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- Polskiego Towarzystwa Chemii Medycznej,
- Komisji Syntezy i Projektowania Nowych Leków Komitetu Terapii i Nauk o Leku PAN,
- Sekcji Chemii Leków Polskiego Towarzystwa Farmaceutycznego
- Uniwersytetu Medycznego w Lublinie.

Plan Konwersatorium

Czwartek, 18.09.2014

15.30-17.00 – Rejestracja uczestników (Hotel Victoria, Ul. Narutowicza 55/60, Lublin)

17.00-18.00 – Wykład inauguracyjny (WI) (Hotel Victoria, Ul. Narutowicza 55/60, Lublin)

Prof. Alexander Tropsha, University of North Carolina at Chapel Hill, USA
„Cheminformatics approaches to drug discovery: challenges, solutions, and opportunities"

18.00-22.00 – Spotkanie powitalne

Piątek, 19.09.2014

9.00-11.00 – Wykłady plenarne (W1-W3) (40')

Prowadzący sesję – *Prof. Katarzyna Kieć-Kononowicz, Prof. Dariusz Matosiuk*

W1

Prof. Katerina Tiligada, Medical School University of Athens, Grecja „GPCRs revisited: Emerging insights into the pharmacological challenges and therapeutic opportunities of the histamine H4 receptor"

W2

Prof. Michael Gütschow, University of Bonn, Niemcy „Inhibition of human cysteine cathepsins by azapeptide nitriles"

W3

Prof. Vladimír Kryštof, Palacký University & Institute of Experimental Botany, Czechy
„Potent cyclin-dependent kinase inhibitors with purine core"

11.00-11.30 – Przerwa kawowa

11.30-13.00 – Komunikaty (K1-K4) (20')

Prowadzący sesję – *Prof. Barbara Malawska, Prof. Krzysztof Jóźwiak*

K1

Prof. Marcin Drąg, Politechnika Wrocławska, Polska „Hybrid Combinatorial Substrate Library in design of specific and active probes for proteolytic enzymes"

K2

Mgr Adam Hogendorf, Uniwersytet Jagielloński, Polska „Systematic study reveals new potent and selective 5-HT6R ligands based on indole scaffold"

K3

Dr Agnieszka Kaczor, Uniwersytet Medyczny w Lublinie, Polska „Recent advances in functioning of G protein-coupled receptors: insight from molecular modelling"

K4

Mgr Paweł Książek, Uniwersytet Warmińsko-Mazurski, Polska „Binding features of estrogen receptor beta selective ligands – docking investigation"

13.00-14.00 – Przerwa obiadowa

14.00-15.00 – Sesja posterowa oraz prezentacje posterów (PP1-PP5) (10')

Prowadzący sesję – *Prof. Zofia Mazerska, Prof. Marek Cegła*

PP1

Mgr Piotr Drączkowski, Uniwersytet Medyczny w Lublinie, Polska „Characterization and screening of enzyme inhibitors using isothermal titration calorimetry"

PP2

Dr Rafał Urniaż, Uniwersytet Medyczny w Lublinie, Polska „X-ray crystallographic structures alignment as a source of fragments for effective drug design"

PP3

Mgr Aleksandra Rak, Uniwersytet Jagielloński, Polska „Arylsulfonamide derivatives of aryloxyalkilamines as new uroselective a1-adrenolitics in the treatment of benign prostatic hyperplasia"

PP4

Dr Anna Zawadzka, Uniwersytet Warszawski, Polska „Synthesis and evaluation of substituted tacrine - melatonin heterodimers as cholinesterases inhibitors"

Komunikat sponsora

Komunikat firmy ABL&E-JASCO Polska

15.30-16.00 – Przerwa kawowa

16.00-17.20 – Komunikaty (K5-K8) (20')

Prowadzący sesję –*Dr hab. Agata Paneth, Prof. Andrzej Bojarski*

K5

Prof. Josef Jampilek, University of Veterinary and Pharmaceutical Sciences, Czechy „Synthesis and in vitro anti-trypanosomal screening of hydroxynaphthalene-2-carboxanilides"

K6

Mgr Marcin Poręba, Politechnika Wrocławska, Polska „Unnatural amino acids as a new tool for investigation of substrate specificity of human caspases"

K7

Mgr Ewelina Węglarz-Tomczak, Politechnika Wrocławska, Polska „Phosphinic inhibitors of aminopeptidases"

K8

Mgr Artur Wnorowski, Uniwersytet Medyczny w Lublinie, Polska „Activation of β2-adrenergic receptor with (R,R')-4'-methoxy-1-naphthylfenoterol inhibits proliferation and motility of a panel of melanoma cell lines"

Sobota, 20.09.2014

09.00-11.00 – Komunikaty (K9-K14) (20')

Prowadzący sesję – *Prof. Jadwiga Turło, Prof. Zbigniew Kamiński*

K9

Dr Robert Czarnomysy, Uniwersytet Medyczny w Białymstoku, Polska „Apoptotic effects of novel pyridine platinum(II) complexes on the Ishikawa endometrial cancer cell lines"

K10

Prof. Grażyna Biała, Uniwersytet Medyczny w Lublinie, Polska „Pharmacological and behavioral characterization of organic cation transporter-2 deficient mice"

K11

Mgr Agnieszka Gornowicz, Uniwersytet Medyczny w Białymstoku, Polska „The molecular mechanism of apoptosis by novel dinuclear platinum(II) complex with anti-MUC1 in estrogen negative MDA-MB-231 breast cancer cells"

K12

Prof. Zofia Mazerska, Politechnika Gdańska, Polska „The II phase metabolism of endogenous and exogenous compounds, including antitumor chemotherapeutics"

K13

Dr Ewa Gibuła-Bruzda, Uniwersytet Medyczny w Lublinie, Polska „Effects of new analogs of enkephalin and deltorphin in antinociceptive tests in animals"

K14

Dr Joanna Katarzyńska, Politechnika Łódzka, Polska „Cyclopeptides with potential immunosuppressive activities"

11.00-11.30 – Przerwa kawowa

11.30-13.00 – Komunikaty (K15-K20) (20')

Prowadzący sesję – *Prof. Monika Wujec, Prof. Zbigniew Karczmarzyk*

K15

Prof. Zbigniew J. Kamiński,, Politechnika Łódzka, Polska
„Arrays of peptides immobilized on the cellulose – new tool in medical diagnostic"

K16

Mgr Jakub Staroń, Instytut Farmakologii Polskiej Akademii Nauk, Kraków, Polska
„Bioisosteric substitution of sulphonyl group in 5-HT_6R ligands - A study on ligand-receptor interactions"

K17

Dr Anita Płazińska, Uniwersytet Medyczny w Lublinie, Polska
„Prediction of the stereochemical effects in the ligand-receptor interactions: metadynamics simulations"

K18

Dr Beata Kolesińska, Politechnika Łódzka, Polska
„Search for new inhibitors of Insulin/Amylin aggregation"

K19

Dr Anna Waszkielewicz, Uniwersytet Jagielloński, Polska
„Synthesis, anticonvulsant activity and mechanism of action of some aminoalkanol derivatives"

K20

Dr Beata Żołnowska, Gdański Uniwersytet Medyczny, Polska „N-Substituted N'-(2-arylmethylthio-4-chloro-5-methylbenzene sulfonyl)guanidines – novel antiproliferatives, antimicrobials and inhibitors of human carbonic anhydrase isozymes I, II, IX and XII"

13.00-14.00 – Przerwa obiadowa

14.00-15.00 – Sesja posterowa oraz prezentacje posterów (PP5-PP10) (10')

Prowadzący sesję – *Prof. Jolanta Kotlińska, Prof. Krzysztof Bielawski*

PP5

Dr Paulina Kasperkiewicz, Politechnika Wrocławska, Polska
„Investigation of specific activity based probes for serine proteases"

PP6

Mgr Paulina Koczurkiewicz, Uniwersytet Jagielloński, Polska „Synthesis and biological properties of 4-chloro-3-methylphenoxyethylamine derivatives of trans-2-aminocyclohexan-1-ol"

PP7

Mgr Katarzyna Wójcik, Uniwersytet Jagielloński, Polska
„Wnt5a attenuates TGF-β induced fibroblasts to myofibroblast trasition in bronchial fibroblasts derived from asthmatic patients"

PP8

Dr Ewa Chmielewska, Politechnika Wrocławska, Polska
„Aminomethylenebisphosphonates as potential drugs against osteoporosis"

PP9

Dr Urszula Kijkowska-Murak, Uniwersytet Medyczny w Lublinie, Polska „The Chemical Database of compounds synthesized in Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances in Medical University in Lublin"

PP10

Dr Agnieszka Potęga, Politechnika Gdańska, Polska „Electrochemical simulation of enzymatic transformations studied for the selected antitumor acridine derivatives"

15.00-15.30 – Przerwa kawowa

15.30-18.30 – Komunikaty (K21-K28) (20')

Prowadzący sesję – *Prof. Anna Bielawska, Prof. Stanisław Ryng*

K21

Prof. Jadwiga Turło, Warszawski Uniwersytet Medyczny, Polska
„Modification of polysaccharides structure and biological activity by selenium incorporation"

K22

Mgr Mateusz Gierszewski, Uniwersytet im. Adama Mickiewicza, Polska
„Investigations of singlet and triplet states for sulfanyl porphyrazines with isophthaloxy substituents"

K23

Dr Waldemar Maniukiewicz, Politechnika Łódzka, Polska „Structural and spectroscopic characterization and Hirsheld surface analysis of major component of antibiotic mupirocin - pseudomonic acid A"

K24

Dr hab. Jarosław Sączewski, Gdański Uniwersytet Medyczny, Polska
„From heterocyclic hydroxylamine-O-sulfonates to Safirinium: a novel fluorescent labeling platform"

K25

Dr Ewa Tykarska, Uniwersytet Medyczny w Poznaniu, Polska „Supramolecular structure of glycyrrhizic acid and carbenoxolone: properties of molecules forming stable micelles"

K26

Dr Marta Ziegler-Borowska, Uniwersytet Mikołaja Kopernika, Polska „Synthesis of chitosan coated magnetic nanoparticles designed for bioligands binding"

K27

Dr Małgorzata Jeleń, Śląski Uniwersytet Medyczny, Polska "Tetracyclic and pentacyclic azaphenothiazines with the quinoline ring"

K28

Dr Jacek Stefanowicz, Warszawski Uniwersytet Medyczny, Polska "Stereoselective synthesis of 3β-aminotropane quinolineamides with potential atypical antipsychotic activity"

20.00-24.00 – Wieczór pożegnalny (Dworek, Ul. Krochmalna, Lublin).

Transport zapewniają Organizatorzy Konwersatorium.

Wykład inaguracyjny (WI)

WI

Cheminformatics approaches to drug discovery: challenges, solutions, and opportunities

Alexander Tropsha

Laboratory for Molecular Modeling, Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

The accumulation of heterogeneous and diverse data concerning chemical effects on biological systems gave rise to the field of cheminformatics. The constituent areas of this rapidly growing and expanding field include data extraction, curation, visualization, and statistical modeling of structure-activity (property) relationships. I shall discuss the unsolved challenges and diverse applications of cheminformatics, especially, statistical (Q)SAR modeling in drug discovery and beyond. Specific examples of unsolved challenges include data quality (chemical and biological data curation) and dataset "modelability", statistical predictive power vs. transparency and interpretability of models, and broader acceptance of QSAR models by experimental scientists and regulatory agencies. Applications include traditional areas of bioactivity prediction as well as the prediction of complex endpoints (e.g., ADMETox modeling), nanotoxicology, drug delivery, modeling of chemical mixtures, and materials informatics. After summarizing best practices in the field of QSAR modeling, I will present examples of model applications to virtual screening resulting in the discovery of novel experimentally confirmed hit compounds. I will discuss novel integrative strategies for predicting *in vivo* effects of chemicals by concordant exploitation of both computed descriptors of chemical structures and their short term biological effects regarded as biological descriptors, which offers new avenues for model interpretation in the context of underlying biological pathways. Finally, recent studies in our group and elsewhere suggest that QSAR modeling can be successfully applied to novel areas of modeling materials and nanomaterials. For illustration, I will discuss the case study of rational design of surface-modified carbon nanotubes with the controlled protein binding and cytotoxicity.

Wykłady plenarne (W1-W3)

W1

GPCRs revisited: Emerging insights into the pharmacological challenges and therapeutic opportunities of the histamine H_4 receptor

Katerina Tiligada

Department of Pharmacology, Medical School University of Athens, M. Asias 75, 11527 Athens, Greece,

Since its discovery at the beginning of the 20th century, histamine has been one of the most studied biological molecules in medicine. Histamine elicits pleiotropic actions, largely but not exclusively through binding to four currently known G protein-coupled receptors (GPCRs), designated as H_1R-H_4R. In the past decade, the identification of the H_4R led to the intense evaluation of its translational potential and to the development of a significant number of selective high affinity ligands. A large body of experimental evidence identifies the H_4R as a central player in the orchestration of immune responses and highlights the therapeutic exploitation of histaminergic systems for a range of poorly treatable chronic inflammatory disorders, including asthma, urticaria and autoimmune diseases. Although some H_4R-targeting agents have already advanced into clinical trials, the cell and tissue variability in H_4R-mediated signals and the profound intra- and inter-species differences in potency, selectivity and off-target effects of H_4R ligands hamper investigations and call for more cautious interpretation of the preclinical H_4R-mediated effects into human clinical settings. Furthermore, the recently documented ability of GPCRs to regulate cellular events in a G-protein-independent manner seems to underlie, at least in part, the complexity of H_4R pharmacology. Beyond its fundamental importance in cellular functioning, this biased agonism (or functional selectivity) provides a challenging framework driving the development of differentiated therapeutics targeting histamine receptors.

W2

Inhibition of human cysteine cathepsins by azapeptide nitriles

Michael Gütschow

Pharmaceutical Institute, University of Bonn, D-53121 Bonn (Germany)

Peptide nitriles have been shown to reversibly react with the active site cysteine under formation of a covalent thioimidate adduct. The structural optimization with respect to the positions P3, P2, P1, P1′, and P2′ resulted in the identification of potent and selective inhibitors of the corresponding cathepsins.

The isoelectronic replacement of the Cα-H group by a nitrogen atom to give azapeptides is a common structural modification in the chemistry of peptides and peptidomimetics. This structural modification has first been applied to the P1 position of peptide nitriles leading to the exploration of a new class of azapeptide-type protease inhibitors [1]. The resulting azadipeptide nitriles were characterized as highly potent inhibitors of cysteine cathepsins, although they possess a methylated P2-P1 peptide bond, which accounts for their stability towards chymotryptic cleavage. Such azadipeptide nitriles showed time-dependent inhibition, and the progress curves were analyzed by slow-binding kinetics, thus providing access to the association and dissociation rate constants of the reversible isothiosemicarbazide adducts, resulting from the attack of the active site cysteine at the aza-nitrile warhead.

Structure-activity relationships for several series of azapeptide nitriles will be presented [2]. Their application as PET tracers and the comparative suitability of this chemotype for the development of activity-based probes will be discussed [3]. Based on the concept of azapeptide nitriles, structural investigations on protease-inhibiting rigidized peptide derivatives are also shown [4].

[1] R. Löser, M. Frizler, K. Schilling, M. Gütschow; *Angew. Chem. Int. Ed.* 2008, *47*, 4331.
[2] M. Frizler, J. Schmitz, A. C. Schulz-Fincke, M. Gütschow; *J. Med. Chem.* 2012, *55*, 5982-5986. [b] M. Frizler, F. Lohr, N. Furtmann, J. Kläs, M. Gütschow; *J. Med. Chem.* 2011, *54*, 396-400. [c] M. Frizler, F. Lohr, M. Lülsdorff, M. Gütschow; *Chem. Eur. J.* 2011, *17*, 11419.
[3] M. D. Mertens, J. Schmitz, M. Horn, N. Furtmann, J. Bajorath, M. Mareš, M. Gütschow; *ChemBioChem* 2014, *15*, 955-959. [b] M. Frizler, I. V. Yampolsky, M. S. Baranov, M. Stirnberg, M. Gütschow; *Org. Biomol. Chem.* 2013, *11*, 5913.
[4] P. A. Ottersbach, J. Schmitz, G. Schnakenburg, M. Gütschow; *Org. Lett.* 2013, *15*, 448. [b] P. A. Ottersbach, G. Schnakenburg, M. Gütschow; *Chem. Commun.* 2012, *48*, 5772.

W3

Potent cyclin-dependent kinase inhibitors with purine core

Vladimír Kryštof

Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR,, Šlechtitelů 11, 78371 Olomouc, Czech republic

The cyclin-dependent kinases (CDKs) are key regulators of the cell cycle, RNA transcription and some other processes. They are activated in a cell cycle-specific manner by cyclins and phosphorylate targets including the transcriptional regulators that in turn induce phase-specific gene expression, stimulate DNA replication or initiate mitosis. Due to their frequent deregulation in cancer cells, CDKs have been viewed as valid drug targets. The potency of CDK inhibitors designed around a purine heterocycle scaffold is largely determined by the purine's 2,6,9- substitution pattern but can also be affected by the nitrogen atom distribution in the heterocyclic core [1]. Although many selective CDK inhibitors have been tested in clinical trials, none have yet been approved, largely due to undesired side effects arising from their unfavourable pharmacological properties. Therefore, the identification of new active compounds and pharmacophores that inhibit CDKs is still a meaningful challenge. We have therefore synthesized and performed a SAR study on novel 2-substituted-6-biarylmethylamino-9-cyclopentyl-9H-purine derivatives and related bioisosteres [2,3]. Novel compounds significantly increased CDK inhibitory activity and cytotoxic effects in cancer cell lines compared to previously described compounds of this class. In addition, several candidates showed promising anticancer activity in vivo [4].

[1] Jorda R, Paruch K, Krystof V. Cyclin-dependent kinase inhibitors inspired by roscovitine: purine bioisosteres. Curr Pharm Des. 2012;18(20):2974-80.

