia (Grant 5442-1/2018/405 to A.B.) and the Slovenian Research Agency (research core financing P1-0208).

References

1. BOLJE, A., GOBEC, S.: Pharmaceutics, 13, 2021, 534–566.
2. BOLJE, A., *et al*.: (unpublished results), 2021.

SC-9

PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERISATION OF NOVEL DERIVATIVES CONTAINING *N*-ARYLPIPERAZINE FRAGMENT

ČURILLOVÁ, J.,[1] PECHÁČOVÁ, M.,[1] PECHER, D.,[2] JAMPÍLEK, J.,[3] POSPÍŠILOVÁ, Š.,[3] MICHNOVÁ, H.,[3] MALÍK, I., [1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovak Republic

[2]Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy,Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovak Republic

[3]Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic

e-mail: curilova2@uniba.sk

The influence of selected physico-chemical and biological characteristics of two pharmaceutically important moieties, namely trifluoromethyl group and variously substituted *N*-arylpiperazine scaffold had been studied in the series of original hybrid molecules 3-[4-(Substituted)phenyl-/4-(diphenylmethyl)phenylpiperazin-1-yl]-2-hydroxypropyl-1-[(3-/4-trifluoromethyl)phenyl]carbamates and their salts. The lipophilicity of novel compounds containing the trifluoromethyl group in the 3-/4- position and of the phenyl ring and substituted *N*-arylpiperazine in the salt-forming part of molecule was examined both experimentally and by *in silico* methods. The selected molecules containing both pharmacophore fragments were tested *in vitro* against *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *M. tuberculosis* H37Ra/ATCC 25177, *M. kansasii* DSM 44162, *M. smegmatis* ATCC 700084, *C. albicans* CCM8186, *C. parapsilosis* CCM 8260, and *C. krusei* CCM 8271. 1-[{(3-trifluoromethyl)phenyl}carbamoyloxy-2-hydroxypropyl]-4-(diphenylmethyl)piperazin-1-ium chloride was the most effective against all the screened mycobacterial strains (MICs ranged from 3.64 to 14.5 μM).

The study was supported by the projects FaF-UK/30/2021 and UK/77/2021

References

1. POSPISILOVA, S., MALIK, I., CURILLOVA, J., *et al.*: Bioorganic Chemistry 102, 2020 104060.

SC-10

DESIGN, SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION OF NEW BORONIC ACIDS DERIVATIVES

ŠLECHTA, P.,[1] KUČEROVÁ-CHLUPÁČOVÁ, M.,[1] JANĎOUREK, O.,[2] KONEČNÁ, K.,[2] DOLEŽAL, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: slechtape@faf.cuni.cz

The presented compounds follow up the research of previously studied series of hybrid compounds, that exerted high *in vitro* antimycobacterial activity. They were designed as hybrids combining pyrazinamide and *para*-aminosalicylic acid.[1] The current study is focused on combination of pyrazinamide with 4-aminophenylboronic acid, bioisoster of *para*-aminobenzoic acid, that is crucial precursor in folate pathway. Bioisosteric replacement of the carboxylic group with boronic acid could afford reversible covalent bonds towards the potential targets in microorganisms.

The compounds were synthesized by condensation of 4-aminophenylboronic acid pinacol ester with variously substituted heteroaromatic acids that underwent the previous activation. The subsequent deprotection of boronic acid pinacol ester proceeded smoothly to afford novel compounds.[2]

The synthetic products and the isolated condensation intermediates were subjected to biological *in vitro* screening against fungi and bacteria, including mycobacteria. Some of the compounds showed moderate antimycobacterial activity.

The study was supported by SVV 260 547. Supported by Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

References

1. ZITKO, J., SERVUSOVA, B., PATEROVA, P., *et al.:* Molecules, 18, 2013, 14807–14825.
2. HINKES, S. P. A., KLEIN, C. D. P.: Org. Lett., 21, 2019, 3048–3052.

SC-11

SYNTHESIS AND ANALYSIS OF BIDENTATE SCHIFF BASES AS POTENTIAL LIGANDS FOR NEW METAL COMPLEXES

OBOŇOVÁ, B.,[1] HABALA, L.,[1] HERICH, P.,[1,2] VALENTOVÁ, J.,[1]

[1] Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia

[2] Institute of Physical Chemistry and Chemical Physics, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

e-mail: obonova@fpharm.uniba.sk

Chemistry of Schiff base ligands and their reduced homologues is still gaining increased attention due to their convenient and straightforward synthetic methods and a wide range of complexation abilites with almost all types of metal ions. Schiff bases and their metal complexes are widely used in industrial field. Recent studies are also showing their promising biological properties, including antibacterial, antifungal, antiproliferative, antioxidant and antivirotic activities.[1, 2, 3] This project presents synthesis of four bidentate Schiff bases and their reduced homologues obtained from condensation reaction of 1,2-cyclohexanediamine and substitued benzaldehydes (4-fluorobenzaldehyde, 4-trifluorobenzaldehyde, 3,5-difluorbenzaldehyde and 3,5-bis-trifluorbenzaldehyde) . The structural features of the synthetized compounds were confirmed by ^{1}H and ^{13}C NMR, infrared and electronic spectroscopic methods and by elemental analysis. Some of the prepared bases were also analyzed by single crystal X-ray crystallography.

The study was supported by Grant from Pharmaceutical faculty (FaF UK/16/2021).

References

1. LIANG, J., SUN, D., YANG, Y., *et al.:* Eur. J. Med. Chem., 224, 2021, https://doi.org/10.1016/j.ejmech.2021.113696
2. UDDIN, M. N., AHMED, S. S., ALAM, S. R.: Journal of Coordination Chemistry, 73, 2020, 3109–3149
3. TADELE, K.T., TSEGA, T. W.: Anti-Cancer Agents in Medicinal Chemistry, 19, 2019, 1786–1795

SC-12

SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SELECTED *N*-HYDROXYCINNAMAMIDE DERIVATES

ONUSCAKOVA, M.,[1] PIZOVA, H.,[1] KAUEROVA, T.,[2] KOLLAR, P.,[2] BOBAL, P.,[1]

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

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Masaryk University, Czech Republic

e-mail: 507453@muni.cz

Histone deacetylases (HDACs) have been long linked with tumor progression, demonstrating their important role in neoplasia. Inhibitors of HDAC (HDACI), which form a complex with Zn^{2+} ions in the active site of enzymes, are novel anticancer agents that induce tumor cell death, differentiation, and/or cell cycle arrest. Zinc-dependent HDAC subtypes display expressions in different cancer types and considerably support oncogenic cell transformation. Therefore, we believe that they are interesting anticancer drug targets. We have designed and synthesized a series of *N*-hydroxycinnamamide derivates based on common HDAC`s pharmacophore with diversely substituted anilides (Fig.1). These new promising HDACIs have a zinc-binding group, which is a hydroxamic acid group. For preliminary investigation of antiproliferative activity, selected monocytic leukemia cell line THP-1 was used. The inhibitory activity of compounds was evaluated by WST-1 analysis. As the positive control in the screening, Vorinostat® (SAHA) was used - the first registered HDACI.

Derivatives with substituents like bromine, fluorine, methyl, or trifluoromethyl group on anilide exhibit significant antiproliferative activity. The most potent compounds from the tested series were the compounds with a methyl substituent on the aromatic ring of anilide with a value IC_{50} <2 μmol/l (SAHA IC_{50} <1 μmol/l).

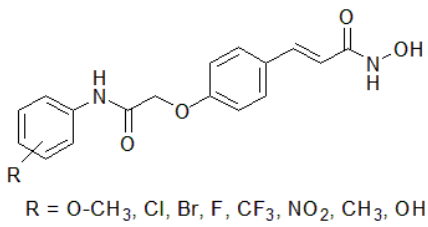


Figure 1. The structure of *N*-hydroxycinnamamide derivates

The study was supported by grants MUNI/A/1682/2020 and MUNI/A/1598/2020.

SC-13

SYNTHESIS OF SCHIFF BASES WITH POTENTIAL THERAPEUTIC ACTIVITY

BALLAYOVÁ, V., ZUBÁČ, P., ŽÁČKOVÁ, R., FARSA, O.

Department of Chemical Drugs, Faculty of Pharmacy in Brno, Masaryk University, Czech Republic

e-mail: 516259@mail.muni.cz

The Schiff bases exhibit a wide range of biological activities including widespread application of their metal complexes due to their interesting physical and chemical properties. The main aim of this research is synthesis of basic thiosemicarbazone and semicarbazone derivatives of acetophenone (Fig. 1). The therapeutic importance is focused on antituberculotic effect, as well as an inhibitory effect on the enzyme aminopeptidase N (APN). Studied compounds show significant similarity with thiacetazone, second-line drug used to treat tuberculosis. Desired products are formed by three-step synthesis using aminoacetophenone as a starting material. The reactions are focused on preparation of Schiff bases - thiosemicarbazone derivatives of acetophenone and semicarbazone derivatives of acetophenone. The most promising compounds undergo testing on *Mycobacterium smegmatis* CCM 4622. The significant values of minimal inhibitory concentration (MIC) exhibit compounds with heterocyclic amine with saturated heterocyclic skeleton such as piperidine or pyrrolidine. The synthetized compounds were also evaluated as potential inhibitors of APN, using optical photometric method of absorbance in the visible and ultraviolet region at wavelength of 405 nm. The highest inhibitory activity showed a compound with N-methylpiperazine fragment.