[2] Jorda R, Havlícek L, McNae IW, Walkinshaw MD, Voller J, Sturc A, Navrátilová J, Kuzma M, Mistrík M, Bártek J, Strnad M, Krystof V. Pyrazolo[4,3-d]pyrimidine bioisostere of roscovitine: evaluation of a novel selective inhibitor of cyclin-dependent kinases with antiproliferative activity. *J Med Chem*. 2011; 54(8):2980-93.

[3] Gucký T, Jorda R, Zatloukal M, Bazgier V, Berka K, Řezníčková E, Béres T, Strnad M, Kryštof V. A novel series of highly potent 2,6,9-trisubstituted purine cyclin-dependent kinase inhibitors. *J Med Chem*. 2013; 56(15):6234-47.

[4] Haider C, Grubinger M, Řezníčková E, Weiss TS, Rotheneder H, Miklos W, Berger W, Jorda R, Zatloukal M, Gucky T, Strnad M, Kryštof V, Mikulits W. Novel inhibitors of cyclin-dependent kinases combat hepatocellular carcinoma without inducing chemoresistance. *Mol Cancer Ther*. 2013; 12(10):1947-57.

Acknowledgements

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Komunikaty (K1-K28)

K1

Hybrid Combinatorial Substrate Library in design of specific and active probes for proteolytic enzymes

Marcin Drąg[1], Marcin Poręba[1], Paulina Kasperkiewicz[1], Wioletta Rut[1], Scott Snipas[2], Heather Parker[3], Christine Winterbourn[3], Guy S. Salvesen[2]

[1]Division of Bioorganic Chemistry, Faculty of Chemistry, Wroclaw University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland; [2]Sanford-Burnham Medical Research Institute, 10901 North Torrey Pines Road, La Jolla, USA; [3]University of Otago, Christchurch, New Zealand

Proteases are known to participate in many cellular processes due to their ability to process the peptide bond. They are key players in several cascades taking place in the cell with apoptosis, fibrinolysis, blood clotting, complement fixation, gastrulation or general cell cycle being only a few examples. However, proteases can be also bad guys and participate in the cellular events, which lead to severe diseases such as cancer, diabetes, pathogens infections or hypertension [1].

Each protease recognizes only substrates, which can fit into the recognition pockets. There are several methods of determination of substrate specificity [2]. One of the most versatile is Positional Scanning Substrate Combinatorial Library approach, which allows fast and reliable determination of substrate preferences for most of the proteases. To date used libraries contained only natural amino acids and only very occasionally unnatural amino acids were incorporated into the structure of synthesized substrates [3]. To get better insight into substrate specificity, we have applied several different tailored to certain type of protease approaches using broad range of unnatural amino acids. This allowed us to obtain for several different endopeptidases much better substrates comparing to natural amino acids derivatives. Full strategy for profiling of substrate specificity for serine and cysteine endopeptidases will be presented [4, 5].

[1] M. Drag, G. S. Salvesen, *Nature Reviews Drug Discovery.* 2010; *9 (9):* 690-701.
[2] M. Poreba, M. Drag. *Current Medicinal Chemistry* 2010; *17(33):* 3968-95.
[3] P. Kasperkiewicz, A. D. Gajda, M. Drag, *Biological Chemistry,* 2012, *393 (9):* 843-51
[4] P. Kasperkiewicz , M. Poreba, S.J. Snipas, H. Parker, C.C. Winterbourn, G.S. Salvesen, M. Drag M., *Proc Natl Acad Sci U S A.* 2014 *111(7):* 2518-23
[5] M. Poreba, P. Kasperkiewicz, S.J. Snipas, D. Fasci, G.S. Salvesen, M. Drag M., *Cell Death & Differentiation (Nature Press)*, 2014, in press (doi: 10.1038/cdd.2014.64.)

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K2

Systematic study reveals new potent and selective 5-HT6R ligands based on indole scaffold

Adam Hogendorf [a,b], Grzegorz Satała [a], Jakub Staroń [a], Dawid Warszycki [a], Agata Hogendorf [a], Ryszard Bugno [a], Andrzej J. Bojarski [a]

[a] Department of Medicinal Chemistry, Institute of Pharmacology Polish Academy of Sciences, 12 Smętna Street, 31-343, Kraków, Poland

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Herein we summarize the development of new series of 5-HT_6R ligands. Based on pharmacological profile of a published compound [2-(4-iodo-2,5-dimethoxyphenyl)ethyl][(2-methoxyphenyl)methyl] amine[1,2] (Cimbi-5), it has been assumed that structural modifications could possibly retain or modify its 5-HT_{2A}R and 5-HT_6R activity while keeping high selectivity over related targets intact (K_i = 0.044 nM at 5-HT_{2A}R, K_i = 73 nM at 5-HT_6R; $K_i \geq$ 500 nM was reported for receptors: 5-HT_{1A}, D_3, H_2, 5-HT_{1D}, α_{1A} adrenergic, δ opioid, 5-HT_{5A}, 5-HT_{1B}, D_2, 5-HT_7, D_1, 5-HT_3, 5-HT_{1E}, D_5, muscarinic M_1-M_5, H_3, and transporters: DAT, SERT[3,4]). Incorporation of 3-methylindole in place of benzyl group was considered. Three pilot compounds AH-120, AH-122 and AH-125 were synthesized and their affinities towards 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7 and D_2 receptors were determined in radioligand assays. While the 5-HT_{2A}R activities were found at least four orders of magnitude lower in comparison to the parent compound, the 5-HT_6R binding affinities were favourable (39 nM $\leq K_i \leq$ 87 nM). New ligands (AH-184, AH-185 and 186) were subsequently synthesized to check the pharmacological profile of raw core substructure of the pilot series. It became clear that the substitution patterns in AH-120, AH-122 and AH-125 do not contribute significantly to their 5-HT_6R activity. In the next step we prepared database of available aromatic aldehydes and arylethylamines; the C-N bond between these reagents can be readily formed via reductive amination to yield more derivatives of the investigated group of ligands. All the 3364 structures of virtual library were docked to 5-HT_{2A}, 5-HT_{2C} and 5-HT_6 receptor homology models using Glide software. Virtual docking will be used to search for additional ligand-aminoacid interactions that can arise after incorporation of specific functional groups. Compounds from the sets are to be synthesized in our search of potent and selective 5-HT_6 receptor ligands; it is likely that further improvements in binding affinity will be reached as soon as appropriate substitution patterns in both aromatic systems are found.

[1] Heim, R., *Synthese und Pharmakologie potenter 5-HT_{2A}-Rezeptoragonisten mit N-2-Methoxybenzyl- Partialstruktur*. Ph.D. Dissertation, Free University of Berlin, 2004.

[2] Braden, M. R.; Parrish, J. C.; Naylor, J. C., Nichols, D. E. Mol. Pharm. 2006, 70, 1956-1964.

[3] Ettrup, A.; Hansen, M.; Santini, M. A.; Paine, J.; Gillings, N.; Palner, M.; Lehel, S.; Herth, M. M.; Madsen, J.; Kristensen, J.; Begtrup, M.; Knudsen G. M. Eur. J. Nucl. Med. Mol. Imaging 2011, 38, 681-693.

K3

Recent advances in functioning of G protein-coupled receptors: insight from molecular modelling

Agnieszka A. Kaczor

[a]*Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Lab, Faculty of Pharmacy with Division for Medical Analytics, Medical University of Lublin, 4a Chodzki St., PL-20059 Lublin, Poland*

G protein-coupled receptors (GPCRs) represent the most important family of drug targets to date. However, state-of-the-art experimental procedures, able to characterize in deep both GPCR modulation in health and disease and the molecular mechanisms of drug action at these receptors, have provided a more nuanced picture than previously expected. Several aspects of GPCR function, which are currently being characterized, clarify some regulatory processes regarding these receptors and, at the same time, introduce novel levels of complexity which should be taken into consideration for rational drug design. In this scenario, computational approaches can help in several ways to rationalize the increasing amount of data on GPCRs and their ligands. On one hand, a set of databases devoted to these receptors provide excellent starting points for data mining. On the other hand, exploitation of the ever-increasing ligand and structure-based information by novel computational techniques can help addressing emerging questions in the GPCR field. Some of these questions comprise the refined modulation of GPCR signaling states by biased agonists, the exploitation of GPCR oligomers as drug targets, the analysis of polypharmacology in GPCR ligands, the development of strategies for receptor deorphanization or the prediction of off-target interactions of known drugs targeting these receptors. During the lecture I will cover some of these strategies for knowledge-based rational design for GPCRs and will discuss the main hurdles which they may need to overcome to yield novel, safer and more efficacious drugs possessing polished mechanisms of action.

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K4

Binding features of estrogen receptor beta selective ligands – docking investigation

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Estrogen receptors exist as two subtypes ERα and ERβ which are characterized by various distribution in human tissues and diverse transcription regulation. Ligands capable of selective ERβ activation show positive effect in treatment of such diseases as certain cancers, endometriosis, inflammatory diseases and assist in maintaining cardiovascular and nervous system health. In order to provide new treatment for such diseases a new generation of selective estrogen receptor modulators is required. This remains unsolved task due to several difficulties. Based on prior studies it is known that minor modifications of ligands can influence selectivity of ER agonists binding. Structural changes responsible for this effect remain unknown due to limited experimental data and its incomplete interpretation. The majority of designed ligands acting on ER possess chiral centers thus exist as stereoisomers. Unfortunately, not every spatial isomer is individually considered in experimental research. In general, stereoisomeric mixtures are tested. It hinders explanation how molecule stereochemistry influences subtype binding affinity and selectivity. In order to reveal differences between compounds with different stereochemistry, molecular modeling techniques might be the only effective method.

The aim of this study is to investigate structural basis of diverse selectivity and binding affinity of chosen estrogen receptor β agonists based on the proposed pharmacophore model. Conducted docking simulations revealed that terminal aromatic rings position in the A- and D-ring regions is a factor determining binding affinity of ERβ agonists. This can be ascribed to two terminal hydroxyl groups, rigid linker, and introduction of aliphatic substituents. Key ERβ selectivity factors are substituents fitted inside characterized cavities I and II, their bulkiness, attachment to linker, and stereochemistry. These molecular features should be considered during search and design of new improved estrogen receptor β agonist.

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K5

Synthesis and in vitro anti-trypanosomal screening of hydroxynaphthalene-2-carboxanilides

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Trypanosoma brucei is a species of salivary trypanosome which causes African trypanosomiasis, known also as sleeping sickness. *T. brucei* has traditionally been grouped into three subspecies: *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense* [1]. *T. brucei* is one of only a few pathogens that can cross the blood brain barrier [2]. In 2009 the number of reported cases was 9878, in 2012 the number of new reported cases decreased to 7216. However, the estimated number of actual cases is 20 000 and the estimated population at risk is 70 million people. Four drugs that show non-negligible undesirable effects are registered for the treatment of sleeping sickness [3]. These facts underline the urgency of searching for new structure types of drugs to achieve effective control of African trypanosomes.

Series of ring-substituted 1-hydroxynaphthalene-2-carboxanilides and 3-hydroxynaphthalene-2-carboxanilides were prepared by microwave-assisted synthesis [4,5]. Primary *in vitro* screening of the synthesized compounds was performed against wild-type S427 (bloodstream form) of *T. b. brucei*. The position of the phenolic moiety on the naphthalene scaffold is significant for the activity. Generally it can be summarized that the compounds with lipophilic and/or electron-withdrawing moieties showed activity comparable with that of suramin.

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K6

Unnatural amino acids as a new tool for investigation of substrate specificity of human caspases

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Cysteine proteases are one of the largest group of proteolytic enzymes that regulate most of physiological processes in all living organisms. This group is divided into several clans, among which CA clan is the largest and well described in the literature. However, recently main efforts regarding cysteine proteases characterization have been shifted toward CA clan of proteases. This clan contains alter alia clostripains (C11), leguamins (C13), caspases (C14), gingipains (C25) and separases (C50). All those members exhibit some structural similarities like His-Cys catalytical dyad that is incorporated in common His-Gly-linker-Ala-Cys motif. Caspases and legumains are believed to have similar protein fold, however their biological functions are different. What`s more interesting caspases and legumain share some similarities in substrate hydrolysis. Legumains have strongly prefer hydrolyzing substrates after asparagnine residue but also display ability to cleave peptides on the carboxyl site of aspartic acid residue, which overlaps with caspases activity. To date many different types of substrates have been synthesized and biologically evaluated toward caspases and legumain. Unfortunately these chemical probes lack specificity, because these enzymes display similarities in their active site architecture. The knowledge regarding subsites preferences can be significantly enlarged with the use of unnatural amino acids. Here we present the full substrate specificity profiles of apoptotic caspases and legumain. These results have been obtained with the use of fluorogenic tetrapeptide combinatorial library containing natural and unnatural amino acids. The use of unnatural amino acids allowed us to design very active and selective caspases substrates, as well as a brand new legumain substrates, that display no overlapping with any tested caspase. This work was supported by the National Sceince Centre grant 2011/03/B/ST5/01048 in Poland to MD, the Ministry of Science and Higher Education in Poland - grant Iuventus Plus to MP and by the European Union as a part of the European Social Found.

K7

Phosphinic inhibitors of aminopeptidases

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Phosphorus-containing transition state analogues represent an interesting class of potent inhibitors of alanyl aminopeptidase (APN), a zinc-dependent exopeptidase that catalyzes the release of neutral amino acids from the N-termini of peptides or proteins. The most significant deregulation of the mammalian ortholog action in the biomedical context is associated with angiogenesis, cancerogenesis, malignancy and metastasis [1,2]. The counterpart enzyme expressed by microorganisms is mostly responsible for proteolysis and nutrition delivery. It participates in proteins digestion in the host body, what is directly responsible for the clinical symptoms of bacterial and parasite infections [3]. Infection of *Neisseria meningitidis*, a gram-negative bacterium that cause meningitis, can be an example of such a bacterial pathology. Meningococcal meningitis is a life-threatening disease, without medical therapy almost always fatal. Blocking the activity of alanyl aminopeptidase from the human pathogen might appear a new strategy in highly specific control of meningococcal meningitis and can allow to recognize new tools to identify the precise roles of APN in the life cycle of *N. meningitidis.* Recently, recombinant *Nm*APN was isolated, its sequence, the crystal structure and the substrate specificity were investigated [4,5].

In this work we present new strategy of rational modifications of canonical organophosphorus inhibitors that led to identify highly effective and selective inhibitors of APNs, with inhibition constants at the nanomolar range. New compounds act in a competitive, non-covalent manner. The phosphonate group closely mimics the high energy transition state of the reaction catalyzed by the enzyme, whereas the P1 and P1′ residues bind specifically to the corresponding S1 and S1′ pockets.

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K8

Activation of β2-adrenergic receptor with (*R*,*R*')-4'-methoxy-1-naphthylfenoterol inhibits proliferation and motility of a panel of melanoma cell lines

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(*R*,*R*')-4'-methoxy-1-naphtylfenoterol (MNF) inhibits proliferation of 1321N1 astrocytoma cell line in a β_2-adrenergic receptor (β_2AR)-dependent manner, while reducing rat C6 glioma growth in a xenograft mouse model. MNF interferes also with proto-oncogenic signaling induced by the orphan G-protein-linked receptor, GPR55, in human HepG2 hepatocarcinoma and PANC-1 pancreatic carcinoma cells.

Here, we utilized a number of cellular and biochemical techniques to investigate the anti-tumorigenic potential of MNF in a panel of human melanoma cell lines expressing varying levels of β_2AR. Scratch assays and real-time analysis using xCELLigence system demonstrated the ability of MNF to dose-dependently reduce the motility and invasiveness of three different melanoma cell lines, namely UACC-647, M93-047 and UACC-903. Degree of the inhibition was significantly higher in cells expressing more β_2AR. Pharmacological inhibition of β_2AR and protein kinase A (PKA) protected against the actions of MNF on cell motility. Forskolin, activator of adenylyl cyclase, and Ro 20-1724, inhibitor of cAMP-hydrolyzing phosphodiesterase 4, mimicked the MNF activity on UACC-647 cells migration.

We concluded that the anti-tumorigenic functions of MNF in melanoma cells rely on β2AR-dependent accumulation of cyclic AMP, subsequent activation of PKA and phosphorylation of downstream signaling molecules.

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K9

Apoptotic effects of novel pyridine platinum(II) complexes on the Ishikawa endometrial cancer cell lines

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The aim of the present study was to examine the impact of a four berenil complexes of platinum(II) of formula $[Pt_2L_4(berenil)_2]Cl_4$, where L is 4-ethylpyridine (Pt10), 3-ethylpyridine (Pt11), 3-(n-butyl)pyridine (Pt12) and 4-(t-butyl)pyridine (Pt13) on viability of Ishikawa endometrial cancer cells, inhibition of [^{3}H]thymidine incorporation into DNA and pro-apoptotic effect [1]. These compounds can constitute potential drugs of high antineoplastic activity. Our results confirm that compounds Pt10-Pt13 are more potent antiproliferative agents than cisplatin on the Ishikawa endometrial cancer cells. The human skin fibroblast cells were used as comparative cells. Moreover, it was shown that all examined compounds Pt10-Pt13 inhibit DNA biosynthesis in neoplastic cells stronger than in human skin fibroblasts.

Flow cytometric analysis after annexin V-FITC and propidium iodide staining confirmed also that apoptosis was the main response of Ishikawa endometrial cancer cells to Pt10-Pt13 treatment. This compounds showed higher ability to induce apoptosis in Ishikawa endometrial carcinoma cells in comparison with cisplatin. Our results suggest that apoptosis of Ishikawa endometrial cancer cell lines in the presence of Pt10-Pt13 follows the mitochondrial pathway, with the decrease in mitochondrial membrane potential and activation of caspase 9, as well as by the external pathway with the significant increase in FADD protein expression and caspase 8. The activation of caspase 3 simultaneously with increase DNA fragmentation confirmed also that apoptosis was the main response of endometrial cancer cells to Pt10-Pt13 treatment. Moreover, activation of caspase 3 showed that caspases cascade was started, particularly effector caspase 3, engaged in the executive phase of apoptosis. The obtained results in the present study demonstrated the cytotoxic activity of new pyridine platinum(II) complexes can be connected with their ability to impair of DNA biosynthesis and induction of apoptosis.

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K10

Pharmacological and behavioral characterization of organic cation transporter-2 deficient mice

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Central serotonergic and noradrenergic pathways control a wide range of functions including mood and emotion, and dysfunction of these circuits has indeed been implicated in the etiology and physiopathology of anxiety and depression. The high-affinity sodium driven transporters for noradrenaline (NE) and serotonin (5-HT), which ensure neurotransmitter clearance at the synapse, are the principal targets of widely used antidepressant drugs. Other monoamine transport systems, with lower affinity, have been detected in the brain, but their role is largely unknown [1,3]. The body of neurochemical and electrophysiological evidence presented herein [2] demonstrates that OCT2, a member of the polyspecific low affinity, high-capacity organic cation transporter (OCT) family, is expressed notably in the limbic system and implicated in anxiety and depression-related behaviors in the mouse. Genetic deletion of OCT2 in mice produced a significant reduction in brain tissue concentrations of NE and 5-HT and in *ex vivo* uptake of both these neurotransmitters in the presence of the dual 5-HT–NE transport blocker, venlafaxine. *In vivo* clearance of NE and 5-HT evaluated using microiontophoretic electrophysiology was diminished in the hippocampus of OCT2 knock out mice in the presence of venlafaxine, thereby affecting postsynaptic neuronal activity. OCT2 knock out mice displayed an altered sensitivity to acute treatments with NE- and/or 5-HT-selective transport blockers in the forced-swim test. Moreover, the mutant mice were insensitive to long-term venlafaxine treatment in a corticosterone-induced, chronic depression model. In conclusion, our results reveal the role of OCT2 in NE and 5-HT uptake in the brain as a complementary system to the high-affinity transporters, important for mood-related behaviors. They emphasize the importance of OCT2 in the acute action of antidepressants and indicate that this transporter is required for chronic antidepressant treatments to exert their full effects. As OCT2 appears to have a similar localization in human and rodent brain, our findings identify OCT2 as an important postsynaptic determinant of aminergic tonus and mood-related behaviors and a potential pharmacological target for mood disorders therapy.

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K11

The molecular mechanism of apoptosis by novel dinuclear platinum(II) complex with anti-MUC1 in estrogen negative MDA-MB-231 breast cancer cells

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Mucin 1 (MUC1) is a type I transmembrane glycoprotein that is expressed on apical membranes in a variety of normal tissues, but aberrantly is overexpressed in various cancer cells especially in breast cancer cells. *In vitro* studies demonstrated that the expression of MUC1 is involved in the invasion and resistance to genotoxic anticancer reagents suggesting that it is closely associated with poor prognosis of patients with breast cancer. Investigators reveal that MUC1 has immense potential as diagnostic or prognostic marker and as therapeutic target in breast cancer [1]. The aim of the study was to evaluate the proapoptotic properties of a new dinuclear platinum(II) complex Pt12 used together with anti-MUC1 monoclonal antibody in human MDA-MB-231 breast cancer cells. The effect of Pt12 with anti-MUC1 was investigated using flow cytometry assessment of annexin V binding, analysis of mitochondrial membrane potential and defragmentation of DNA by TUNEL assay. The cellular response of human breast cancer cells to Pt12 with anti-MUC1 has been studied using cisplatin as a reference. Flow cytometric analysis of breast cancer MDA-MB-231 cells was performed after 24 and 48 h of incubation with anti-MUC1 (10 μg/mL), Pt12 (10 μM), Pt12 + anti-MUC1 (10 μM + 10 μg/mL), cisplatin (10 μM), cisplatin + anti-MUC1 (10 μM + 10 μg/mL) and staining with Annexin V and propidium iodide (PI). We have found that the apoptotic effect of Pt12 with anti-MUC1 was stronger than evoked by cisplatin, anti-MUC1, Pt12, and cisplatin used with anti-MUC1. We have also investigated the effect of compounds on mitochondrial membrane potential. The highest decrease of the mitochondrial potential was observed after 48h of incubation with Pt12 and anti-MUC1. To detect the yield of DNA strand breaks, which are intimately associated with an apoptotic response, the TUNEL assay was performed after treating cells with compounds for 24 and 48 h. We determined whether Pt12, cisplatin, Pt12 with anti- MUC1, and cisplatin with anti-MUC1 induced DNA fragmentation in MDA-MB-231 breast cancer cells. A highest increase in the percent of TUNEL positive cells was observed after incubation of Pt12 with anti-MUC1 in comparison to cisplatin and combined treatment of reference compound with anti-MUC1 after 24 and 48 h. These results indicate that Pt12 with anti-MUC1 induces apoptosis, decreases mitochondrial membrane potential and induces DNA fragmentation in estrogen negative MDA-MB-231 breast cancer cells and it may go through the mitochondrial pathway.

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K12

The II phase metabolism of endogenous and exogenous compounds, including antitumor chemotherapeutics

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The II phase metabolism, it is a set of metabolism and excretion pathways of endogenous as well as exogenous compounds including xenobiotics. UDP-glucuronyltransferases (UGTs; EC 2.4.1.17) are the most crucial representatives of II phase enzymes, which are responsible for the transformation of bilirubine and bile acids, steroids and thyroid hormones and lipids. Exogenous compounds, including drugs, carcinogens, environmental pollutants and nutrient components are also the substrates of UGTs. Deactivation of xenobiotics and the following excretion of hydrophilic conjugates is the main task of glucuronidation [1]. However, one can found glucuronides of comparable or even higher reactivity than that of the native compound. For example, morphine 6-O-glucuronide, and glucuronides of retinoids and nonsteroid anti-inflammatory drugs represent the group of active glucuronides [2]. Nearly 35% of all drugs are metabolized by UGTs. Major sites of these reaction include the liver, intestine and kidney. There were found 22 functional UGT isoforms that belong to 5 subfamilies (UGT1A, 2A, 2B, 3A and 8A). Among variety of drugs conjugated by UGTs, anticancer agents are of special interest, because of the reported differences in UGT expression in normal and tumour tissues. On the other hand, glucuronidation may also represent a mechanism of intrinsic drug resistance, as it was observed for irinotecan and methotrexate glucuronides in colon and breast cancer, respectively [3]. It has also been shown that new types of glucuronides would play a role of prodrugs, that are hydroxylyzed selectively in tumour cells [4].

Studies of our group indicated that triazolo- and imidazoacridinone antitumor agents, C-1305 and C-1311 were glucuronidated with human liver and intestine microsomes and selectively with UGT1A10 isoform *in vitro* [5]. Furthermore, C-1305 glucuronide gave higher cytotoxicity than the parent drug [6]. We also demonstrated that another acridine antitumor agent, C-1748, was transformed to specific glucuronide on aliphatic hydroxyl group. Furthermore, our current results indicate that the studied antitumour acridines are able to modulate enzymatic activity of UGT. In conclusion, we demonstrated that not only drug metabolism but also drug-drug interactions should be considered in the design of acridine mediated antitumor therapy in combination with other chemotherapeutics.

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K13

Effects of new analogs of enkephalin and deltorphin in antinociceptive tests in animals

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The aim of study was to exam the antinociceptive activity of new analogs of enkephalin (cUENK6) and deltorphins (DK-4, DEL-6, EW1 and EW2) in two thermal nociceptive tests: tail-immersion and hot-plate in animals. The antinociceptive effect of peptides was compared to morphine effects. All peptides are either a cyclic analog of enkephalin (cUENK6) or deltorphin (DEL-6, EW1 and EW2), except DK-4 that was linear analog of deltorphin II. Furthermore, all peptides have incorporated either ureid bridge or carbonyl bridge in their structures. *In vitro* cUENK6 was preferential (but not selective) functional MOR agonist [Pawlak et al., 2001] but deltorphin analogs (DK-4 and DEL-6) were preferential DOR agonists [Pawlak et al., 2001; Kotlinska et al., 2010]. To determine *in vivo* activity of peptides towards opioid receptors, the selective antagonists of MOR, DOR and KOR were used. In our study, cUENK6 showed stronger and longer antinociceptive effect than morphine. Although analgesic effect of this peptide was preferentially inhibited by selective DOR antagonist, a cross-tolerance between morphine and cUENK6 was shown. In turn, deltorphin analogs revealed antinociceptive effect comparable or weaker than morphine. DEL-6 effect was inhibited more efficiently by MOR antagonist, while DK-4 activity was inhibited mainly by DOR antagonist. Moreover, combined administration of subanalgesic dose of morphine and DEL-6 indicated strong additive analgesic effect. The cross-tolerance between morphine and deltorphins analogs was also showed. The obtained results suggest, that although MOR and DOR are involved in antinociceptive effects of deltorphins analogs, they preserve MOR-independent analgesic activity (probably DOR-dependent). Our animal *in vivo* studies did not confirm *in vitro* receptor affinities of these peptides.

K14

Cyclopeptides with potential immunosuppressive activities

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Cyclic peptides have a variety of biological functions, most of them are hormones, antibiotics or toxins (e.g. insulin, oxytocin, vasopressin, calcitonin, gramicidin, cyclosporine). They are more resistant to proteases than their linear counterparts. Due to the reduced conformational freedom cyclopeptides represent a convenient tool for conformational studies to establish the structural requirements for ligand – receptor interactions [1].

Here we present the results of work on analogues of natural cyclononapeptide - cyclolinopeptide A (CLA). Cyclolinopeptide A, cyclo-(Leu-Ile-Ile-Leu-Val-Pro-Pro-Phe-Phe-), was isolated from linseed oil. CLA possesses a strong immunosuppressive activity comparable with that of cyclosporin A. It has been postulated that CLA inhibits calcium-dependent activation of limfocytes T through interaction with cyclophilin A - the peptidyl-prolyl cis–trans isomerase (PPIase) [2]. However, our research using SPR methodology shows that the effects of CLA with the enzyme are nonspecific in order to its low complexity suggesting a different mechanism of action [3]. The series of CLA analogs modified with pseudoprolines derived from serine, threonine and methylserine were synthesized manually by standard solid-phase procedure and high dilution based cyclization method. Some peptides strongly suppressed mitogen-induced proliferation of T and B cells to a similar degree as well as the humoral immune response *in vitro*. CD spectra indicate that the conformational freedom of active compounds is largely increased in comparison to other peptides. A moderate toxicity with regard to mouse and human lymphocyte suggest potential therapeutic application of selected analogs in the treatment of some diseases of the immune system.

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K15

Arrays of peptides immobilized on the cellulose – new tool in medical diagnostic

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Arrays of peptides immobilised on cellulose [1] were found useful for epitope mapping, molecular receptors, catalysts and many others. We used the classic SPOT methodology [2] to obtain peptides arrays for identification of immunologically active fragments (epitopes) of ureases excreted by typical human pathogens. For several of them, homology with fragments of human proteins was found, strongly suggesting participation of the molecular mimicry mechanism in autoimmune diseases. Selected epitopes were found useful for diagnosing patients suffering atherosclerosis and/or rheumatoid arthritis [3].

On the other hand, we observed for peptides immobilised on the cellulose surface the process of self-organization, leading to formation of molecular receptors, which are prone to binding ligands in cavities formed in-between the peptide chains. Receptors formed on this way recognize shape, size, charge distribution and chirality of ligands very efficiently and moreover, docking procedure is reversible. This enabled application of peptide arrays in studies on the binding profile of pharmaceutically active compounds [4], as a new platform for preliminary screening new drug candidates [5], for differentiation of human healthy and thyroid tumour tissue and for monitoring metabolite profile for non-invasive cancer diagnostic in mice model [6].

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K16

Bioisosteric substitution of sulphonyl group in 5-HT$_6$R ligands - A study on ligand-receptor interactions

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Bioisosterism is a method extensively used by medicinal chemists to design new molecules by structural modifications of known biologically active compounds, to obtain substances with improved pharmacokinetic and/or pharmocodynamic profile.[1]Additionally, bioisosterism can be used for analysis of ligand-receptor interactions.

Most (86%) of known 5-HT$_6$R ligands can be described by a pharmacophore containing four elements: hydrophobic/aromatic group (e.g. phenyl), double hydrogen bond acceptor (e.g. sulphonyl group), hydrophobic core (e.g. indole, naphthalene) and basic nitrogen atom.[2,3,4,5] The remaining 14% of ligands does not possess either sulphonyl group or basic nitrogen but still are very active towards 5-HT$_6$R. In order to investigate the influence of sulphonyl group for interactions with receptor binding pocket, a series of ligands with bioisosteric substitution of sulphonyl group with carbonyl or methylene group were designed and synthesized. These groups either conserve hydrogen bond acceptor or spatial properties of sulphonyl group.

With the use of molecular modelling techniques and crystal structure analysis, a detailed analysis of binding mode was performed. A new model of interactions between ligand and amino acid residues in receptor binding pocket was proposed.

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K17

Prediction of the stereochemical effects in the ligand-receptor interactions: metadynamics simulations

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Understanding the chiral recognition mechanism underlying the stereoselectivity is extremely important for many fields of life sciences, including proteins and biomimetic receptors design and drug discovery. The asymmetrically bound ligand may produce greater variance in the receptor cavity that could further elevate the conformation heterogeneity and different downstream signaling pathways in the cell. Due to the significance of this problem, numerous experimental and theoretical studies are devoted to study the stereoselectivity-related phenomena. We developed the fast, screening computational approach designed to study the influence of the stereoconfiguration-related effects on the various chemical and biochemical processes. The method is aimed at calculating the free energy profiles using the molecular dynamics (MD) simulations combined with the enhanced sampling algorithms. The general idea is to describe all stereoisomers in terms of a single, common set of force fields parameters, allowing for the interconversion between particular stereoconfigurations. A large energetic barrier inherent in the modified terms of potential ('improper dihedral' type) prevents the spontaneous interconversion of the chiral centre during unbiased simulations but can be easily overcame when using the enhanced sampling techniques. The extension of the described technique is straightforward; the modified potential describing the stereoconfiguration of the chiral center(s), can be used directly in other types of enhanced-sampling simulations (e.g., parallel tempering, umbrella sampling) to provide not only the free-energy profile(s) but also some more information of other types. Furthermore, all chiral centers of interests can be tackled simultaneously, during a single, short MD simulation. The proposed method combined with metadynamics technique appeared to be most effective for studying the trends in ligand–receptor affinity values; the mechanistic aspects of ligand–receptor interactions (e.g. for determining the position of bound ligand) require additional simulations. We were able to predict the stereoselective effects present during binding of fenoterol stereoisomers to the β2-adrenergic (the simulation lasting only 2 ns allows to estimate the order of affinities for four stereoisomers). The trend in the binding affinities of all stereoisomers is reflected properly, including the highest and lowest affinities characteristic of (R,R)- and (S,S)-fenoterol, respectively, Fig. 1.

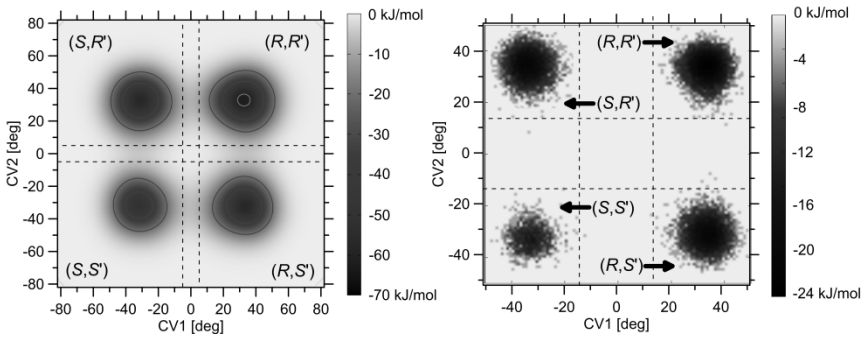

Fig. 1. Fig. 3. (left) A typical free energy landscape obtained as the result of single, metadynamics-based simulation. (right) The free energy landscape obtained as the result of REMD-based simulation.

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K18

Search for new inhibitors of Insulin/Amylin aggregation

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Amyloidosis includes a variety of unrelated disease entities, characterized by one common element - the formation of insoluble deposits, known as amyloid deposits or amyloids. Between 1,500 and 3,500 new cases are diagnosed every year. Currently known are at least 21 proteins that are precursors of different forms of amyloidosis. This group includes cystatin C, α-synucleine, β2-microglobulin, immunoglobulin light chain, transthyretin, apolipoprotein I, apolipoprotein II, gelsolin, lysozyme, fibrynogen α and others. Diabetes is a chronic disease characterized by either the inability of the body to produce insulin (type 1) or the failure to respond to it (type 2) [1]. Insulin and amylin aggregation resulting in the formation of amyloid fibrils is combined with the development of diabetes type 1 and 2. Amyloid fibrils contain the polypeptide chains organized in a "cross-β-sheet" conformation [2]. It is well known that mechanism of amyloid formation is a common for all different polypeptides (proteins). It includes a conformational transition of α-helical or non-β-sheet soluble forms into β-sheet aggregates [3]. There is also increasing evidence that fibrillar and/or prefibrillar aggregates are cytotoxic and that their formation process is directly associated with the pathologic sequel of the corresponding disease [4]. The investigation and development of fibrillogenesis inhibitors is an important scientific and therapeutic goal for at least three reason. First, it is understood that both the soluble monomers, oligomers and insoluble deposits are responsible for the cytotoxicity. Second, fibrillogenesis inhibitors are usually identified experimentally as such, but these compounds may also bind to intermediates in the fibrillogenesis pathway and have hard-to-predict consequences, including improved clearance of more cytotoxic soluble oligomers. Third, inhibitors are valuable structural probes. Currently, the most efforts are dedicated to the rational design of fibrillogenesis inhibitors. Among these, there are internal segments of fibril-forming peptides modified by incorporation modified amino acids which lead to modifications of fibillogenic domains, insertion of prolines into fibrillogenic domains, modification of peptide termini, peptide cyclization. In our studies we applied methods of modification of peptide backbone atoms by incorporation of *N*-methylated amino acids and α,α-disubstituted residues.