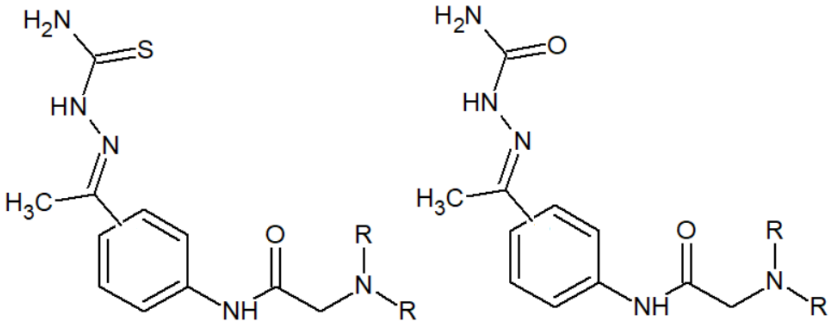


Figure 1. Thiosemicarbazone and semicarbazone derivatives of acetophenone

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

SC-14

HYBRID COMPOUNDS CONTAINING 4-AMINOSALICYLIC ACID AS POTENTIAL ANTITUBERCULOTICS

NAWROT, D., BOUZ, G., ZITKO, J., DOLEŽAL, M.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: nawrotd@faf.cuni.cz

Tuberculosis, caused by *Mycobacterium tuberculosis*, is the leading cause of death worldwide from a single infectious organism and a major threat to public health due to growing antimicrobial resistance.[1] Tuberculosis was effectively treated with first-line anti-TB drugs; however, due to the raising antimicrobial resistance, newer approaches to eradicate the disease are needed.

4-Aminosalicylic acid is a second line agent for tuberculosis. In presented series we attempted hybrid compounds bearing this moiety. Title compounds are based on positional derivatives of picolinic acid linked to 4-aminosalicylic acid or 4-aminobenzoic acid by amidic bond. Compounds were tested for biological activity against selected strains of *Mycobacterium* (*M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra, *M. kansasii*, *M. avium*, *M. smegmatis, M. aurum)*. The minimum inhibitory concentration (MIC) for tested mycobacterial strains was determined for all tested compounds beside isoniazid, ciprofloxacin and rifampicin as a reference. Results of the biological testing and structure-activity relationships are discussed in the presentation.

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 547) and EFSA-CDN (Grant CZ.02.1.01/0.0/0.0/16_019/0000841) cofunded by ERDF (Dr. Ghada Bouz).

References

1. World Health Organization, Global Tuberculosis, Report 2020. www.who.int/tb/publications/global_report/en/ accessed: 15.08.2021

SC-15

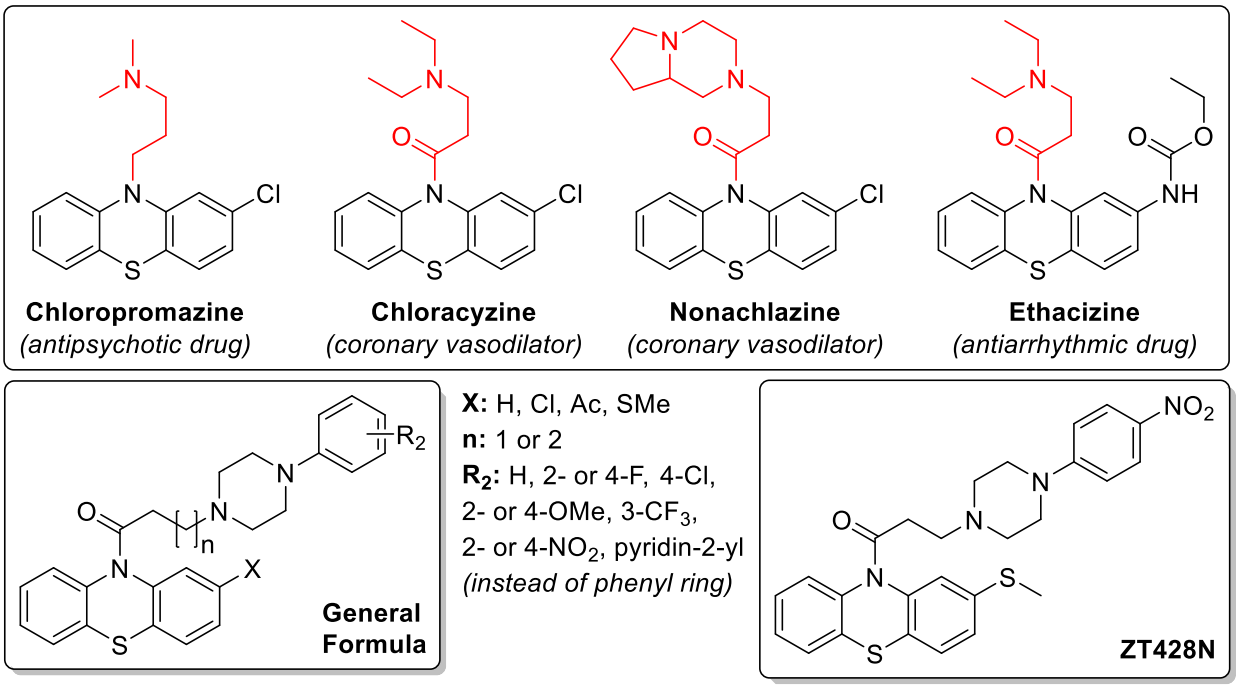
DESIGN, SYNTHESIS AND STRUCTURE-BASED OPTIMIZATION OF POTENTIAL PHENOTHIAZINE ANTIMICROBIALS

ZUBAC, P.,[1] FARSA, O.,[1]

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

e-mail: peter.zubac@gmail.com

Tuberculosis and related infections (e.g. Mycobacterium avium complex) are of grave importance for recent antimicrobials drug discovery. Inhibition of NADH dehydrogenase type II (NDH-2) seems to be more than suitable, as this enzyme is absent in mammalian mitochondria, so only (myco)bacteria will be prevented from respiration. Phenothiazines (PHTZs), commonly known as major tranquilizers and antipsychotics, can be used as NDH-2 inhibitors (especially thioridazine seems to be very potent), but their use as anti-TB drugs is currently limited by their potent neuroleptic and sedative effects.



This study aims to remove these unwanted CNS-based effects through some structural modifications. Replacement of the commonly used N,N-dimethylaminopropyl side chain with a more complex one - phenylpiperazine-based acyl group (e.g. aromatic-ring-substituted 3-(4-phenylpiperazin-1-yl)propanoyl moiety) will lead to the ultimate loss of dopaminergic receptor affinity, responsible for their antipsychotic and sedative properties. Known examples of this loss-of-effect are ethacizine and

moracizine (antiarrhythmic drugs) or chloracyzine and nonachlazine (coronary vasodilators) - all without unwanted CNS effects. Derivatives containing phenylpiperazine moiety are a subject of extensive anti-TB drug research at our institute, but also at the Faculty of Pharmacy in Hradec Králové, Czech Republic, and Bratislava, Slovakia, so they were chosen as a convenient alternative to N,N-dialkylamino group. From all possible modifications of the side chain, the 3-[4-(**4-nitrophenyl)piperazin**-1-yl]**propanoyl** moiety appeared to be one of the most promising, but the research is still in progress.

The study was supported by IGA VFU Brno 312/2019/FaF.

References

1. NIZI, M. G., DESANTIS, J., NAKATANI, Y., *et al.*: Eur. J. Med. Chem., 201, 2020, 112420.

SC-16

ANTIMICROBIAL ACTIVITY OF SEMISYNTHETIC DERIVATIVES OF MONTANINE-TYPE ALKALOIDS

MAAFI, N.,[1] KONEČNÁ, K.,[2] JANĎOUREK, O.,[2] DIEPOLTOVÁ, A.,[2] CAHLÍKOVÁ, L.,[1]

[1]ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: negarm@faf.cuni.cz

The Amaryllidaceae plant family is one of the most important alkaloid containing plant families with potent biological properties such as: antitumor, antimicrobial, antimalarial, and significant antineurodegenerative activities. Among all Amaryllidaceae alkaloids, montanine-type alkaloids are characterized by 5,11-methanomorphanthridine ring system and are known for their potential antiproliferative, antimalarial, antirheumatic, anticholinesterase and most recently for their antimicrobial activity. [1] Montanine itself, demonstrated activity against pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermis*, giving values of 5, 20, 5 and 15 µg respectively, as minimum quantities for inhibitory activity.[2]

In this study, 22 new derivatives of montanine-type alkaloids were synthesized and evaluated for their antibacterial and antimycobacterial activity on a panel of four Gram-positive, four Gram-negative and 3 avirulent strains of mycobacterium (*Mycobacterium smegmatis*, *M. aurum* and *M. tuberculosis* H37Ra). Among all, 4 derivatives demonstrated significant activity against *Klebsiella pneumoniae* with MIC less than 0.031 $mg.ml^{-1}$ and 3 derivatives showed MIC less than 0.008 $mg.ml^{-1}$ on all tested strains of *mycobacterium*.