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K19

Synthesis, anticonvulsant activity and mechanism of action of some aminoalkanol derivatives

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Among most frequent disorders of neurological system there are epilepsy and neuropathic pain. Epilepsy is defined as occurrence without identifiable cause recurrent epileptic seizures, while neuropathic pain is connected with abnormal function of neurons. Common pathomechanisms of those two disorders, i.e. pathological changes occurring within nervous system enable simultaneous development of drugs for those kind of disorders. Both epilepsy and neuropathic pain, despite available pharmacotherapy, remain insufficiently treated in significant groups of patients. It is estimated that about 30% of epilepsy patients still suffer from seizures, while among patients experiencing partial seizures (simple, complex, and secondarily generalized) the percentage is even higher and reaches 40%. Neuropathic pain is insufficiently treated in about 50% of patients. [1]

Herein we present that incorporating ether moiety between phenoxy and alkylaminoalkanol groups of phenoxyalkylaminoalkanols influenced the specificity of mechanism of action of the last, resulting in very beneficial results in in vivo pharmacological tests. The presented N-[(phenoxy)ethoxy]alkylaminoalkanols similary to formerly described phenoxyalkylaminoalkanols [2] are characterized by beneficial pharmacological properties in in vitro and in vivo testing for potential use in treatment of disorders and symptoms with neurological etiology, such as epilepsy and pain. Moreover, enantioselectivity in terms of mechanism of action of the most active compounds, for several targets, has been shown. [3]

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K20

N-Substituted *N'*-(2-arylmethylthio-4-chloro-5-methylbenzene sulfonyl)guanidines – novel antiproliferatives, antimicrobials and inhibitors of human carbonic anhydrase isozymes I, II, IX and XII

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The sulfonamides are an important class of compounds with a broad spectrum of applications in medicinal chemistry. Depending on the modification of their structure, they exhibit antibacterial, hypoglycemic, diuretic, anticarbonic anhydrase, antithyroid, anti-inflammatory and anticancer activities [1]. It has been known that sulfonamides may act as antitumor agents through a variety of mechanisms, and the most prominent mechanism is the inhibition of carbonic anhydrase isozymes [2].

Meanwhile, sulfonamides has played important role in treatment of antimicrobial infections since over 70 years ego. This class of sulfa drugs are popular due to good tolerance by patients, ease of administration, wide spectrum of antibacterial activity and relatively low costs [3]. Sulfonamide derivatives as the structural analogues of *p*-aminobenzoic acid, inhibit the 6-hydroxymethyl-7,8-dihydropteroate synthase and limit of folic acid synthesis in prokaryotes, that is essential to cell growth [4].

We would like to present results of our studies on synthesis and biological activities of novel *N*-substituted *N'*-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl)guanidine. The most active antitumor guanidine derivatives, inhibiting 32-35 human tumor cell lines with GI_{50} in the range of 2.1-5.0 μM also showed relatively high inhibitory activity toward transmembrane tumor-associated isoforms hCA IX and XII with K_i from 18-40 nM. We also obtained compound which presented high selectivity toward isozymes hCA IX and XII and displayed significant inhibitory activity against A498 cell line of renal cancer [5].

The majority of compounds exhibited antibacterial potency against isolates from patients with infections of oral cavity, respiratory tract and intestinal tract. The most active compound showed very strong activity, with MIC ≤ 6.2 μg/ml against eleven bacteria strains belonged to Gram-positive anaerobes and aerobes and also exhibited promising activity toward resistant microorganisms such as *methicillin-resistant Staphylococcus aureus* and *Enterococcus faecalis*.

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K21

Modification of polysaccharides structure and biological activity by selenium incorporation

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Described in the scientific literature mushroom-derived exopolysaccharides, especially β-glucans display anticancer activity which results from their immunostimulating properties. On ground of the mechanism of the pharmacological activity, the mushroom-derived polysaccharides are classified as the biological response modifiers (BRM). We hypothesized, that enriched in selenium polysaccharides derived from mushroom would possess higher biological activity than non-enriched, currently used as immunomodulators. In our previous studies we have optimized a method for biosynthesis of the selenium-enriched polysaccharides in medicinal mushroom *Lentinula edodes*.

Currently we have isolated a Se-enriched exopolysaccharide fraction from Se-enriched mycelium of *L. edodes* and compared its structure and biological activity with not enriched reference fraction. The isolated Se-polysaccharide proved to be a mannoglucan of high (>900 kDa) molecular weight. The type of glycosidic bounds, identified by IR and NMR spectra, was mainly β, but also α. X-ray absorption spectrometry showed that the degree of Se oxidation in the polysaccharide was equal to –II. XANES and XAFS spectra (X-ray absorption spectrometry) show, that selenium in the structure of polysaccharide with great probability is glycosidically linked, to form a Se-glycoside.

When assayed in concentrations 1-100 μg/mL, the Se-polysaccharide caused significant inhibition of human T lymphocyte activation induced by mitogens, without any effect on reactive oxygen species production by granulocytes. This selective immunosuppressive activity, non-typical for mushroom derived polysaccharides, was significantly higher for Se-containing polysaccharides than for reference selenium-free fraction. *In silico* modeling of Se-polysaccharide molecule indicate significant differences in the structure, as compared with the reference fraction. Differences in the structure are probably responsible for the higher activity of Se-containing fraction.

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K22

Investigations of singlet and triplet states for sulfanyl porphyrazines with isophthaloxy substituents

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Porphyrazines have attracted considerable interest due to their potential as photosensitizers in Photodynamic therapy (PDT) and Photodynamic diagnosis (PDD) [1,2]. Therefore, photophysical and biological properties of many porphyrazines have been investigated [3,4]. In our study, the absorption, emission and synchronous fluorescence spectroscopy was applied to describe the singlet state of three sulfur porphyrazine derivatives possessing different peripheral substituents: isophthaloxyethylsulfanyl (**1**), isophthaloxybutylsulfanyl (**2**) and isophthaloxypentylsulfanyl (**3**). Absorption spectra for **1**, **2** and **3** in different organic solvents revealed strong Q-band at about 670 nm and sharp intense Soret band at about 380 nm. The fluorescence emission spectra of **1** show only one red emission (so-called $S_1 \rightarrow S_0$ emission), whereas for compounds **2** and **3** blue emission (so-called $S_2 \rightarrow S_0$ emission) was additionally observed. The fluorescence lifetimes for S_1 emission in methanol and acetonitrile and for S_2 emission in acetonitrile were measured for **2**, whereas for **1** in different organic solvents. The transient absorption spectroscopy was employed to describe the triplet states of **1**, **2** and **3** in acetonitrile and dichloromethane.

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K23

Structural and spectroscopic characterization and Hirsheld surface analysis of major component of antibiotic mupirocin - pseudomonic acid A

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Mupirocin is a mixture of several pseudomonic acids, with pseudomonic acid A constituting 95% of the mixture. As the main ingredient of mupirocin the pseudomonic acid A, (9-[(E)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl] oxynonanoic acid), *see Fig 1*, is extensively used worldwide as naturally occurring polyketide antibiotic, inhibitor of Gram-positive bacterial and mycoplasmal pathogens, produced by the Gram-negative soil bacterium *Pseudomonas fluorescens* NCIMB 10586 [1].

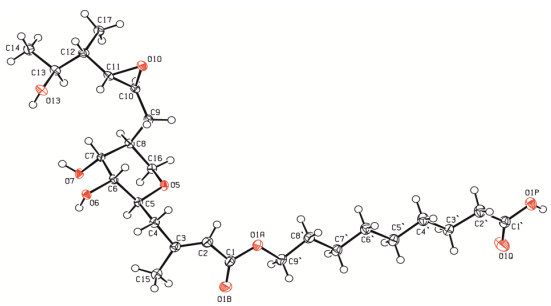

Fig. 1. Molecular structure of pseudomonic acid A.

The crystal structure of pseudomonic acid A was determined from single-crystal X-ray diffraction data at low temperature (100 K). The compound crystallizes in the monoclinic system, space group $P2_1$, with unit cell dimensions a = 12.4844(5), b = 5.0313(2), c = 21.5251(9) Å and β = 101.730(2)°. The molecules associate in dimers in head-to-tail motif through strong O-H···O hydrogen bonds packed in the parallel arrangement along crystallographic axis *b*. Additionally, relatively weak C-H···O and C-H···π interactions form 3-D hydrogen bond framework. From the Hirshfeld surfaces and 2-D fingerprint analysis it was found that the subtle interactions, such as H···H, associating two-thirds of the all intercontacts, provide extra stabilization in addition to the presence of the mentioned above strong hydrogen bonds. The electrostatic potential mapped over the Hirshfeld surface visualises electrostatic complementarities in the crystal packing. Results of X-ray diffraction and Monte Carlo methods reveal two conformations of *n*-alkyl chain of pseudomonic acid A, extended in the single-crystal and folded in the liquid state. A detailed interpretation of the FT-IR and NMR spectra will be also presented. The TG and DTG results indicated that pseudomonic acid A is stable up to 210°C .

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K24

From heterocyclic hydroxylamine-*O*-sulfonates to Safirinium: a novel fluorescent labeling platform

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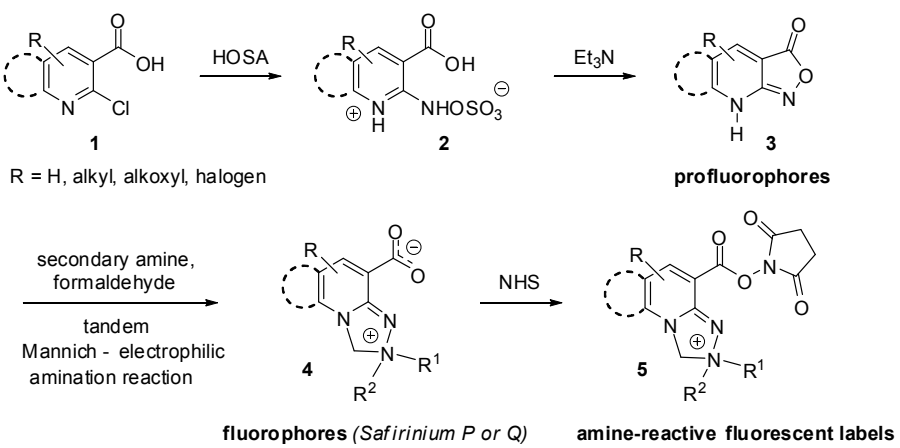
Fluorogenic reactions and reaction-based small molecule fluorescent probes making use of selective, bioorthogonal chemistry find practical applications in environmental analysis, occupational medicine and biological research [1].

New fluorescent dyes **4** and probes **5** were synthesized based on tandem Mannich – electrophilic amination reaction. First, the reaction of 2-chloroazine-3-carboxylic acids (**1**) with hydroxylamine-*O*-sulfonic acid (HOSA) gave corresponding betaines **2** which upon treatment with Et_3N afforded 2,1-isoxazolo-3-ones **3**. Reacting profluorophores **3** with secondary amines and formaldehyde gave 'click-on' fluorescent betaines **4** (*Safirinium*-P and *Safirinium*-*Q*), and hence, provided a new platform for the direct, selective and sensitive detection of formaldehyde and/or secondary aliphatic amines. Hydrophilic, water-soluble fluorescent 1,2,4-triazolo[4,3-*a*]pyridin-2-ium-8-carboxylates **4** were further converted into the amine-reactive *N*-hydroxysuccinimide esters **5** which, in turn, served for labeling lysine-containing proteins and cells [2,3].



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K25

Supramolecular structure of glycyrrhizic acid and carbenoxolone: properties of molecules forming stable micelles

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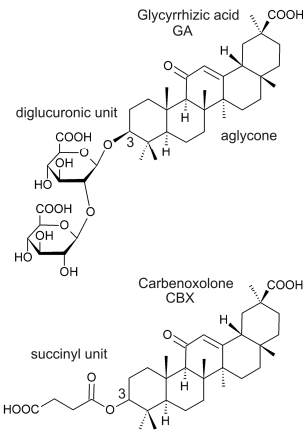
Nanostructures such as micelles, liposomes, vesicles, etc. are used in modern drug delivery system (DDS) to facilitate targeted transport and release of the drug in the body, what increases the effectiveness of therapy.[1,2] Traditionally, an active ingredient is almost exclusively considered as cargo in nanoscale drug delivery. Lately, the use of natural and synthetic drug amphiphiles has been proposed as an effective strategy for creating self-delivering drug carriers with improved loading capacity of medicines.[3] The assembly behavior of drug amphiphiles is critical in designing supramolecular drug carriers. However, the interplay between weak hydrophobic forces and strong hydrophilic interactions leading to the construction of well-defined, stable nanostructures is poorly understood.



Glycyrrhizic acid (GA) – the molecule known for its ability to form micelles in aqueous solutions and its less hydrophilic derivative - carbenoxolone (CBX) are perfect candidates to study the possible factors influencing the self-assembly of pharmaceutically relevant compounds into stable nanostructures. X-ray crystallography revealed that association of CBX and GA results in a layered 3D-architecture with hydrophobic areas created by interdigitated triterpene moieties. Despite those similarities, the aggregation of host undergoes significant changes in response to the replacement of highly hydrophilic disaccharide unit of GA for the succinyl moiety of CBX. Comparison of crystal structures of both compounds has demonstrated the role of hydrophilic interactions on the stabilization of self-assembly of amphiphilic GA molecule. In GA, the presence of a sugar area formed by hydrogen-bonded disaccharide units at the interface of neighboring hydrophobic layers results in 2D-isomorphism of GA and its mono- and dibasic salts and different solvates. The reduced hydrophilicity of succinyl moiety of CBX and lack of the stable hydrogen-bonded network increases architectural diversity of CBX structures.

The similar construction of hydrophobic areas created by reversely organized triterpene moieties suggest that hydrophobic interactions are the driving force for association of these two related compounds. Nevertheless, the hydrogen-bonded sugar area might be responsible for formation of stable GA micelles.

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Acknowledgements

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K26

Synthesis of chitosan coated magnetic nanoparticles designed for bioligands binding

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Over the last decade the magnetic nanoparticles research has rapidly increased. This type of nanoparticles has been highly employed in chemistry and biomedical applications such as catalysis, magnetic hyperthermia, magnetic resonance imaging (MRI), bioseparation, diagnostic agents and especially for biomolecule immobilization and drug delivery.[1-3] Development and investigation of new iron-oxide multifunctional magnetic nanomaterials designed for different application has gained a great attention in current materials science.[4,5]

In this study magnetic nanoparticles based on Fe_3O_4 and coated with pure and chemically modified chitosan have been prepared [6]. Nanoparticles coated with mixture of chitosan and amphiphilic polymer were also synthesized.[7,8] The surface of prepared nanoparticles was designed for good dispersion in several types of solvents: polar and nonpolar and especially for effective proteins immobilization. Two bioligands were chosen: lipase from *Candida rugosa* and human serum albumin (HSA). As expected such supports resulted in a higher immobilization yield with much larger concerning biomolecules loads on the surface than that known in literature. The size and structure of prepared nanoparticles were characterized by ATR spectroscopy, XRD and TEM analysis.

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[8]. Ziegler-Borowska et al., Appl. Surf. Sci., 2014; 288: 641-648.

Acknowledgements

Financial support from Government wherewithals for science (years 2013-2015) IP2012034472 is gratefully acknowledged. The project was also partially supported by National Science Centre research grant: 2013/09/N/NZF/03557.

K27

Tetracyclic and pentacyclic azaphenothiazines with the quinoline ring

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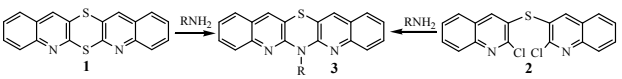
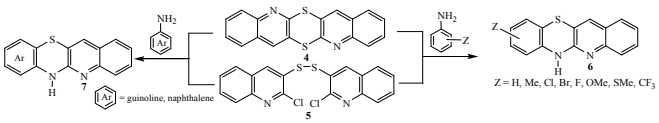
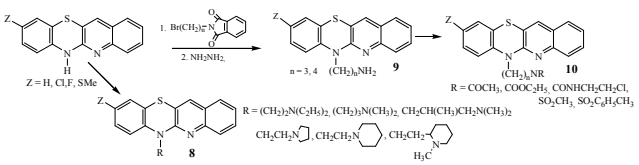
Phenothiazines constitute the largest group of psychoactive clinically used compounds. New phenothiazine derivatives possess several other biological activities including antibacterial, antifungal, antiproliferative, anti-inflammatory**,** antiparkinsonian activities, reversal of multidrug resistance and potential treatment in Alzheimer's, Creutzfeldt-Jakob and AIDS diseases [1,2]. We modified the phenothiazine structure with the quinoline ring to form new azaphenothiazine being 6-substituted diquinothiazines 3. Some of these compounds exhibit significant anticancer activities against human cell lines of lung, colon, breast, renal, ovarian, prostate and CNS cancers, melanoma and leukemia determined in National Cancer Institute in Bethesda, in USA [3].



Reactions of the 1,4-dithiin ring opening in diquinodithiin 4 with various m- and p-substituted anilines, aminoquinolines and naphthylamines led to substituted 6H-quinobenzo[3,2-b][1,4]-thiazines 6 and diquinothiazines and quinonaphthothiazines 7. The same products were obtained using 2,2'-dichloro-3,3'-diquinolinyl disulfide 5 [4].



6H-quinobenzothiazines 6 were transformed into the dialkylaminoalkyl, acylaminoalkyl and sulfonylaminoalkyl derivatives 8 and 10 in the reactions with dialkylaminoalkyl chlorides and phthalimidoalkyl bromides, followed by hydrolysis to the aminoalkyl derivatives 9 and N-acylation and N-sulfonylation [5, 6].



The identification of the product was based on the NMR analysis (^{1}H, ^{13}C, 2D experiments COSY, NOESY, ROESY, HSQC and HMBC), MS and X-ray analysis of selected derivatives. Compounds 8 and 10 exhibited antiproliferative and anticancer activity (compounds selected based on their strong

antiproliferative effects were tested against leukemia L-1210, epidermal carcinoma A-431 and colon carcinoma SV-984 cells). N-substituted quinonaphthothiazines exhibited anticancer activites against human cell lines of glioma (SNB-19), melanoma (C-32) and breast cancer (T47D). The most active compounds showed activities comparable to cisplatin.

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K28

Stereoselective synthesis of 3β-aminotropane quinolineamides with potential atypical antipsychotic activity

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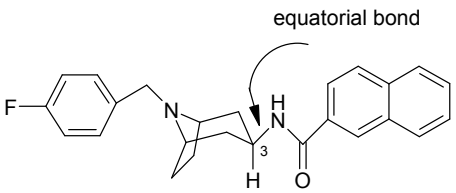
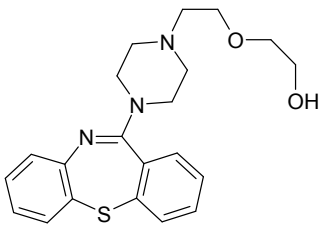
Many naftamide derivatives of 3-aminotropane that have been obtained in our research show a very high affinity to D_2, 5-HT_{1A} and 5-HT_{2A} receptors [1,2]. This profile of affinity is characteristic of several atypical antipsychotic drugs. For example, the profile of the compound **4F-Nb₂** is almost identical to Quetiapine – a valued drug used in schizophrenia and bipolar affective disorder therapy.



4F-Nb$_2$



Quetiapine

K_i [nM] D_2 : 137 ± 15.0
5-HT_{1A}: 218 ± 22.0
5-HT_{2A}: 209 ± 7.8
Meltzer Index (pK_i5-HT_{2A} / $pK_i D_2$) = 0.97

D_2 : 180
5-HT_{1A}: 230
5-HT_{1A}: 220
Meltzer Index = 0.99 [3]

In this work we synthesised a series of new derivatives of 3β-aminotropane with introduced quinolines instead of a naphtalene system. Isomerism at C3 of tropane bicyclic system has fundamental importance for the biological activity of these compounds. We have previously shown that equatorial (β, *egzo*) stereoisomers are incomparably more active [1]. For this reason, stereochemical purity of some compounds was determined by HPLC method. The results of this investigation showed high stereoselectivity and stereospecificity of applied synthetic methods. At the end SAR analysis for newly synthesised derivatives was performed.

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Prezentacje posterów (PP1-PP10)

PP1

Characterization and screening of enzyme inhibitors using isothermal titration calorimetry

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Given that 47% of marketed small-molecule drugs act on enzymes, this group of proteins appears to be one of the most pharmacologically important class of biomolecules. The regulation of enzymes activity as a therapeutic strategy has attracted the attention of researchers for decades. Nevertheless, the development of new techniques for characterization, effective screening and selection of new drug candidates acting as enzyme modulators is still an important issue.

Here we present an application of isothermal titration calorimetry (ITC) for evaluation of inhibitor-enzyme interactions. Two different enzyme proteins were used for this purpose: acetylcholinesterase and dipeptidyl peptidase-4.

The ITC assay is widely used in medicinal chemistry and become a gold standard in characterization of ligand-target binding. As the only technique able to directly determine enthalpic and entropic contribution to the total energy of binding, ITC is the most commonly used to derive thermodynamic parameters of complex formation. However, our results show that proper experimental setup allows also for determination of potency of enzyme-targeting compounds and evaluation of their influence on kinetic parameters of enzyme-catalysed reaction. The technique is highly precise what stems from the fact that the assay is label-free and there is no need to use radioactive, chromo- or fluoro-phore tagged reactants or immobilized proteins. Therefore, interactions were studied in conditions resembling physiological with macromolecules in their native forms. Thus, irreproducibility resulting from secondary reactions and protein chemical modifications was avoided. Due to universal character of the detectable signal (heat) ITC can be applied to evaluation of various inhibitor-enzyme systems.

PP2

X-ray crystallographic structures alignment as a source of fragments for effective drug design

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One of the main challenges for nowadays medicine are novel drugs for a memory deficits and cognitive disabilities treatment. New drugs may improve life quality of people suffering from neurodegenerative diseases. Thus, since It has been demonstrated that the AMPA receptors appear to be a critical to mediating synaptic plasticity and long-term potentiation, the allosteric positive modulation of the receptor became one of the promising direction for Parkinson's and Alzheimer's diseases treatment.[1]

The ligand binding domain of the receptor was co-crystallized with almost 50 different allosteric modulators. It allowed a unique opportunity to evaluate the native orientation and bio-active conformation of high number of congeneric compounds in terms of the same binding domain conditions. The main advantage of this kind of ligands' alignment is fact that the alignment is obtained only by the overlay of the crystal structures and neither molecular docking nor receptor-based alignment methods need to be applied for the ligands.[2]

The high-resolution X-ray structures provide a rare opportunity to sample ligands' orientations as a fragments' library for rational drug design process [4]. The strategy was successfully applied to design and optimize new class of compounds base on well-recognized scaffold.[4]

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PP3

Arylsulfonamide derivatives of aryloxyalkilamines as new uroselective α_1-adrenolitics in the treatment of benign prostatic hyperplasia

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α_1-adrenoceptor blockers are currently the first-line treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia (BPH). α_1-adrenoceptors (αAR) antagonists have high therapeutic efficacy, as they relax prostate smooth muscle and decrease urethral resistance. In contrast, the use of αARs blockers may promote blood pressure–related side-effects, particularly hypotension. It was shown that blockade of subtypes A and D of αAR is important in relieving obstructive and storage symptoms, whereas blockade of subtype B is responsible for side effects, respectively. Taking above-mentioned mechanisms into consideration we are looking for new, highly selective compounds, targeting both prostate α_{1A} ARs and bladder α_{1D} ARs [1,2].

A series of arylsulfonamide derivatives of aryloxyalkilamines was synthesized and evaluated in preliminary pharmacological tests. We have assessed the affinity to both α_1 and α_2 ARs, the preferential activity to the subtypes A and B of αARs, antihypertensive properties and the impact on the pressor response elicited by catecholamines. In the first step, radioligand binding studies showed that most of the newly synthesized derivatives have significant affinity for α_1 ARs (Ki = 19 - 130 nM), therefore they displayed approximately 10-13 fold selectivity to α_1 ARs versus α_2 ARs (Ki ca. 300-800 nM). Subsequently it was demonstrated that all selected compounds have antagonistic effect on the pressor response elicited by methoxamine in the range of 90-95%, what confirmed their adrenolitic activity. Furthermore the influence on blood pressure for selected compounds after one-time *i.v.* administration was assessed. Several derivatives did not significantly decrease systolic and diastolic blood pressure in normotensive anesthetized rats within a dose range of 2-5 mg/kg *i.v.* In comparison, tamsulozin decreased both blood pressure parameters already in a dose of 2 mg/kg *i.v.* In the end the calculated EC 50 values for α_{1A}/α_{1B} showed that selected compounds have preferential activity to the subtype A of α_1 ARs (in the range of 1.5 - 6 fold).

The obtained results may indicate that the tested structures are uroselective which may be a result of their preferential activity within a specific receptor subtypes of α_1-adrenoceptor (α_{1A}/α_{1D}). In order to confirm this hypothesis there is a huge need to perform further pharmacological studies. The evaluation of urodynamic parameters in rats with testosterone- induced prostate hyperplasia is the next step of research for the confirmation of the therapeutic efficacy of new α_1-adrenolitics.

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Acknowledgments

Partly supported by Polish National Science Center founding, NCN 2011/03/B/NZ7/00724.

PP4

Synthesis and evaluation of substituted tacrine - melatonin heterodimers as cholinesterases inhibitors

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Despite intensive research, there currently is no effective causal therapy of AD, and the treatment focuses only on the relief of symptoms. One of the leading therapeutic strategies is based on the assumption that increasing the amount of the neurotransmitter acetylcholine ACh would lead to more active cholinergic neurons, which in turn would slow down the progression of the disease. This objective can be achieved by the inhibition of acetylcholinesterase (AChE) [1]. Recently, it became clear that butyrylcholinesterase (BuChE) is also capable of hydrolizing acetylcholine, and therefore its inhibition may further enhance cholinergic transmission in AD [2-3].

Currently, melatonin is a very intensely studied for its use of in the treatment of AD [4]. The potential effectiveness of therapy is associated mainly with the effects of melatonin on three factors crucial for the development of the disease: increased production of reactive oxygen and nitrogen species, inflammation and formation of β-amyloid plaques [5].

As a continuation of our studies [6-7], we designed compounds with inhibitory activity against cholinesterases. In this study we report synthesis and biological activity of several series of hybrid molecules, derivatives which include compounds substituted in the tacrine ring with one or two halogen atoms. That heterodimers combine tacrine and melatonin with a *n*-methylene units linker between the amine and the carbamate groups.

The activity of the synthesized compounds against human cholinesterases was evaluated with spectrophotometric Ellman's method.

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Acknowledgements

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PP5

Investigation of specific activity based probes for serine proteases

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Human Neutrophil Elastase (HNE), Proteinase 3 (PR3), Cathepsin G (CatG) and recently discovered Human Neutrophil Serine Protease 4 (NSP4) belong to Human Neutrophil Serine Proteases (NSP's). NSP's, stored in active form in neutrophils, are involved in host defense and are secreted during inflammatory response. They are involved in bacterial proteins cleavage during inflammation. On the other hand, because these enzymes are able to destroy almost every extracellular component, they uncontrolled activity leads to several civilization diseases, including lung cancer [1-3].

To date there are not available specific synthetic substrates sequences, which could differentiate each NSPs. One of the most versatile approaches to determine substrate specificity of proteases is Positional Scanning Substrate Combinatorial Library approach, which allows fast and reliable determination of substrate preferences for most of the proteases. To get better insight into substrate specificity of selected human neutrophil serine proteinases, we have extended classic PS-SCL approach by using broad range of unnatural amino acids. Screening of this library yielded several candidates for optimal and selective peptide sequences. Validation of the screening results with optimal individual substrates confirmed that specific recognition element can be found for investigated proteases. Additionally, we have used these optimal sequences for design of specific Activity Based Probes [4, 5].

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PP6

Synthesis and biological properties of 4-chloro-3-methylphenoxyethylamine derivatives of trans-2-aminocyclohexan-1-ol

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Epilepsy is one of the most common chronic neurological problems characterized by seizures. Although there is a number of antiepileptic drugs there is a significant group of patients (up to 30%) who are resistant to the available AEDs. Therefore, there is an unmet need to develop AEDs with lower cytotoxicity and higher selectivity. In order to make this development more rational structural fragments that may enhance anticonvulsant activity were identify.

While searching for new substances with anticonvulsant activity we directed our attention to derivatives of aminocyclohexanol. Many pharmacological activities of aminocyclohexanol were described such as: secretolytic, analgetic, antidepressant. Our former research proved that aminoalkanol derivatives can also act as anticonvulsant – they provided protection against seizures, mainly MES-induced seizures and at the same time exhibited low neurotoxicity [1], [2].

Herein we report the synthesis, *in vivo* anticonvulsant activity, *in vitro* biotransformation, determination of the safety profile in procariotic or eucariotic models of the trans-1,2-aminocyclohexanol derivatives – compound 1,2 and 3 (two enantiomers and the racemat, respectively). Those compounds were found to have anticonvulsant activity against MES test in studies carried out in National Institute of Health (Rockville, USA). Rat hepatic microsomal fraction in the presence of an NADPH- generation system were used to evaluate the metabolism of analyzed compounds. The result obtained from liver fractions applications were compared to the result from as the microbiological biotransformation assay in which *Cunninghamella* model was involved. The cytotoxic effect titled compounds were investigated on four types of cancer and normal cell human origin (DU-145, PNT2, WM 793, HSF) by use trypan blue exclusion test. While their mutagenic activity were studied in *Vibrio harveyi* and Ames tests. While their mutagenic activity were studied in *Vibrio harveyi* and Ames test. Results were confirmed that compounds 1-3 did not cytotoxic effect on cells and are safe to use.

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PP7

Wnt5a attenuates TGF-β induced fibroblasts to myofibroblast trasition in bronchial fibroblasts derived from asthmatic patients

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Bronchial asthma is one of the most common chronic lung diseases. It is inflammatory disorder of the lower airways manifested by reversible constriction of bronchi but also progressing with an irreversible bronchial wall modeling. Despite basic pathomechanism of asthma, characterized by influx of blood cells, many evidences suggest that extracellular matrix thickening and increased number of smooth muscle cells is an early event. Growth factors and cytokines like transforming growth factor type β (TGF-β) are known to induce fibroblast to myofibroblast transition (FMT) during the course of asthma.

Our previous studies demonstrated that human bronchial fibroblasts (HBFs) derived from patients with diagnosed asthma display predestination towards TGF-β-induced phenotypic switches. Here we show that activation of WNT pathway by using Wnt5a ligand significantly attenuated TGF-β induced FMT in HBFs from asthmatics, whilst an opposite effect was observed in HBF isolated from non-asthmatics. Extracellular addition of Wnt5a caused nearly 50% inhibition in TGF-β dependent FMT in asthmatic fibroblasts.

The observed cross-talk between WNT and TGF-β signaling pathways in HBFs isolated from patients with bronchial asthma is a possible target for therapeutic intervention. Our model, based on primary cultures of bronchial fibroblasts in vitro, can be a valuable experimental system to study mechanisms underlying bronchial wall remodeling. In the future it can help in development of new therapeutic strategies against asthma.

PP8

Aminomethylenebisphosphonates as potential drugs against osteoporosis

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Bisphosphonates are an important class of drug molecules, used to treat osteoporosis, Paget's disease, and hypercalcemia due to malignancy because they are powerful inhibitors of bone resorption [1]. They act by inhibiting the enzyme farnesyl diphosphate synthase (FPPS) [2]. A subclass of bisphosphonates, derivatives of aminomethylenebisphosphonic acid, has been also described to exhibit promising antiparasitic [3] and herbicidal activities [4]. The simplest procedure for preparation of aminomethylenebisphosphonates relays on three-component reaction between amine, triethyl orthoformate and diethyl phosphite, followed by acid hydrolysis. We synthesized more than two hundred aminomethylenebisphosphonates, with structural variety. The aim of this studies is the preselection *in vitro* of bisphosphonates in the model cell line to choose potent compounds. The mouse macrophage-like cell line J774E used in this study is a convenient model to identify the activity of bisphosphonic acids. The *in vitro* cytotoxic effect of all agents was examined after 72-hours of exposure of the cultured cells to varying concentrations of the tested compounds, using the MTT assay. The *in vitro* results were presented in terms of inhibitory concentration 50% (IC_{50}) values. We choose six the most potent compounds selected in this examination. Its activity is higher that that of referential compound incadronate (a commercial representative of the latest generation of antiosteoporotic drugs) The most active compounds were testing *in vivo* test in induced osteoporosis on sheeps. They activity is promising and the results of our research where just published in patent

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PP9

The Chemical Database of compounds synthesized in Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances in Medical University in Lublin

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Every year a lot of new compounds is synthesized in our Department. Working with increasing amount of data requires legible way to organize them so we decide to create database which is available on website: www.bdz.umlub.pl. The database makes research easier because of improvement of access to information and saving time spending to collect them. This type of ordering data help us to share information with broad community of scientists.

The main aim of presented work was to create database of chemical compounds. The first group of compounds which was listed in our database was imidazoline derivatives. We collected 251 compounds which were obtained in series of master thesis from 2004 to 2013. Every compound have acquired its own number in this database and short description of its basic properties. We prepared optimized three – 3D structure of every compound with the aid of the latest version of the Spartan software using *ab initio* method with 3-21G* basis function. They were generated with use of experimental data – constructed from fragments of crystallographic structures which are deposited in CDCC database. These structures can be downloaded from our database as a mol2 file which could be useful in exploring their properties by methods of computer modelling of drugs.

We also worked out the procedure of *in silico* experiments, which could be used in discovering potential activity of our compounds. We conducted reverse virtual screening which is one of new method of drugs discovering. For the calculation we chose 17 compounds which were selected by Selector™ of SYBYL x1.2 (TRIPOS) as representatives of smaller groups of compounds (clusters). Although we used only web servers (idTarget, PharmMapper) which are basic instruments for this type of research, we validated results by docking (using Surflex™module of SYBYL x1.2 (TRIPOS)) successfully. We obtained a lot of new data which have to be analyzed carefully.

These days pharmaceutical industry has a lot of difficulty in discovering new drugs so we cannot afford to wasting potential of compounds which have been already synthesized.

PP10

Electrochemical simulation of enzymatic transformations studied for the selected antitumor acridine derivatives

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The elucidation of the metabolic pathways and the biotransformation mechanisms of potential drugs is a crucial point in drug development. It allows to know the activation routes of the new biologically active compounds, especially in respect to their possible toxicity. Generally, *in vivo* or *in vitro* experiments with liver microsomes or hepatocytes are performed. However, these testing schemes are tedious, time consuming and of limited reproducibility [1]. The combination of electrochemistry coupled on-line to mass spectrometry (EC-MS) forms a powerful analytical technique with unique applications in the fields of drug metabolism. In an electrochemical (EC) flow-through cell, the oxidative metabolism of xenobiotics (mostly catalysed by cytochrome P450 isoenzymes) is simulated. The cell is used in on-line set-up with electrospray mass spectrometry (ESI-MS), so that an identification of both reactive and stable metabolites is possible [2].Here, we present the benefits of electrochemical drug metabolism simulation for C-1311 and C-1748, representative antitumor acridine derivatives developed in our laboratory. The studied compounds exhibit high cytotoxic activity against a broad spectrum of cell lines *in vitro* and of transplantable animal tumors. The previous studies on molecular mechanisms of their biochemical action showed that metabolic activation might be a prerequisite stage for the following interactions of these agents with cell macromolecules. Next, we showed that both of them were metabolized by enzymes originating from rat and human liver microsomes (RLMs and HLMs). In the presented work C-1311 and C-1748 were chosen as the model drugs to investigate oxidative metabolism under electrochemical conditions (the ROXY™ EC System, Antec, USA) coupled to mass spectrometry. The results obtained by EC/MS were then compared with conventional *in vitro* studies with RLMs as well as HLMs.Electrochemical conversion of C-1311 and C-1748 into phase I metabolites was successfully achieved. Comparison of MS results from liver cell microsome incubations with MS results from electrochemical studies allowed to demonstrate that some of the most important metabolic products of the studied compounds were detected both in the conventional enzymatic approach and in the electrochemical simulation. Furthermore, newly reported metabolic reaction product of C-1748 become accessible. Summing up, the obtained results confirmed that EC/MS is very well-suited for the simulation of the oxidative metabolism of antitumor acridines. It can be a versatile and user friendly tool in drug discovery and development when applied complementary to established *in vitro* or *in vivo* approaches.