The study was supported by SVV 260548 and 260549 project.

References

1. KOUTOVÁ, D., MAAFI, N., HAVELEK, R., *et al.*: Molecules, 25, 2020, 2337.
2. CASTILHOS, T.S.; GIORDANI, R.B.; HENRIQUES, A.T.; *et al.*: Rev. Bras. Pharmacogn., 17, 2007, 209–214.

SC-17

INDOLE ALKALOID FROM *VINCA MINOR* L. WITH PROMISING ACTIVITY AGAINST ALZHEIMER'S DISEASE

VRABEC, R.,[1] MARIKOVA, J.,[2] KORABECNY, J.,[3] HULCOVA, D.,[1] LOCAREK, M.,[1] KUCERA, T.,[3] JUN, D.,[3] HRABINOVA, M.,[3] HAVELEK, R.,[4] SOUKUP, O.,[4] ANDRISANO, V.,[5] OPLETAL, L.,[1]

[1] Department of Pharmaceutical Botany, ADINACO Research Group, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

[4] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

[5] Department of Life Quality Studies, University of Bologna, Rimini, Italy

e-mail: vrabecr@faf.cuni.cz

Based on our previous research, alkaloids of *Vinca minor* L. possess a selective inhibition activity against human butyrylcholinesterase (*h*BuChE), a less known but crucial enzyme in the pathology of Alzheimer's disease (AD). One of the compounds, namely 2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]]-1*H*-indole, isolated from this species for the first time, exerted unusual inhibitory *h*BuChE activity (IC_{50} 0.65 μM). The alkaloid also exhibited a good inhibition of prolyloligo-peptidase (IC_{50} 58 μM), another enzyme involved in AD's pathogenesis. These results led us to further examination. The enzyme kinetics study revealed the binding mode to the active site of the *h*BuChE to be as reversible competitive, while *in silico* simulations, such as molecular docking and dynamics, clarified the binding pose. Parallel artificial membrane permeability assessment *in vitro* predicted this compound's ability to penetrate the blood-brain barrier by passive diffusion. This alkaloid also tentatively seemed non-cytotoxic, as showed by a cytotoxicity test on the panel of ten tumorous cell lines. Since this structure can be prepared synthetically, these compelling results support the future exploration of potentially better analogues.

This study was supported by projects SVV 260 548, Progres Q42, InoMed, Czech Science Foundation project No. 20-29633J, Long-term development plan (Faculty of Military Health Sciences), and by University of Hradec Kralove (Faculty of Science, no. VT2019-2021).

SC-18

DETECTION AND CHARACTERIZATION OF TRIMETAZIDINE METABOLITES IN HUMAN URINE BY LC-MS/MS

CHOMANIČOVÁ, K.,[1] ALECHAGA, E.,[2] TERRERO, A.,[2] VENTURA, R.,[2]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy in Bratislava, Comenius University, Slovak Republic

[2] Catalonian Anti-Doping Laboratory, Doping Control Research Group, Institut Hospital del Mar d'Investigacions Mèdiques in Barcelona, Spain

e-mail: chomanicova1@uniba.sk

Trimetazidine (TMZ), well known anti-anginal drug, has been since January 2014 added to the World Anti-Doping Agency (WADA) Prohibited List as a substance prohibited in competition.[1] Also, since 2016 guidelines for the diagnosis and treatment of acute and chronic heart failure have established the position of trimetazidine as a new therapeutic strategy for the management of patients with angina and heart failure with reduced ejection fraction.[2] In the present study, the application of LC-MS/MS allowed us to identify trimetazidine metabolites in human urine. After a single dose of trimetazidine were analysed urine samples of one healthy volunteer at baseline and four time intervals. Accurate mass of possible protonated / deprotonated ions of trimetazidine metabolites from the created database was used to look for possible candidates in the full-scan data of the post-administration samples that were not present in the basal samples. Full-scan spectra and product ion scan spectra at two collision energies of detected possible metabolites were acquired and as a major metabolite was observed intact trimetazidine. Moreover, five minor metabolites have been observed, and one of them was up to now unreported. Considering the detector response in MS analysis to be proportional to the metabolite concentrations, pharmacokinetic curves were constructed by comparing the chromatographic peak areas associated with each *m/z* value of detected metabolites with their maximum peak area.

The study was supported by the Comenius University Grant no. UK /94/2021

References

1. WADA: International standard, 2014 Prohibited list, Montreal, 2014
2. LOPATIN, Y.: Heart metab., 71, 2016, 23-26.

SC-19

REAL-TIME MONITORING IN SEQUENTIAL INJECTION SYSTEM

SKLENÁŘOVÁ, H.,[1] HORSTKOTTE, B.,[1] PÁVEK, P.,[2] MIRÓ, M.,[3] SOLICH, P.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] FI-TRACE Group, Department of Chemistry, University of Balearic Islands, Carretera de Valldemossa Km 7.5, 07122, Palma de Mallorca, Spain

e-mail: sklenarova@faf.cuni.cz

Sequential injection analysis (SIA) can be easily applied to handle samples taken from various long-term processes in a dynamic mode together with on-line analysis in a fully automated closed system. Applications of such as monitoring in our research group involve determination in *real-time* of dissolution/release of active substances from pharmaceutical formulations (including nanoparticles[1]) and release of active substances from nanofibers used as a support to deliver substances for therapeutic reasons on human skin in the area of pharmaceutical analysis. Another field relates to *on-line* monitoring of the interaction of a luminescent marker (single or in combination with inhibitors) with cell membrane transporters[2,3] in the field of toxicological/pharmacological studies. These studies are based on evaluation of the permeation of a fluorescent marker (Rhodamine 123) or of secreted luciferase as a chemiluminescent marker. These applications will be presented and discussed in detail.

This work was supported by the project STARRS reg. No.: CZ.02.1.01/0.0/15_003/0000465 co-funded by ERDF and project CTM2017-84763-C3-3R (AEI/MICINN/FEDER) from the Spanish State Research Agency/Spanish Ministry of Science and Innovation.

References

1. ALVES, A.C., RAMOS, I.I., NUNES, C., *et al.*: Talanta, 146, 2016, 369-374.
2. ZELENÁ, L., MARQUES, S.S., SEGUNDO, M.A, *et al.*: Anal. Bioanal. Chem., 408, 2016, 971-981.
3. SKLENÁŘOVÁ, H., ROSECKÁ, M., HORSTKOTTE, B. *et al.*: Anal. Chim. Acta, 1153 2021, 338296.

SC-20

THE APPLICATION OF GAS CHROMATOGRAPHY FOR DETERMINATION OF RESIDUAL SOLVENTS IN MELATONIN

KASSAB, J., AMIĆ, A.

Department of Chemistry, Josip Juraj Strossmayer University of Osijek, Croatia

e-mail: aamic@kemija.unios.hr

Melatonin is a hormone secreted by the pineal gland and has many various effects on the body, hence it is used as an active component in various pharmaceutical products. Even though many exogenous sources of melatonin can be found, the concentration of melatonin in them is usually low, so synthetic melatonin is used in the preparation of before mentioned pharmaceutical products. One of major problems with synthetized compounds are residual solvents, which may be dangerous to health. Therefore, the main goal of this work was to develop and validate a HSS-GC-FID method for determination of residual solvents (methanol, 2-propanol, *tert*-butyl methyl ether and ethyl acetate) in synthetized melatonin. Key validation parameters were selectivity, linearity, range, precision, accuracy, stability, and limits of detection and quantification. Method has been validated in accordance with ICH guidelines[1,2] and quantification and detection limit for all studied solvents was determined. Results of validation experiments confirmed this method as highly selective, linear in working range, accurate and precise. Standard and sample solutions were stable for two days at room temperature and in the fridge. Since all conditions for validation were met, it can be concluded that the developed HSS-GC-FID method for the determination of residual organic solvents in melatonin raw material is suitable for its intended purpose.

The study was supported by Yasenka d.o.o.

References

1. ICH, Impurities: guideline for residual solvents Q3C(R6), ICH Harmonised Guideline, 2016.
2. ICH, Validation of analytical procedures: Text and methodology Q2(R1)), Current Step 4, ICH Harmonised Tripartite Guideline, 2005

SC-21

CHIRAL SEPARATIONS OF DICARBA-7,8,-*NIDO*-UNDECABORANE AND COBALT BIS(DICARBOLIDE) DERIVATIVES

HORÁČEK, O.[1], NOVÁKOVÁ, L.[2], KUČERA, R.[1]

[1]Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: horaceko@faf.cuni.cz

Boron clusters are artificial three-dimensional structures, which exhibit unique physicochemical properties. Carboranes as a subgroup of boron cluster compounds were derived by substituting BH units for CH units. Initially, the similar steric volume occupied by a rotating phenyl ring and an icosahedral carborane cage has led to the extensive research of carborane moieties as new pharmacophores. Recently, new potential antitumor-drugs containing carborane derivatives dicarba-7,8-*nido*-undecaborane[1] and cobalt bis(dicarbollides)[2] have been prepared, and their activities have been tested.

Chirality of carboranes is caused by introducing endo-/exoskeletal substituents, which impair the symmetry of the cage. Hence, it is vital to investigate analytical methods for chiral separations of carboranes concerning their potential as drugs. The successful chiral separations of neutral, zwitterionic, and anionic carborane derivatives were achieved in CE by beta- and alfa-cyclodextrins. In contrast, the anions, especially dicarba-7,8-*nido*-undecaborane(1-) derivatives, proved to be challenging analytes in HPLC. Recently, we have been able to enantioseparate these anions in HPLC on beta-cyclodextrins for the first time.[3] To achieve faster analyses than in HPLC, we tested to employ SFC. Our study aims to develop suitable CE, HPLC, and SFC methods for chiral separations on an analytical scale using cyclodextrins, polysaccharide derivatives, and quinidine/quinine derivatives. The results will be a basis for the purity assessment methods and analyses on a preparative scale.

This work was supported by the Charles University (GA UK 168 120 and SVV 260 547) and STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/ 0000465).

References

1. DVORANOVA, A., KUGLER, M., HOLUB, J., *et al.*: Eur. J. Med. Chem., 200, 2014.

2. GRÜNER, B., KUGLER, M., EL ANWAR, S., *et al.*: ChemPlusChem, 352, 2021.

3. HORÁČEK, O., PAPAJOVÁ-JANETKOVÁ, M., GRÜNER, B., *et al.*: Talanta, 222, 2021.

SC-22

ANALYTICAL AND SEMIPREPARATIVE SEPARATION OF CHIRAL PHARMACEUTICALS

KURKA, O., KUCERA, L., BEDNAR, P.

Department of Analytical Chemistry, Faculty of Science, Palacky University in Olomouc, Czech Republic

e-mail: ondrej.kurka@upol.cz

Separation of chiral pharmaceuticals is critical when assessing formulations with different activity of individual stereoisomers. Also, active pharmaceutical ingredients consisting of individual enantiomers can be prepared using chromatographic isolation from a racemic mixture. For this purpose, polysaccharide coated chiral stationary phases (CSPs) were used to separate pharmaceuticals of different classes (i.e. antifungals, calcium channel blockers) both in analytical and in semipreparative scale. Different modes of separation (i.e. polar organic, reverse phase, normal phase) can be utilized based on the specific combination of the analyte and the chiral selector of the CSP and a two-step separation can be utilized in case of compounds with more chiral centers[1].

Another crucial part of analysis of chiral compounds is identification of individual enantiomers. Aside from costly approaches enabling determination of absolute configuration, such as nuclear magnetic resonance and X-ray diffraction, determination of the optical rotation sign can be sufficient in some cases. In HPLC, this task can be achieved online using a flow-through optical rotation detector. However, the magnitude of the signal may vary based on the nature of the analyte and on the mobile phase. A flow-injection analysis study of previously isolated enantiomers of a chiral pharmaceutical (felodipine) proves different mean peak areas are obtained using an optical rotation detector depending on the solvent used[2].

References

1. KURKA, O., KUCERA, L., BEDNAR, P.: J. Sep. Sci., 39, 2016, 2736–2745.
2. ZAMPACHOVA L.: Studium chirality s využitím výpočetní chemie a chromatografického experimentu; Diploma Thesis, Palacky University, 2019, Olomouc.

SC-23

COMPLEX SCREENING OF RETENTION BEHAVIOUR OF CATECHOLAMINES

HEBLÍK, D., KASTNER, P., KUČERA, R.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: heblikd@faf.cuni.cz

Nowadays, in the light of relatively new information about the effect of serotonin and its metabolites level on fetal brain development,[1] the question arises as to whether catecholamines also affect fetal development. Despite many published methods, the determination of catecholamines is still a challenge due to their chemical nature and low concentrations in complex biological materials.[2]

As part of the development of an HPLC method for the determination of catecholamines in placental tissue, the screening of retention behaviour of tyrosine, L-DOPA, dopamine, noradrenaline, and adrenaline is presented. The most used chromatographic system in the determination of catecholamines is a reversed phase chromatographic system. This screening includes the retention behavior of catecholamines using eight mobile phases with different compositions and pH, on commercially available stationary phases (C18, C18-PFP, PFP and others). Detection of catecholamines is based on their absorption in the UV range and their fluorescence. This screening further describes the behaviour of catecholamines under HILIC conditions focusing on different concentration of organic solvent.

References

1. BONNIN, A., LEVITT, P.: Neuropsychopharmacology, 37(1), 2012, 299–300.
2. GORBUNOVA, M. V., GUTOROVA, S. V., BERSENEVA, D. A., *et al.*: Applied Spectroscopy Reviews, 54, 2019, 631–652.

SC-24

DEVELOPMENT OF LC-MS METHOD FOR SIMULTANEOUS DETERMINATION OF DRUGS USED IN TREATMENT OF INFLAMMATORY BOWEL DISEASES

PECHER, D.,[1,2] MIKUŠ, P.,[1,2]

[1]Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia.

[2]Toxicological and Antidoping Center, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia.

e-mail: pecher1@uniba.sk

Thiopurines (TP) are therapeutic agents commonly used in the treatment of inflammatory bowel diseases (IBD). The treatment with these drugs is associated with low efficacy, high occurrence of adverse effects, and non-compliance in some patients. Monitoring of the drugs and their metabolites is often used to diminish these issues. This work represents the development of the LC-MS method for simultaneous determination of TP drugs used in IBD and their metabolites. The chromatographic behavior of azathioprine, 6-mercaptopurine, 6-thioguanine, 6-methylmercaptopurine, 6-methylthiopurine, and thiouric acid on various stationary phases was evaluated. Based on the obtained capacity factors, peak heights, and peak shapes the most suitable stationary phase was chosen - Accucore aQ column. Next, the mobile phase was optimized. For the aqueous part, various concentrations of ammonium bicarbonate (basic pH) and formic acid (acidic pH) were tested. For the organic part, the suitability of methanol and acetonitrile was evaluated. The basic pH of aqueous part provided ca. 2-3 times better peak heights than the acidic one. The mixture of 2.5 mM ammonium bicarbonate and methanol was chosen as the best compromise for optimal retention and sensitivity for all analytes. After the validation, the developed method is suitable for control of patient's compliance to thiopurine treatment or for therapeutic drug monitoring of TP and their metabolites, which after the implementation to clinical practice can lead to optimization of the IBD therapy.

The study was supported by projects APVV-15-0585, VEGA 1/0463/18, KEGA 027UK-4/2020, and FaF UK/31/2021. The analytical experiments were carried out in the Toxicological and Antidoping Center at the Faculty of Pharmacy Comenius University in Bratislava.

SC-25

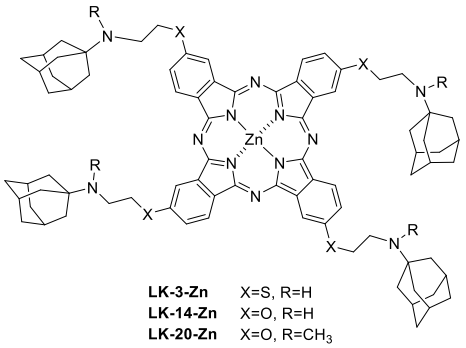
IMPROVING MONOMERIZATION OF PHTHALOCYANINES IN WATER BY CREATING SUPRAMOLECULAR COMPLEX WITH CUCURBITURIL

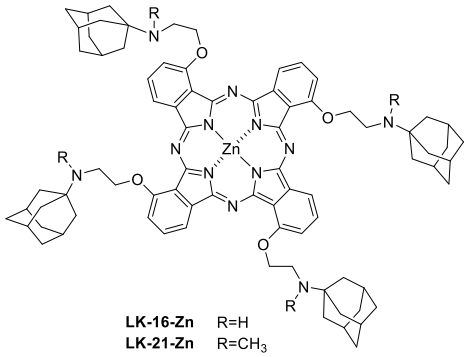
KOČIŠČÁKOVÁ, L., ZIMČÍK, P.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: kociscal@faf.cuni.cz

Phthalocyanines (Pcs) are macrocyclic compounds which are used e.g., as photosensitizers in photodynamic therapy. Because of their planar structure they tend to form aggregates and then lose their desired photochemical and photophysical properties. Formation of supramolecular complexes with cucurbituril (CB, macromolecules composed of methylene bridged glycoluril oligomers[1]) should improve those properties. One of the strongest interaction reported is between CB[7] (cucurbituril with 7 monomers) and aminoadamantane.[2] Five zinc Pcs, peripherally or non-peripherally substituted with aminoadamantane, were prepared. Their absorption spectra were studied and they showed substantially improved (but not complete) monomerization in water after addition of four equivalents of CB[7]. This ratio confirms previously obtained crystallographic structure and computer model of phthalonitrile precursor, where one molecule of aminoadamantane is complexed by one molecule of CB[7].