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Acknowledgements

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Postery (P1-P122)

P1

Microwave synthesis of imidazo[2,1-c][1,2,4]triazole

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Microwaves are increasingly being used in the methodology of organic synthesis due to the shorter reaction time and higher reaction yield.

Derivatives of imidazo[2,1-c][1,2,4]triazole were prepared by the classical method where hydroiodide derivatives of 1-aryl-2-hydrazynoimidazoline react with carbon disulfide [1,2]. The same reaction employing microwave radiation was 60 times faster than the classical method and allowed to obtain purer products. The resulting compounds were analyzed using IR, MS, ^{1}H NMR and ^{13}C NMR.

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P2

Solid phase synthesis of DNA ligands containing in the structure an amine acid derivatives

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Most of the used in anticancer therapy drugs inhibit replication of DNA, both by covalent and non-covalent binding. The mechanisms of these interactions are subjected to detailed research [1].

Lexsitropsins is the name of a group of synthetic DNA ligands – "minor groove bionders" - heteroaromatic oligopeptides, design on the model of netropsin and distamycin. The analogues of these natural antibiotics, as well as other minor groove binders, have found substantial applications in anti-cancer therapy. Also compounds with antibacterial, antifungal, antiviral, and antiparasitic activity have been identified. Moreover, their importance in anti-infective therapy also has been significant and its importance is growing [2].

A simple and general procedure for solid phase synthesis of distamycin analogues, using the different strategy than most of described approaches based on traditional synthesis of peptides, has been developed [3]. This method makes it possible to parallel syntheses of many new compounds, permitting automation of the process with using several different aromatic nitro amines, as well as acyl chlorides with nitro group, so it allows obtainment of a lot of new compounds. Distamycin analogues obtained by this method bound to DNA [4].

This method was used to prepare netropsin and bis-netropsin containing benzene and heteroaromatic rings [5]. Here we presented application of this method to obtain netropsin analogues containing lysine fragment (Fig. 1)

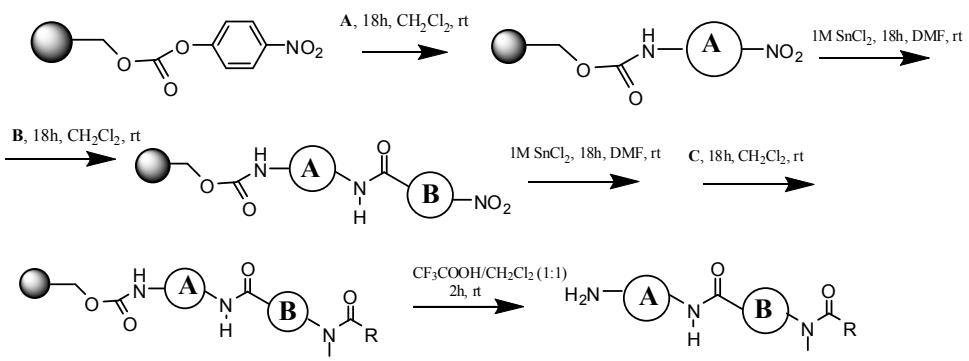

Fig.1. Synthesis of netropsin analogues with lysine fragment (R= lysine).

Introduction to the synthetic DNA ligands structure of an amino acid fragment should broaden the scope of action of these compounds and cause an increase in their antitumor activity.

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P3

Determination of lipophilicity of cholinesterase inhibitors, heterodimeric isoindoline-1,3-dione derivatives, using micellar electrokinetic chromatography

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Nowadays, drug discovery projects focus on the improvement of both, the activity towards selected targets and drug-like properties of molecules. Drug-like properties are viewed as properties, which provide desirable pharmacokinetic characteristics, independent of pharmacological or therapeutic targets. Among different drug-like properties, lipophilicity of drugs seems to be essential for their activity in central nervous system. Therefore we decided to investigate lipophilicity of the compounds of a potential use in the treatment of Alzheimer's disease synthesized in our group. Currently, we focus on search for multifunctional compounds, which are cholinesterase and amyloid β fibril formation inhibitors [1, 2]. The presented work is a part of our studies on physicochemical properties of bioactive compounds [3, 4]. The aim of this study was to apply micellar electrokinetic chromatography (MEKC) analysis to indirectly determine log P_{oct} values of ten heterodimeric isoindoline-1,3-doine derivatives, and to compare them with lipophilicity descriptors calculated by computational methods and with their inhibitory activity against cholinesterases. The first phase of the study was to determine the log *k* parameters (MEKC) for seven standard compounds in order to define the relationship between log *k* and log P_{oct} coefficients (calibration curve). Selection of the standard compounds was based on their published log *P* values, which should be in the range of log *P* expected for the tested compounds (0.65 – 3.21). The second stage was the MEKC analysis of the tested compounds and interpolation of the obtained log *k* values on the calibration curve in order to obtain estimated log P_{oct} coefficients. The third stage was a calculation of lipophilicity parameters (calculated log *P* values) by using different programs, comparison and assessment of the results, and in the end, a comparison of lipophilicity parameters with the cholinesterases inhibitory activity. The performed pilot study enabled us to characterize the lipophilicity of a small set of heterodimeric isoindoline-1,3-dione cholinesterase inhibitors. The MEKC method will be applied for the determination of lipophilicity of a larger set of cholinesterase inhibitors. It could be also used for lipophilicity characterization of other derivatives.

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P4

Molecular dynamics approach to GPCR allosteric modulation

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The necessity of improvements in a number of therapies makes it important to investigate drug action mechanisms more sophisticated than classical orthosteric agonism/antagonism. An increasing interest in compounds exerting biased effects on their molecular targets is noticeable in recent years. Advantages such as activation pathway specificity and preservation of physiological spatial and temporal patterns of activation can be achieved by use of allosteric modulators. In particular, targeting opioid receptors with such modulators could result in number of benefits e.g. in pain, depression, respiratory and immune disorders treatment, with less side effects due to receptor- and pathway-specific action. There are reports on positive and negative opioid receptors' modulators with limited, micromolar efficacy. The data can be a starting point for identification of possible allosteric binding sites and mechanisms of modulation of opioid receptors. In this work molecular modeling, docking and molecular dynamics studies are used to investigate interactions of known negative allosteric modulators: salvinorin A, THC and cannabidiol on mu opioid receptor [1,2]. An attempt to describe allosteric binding site and identify key residues responsible for modulation is undertaken. The analysis of the molecular dynamics results may give a deeper insight into GPCR allosteric modulation mechanisms and contribute to development of novel active compounds.

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P5

Synthesis and in vitro antiproliferative activity of novel thiazolo[4,5-*d*]pyrimidines containing trifluoromethyl moiety

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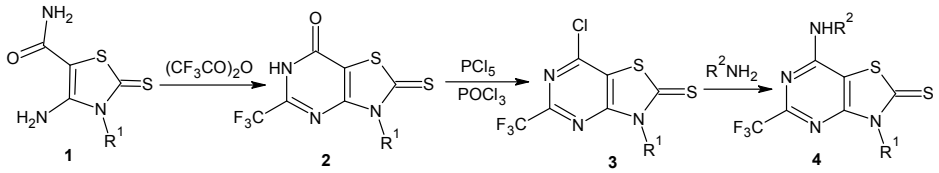
Effect of the 5-trifluoromethyl moiety on the biological activity of the chemical compound has been known for many years. The enhanced activity can be attributed to the presence of highly electronegative fluorine atoms in the molecules which increase the lipophilicity and facilitates hydrophobic interactions of the molecules with specific binding sites on receptors. A well-known drugs include CF3 group eg. fluoxetine (an antidepressant), mefloquine (used in the prevention and treatment of malaria), celecoxib (a non-steroidal anti-inflammatory), sorafenib (used in kidney and liver cancer), efavirenz (an HIV reverse transcriptase inhibitor), bicalutamide (used in prostate cancer). The beneficial effect of the substituent on the activity of the newly synthesized 5-trifluoromethyl-thiazolo[4,5-*d*]pyrimidines **2**-**4** was also confirmed by the results of in vitro screening tests. Previously received thiazolo[4,5-*d*]pyrimidines showed anti-tumor activity [1]. The new derivatives **2** and **3** were obtained by the cyclocondensation of 3-substituted 4-amino-5-carboxamido-2,3-dihydrothiazole-2-thiones with trifluoroacetic anhydride followed by chlorination. Treatment of compounds **3** with primary amines gave 7-amino derivatives **4**. Anticancer activity of the selected compounds was determined at a single dose towards the full panel of 60 human cancer cell lines by the National Cancer Institute (NCI). Most active compound showed a remarkable cytostatic activity at 10^{-5} M concentration (mean growth percent=29.5) and in extended studies demonstrated a strong cytotoxicity against 47 cell lines($logLC_{50}$<-4.00, up to a value -5.82 for line NCI-H522 of lung cancer).



R^1 = alkil, aryl R^2 = aryl, haloaryl

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P6

Cycloaddition reactions acetylenic derivatives of betulin with benzyl azide

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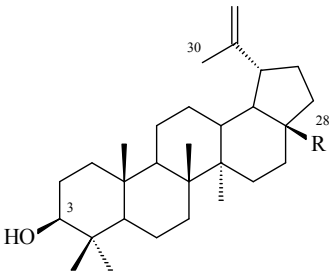
Betulin 1 and betulinic acid 2 are natural compounds, which can be easy converted to various semisytnthetic derivatives. These compounds and their derivatives were reported, as potential therapeutics for anticancer and HIV therapy [1-3].



Betulin **1** R= CH_2OH

Betulinic acid **2** R=COOH

The aim of this study was the synthesis of derivatives of betulin bearing an acetylenic side chain at the C3, C28 and C30 positions. New acetylenic derivatives were used for the synthesis of 1,2,3-triazoles by 1,3-dipolar cycloaddition. Cycloaddition reactions were carried in dry dichloromethane in the presence of catalytic amount of copper iodide (CuI). The chemical structures of new compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, MS and IR spectroscopy. The obtained triazoles, as well as betulin **1**, were tested for their antiproliferative activity against three human cancer cell lines: T47D breast cancer, SNB-19 glioblastoma and C-32 melanoma.

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P7

The effect of anti-MUC1 used with novel dinuclear platinum(II) complex in human skin fibroblasts

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The mucin MUC1 is a type I transmembrane protein that is expressed by normal and cancerous epithelial cells in which it plays roles in regulating adhesion and cell signaling. The presence of full-length MUC1 was proved in normal human skin fibroblasts by using reverse-transcription polymerase chain reaction and Western blot [1]. Mucin 1 directly interacts with variety of transcriptional factors and it causes transcriptional alterations that result in metabolic reprogramming of the cells. MUC1 also takes part in modulating enzymatic functions of metabolic enzymes. Recently we showed that novel platinum(II) complex used with anti-MUC1 had strong antitumour activity against estrogen positive and negative breast cancer cell lines (MCF-7 and MDA-MB-231) in comparison to cisplatin used with anti-MUC1 [2]. The aim of the study was to evaluate the effect of a monoclonal antibody against MUC1 used with novel platinum(II) complex on cytotoxicity of human skin fibroblasts. The effect of that compounds was compared with monoclonal anti-MUC1 used in combination with reference cisplatin. Cell viability was performed according to the method of Carmichael using tetrazolium salt (MTT). The biosynthesis of DNA was measured by [^{3}H]-thymidine incorporation. Collagen biosynthesis was assessed by incorporation by 5-[^{3}H]-proline into proteins susceptible to the action of bacterial collagenase of fibroblasts. Our experimental studies demonstrated that anti-MUC1 used with novel platinum(II) complex was less cytotoxic compared to monoclonal anti-MUC1 used with reference cisplatin in human skin fibroblasts. We proved that monoclonal antibody had very low cytotoxic potency compared to other compounds in normal skin fibroblasts. The number of viable fibroblasts was higher than 90%. Although a combination therapy (platinum(II) complex + anti-MUC1) weaker inhibited the incorporation of [^{3}H]-thymidine into DNA of human fibroblasts compared to anti-MUC1 used with cisplatin. The collagen biosynthesis was more inhibited by anti-MUC1 and cisplatin compared to novel platinum(II) complex used with anti-MUC1. Our results showed that novel platinum(II) complex used in combination with monoclonal antibody against MUC1 was less cytotoxic and its antiproliferative potential was weaker compared to cisplatin used with anti-MUC1 in normal cells represented by skin fibroblasts.

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P8

Topoisomerase inhibitory properties of octahydropyrazin[2,1-a:5,4-a']diisoquinoline derivatives

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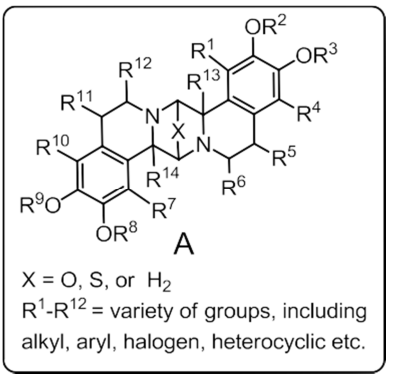
The project is part of a leading trend research on the search for new anticancer drugs. The basis of this project is our discovery of a new class of compounds with octahydropyrazin-[2,1-a:5,4-a']- diisoquinoline skeleton that exhibit high antitumor activity in MCF-7 and MDA-MB-231 breast cancer cells. Molecular mechanism of their action is as yet unknown, although our preliminary studies have shown that they are inhibitors of both topoisomerase I and topoisomerase II - important enzymes involved in DNA replication. Simultaneous targeting of both topoisomerase I and topoisomerase II by octahydropyrazin[2,1-a:5,4-a']diisoquinoline derivatives may be an important contributing factor for circumventing resistance mechanisms due to alteration of a single target enzyme. This research programme can make a significant contribution to an understanding of the structural requirements for dual enzyme inhibition. We assume that due to the structural similarity of examined compounds to the natural products with anticancer activity is a good chance of finding an optimal candidate for an anticancer drug.

We synthesized derivatives in both optical forms bearing a variety of substituents as presented on structure **A**. The impact of substitution pattern and absolute configuration on bioactivity was analyzed to contribute to the rational design of more selective drugs to target topoisomerase proteins.



A

X = O, S, or H_2

R^1-R^{12} = variety of groups, including alkyl, aryl, halogen, heterocyclic etc.

Acknowledgement

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P9

Phytochemical and biological properties of essential oil isolated from *Citrus hystrix*

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Currently, there is observed an increasing interest in natural plant-delivered substances in the researches for therapeutic solutions such as infectious diseases. Based on the wide spectrum of biological activities, citrus essential oils may be an alternative to presently used therapies, which give insufficient effects, as well as number of side effects. Further research on a larger scale are necessary to confirm and prove their effectiveness and to identify the mechanisms of their biological and pharmacological activity *in vitro* and *in vivo*.

This report describes scientific examination about biological properties of citrus essential oil isolated from *Citrus hystrix*, also known as Kaffir lime, obtained for analisis from Apipol Farma Japan company. We analized the chemical composition of essential oil, its antibacetrial activity and also influence on viability and proliferation of normal cells – human skin fibroblasts (HSF). 15 strains of *Acinetobacter baumani* were examined by disc diffusion method and MIC (MIC – Minimal Inhibition Concentration) values was determined. There was observed a very high activity of the essential oil of *Citrus hystrix* against all tested strains and MIC values obtained ranged from 0.125 µl/ml to 1 µl/ml. Presented in this paper results on the effects on fibroblasts HSF show that oil extracted from *Citrus hystrix* does not cause cytotoxic effect against these cells, however, apparently reduces the proliferation of HSF at a dose-dependend manner.

Shown biological properties militate in favor of conducting further research on the use essential oil from *Citrus hystrix* in therapy.

P10

Synthesis of novel group of multireceptoral ligands with antipsychotic and pro-cognitive properties

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First-generation antipsychotics, like e.g. haloperidol or chlorpromazine, allowed to effectively treat positive symptoms of schizophrenia and related psychotic disorders, but they also revealed high rate of side effects, e.g. extrapyramidal symptoms [1]. Discovery of second-generation antipsychotics (olanzapine, risperidone, etc.) significantly reduced the range of the observed adverse effects, but those drugs did not eliminate cognitive deficits in schizophrenia [1]. Therefore new therapeutic agents with dual effect i.e. suppression of psychotic symptoms and elimination of cognition impairment are still needed [2].

We aimed at development of effective antipsychotic agents that would also ameliorate the cognitive deficits. The consistent series of about fifty compounds was synthesized and studied *in vitro* in binding and functional assays to identify compounds with receptor profile that could provide both antipsychotic and pro-cognitive features. The most promising lead compound showed high affinity for adrenergic α_1, α_{2c}, serotoninergic 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and dopaminergic D_1, D_2, D_3 receptors; and behaved as an 5-HT_{2A}/5-HT_6/D_2 antagonist. Antipsychotic and cognitive models assessing *in vivo* activity of these compounds included locomotor activity assays and novel object recognition assays. Like other antipsychotic agents, the lead compound reversed PCP-induced hyperactivity in animals and, in addition, it demonstrated pro-cognitive actions in the novel object recognition assay.