The study was supported by SVV 260 547 and Czech Science Foundation (20-09212S).

References

1. WIEMANN, M.; JONKHEIJM, P.: Isr. J. Chem. 58 (3-4), 2018**,** 314-325.
2. LIU, S. *et al.*: J. Am. Chem. Soc. 127, 2005, 15959-15967.

SC-26

CATIONIC VERSUS ANIONIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY: WHAT A DIFFERENCE THE CHARGE MAKES

KOLLAR, J.[1], MACHACEK M.[2], HALASKOVA M.[2], DEMUTH J.[1], ROHLICKOVA M.[2], NOVAKOVA V.[1], ZIMCIK P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: kollarj@faf.cuni.cz

Phthalocyanines (Pcs) and their aza-analogues represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area over 600 nm and strong singlet oxygen production) that can be used as photosensitizers in photodynamic therapy (PDT) of cancer. The literature reports on anionic and cationic Pcs for PDT suggest systematically significant differences in their photodynamic activity.

The aim of this work was to find the parameters and/or factors that may contribute to the substantial difference in photodynamic activity between zinc(II) Pcs bearing opposite charges on peripheral substituents (carboxylate functions or quaternary nitrogens). Four different sets of compounds were introduced into the study, namely anionic hydrophilic, cationic hydrophilic, anionic amphiphilic and cationic amphiphilic to compare both the influence of the charge type and its distribution on the macrocycle core. All Pcs were tested on photodynamic activity *in vitro* on HeLa cells with different activity for anionic Pcs (EC_{50} ~ 0.3-10 μM) and cationic Pcs (EC_{50} ~ 3-50 nM). The effect of pH, binding to serum proteins, interaction with biomembranes, subcellular localization and relocalization after irradiation were discovered to be the main factors responsible for lower photoactivity of anionic Pcs.[1]

The work was supported by Czech Science Foundation (19−14758Y), Charles University (projects SVV 260 547 and PRIMUS/20/SCI/013),

References

1.KOLLAR, J., MACHACEK, M., HALASKOVA, M., *et al.*: J. Med. Chem., 63, 2020, 7616−7632.

SC-27

SEMISYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

PERINOVA, R.,[1] KOHELOVA. E.,[1] SPULAK, M.,[2] MARIKOVA, J.,[2] HULCOVA, D.,[1] CAHLIKOVA, L.[1]

[1] ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Bioorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

e-mail: perinovr@faf.cuni.cz

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology and data indicate an exponential rise in the number of cases of this disease. The well-known Amaryllidaceae alkaloid (AA) galanthamine is marketed drug for AD therapy under the commercial name Reminyl© (galanthamine hydrobromide).

Studies also pointed out various pharmacological properties of semisynthetic derivatives of some AA, such as alkaloid haemanthamine (HMT), which is widely distributed through Amaryllidaceae plants. Based on our previous results, where several HMT derivatives demonstrated promising *h*AChe/*h*BuChe inhibition potency, we continued the preparation of further HMT semisynthetic derivatives.[1]

Several new esters and ethers showed interesting inhibition of both studied cholinesterases, thus structure-activity relationship (SAR) was also studied.[2] Newly prepared compounds were identified by 1D-, 2D- NMR and ESI-MS methods. The most active compounds were studied in more detail (e.g. type of inhibition, docking studies, logBB etc.).

This project was supported by Charles University grant SVV UK 260 548.

References

1. KOHELOVÁ, E., PEŘINOVÁ, R., MAAFI, N., *et al.*: Molecules, 24, 2019, 1307.
2. PEŘINOVÁ, R., MAAFI, N., KORÁBEČNÝ, J., *et al.*: Bioorganic Chemistry, 2020, 100.

SC-28

SYNTHESIS OF NOVEL 2-(PHENYLCARBAMOYL)NAPHTHALENE-1-YL ALKYLCARBAMATES POSSESSING EXCELLENT ANTIMICROBIAL ACTIVITY AGAINST MRSA

GONEC, T.,[1] MICHNOVA, H.,[2] KLEINEROVA, K.,[1] BUCKOVA, G.,[1] CIZEK, A.,[2] JAMPILEK, J.[3]

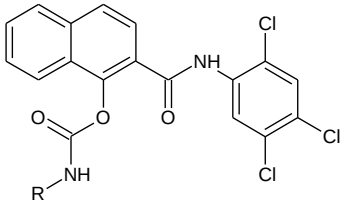
[1] Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University, Czech Republic

[2] Department of Infectious Diseases and Microbiology, Faculty of Veterinary Sciences, University of Veterinary Sciences Brno, Czech Republic

[3] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Slovakia

e-mail: *gonect@pharm.muni.cz*

Recently, we have synthesized novel series of *N*-phenylhydroxynaphthalene carboxamides possessing promising antimicrobial activities.[1] Introduction of alkylcarbamate moiety lead to enhancement of physical-chemical properties (especially of solubility) and the biological activity was preserved.[2] Series of 13 novel compounds (Picture 1) was synthesized and evaluated for antimicrobial activity. Preliminary results showed that all compounds have comparable or much better MIC than standards ciprofloxacin and ampicillin. Best compounds showed MIC in a range of few nM.



Picture 1 Studied compounds. R: ethyl-octyl; isopropyl; cyclopentyl-cycloheptyl; phenylethyl; phenylbutyl

The study was supported by MUNI/A/1682/2020

References

1. MICHNOVÁ, H., *et al.*: Molecules, 24, 2019, 2991
2. GONĚC, T., *et al.:* Molecules, 21, 2016, 1089

SC-29

PYRIDINIUM OXIMES WITH ORTHO-POSITIONED HALOGEN MOIETY ARE EFFECTIVE REACTIVATORS OF CHOLINESTERASES INHIBITED BY NERVE AGENTS

MUSILEK, K.,[1*] MALINAK, D.,[1] KOHOUTOVA, Z.,[1] PRCHALOVA, E.,[1] ANDRYS, R.,[1] SKARKA, A.,[1] ZORBAZ, T.,[2] KOVARIK, Z.,[2] REX, S.,[3] SUKUPOVA, M.,[3] HEGER, Z.[3]

[1] Department of Chemistry, Faculty of Science, University of Hradec Kralove, Czech Republic

[2] Institute for Medical Research and Occupational Health, Zagreb, Croatia

[3] Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic

*e-mail: kamil.musilek@uhk.cz

The cholinesterase antidotes (co called "oximes") are lifesaving antidotes in case of organophosphate intoxications (e.g. pesticide or nerve agent exposure). In about past two decades, our team has developed hundreds of charged oximes of which some were found to be very powerful acetylcholinesterase reactivators (e.g. K027 and K203).[1] The reactivation effect can be further modified by the presence of nucleophilic moieties (i.e. halogens) which influence the pK_a properties of the molecule and help to the formation of functional nucleophile. This phenomenon was proved by the chlorinated oximes as one of the most broad-spectrum reactivators of acetylcholinesterase or butyrylcholinesterase available to date (e.g. K868).[2]

However, the charged oximes are minimally penetrating to CNS. For this reason, the charged molecules can be encapsulated by apoferritins (apoFRTs) that are able to transfer and release them in the brain.[3] The engineered apoFRTs are currently under development together with fluorescent-labeled oxime reactivators that valuably allow to detect their deposition and release *in vitro* or *ex vivo*.

The study was supported by the Czech Science Foundation (no. 19-13628S), University of Hradec Kralove (no. SV2104-2021, VT2019-2021), Croatian Science Foundation (no. HRZZ-IP-2018-01-7683) and CEITEC2020 LQ1601.

References

1. GORECKI, L., SOUKUP, O., *et al.*: Arch. Toxicol. 93, 2019, 673-691.
2. ZORBAZ, T., MALINAK, D., *et al.*: J. Med. Chem. 61, 2018, 10753−10766.
3. TESAROVA, B., MUSILEK, K., *et al.*: J. Control. Release. 325, 2020, 176-190.

SC-30

TRANSITION METAL CARBOXYLATE COMPLEXES WITH BIOLOGICAL ACTIVITY

HABALA, L.,[1] VALENTOVÁ, J.,[1] PAŠKOVÁ, Ľ., [2] OBOŇOVÁ, B.[1]

[1] Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic

[2] Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic

e-mail: habala@fpharm.uniba.sk

Metal complexes with carboxylate ligands represent an interesting category of potentially bioactive compounds. Metal carboxylates exhibit a variety of coordination modes and a great structural diversity, with important implications for biology and pharmacy. They offer possibilities for the development of new metallopharmaceuticals, such as antimicrobial and anticancer agents. Among them, special importance can be attached to metal complexes of non-steroidal anti-inflammatory drugs (NSAIDs), due to the manifold biological activities of these compounds. E.g., the copper complex of indomethacin is an established medication with antiinflammatory properties used in veterinary medicine. On the other hand, amphiphilic complexes containing hydrophobic moiety are a category of substances with specific biological activity, capable of interacting with hydrophobic domains of DNA and proteins as well as with lipid membranes. Here we present examples from our work concerning metal complexes with NSAIDs (indomethacin), amphiphilic carboxylates (agaric acid) and various other carboxylate ligands.

The study was supported by the Scientific Grant Agency of the Slovak Republic, grant VEGA 1/0145/20, and Faculty of Pharmacy grant FaF UK/16/2021.

SC-31

DE NOVO DESIGN OF 2-AMINOOXAZOLES AS INHIBITORS OF BACTERIAL β-KETOACYL-ACYL CARRIER PROTEIN SYNTHASE III (FabH)

JUHÁS, M.,[1] PALLABOTHULA, V. S. K.,[1] NAWROT, D.,[1] HATOKOVÁ, P.,[1] DIEPOLTOVÁ, A.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: juhasm@faf.cuni.cz

Antibacterial drug resistance is one of the most critical health problems. The pathogens resistant to even last-resort antibiotics are increasing, and very few new potent antibacterial compounds have been introduced in recent years. Even the promising drug candidates are often discontinued in the primary stages of the drug discovery pipeline due to their unspecific reactivity (PAINS), toxicity, insufficient stability, or low water solubility.

In this work, we designed a new series of substituted 2-aminooxazoles to potentially overcome the above-mentioned problems.

The design is based on the hit compounds obtained from the high-throughput virtual screening (HTVS). The virtual library used for the HTVS contained approx. 53k diverse compounds, and was constructed from the building blocks obtained from several available suppliers (Enamine, Fluorochem and Molport) and our in-house compounds (see **Figure 1**).

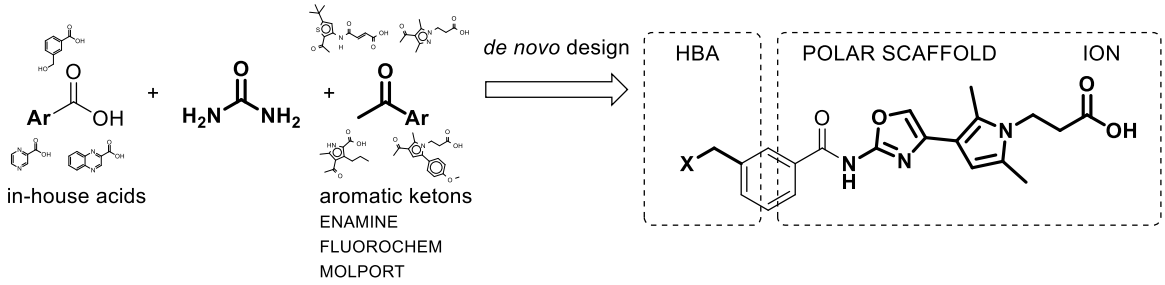


Figure 1. The general structure of synthesized derivatives obtained from the HTVS

All compounds were docked to the *Escherichia coli* FabH (PDB ID: 5BNM and 5BNS), serving as a prototype for other G- bacteria. HTVS was done in three successive stages with increasingly more accurate docking settings, each stage with ten percent best results from the previous one. Finally, we

assessed the best obtained hits and devised the general structure as presented in **Figure 1** that will be used for synthesis, further optimizations, and biological evaluation.

This research was supported by the Grant Schemes at CU (reg. no. CZ.02.2.69/0.0/0.0/19_073/00169359).

SC-32

STATISTICAL EVALUATION OF THE RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND ANTIMYCOBACTERIAL ACTIVITY *IN VITRO* OF SELECTED PHENYLCARBAMIC ACID DERIVATIVES

PECHÁČOVÁ, M.,[1] MALÍK, I.,[1] ČURILLOVÁ, J.,[1] ČIŽMÁRIK, J.,[1]

[1] Department of Pharmaceutical chemistry, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic

e-mail: pechacova10@uniba.sk

This research focuses on the relationships between a structure of 4-(2-{[(2-/3-(4-alkoxyphenyl)carbamoyl]-oxy}ethyl)pyrrolidin-4-ium-chlorides (alkoxy = methoxy to decyloxy) and their activity *in vitro* against some strains of *Mycobacterium* spp. by chemometric Principal Component Analysis. Input parameters of the analysis are molecular descriptors characterizing i) lipophilicity – logarithms of partition coefficients (log P_{exp}) estimated by a shake-flask method in the octan-1-ol/phosphate buffer system, ii) acidity – acid-base dissociation constants (pK_a) estimated by a potentiometric titration method, iii) the minimum inhibitory concentration (*MIC*) values of the compounds under the study determined after 14- and 21-day cultivation *in vitro* against *M. tuberculosis* CNCTC My 331/88 (identical with the $H_{37}R_v$ and ATCC 2794 strain, respectively), *M. avium* CNCTC My 330/80 (identical with the ATCC 25291 strain), *M. kansasii* CNCTC My 235/80 (identical with the ATCC 12478 strain) and *M. kansasii* My 6509/96 (clinical isolate), respectively. As calculated, the activity of given phenylcarbamic acid derivatives is relatively independent on their acid-base properties and, in addition, lipophilicity might be considered more substantial parameter influencing such type of their biological action *in vitro*.

The study was supported by the Grant of the Comenius University UK/77/2021.

SC-33

ANTIMICROBIAL SYNERGY STUDY – CHECKERBOARD ANALYSIS

DIEPOLTOVÁ, A., KONEČNÁ, K., JANĎOUREK, O.,

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: diepolta@faf.cuni.cz

Interaction determination by checkerboard analysis is used to study the impact on the potency of the antibiotic drug combination in comparison to their individual activities. Such relation is represented as the Fractional Inhibitory Concertation index.[1] This methodology is a promising strategy, especially in a search for molecules with an antibacterial boosting effect, which can contribute to the effort of partial restriction of antibiotic resistance and achievement of lower drug dosing in the treatment regimens. The checkerboard studies belong to an integral part of the preclinical research in anti-infective drug discovery. In this research, the activity of a candidate molecule with antibiotic potential is mostly determined in combination with commercially available antibiotic drugs, preferably with different mechanisms of action. Different methodical approaches can be chosen to monitor the synergistic effect of drugs in combination of two or three antibiotics (2D and 3D modes).[2] In addition, the checkerboard analysis is able to reveal the undesirable antagonistic effect. This technique can be efficiently arranged in microtiter plates and results are evaluated using data obtained by spectrophotometric measurement.

The study was supported by the SVV Project No. 260549 of Charles University, Faculty of Pharmacy in Hradec Králové, Grant Schemes at CU (reg. no. CZ.02.2.69/0.0/0.0/19_073/00169359) and Ministry of Health of the Czech Republic, grant nr. NU21-05-00482 and by The Czech Science Foundation project No. 20-19638Y.

References

1. ORHAN, G., BAYRAM, A., ZER, Y., *et al.*: Journal of Clinical Microbiology, 43(1), 2005, 140–143.
2. STEIN, C., MAKAREWICZ, O., BOHNERT, J.A., *et al.*: PLoS One, 10(6), 2015, e0126479.

SC-34

A CLICK REACTION IN OLIGODEOXYNUCLEOTIDE PROBES LABELLING

KOLAROVA, M., VADALA, A., KUCERA, R., MILETIN, M.

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: michaela.kolarova@faf.cuni.cz

Oligodeoxynucleotide (ODN) probes are synthetized sequences of nucleic acids modified with molecule label, which determines their main use typically as an imaging or detection tool in biochemistry and medical field. This fact defines the labelling as a crucial step in the oligonucleotide probes preparation. Unfortunately, this procedure is nowadays the most problematic part in otherwise highly efficient process carried out on solid phase in oligo synthesizers. A multitude of molecules can serve as a label, namely fluorophores, quenchers, or drugs. The wide diversity of molecules together with large ODN chain demands highly specific, mild conditions during the labelling. One of the newest approaches of the ODN labelling is the Huisgen's cycloaddition, better known as the "click" reaction.[1] Exceptional reaction kinetic provides a uniquely stable yet extraordinarily wide possibilities of the reaction enviroment.