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Acknowledgements

This study was partially supported by the project UDA-POIG.01.03.01-12-063/09-00 co-financed by European Union from the European Fund of Regional Development (EFRD).

P11

Synthesis of magnetite nanoparticles coated by blends of chitosan and poly (quaternary ammonium) salt for biomedical applications

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In the last decade, extensive research that has been carried out on nanotechnology resulted in the development of nanomaterials that have found application in various fields of science, including the biological and medical sciences. Magnetic nanoparticles based on the magnetite (Fe_3O_4) seem to be currently considered as the most crucial nanomaterials of the future medicine, due to the possibility of binding of drugs, proteins (enzymes), antibodies, or nucleotides. These nanoparticles have a superparamagnetic properties, which makes them an excellent contrast agent for applications in medical diagnostics [1-3].

This work is focused on describing synthetic routes for the preparation of magnetic nanoparticles coated by blends of chitosan and poly(quaternary ammonium) salt with various polymers weight ratios, useful for lipase immobilization [4]. For effective enzyme immobilization, magnetic nanoparticles coated by chitosan with surface rich of free amino groups distanced from the polymer chain was also prepared. The degree of immobilization, lipolytic activity and operational stability of the obtained nanomaterials was examined [5]. Furthermore, adsorption isotherms were measured in order to typify the pore structure of magnetic nanoparticles. The influence of the magnetic field on the properties of magnetite nanoparticles was determined by SEM and TEM photographs, XRD and FT-IR. The thermal stability of magnetic materials has been investigated by thermogravimetric analysis (TG/DTG/DTA) in air and nitrogen atmosphere [6].

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P12

New amine and hydrazide derivatives of 8-alkoxy-purine-2,6-dione with diversified biological activity

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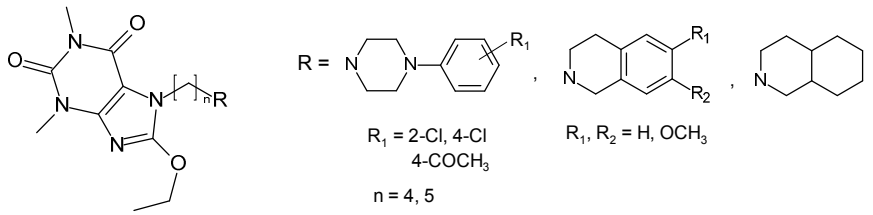
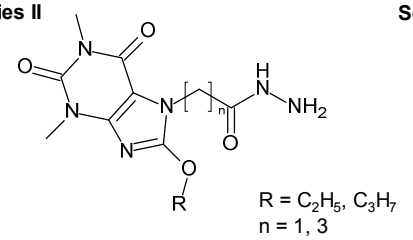
The compounds possessing purine-2,6-dione system characteristic e.g. for theophylline and its derivatives display the wide range of biological activities [1]. The modification in a 7 and 8 position of 1,3-dimethyl-purine-2,6-dione core give possibility to obtain compounds with different pharmacological profile and potential application as various therapeutic agents. For the recent years our research have been focused on the development the 8-alkoxy-1,3-dimethyl-purine-2,6-diones with different substituents in a 7 position, which have been pharmacologically investigated as a potential psychotropic or analgesic agents [2]. As a continuation of our research we designed and synthesized new series of 8-alkoxy-purine-2,6-dione derivatives with amine: arylpiperazine, 6,7-dimethoxy-1,2,3,4,-tetrahydroisoquinoline, perhydroisoquinoline (series I) or hydrazide moieties (series II and III) in a 7 position of purine-2,6-dione system.

Series I



Series II



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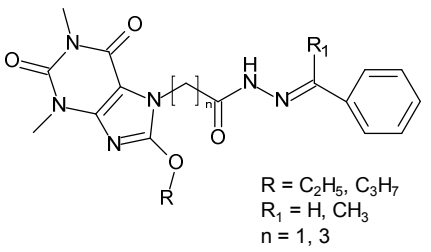

The compounds of series I were designed as potential hybrid ligands which may effect on various biological targets. The research will be carried out on potential 5-HT_{1A}, and 5-HT_7 receptors ligands and inhibitors of PDE4/PDE7 due to their presence in areas of the brain associated with the site of action of psychotropic drugs (antidepressants and atypical antipsychotic). Compounds of series II and III will be evaluated as a potential analgesic agents in some behavioral models (writhing test, hot plate test). Biological data and SAR studies will be presented.

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Acknowledgements

The project was supported by National Science Centre grant No UMO-2012/07/B/NZ7/01173 and grant No K/ZDS/003299

P13

Synthesis and evaluation of cytotoxic activity of new derivatives of betulin containing acetylene groups

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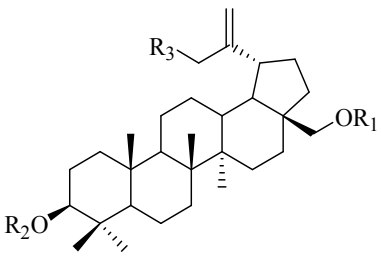
Betulin is a naturally occurring pentacyclic triterpene of the lupane type. It is known that betulin and its derivatives have a broad spectrum of biological activities such as: anticancer, antiviral, antimalarial, antibacterial, antiinflammatory, and hepatoprotective properties [1-2].

Finding of new betulin derivatives with higher anticancer activity and selectivity, that could be applied as potential drugs, is still in an increasing interest. It was reported that acetylenic derivatives of betulin shows cytotoxic activity [3]. On the other hand, in some cases, introduction of a moiety containing phosphorus atom into a molecule is favorable to enhancement of antiproliferative properties [4,5].

In our work we combine betulin with the both structural constituents (phosphorus and carbon-carbon triple bond) in order to create a new class of betulin derivatives.



R_3=H
R_1, R_2: $P(O)(OEt)_2$, C(O)CCH, $C(O)CCCH_3$, $C(O)CCC_3H_5$, $C(O)OCH_2CCH$

R_3= $OP(O)(OEt)_2$
R_1, R_2: H, C(O)CCH

The new synthesized compounds were tested for their antiproliferative *in vitro* activity against two human cancer cell lines: T47D (breast cancer) and SNB-19 (glioblastoma).

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P14

Synthesis of medium-chain-length polyhydroxyalkanoates by *Pseudomonas putida* KT2440 and *Pseudomonas putida* KT2440 RpoN mutant

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Polyhydroxyalkanoates (PHAs) are biodegradable polymers accumulated by bacteria as an intracellular carbon storage material. PHAs are accumulated in response to inorganic nutrient limitation in the presence of carbon excess. Because of their properties, PHAs are desirable polymers with a wide range of applications. Recent findings suggest that PHAs are the suitable materials for fabrication of resorable medical devices, such as sutures, meshes, implants, and tissue engineering scaffolds. Despite the basic attractiveness as a substitute for synthetic plastic, the major barrier for commercial production of PHAs is high cost of their production. Basic research at the molecular level could provide information that might be useful for the improvement of PHA production process.

One of the best known PHAs producers is *Pseudomonas putida* KT2440, which is able to synthesize medium-chain-length PHA (mcl-PHAs) when nitrogen is limited. Therefore, it is suggested that *RpoN* gene is a factor which is engaged in the process of PHA synthesis.

In this study, mcl-PHAs synthesis by *P. putida* KT2440 and its *RpoN* mutant was compared. For PHAs production, two external carbon sources were used: sodium gluconate and oleic acid. Higher PHAs concentration was observed when nitrogen limitation was employed. The wild strain accumulated PHAs at the level of 23% of CDW when sodium gluconate was used, and 15% of CDW when the medium was supplemented with oleic acid. Whereas, RpoN mutant accumulated 16% and 14% PHAs of CDW during growth on sodium gluconate and oleic acid, respectively.

P15

Synthesis of new diazaborininole-thiazoles as potential antimicrobial and anticancer drugs

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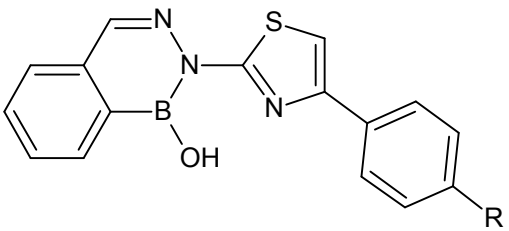
Number of identified fungal infections in human population has been increasing significantly within the last years – this phenomenon is caused *inter alia* by an increase in fungal resistance to drugs that are in common use [1]. It is extremely important to search for new antifungal drugs that demonstrate high antifungal activity and, at the same time, low toxicity profile.

An important group of compounds from the point of view of possible pharmaceutical application are thiazoles. They have been subject to numerous scientific studies over the years because of their wide biological activity, such as antibacterial, antifungal and anticancer [2, 3]. In the present work we turn our attention to synthesis of thiazole-based drugs containing diazaborininole ring and *para*-substituted phenyl group (Figure 1). Compounds containing boron in their structure are known to accumulate in cancer cells, achieving there higher concentration than in healthy cells. This can significantly increase therapies targeting and have a less harmful influence on healthy cells during anticancer treatment [4]. We anticipate that the obtained compounds may demonstrate antimicrobial and/or anticancer activity.



R = Br, F, CN, OMe, Ph, $NHCOCH_3$, NHCONHPh

Figure 1

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P16

Looking for GPR18 ligands

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GPR18 is an orphan G protein-coupled receptor (GPCR). It is very similar to the GPR55 GPCR, is another receptor which has been found to be activated by certain cannabinoid CB receptor ligands. The cannabinoid receptors are part of the endocannabinoid system, which is involved in a variety of physiological and pathophysiological processes. GPR18 and GPR55 show little structural similarity to CB_1 and CB_2 receptors, but they respond to endogenous agents analogous to the endogenous cannabinoid ligands, as well as some natural synthetic cannabinoid receptor ligands. Screened was a part of compounds library (general structure (**1**), Fig.1) (http:mueller-group.pharma.uni-bonn.de/bibliothek) of bicyclic imidazole-4-one derivatives synthesized in Cracow, Poland. A compounds evaluated in β-arrestin recruitment assay followed by cAMP accumulation assay appeared to be a novel class of antagonists at both receptors. Then we investigated their structure-activity relationships at GPR18 and GPR55, and rated their selectivity versus related receptors, including CB_1 and CB_2. (Z)-(2,3-Difluorobenzylidene)-6,7-dihydro-2H-imidazo[2,1b][1,3]thiazin-3(5H)-one (**(2)**, Fig.2) was found as a selective GPR55 antagonist with an IC_{50} of 3.15 μM). Whereas (Z)-2-(3-(4-chlorobenzyloxy)benzylidene)-6,7-dihydro-2H-imidazo[2,1b][1,3]-thiazin-3(5H)-one (**(3)**, Fig.2) was found as potent and selective GPR18 antagonist with IC_{50} of 0.279 μM , >36-fold selective vs. CB_1 and GPR55, 14-fold selective vs. CB_2.

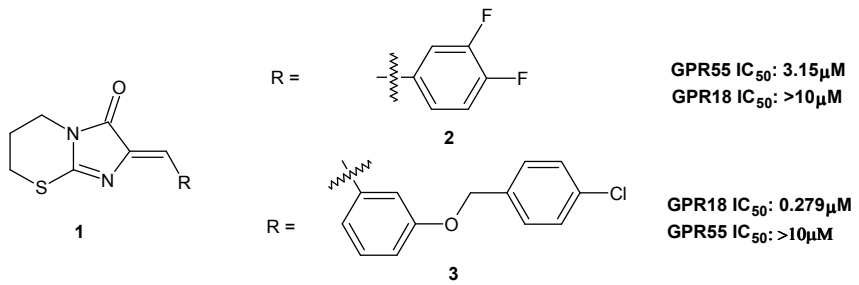

Fig 1. General structure. Fig 2. Ligands of GPR55 and GPR18.

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Partly support by Polish National Science Center DEC. 2013/11/8/N27/04865, GLISTEN: COST Action CM1207 is greatly acknowledged.

P17

Sulfanyl porphyrazines as potential agents for photodynamic therapy – synthesis and physicochemical properties

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Dendrimers constitute a relatively new class of polymeric compounds classified as branched polymers of potential applications in nanotechnology and medicine. They are spherical macromolecules of a diameter nanometer size. Porphyrazines are macrocyclic compounds, derivatives of porphyrins that exhibit good solubility and considerably high values of singlet oxygen generation quantum yields [1] and therefore are considered as potential photosensitizers for photodynamic therapy.

The aim of our study was to synthesize novel mercaptomaleonitrile derivatives possessing isophthaloxy substituents in their periphery. These molecules were subjected to Linstead macrocyclization reactions using magnesium butanolate in butanol what resulted in symmetrical magnesium porhyrazines. Noteworthy, the obtained porphyrazines possess G0 Frechet-type dendrimeric substituents. Novel compounds were characterized using UV-Vis, NMR spectroscopy, including two dimensional NMR techniques: COSY, HMBC, HSQC, and MS MALDI spectrometry.

This study was supported by the National Science Centre under grant No 2012/05/E/NZ7/01204 and by the National Centre for Research and Development under grant No PBS1/A9/13/2012.

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P18

Gramicidin – structures of natural antibiotic

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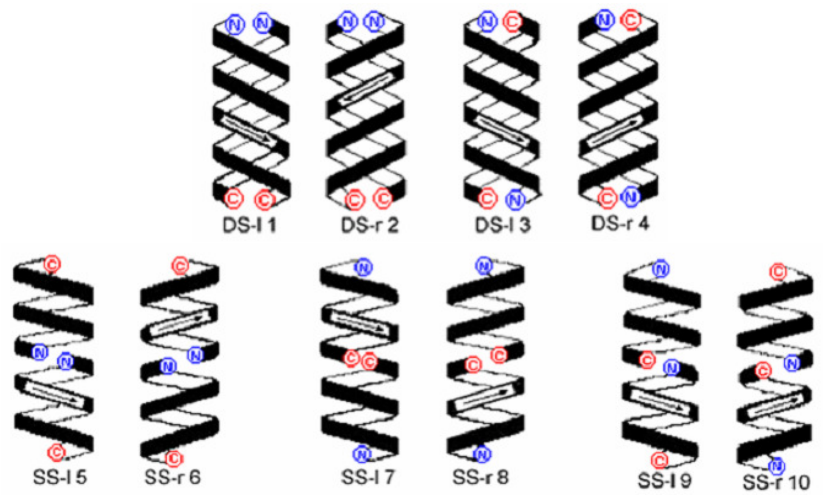
Gramicidin was discovered by Dubos who isolated it from *Bacillus brevis* [1]. Gramicidin contains 15 amino acids, so it is the smallest know membrane transport protein. Its antibiotic activity is based on its property of formation of intramembrane channels, causing monovalent cations, such as Na^+, K^+, to cross the cell wall with no control. It results in cell death. Gramicidin action is restricted to $Gram^+$ bacteria. The most characteristic feature of gramicidin is alternating sequence of L- and D-amino acids, with an ethanolamine residue at carboxyl terminus and formyl group at amino end. The sequence of the main component of gramicidin A is: formyl-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-Trp-ethanolamine.[2]

Theoretically there are 10 different gramicidin dimeric structures [2], but there are only 4 of them observed among structures deposited in Protein Data Bank [3]. In Protein Data Bank there are 22 structures of gramicidin from NMR and X-ray diffraction studies. 14 of them form double helix (one DS-l 1 structure, seven DS-l 3 structure, six DS-r 4 structure). Rest of them forms single helix SS-r 6 (Figure) [4].



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Acknowledgements

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P19

Optimization of tacrolimus biosynthesis by the use of *Streptomyces tsukubaensis* metabolic route precursors

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Tacrolimus (FK-506) is a 23-membered macrolide lactone with a hemiketal-masked α, β-diketoamide function with immunosuppressive activity. It is isolated as a metabolite from the whole fermentation broth of *Streptomyces* species. This potent immunosuppressive calcineurin inhibitor has been widely used in various fields of medicine, including transplantology, dermatology and pharmacotherapy of autoimmune diseases.

Although the total synthesis of FK-506 was described in 1990, due to low efficiency and high cost it proved to be unprofitable and currently, for industrial production, tacrolimus can only be obtained in the biotechnological way [1, 2, 3].

As the biosynthesis of secondary metabolites in *Streptomyces* is dependent on the availability of biosynthetic precursors, we hypothesised that exogenous three-carbon chain compounds may enhance tacrolimus production, acting as precursors of metabolic pathway of FK-506. Our study was aimed at both the verification of the above hypothesis and the optimisation of the medium composition to enhance the productivity of tacrolimus in *Streptomyces tsukub*aensis strain in submerged cultures [2].

For this study shake flask cultures of *Streptomyces tsukubaensis* (FERM BP-0927) were used. The culture media were composed using three different effectors with a three-carbon chain as the leading structure: propanol, propylene glycol and propionic acid. We investigated the response of FK-506 biosynthesis to 0.25%, 0.5%, 0.75%, and 1.0% (v/v) concentrations of media additives in submarged cultures [2]. FK-506 concentration was determined by RP-HPLC method [3].

Enrichment of the fermentation medium with propanol, propionic acid or propylene glycol resulted in a 4-, 3- and 2-fold, respectively, increase in tacrolimus production. The optimal concentration of the precursors was 0.25% for both propanol and propionic acid and 0.75% for propylene glycol.

Our study also demonstrated that the biosynthesis-stimulating mechanism of 3C agents was not related to the stimulation of the strain growth, proving that they are promising precursors of FK-506 biosynthesis path way which specifically enhance tacrolimus biosynthesis in *S. tsukubaensis* submerged cultures. Despite structural similarities, the mechanisms of action of the tested compounds are diverse. The application of the findings can optimise the productivity leading to the improvement of tacrolimus yield in industrial fermentation processes and reducing the cost of this clinically important immunosuppressive agent.