So far nobody has examined a significant yield fluctuation occuring in praxis. We decided to investigate the influence of different conditions on the efficiency of the click reaction in ODN probes preparation. We labeled, deprotected and purified using HPLC method over 250 probes. Three types of molecules (azaphtalocyanine, BODIPY, and acridine derivative) were used for the labelling in four different concentrations (0,1; 1; 10; 100 mM). Three positions in the strand of a 24-base identical sequence were tested (2^{nd}, 13^{th}, and 24^{th}, counted from 3´-end of oligonucleotide, where it is bound to the solid phase). An influence of solid phase support was observed using two most frequently used types with various porosity (controlled pore glass – 500; 1000; 2000 Å and polystyrene 1000 Å).

Experiments proved that hydrophilic/-phobic compatibility of solid phase support and the label, as well as the size of the label, have the crucial impact on the concentration of label needed for fully labelled ODN probe preparation.

The study was supported by Charles University (reg. No. SVV 260547).

References

1. JEWETT, J. C., SLETTEN, E. M., BERTOZZI, C. R.: J. Am. Chem. Soc., 132, 2010, 3688–3690.

SC-35

TARGETING CLASS I AMINOACYL-TRNA SYNTHETASES WITH PYRIMIDINE SUBSTITUTED AMINOACYL-SULFAMOYL NUCLEOSIDES

NAUTIYAL, M.,[1] DE GRAEF, S.,[2] PANG, L.,[1,2] GADAKH, B., [1] STRELKOV, S.V., WEEKS, S.D.[2] AND VAN AERSCHOT, A. [1]

[1]Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Herestraat 49 Box 1041, B-3000, Leuven, Belgium

[2] Laboratory for Biocrystallography, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Herestraat 49 Box 822, B-3000, Leuven, Belgium

e-mail : manesh.nautiyal@umu.se

Aminoacyl-tRNA synthetases (aaRSs) catalyze the ATP-dependent coupling of an amino acid to its cognate tRNA. aaRSs are vital for protein translation and are considered a promising target for developing novel antimicrobial agents. 5'-O-(*N*-aminoacyl)-sulfamoyl adenosines (aaSAs) are non-hydrolyzable analogs of the aaRS reaction intermediate, a potent inhibitor of this enzyme family but are prone to chemical instability and enzymatic modification[1,2]. In an attempt to improve this scaffold's molecular properties, we synthesized a series of base substituted aaSA analogs comprising cytosine, uracil, and N^3-methyluracil targeting leucyl-, tyrosyl- and isoleucyl-tRNA synthetases3. In *in vitro* assays, seven out of the nine inhibitors demonstrated K_i^{app} values in the low nanomolar range[2]. To complement the biochemical studies, X-ray crystallographic structures of *Neisseria gonorrhoeae* leucyl-tRNA synthetase and *E. coli* tyrosyl-tRNA synthetase in complex with the newly synthesized compounds were determined[3]. These highlighted a subtle interplay between the base moiety and the target enzyme in defining relative inhibitory activity. Combined the various insights resulting from this study will pave the way for the further rational design of aaRS inhibitors.

This work was supported by the Research Foundation Flanders G077814N to S.V.S. and A.V.A., G0A4616N.

References

1. SEREBRYAKOVA, M., TSIBULSKAYA, D., MOKINA, O., *et al.*: JACS, 138 (48), 2016, 15690-98.
2. GADAKH, B., VONDENHOFF, G., LESCRINIER, E., *et al.*: BMC, 22 (10), 2014, 2875-86.
3. NAUTIYAL, M., DE GRAEF, S., PANG, L., *et al.*: EJMC, 173, 2019, 154-66.

SC-36

SYNTHESIS OF ACRIDINE-4-CARBOXAMIDES FOR THERMAL STABILIZATION OF SHORT DNA DUPLEXES

KOSTELANSKY, F.,[1] ZIMCIK, P.,[1] MILETIN, M.,[1] HAVLINOVA, Z.,[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] GENERI BIOTECH s.r.o., Hradec Králové, Czech Republic

e-mail: kostelaf@faf.cuni.cz

Melting temperature difference (ΔT_m) between complementary and mismatched duplex has a crucial role for discrimination of point mutations or single nucleotide polymorphisms. The ΔT_m is decreasing with the length of oligodeoxynucleotide. From this point of view, shorter oligodeoxynucleotide probes are advantageous in comparison with longer probes due to higher ΔT_m. On the other hand, their low melting temperature is the main disadvantage that makes the assays based on polymerase chain reaction (PCR) hard to perform. Melting temperature modifiers are used for elimination of the disadvantage. There are three types of modifiers that can be used for thermal stabilisation of oligodeoxynucleotide duplexes: polyamines[1], minor groove binders[2] and intercalators[3]. In our work we focused on synthesis of acridine-4-carboxamide derivatives. They were prepared by modified published procedures. Our acridine derivatives were tested for their ability to stabilize DNA duplex in solution. Melting temperature of dsDNA-acridine complex was compared to melting temperature of dsDNA. Most promising acridine-4-carboxamide derivatives were selected for covalent attachment to the oligodeoxynucleotide by copper-free click chemistry. Modified ODNs were tested at PCR conditions for discrimination of single-base mismatch.

The study was supported by The Grant Agency of Charles University (reg. No. 994218).

References

1. NOIR, R., KOTERA, M., PONS, B., *et al.*: J. Am. Chem. Soc., 130, 2008, 13500-13505.
2. LUKHTANOV, A., LOKHOV, S. G., GORN, V. V., *et al.*: Nucleic Acids Res., 35, 2007, e30.
3. ASSELINE, U., TOULME, F., THUONG, N. T., *et al.*; EMBO J., 3, 1984, 795-800.

SC-37

NOVEL SYNTHETIC APPROACH FOR KINASE INHIBITORS CONTAINING (*Z*)-3-[AMINO(PHENYL)METHYLIDENE]-1,3-DIHYDRO-2*H*-INDOL-2-ONE STRUCTURE MOIETY

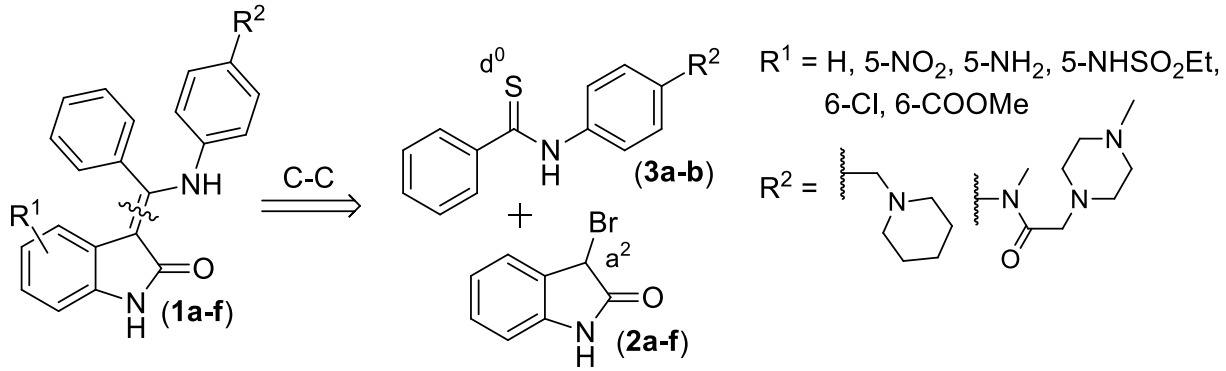
MAREK, L, HANUSEK, J.

Institute of Organic Chemistry and Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic

e-mail: Lukas.Marek3@student.upce.cz

Compounds containing 3-[phenylamino(phenyl)methylidene]indol-2-one (**1**) structure moiety are established in the treatment of several autoimmune diseases and diverse malignancies[1].

In our group, we recently prooved[2] that the thiophile-free *Eschenmoser* reaction (d^0 + a^2) of 3-bromoindol-2-ones with various thioamides respresents a versatile synthetic approach for preparation of 3-[amino(phenyl)methylidene]indol-2-ones (**1**). In this work, we would like to further demonstrate the usefulness of our synthetic approach for the preparation of known kinase inhibitors (**1a-f**), especially *Nintedanib* and *Hesperadin*. Reaction of several subst. 3-bromoindol-2-ones (**2a-f**) and thiobenzanilide derivatives (**3a-b**) was carried out in polar aprotic solvents under mild reaction conditions. Desired compounds were obtained in good to excellent yields (76 – 97 %).



References

1. ROSKOSKI JR, R.: Pharm. Res, 144, 2019, 19–50.
2. MAREK, L., KOLMAN, L., HANUSEK, J., *et al.*: Beilstein J. Org. Chem., 17, 2021, 257–239.

SC-38

SUBPHTHALOCYANINES AS PHOTOSENSITIZERS IN PHOTODYNAMIC THERAPY

DEMUTH, J.,[1,2] MARTÍNEZ-DIAZ, M. V.,[2] MACHACEK, M.,[1] KOZLIKOVA, M.,[1] NOVAKOVA, V.,[1] TORRES, T.[2]

[1] Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Facultad de Ciencias, Universidad Autónoma de Madrid, Spain

e-mail: presenting.author@faf.cuni.cz

Treatment of cancer with non-invasive methods is still challenging. Modern trend is to find efficient drugs, which could be specifically targeted to the tumor to prevent side effects of cancer treatment. The targeted therapy could be mediated by special delivery systems, analogs of biomolecules or locally activated drugs.[1] Photodynamic therapy (PDT) belongs to the latter category, because photosensitizer is only activated in specific body location upon irradiation.

Subphthalocyanines (SubPcs) are well-known macrocyclic compounds with 14-π electron aromatic system. They consist of three isoindole units connected by aza bridges with coordinated central boron (III). SubPcs have tetrahedral structure formed by cone-shape SubPc and axial substituent on boron.[2] This tetrahedral shape helps them to prevent aggregation, which is typical drawback of large aromatic macrocycles. SubPcs have ability to absorb light from 300 – 600 nm and convert absorbed energy into fluorescence or production of singlet oxygen ($^1O_2$). Therefore, they could be used for therapeutic imaging or treatment of tumorous diseases.[3]

The aim of this project was to compare the effect of peripheral substitution on PDT activity. Suitable axial substitution should increase hydrophilicity of SubPcs to enable in vitro evaluation. Target derivatives bearing peripheral substituents with either electron donating or withdrawing properties have been prepared. All prepared compounds were subjected to photophysical evaluation (UV/vis, fluorescence, production of singlet oxygen). Some compounds were under testing for PDT activity on HeLa and SK-MEL-28 cells.

The study was supported by Czech Science Foundation (reg. No. 20-09212S).

References

1. TEPPO, H. R.; SOINI, Y.; KARIHTALA, P.: Oxidative Medicine and Cellular Longevity, 2017, 11.

2. CLAESSENS, C. G.; GONZÁLEZ-RODRÍGUEZ, D.; TORRES, T.: Chem. Rev., 102 (3), 2002, 835-854.
3. XU, H.; JIANG, X. J.; CHAN, E. Y. M. *et al.*: Org. Biomol. Chem., 5 (24), 2007, 3987-3992.

PRE-RECORDED SHORT COMMUNICATIONS

RSC-1

SYNTHESIS, BIOLOGICAL EVALUATION, AND IN SILICO MODELLING OF *N*-SUBSTITUTED QUINOXALINE-2-CARBOXAMIDES

BOUZ, G.,[1] VINŠOVÁ, J.,[2] DOLEŽAL, M.,[1] ZITKO, J.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: bouzg@faf.cuni.cz

Despite the established treatments, tuberculosis remains an alarming threat to public health according to WHO.[1] Novel agents are urgently needed to overcome the increasing rates of resistance and achieve eradication at last. As a part of our long-term research on pyrazine derivatives, we prepared a series of *N*-substituted quinoxaline-2-carboxamides and evaluated their *in vitro* antimycobacterial activity. Several quinoxaline derivatives were found in the literature to possess antitubercular activity.[2] In addition to activity assessment, final compounds were screened for their *in vitro* cytotoxicity on HepG2 liver cancer cell lines. *In vitro* activity against *Mtb*H37Ra (represented by MIC) ranged between 3.91–500 μg/mL, with most compounds having moderate to good activities (MIC < 15.625 μg/mL) and low toxicity. *N*-(naphthalen-1-ylmethyl)quinoxaline-2-carboxamide was identified as a potential antineoplastic agent with selective cytotoxicity against hepatic (HepG2), ovarian (SK-OV-3), and prostate (PC-3) cancer cells lines. Molecular docking studies suggested DNA-intercalating properties of this compound and pointed to vascular endothelial growth factor receptor 2 (VEGFR2) as a potential target.

This research was funded by EFSA-CDN, grant number CZ.02.1.01/0.0/0.0/16_019/0000841, co-funded by ERDF; and by the Czech Science Foundation, grant number 20-19638Y.

References

1. World Health Organization, Global Tuberculosis, Report 2020. www.who.int/tb/publications/global_report/en/ accessed: 04.01.2021
2. PERAMAN, R., KUPPUSAMY, R., KILLI, S.K., *et al.*: Int. J. Med. Chem., 2016, Article No. 6471352.

RSC-2

SYNTHESIS AND EVALUATION OF SMALL MOLECULES ACTIVE AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

KRÁTKÝ, M.,[1] KONEČNÁ, K.,[2] JANĎOUREK, O.,[2] VINŠOVÁ, J.[1]

[1] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: martin.kratky@faf.cuni.cz

A growing acquired resistance to antimicrobial drugs has become a global healthcare problem. Thus, the development of new agents for combating resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), is essential. MRSA also causes life-threating or fatal infections and treatment options are limited.[1]

Our approach is based on a combination of two antimicrobial scaffolds in one molecular entity to affect more bacterial targets to overcome resistance or prevent its development. The scaffolds cover both known antistaphylococcal drugs (e.g., sulfonamides[2]) and new compounds with *in vitro* identified antibacterial effect (e.g., salicylic,[2] guanidine or thiocarbamate derivatives).

The compounds were evaluated *in vitro* for their activity against methicillin-susceptible and resistant *Staphylococci* and to determine their selectivity also against other bacteria, mycobacteria, and fungi. We also investigated their cytotoxicity.

The most promising derivatives showed MIC of ≤0.49 μM together with no significant toxicity to eukaryotic cells. They share bactericidal activity against various Gram-positive cocci, and they are perspective candidates for advanced preclinical studies.

The study was supported by the Czech Science Foundation (reg. No. 20-19638Y), the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF and by Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

References

1. ZHEN, X., LUNDBORG, C.S., ZHANG, M., *et al.*: Sci. Rep., 10, 2020, 3900.
2. KRÁTKÝ, M., KONEČNÁ, K., JANOUŠEK, J., *et al.*: Future Med. Chem., 2021, accepted.

RSC-3

3-(PYRAZIN-2-YL)-1,2,4-OXADIAZOLES AS ANTIMYCOBACTERIAL AGENTS OR ETHIONAMIDE BOOSTERS?

KUČEROVÁ-CHLUPÁČOVÁ, M.,[1] KONEČNÁ, K.,[2] KUNEŠ, J.,[3] PATEROVÁ, P.,[4] JANĎOUREK, O.,[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[4] Department of Clinical Microbiology, Faculty Hospital, Hradec Králové, Czech Republic

e-mail: kucerom@faf.cuni.cz

1,2,4-Oxadiazole ring represents a privileged scaffold in drug discovery.[1] 1,2,4-Oxadiazole derivatives exerted direct antimycobacterial effect,[2] as well potentiation of ethionamide activity *via* inhibition of EthR, that negatively regulates the expression of ethionamide activator EthA.[3] 3-(Pyrazin-2-yl)-1,2,4-oxadiazoles were prepared from 5-alkylated pyrazine-2-carbonitrile *via* the amidoxime route.[4] The products were screened against a panel of mycobacterial strains. The booster activity was tested against *M. tuberculosis* H37Ra in combination with ethionamide. The results will be discussed.

The study was supported by the project Progres Q42 (Charles University, Czech Republic).

References

1. BIERNACKI, K., DAŚKO, M., CIUPAK, O.: Pharmaceuticals, 13, 2020, ph13060111.
2. DE, S. S., KHAMBETE, M. P., DEGANI, M. S.: Bioorg. Med. Chem. Lett., 29, 2019, 1999–2007.
3. COSTA-GOUVEIA, J., PANCANI, E., JOUNY, S.: Nature Sci. Rep., 17, 2017, 5390.
4. HEMMING, K.: J. Chem. Res. (S), 2001, 209–216.

RSC-4

BIOLOGICAL PROPERTIES OF PYRAZINAMIDE DERIVATIVES - BEYOND ANTIMYCOBACTERIAL ACTIVITY

ZITKO, J.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: jan.zitko@faf.cuni.cz

In recent years, the perception of the first-line antitubercular pyrazinamide (PZA) and its metabolite pyrazinoic acid (POA) has changed from a non-specific cytosol acidifier to a multi-target inhibitor of specific mycobacterial enzymes and processes. Since 2000, at least four specific subcellular targets of PZA and/or POA have been reported, including Fatty Acid Synthase I (FAS I), ribosomal protein RpsA, aspartate decarboxylase (PanD), and quinolinic acid phosphoribosyltransferase (QAPRTase). In this presentation, I will recapitulate other than antimycobacterial activities of pyrazinamide derivatives which we have explored in recent years and I will briefly present the projects currently ongoing in our working group. One of the most significant projects deals with 3-aminopyrazine-2-carboxamide derivatives as ATP competitive binders of aminoacyl-tRNA synthetases.[1]

The study was supported by Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

References

1. PANG, L., WEEKS, S.D., JUHÁS, M., *et al.*: Int. J. Mol. Sci. 22, 2021, Article Number 7793. https://doi.org/10.3390/ijms22157793

THANK YOU AND SEE YOU NEXT YEAR!

50th Conference Synthesis and Analysis of Drugs
Faculty of Pharmacy, Masaryk University, Brno,
September 2022