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P20

Novel 2-nonyl-1*H*-benzo[*d*]imidazoles: synthesis, characterization and tuberculostatic activity

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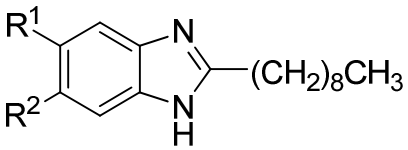
Benzimidazoles have a wide spectrum of biological activity. The direction of their activity is closely associated with the individual elements of the structure. We have already described a significant tuberculostatic activity of some 2-phenalkyl- and 2-cyclohexylalkylbenzimidazoles [1]. We also reported synthesis and activity of 2-(2-cyclohexylethyl)-1*H*-benzo[*d*]imidazole analogues with benzimidazole type of structure. Their tuberculostatic activity *in vitro* was at the level appropriate for the administrated chemotherapeutics [2]. Here we disclosed the synthesis of 2-nonyl-1*H*-benzo[*d*]imidazole derivatives. We synthesized structures possessing different substituents at the benzene ring of benzimidazole system including halogen atoms or methyl groups at C-5 position and in few cases also at C-7 positions.



R^1: H, CH_3, F, Cl, Br
R^2: H, CH_3, Cl

Target benzimidazoles were obtained by heating of decanoic acid with *o*-phenylenediamine derivatives at 160-180°C without solvent. The fusion process was elaborated earlier by our research team [1]. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, and ^{13}C NMR spectra. They have been also tested for tuberculostatic activity *in vitro* against *M. tuberculosis* standard strain H_{37}Rv and two wild strains isolated from tuberculosis patients, one strain resistant towards applied chemotherapeutics (Spec. 210) and the another strain fully sensitive (Spec. 192). Investigations were performed by a classical test-tube method of successive dilution. As a result of the synthesis eight novel derivatives of 2-nonyl-1*H*-benzo[*d*]imidazole have been obtained. Some of the compounds exhibited very good activity towards *M. tuberculosis* sensitive and resistant "wild" strains and the standard strain. The most active 5,6-dimethyl-2-nonyl-1*H*-benzo[*d*]imidazole showed tuberculostatic activity with MIC value 3.1 μg/mL comparable towards all three strain types. Obtained results suggest that the synthesized compounds are good candidates for new class of tuberculosis drugs. It would be intentional to conduct the study of their cytotoxic activity against eukaryotic cells.

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Acknowledgements

This study is supported by the National Science Centre, Cracow (grant no. *2011/0 1/B/NZ4/01187)*.

P21

Synthesis of 4-aryl-pyrido[1,2-c]pyrimidine derivatives– 5-HT_{1A}R ligands and serotonin transporter inhibitors

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The aim of this research is to synthesize and evaluate biological activity of novel pyrido[1,2-*c*]pyrimidine derivatives, containing 2-(piperazin-1-yl)quinoline moiety and its 6-nitro- and 6-bromo-substituted analogs. The results of the study will allow for analyzing structure-activity relationship (SAR) and the influence of lead structure modifications on the pharmacological activity in the tested group of compounds.

Tested ligands were designed and obtained in the way of a multi-step chemical synthesis. The chemical structure and the purity of the analytical samples were confirmed using the methods of ^{1}H NMR and ^{13}C NMR, FT-IR, elemental analysis and HRMS. Obtained compounds were subjected to radioligand binding assays, determining their affinity for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{6}, 5-HT_{7} receptors and serotonin transporter (SERT) protein. Selected ligands were subsequently evaluated in *in vivo* tests, such as: induced hypothermia and forced swimming test in mice. Metabolic stability studies were performed as well.

The obtained ligands displayed moderate to high affinity for 5-HT_{1A} receptors and SERT, and low affinity for 5-HT_{2A}, 5-HT_{6}, 5-HT_{7} receptors. All of them proved to be pre- or postsynaptic agonists of 5-HT_{1A}R. The metabolic stability was significantly affected by substitution in benzene ring of 4-aryl-pyrido[1,2-*c*]pyrimidine moiety. *para*-substitution (especially with fluorine) increased stability, whereas *ortho*-substitution – significantly decreased it.

Searching for dual acting ligands is currently one of the main trends in development of new antidepressants. Combining SERT inhibition (typical for SSRI) with 5-HT_{1A} agonism leads to reduction of latency period, which is typical for most of currently used antidepressants [1,2]. The validity of such approach was confirmed by registration of vilazodone (Viibryd) in 2011, and vortioxetine (Brintellix) in 2013, which were the first dual acting (SSRI+5-HT_{1A}R) antidepressant drugs.

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Acknowledgements

The research has been co-financed with the European Union funds by the European Social Fund as a part of the project "Rozwój nauki - rozwojem regionu - stypendia i wsparcie towarzyszące dla mazowieckich doktorantów".

P22

Evaluation of influence of selected organic compounds on melanogenesis process

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Melanin is synthesized in multi-step process called melanogenesis which takes place in specialized organelles (melanosomes) of pigment cells (melanocytes). Two initial steps of the process *i.e.* oxidation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and then L-DOPA to L-dopachinone are catalysed by metaloenzyme tyrosinase. Further steps depend on cysteine content. In case of high concentration of cysteine the feolemanin (red-yellow pigment) is synthesized, while low concentration of cysteine results in production of eumalanin (brown-black pigment). Melanin content and feumelanin/eumelanin ratio determine colour of the skin, hair and iris in humans. Moreover, proper skin pigmentation results from number and size of melanocytes, as well as transport and distribution of melanin in keratinocytes.

The main function of melanin, besides determination of human phenotype, is protection the organism from UV light and reactive oxygen species. After exposure to sun the synthesis of melanin increases what results in darkening of the skin described as suntun. In case of abnormal melanin synthesis one can recognize skin pigmentation disorders: hyperpigmentations when the melanin is accumulated in some regions or hypopigmantations when there is lack of melanin. Hyperpigmentations such as melasma, freckles, or senile lentigines belong to most common dermatological problems They can be treated by means of advanced instrumental methods together with cosmeceuticals containing skin lightening ingredients like arbutin or kojic acid. Searching for new active ingredients constitute important aspect of medicinal chemistry because of insufficiency of currently used substances and their adverse effects like hyperactivity reactions.

We screened 20 organic compounds synthesized at Department of Bioorganic Chemistry Faculty of Pharmacy JUMC for their ability of tyrosinase inhibition and influence on melanogenesis process. The compounds belong to various groups: xanthone, cinnamic acid and/or aminoalkanol derivatives. At concentration of 50 μM they inhibited the enzyme in the range of 0% to 99.00±1.00% (for kojic acid tyrosinase inhibition was 53.26±1.12%). After kinetic studies we concluded that in the tested group there are both noncompetitive and mixed-type inhibitors

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Acknowledgements

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P23

Synthesis methods of PET radioparasympathomimetic agents

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In the course of our previous research [1, 2] we have obtained a compounds able to inhibit the cholinesterases. In order to evaluate the possibility of overcoming of these molecules through blood-brain barrier there could be used the PET analysis. This method allows to monitor the location of the molecules labeled with β^+ emitter like ^{18}F, ^{11}C or ^{18}O.

We have chosen ^{18}F in fact of the almost two hours long half-life of this isotope. Depending on requirements, there are two types of organic compounds labeling: a nucleophilic substitution, based on $^{18}F^-$ ions, and the electrophilic substitution, requiring generally gas $^{18}F_2$.

Thus we have developed a technique of introducing a fluorine atom into the selected cholinomimetic molecules, which is the theoretical basis of insertion reaction of ^{18}F. This will allow the preparation of isotopically labeled compounds active in PET studies.

Studies of the biological activity based on the Ellman's colorimetric analysis have been conducted to compare the inhibitory activity of human cholinesterases newly formed products with the previously obtained compounds.

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P24

Synthesis of new imidazole-thiazoles as potential antifungal and antibacterial agents

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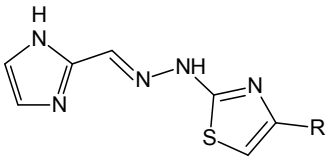
In recent years the emergence of multi-drug resistant bacteria and also resistant fungi is reported worldwide. Therefore novel, effective antifungal and antibacterial drugs are required. For several years, azoles and their derivatives have been widely studied in medicinal chemistry because of their varied biological activities such as antimicrobial [1, 2], antitumor [3, 4].

Since the azole moiety seems to be a possible pharmacophore in various pharmacologically active agents we developed several novel imidazole-thiazoles as potential antimicrobial agents which could furnish better therapeutic activity (Figure 1).



R = 4-Br-Ph, 4-NO_2-Ph 4-F-Ph, 4-CH_3O-Ph, 3,4-di-Cl-Ph, 4-CH_3CONH-Ph, 4-CF_3CONH-Ph, 2*H*-chromen-2-on, 1-adamantanyl

Figure 1

The examined compounds were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method. The microbiological studies have shown that the type of substituent in the *para*-position in benzene ring is very important for their activity. Our results indicate that compounds with Br, F, and 1-adamantanyl substituents possess the widest spectrum of antimicrobial activity against both, reference Gram-positive bacteria (MIC = 1.95 – 125 μg/ml) and fungi belonging to yeasts (MIC = 3.91 – 31.25 μg/ml).

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P25

New diquinothiazines and quinonaphthothiazines - syntheses and structures

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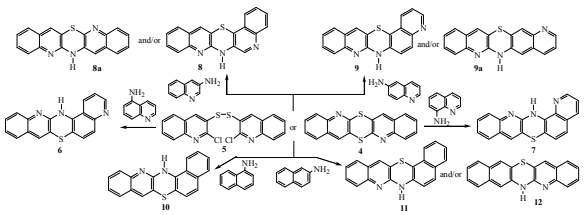
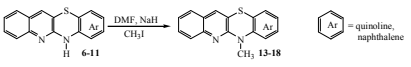
Phenothiazines are a family of heterotricyclic compounds with two o-phenylene rings bridged by the nitrogen and sulfur atoms. They have been used for over a century as well-tolerated drugs against a psychosis. New phenothiazines exhibit anticancer activities, reversal of multidrug resistance and potential treatment in Alzheimer's and Creutzfeldt-Jakob diseases [1]. The most perspective modifications of the phenothiazine structure can be achieved by substitution of the benzene ring with an azine ring to form azaphenothiazines [2]. In continuation of our search for pharmacoactive diquinothiazines, we modified the phenothiazine structure to form a new type of pentacyclic azaphenothiazines in reactions of the 1,4-dithiin ring opening in diquinodithiin **4** with 3-, 5-, 6-, 8-aminoquinoline hydrochlorides and 1-, 2-naphthylamine hydrochlori-des. The same diquinothiazines **6**-**9** and quinonaphthothiazine **10**-**11** were obtained using 2,2'-dichloro-3,3'-diquinolinyl disulfide **5** and 3-, 5-, 6-, 8-aminoquinolines and 1-, 2-naphthylamines. The reaction with 5- and 8-aminoquinolines ran to diquinothiazine **6** and **7**. The reactions with 3- and 6-aminoquinolines were more complex as two ways of the thiazine ring formation were possible (**8** and **8a**, **9** and **9a)**. The ^{1}H NMR analysis of the reaction products pointed at compounds **8** and **9** excluding compounds **8a** and **9a**. The similar reaction with 1-naphthylamine gave pentacyclic 14H-quinonaphthothiazine **10**. In the reactions with 2-naphthylamine two ways of the thiazine ring formation were also possible. The identification of the product structures pointed at compound **11** excluding compound **12**.



As some syntheses of phenothiazines can proceed via the Smiles rearrangement of the S-N type of the appropriate sulfides to more complex products, the identification of the product structures was based on the spectroscopic NMR analysis (^{1}H, ^{13}C, two-dimensional experiments COSY, NOESY, ROESY, HSQC and HMBC) of their N-methyl derivatives.



New quinonaphthothiazines were transformed into N-substituted derivatives with allyl, propargyl and diethylaminoethyl groups and were examined for their anticancer activities against human cell lines of glioma (SNB-19), melanoma (C-32) and breast cancer (T47D).

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P26

Synthesis end evaluation of biological activity of enediyne derivatives of 5,8-quinolinedione

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[b] Department of Cell Biology, Medical University of Silesia, Jedności St 8, 41-200 Sosnowiec, Poland

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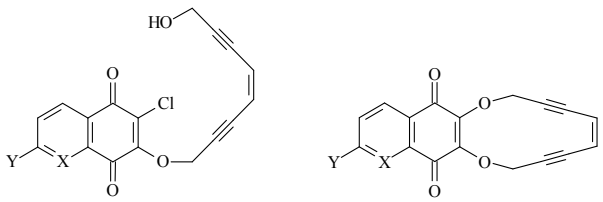
During the last two decades, quite a few active compounds having an enediyne unit have been isolated and identified from soil bacteria. Enediynes are highly potent antitumor-pharmacophores with an activity exceeding that of any other anticancer drug by a factor of up to a 1000-fold. The characteristics element of this group is an unsaturated core with two acetylenic groups conjugated to a double bond [1-2].

The next interesting group of natural products were 5,8-quinolinediones antibiotics with have a wide spectrum of biological activity. Compounds show high toxicity and for this reason their application in chemotherapy is strongly limited [3].

Purpose of this work was to synthesize enediyne derivatives of 5,8-quinolinediones and then, to study the structure – activity relationship.



X = N, Y = H
X = N, Y = CH_3
X = C, Y = H

The obtained conjugates were tested for their antiproliferative activity against human cancer cell lines: breast cancer (MDA-MB-231), glioblastoma (SNB-19) and melanoma (C-32).

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[3] Cai W.; Hassani M.; Karki R. et al. Bioorg. Med. Chem. 18 (2010) 1899-1909

Acknowledgements

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P27

Structural studies aromatic imines – substrates in benzimidazole derivatives synthesis

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Tuberculosis is an infectious disease, caused by *Mycobacterium tuberculosis*, especially active in poor countries. This disease remains a serious problem, because of increasing resistance of the bacteria to known drugs [1]. For this reason, researches aimed at finding new tuberculostatic drugs have been undertaken in many laboratories. Among others also some benzimidazole derivatives synthesized by H. Foks group from Medical University of Gdańsk show tuberculosis activity (Fig 1.).

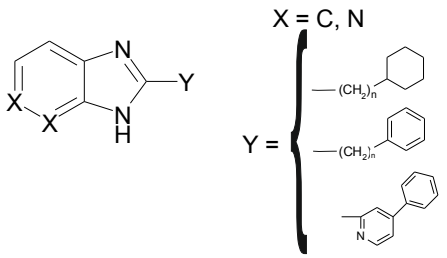

Fig. 1: Structures of benzimidazole derivatives of interest

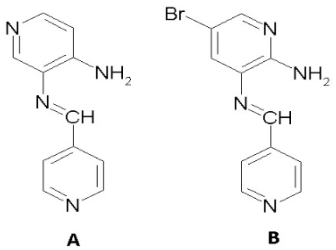

Fig. 2: Structures of the examined compounds

Intermediate products in benzimidazole synthesis were aromatic imines. Crystals of two of them: (N^3-[1-pyridin-4-yle-methylidene]-pyridine-3,4-diamine (A) and 5-bromo- N^3-[1-pyridin-4-yl-methylidene]-pyridine-2,3-diamine (B) - Fig.2 were studied by X-ray methods.

Compound A crystallizes in C2/c space group and the parameters of its unit cell are: a=15.614(2) Å, b=8.896(1) Å, c=13.886(2) Å, β= 90.57(1)°. In the case of structure B (space group is P1) the parameters are: a=7.286(3)Å, b=8.702(4)Å, c=9.310(4)Å, α=91.73(2)°, β=94.83(1)°, γ=112.77(1)°. The theoretical powder diffraction patterns of compounds A & B proof that they are main products.

Both of the examined compounds form strong intermolecular NH...N hydrogen bonds in their crystals. Molecules A form chains running in [010] direction utilizing hydrogen bonds between amine groups and nitrogen atoms of unsubstituted aromatic ring. In the case of compound B, intermolecular hydrogen bonds bind amine groups and nitrogen atoms in aromatic rings in two antiparallel molecules, forming another rings. In CSD [3] we have found 35 structures similar to the examined ones.

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P28

Histamine H_4 receptor activity of the 1,3,5-triazine derivatives

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Histamine plays its function through binding with four already known histamine receptors, designed as H_1-H_4. It is assumed, that the youngest member of the family – histamine H_4 receptor (H_4R), which was discovered and cloned in 2000/2001 by several independent research groups [1], is involved in inflammatory processes and immune responses, because of its mainly expression in various cells of the immune system (monocytes, mast cells, dendritic cells, eosinophils and basophils) [2]. Potential therapeutic effect of H_4R antagonists/inverse agonists in animal models of acute inflammations, allergic rhinitis, asthma or pruritus was confirmed [3]. As physiological role of H_4R is not clear - new, potent and selective ligands are required to investigate its action. Among H_4R ligands already described in the literature and patent data there can be found a large group of triazine derivatives [4,5,6].

The aim of this study was to evaluate *in vivo* activity of 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives (TR-7, KB-4 and JN-35), and compare them with results obtained for its hydrochloride analogues (TR-7xHCl, KB-4xHCl and JN-35xHCl). Compounds were tested in croton oil-induced ear edema model, indomethacin-induced gastric lesions model and pruritus model *in vivo* in mice. Compounds examined in the presented studies were selected from the library of compounds synthesized in our Department.

The obtained results showed that pre-treatment with TR-7, KB-4 or JN-35 has strong to moderate influence on ear weight, gastric lesion index and scratching busts. In some cases hydrochloride forms of examined compounds were more active than their base analogues. The results in detail will be presented and discussed.

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[5] Łażewska D, Kieć-Kononowicz K, *Frontiers in Bioscience.* 4 (2012), 967.
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Acknowledgements

This work was kindly supported by National Science Center DEC-2011/02/A/NZ4/00031 and GLISTEN: COST Action CM1207.

P29

Synthesis and anticonvulsant activity of new 2-(2,5-dioxopyrrolidin-1-yl)propionamides and 2-(2,5-dioxopyrrolidin-1-yl)butanamides

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Even though significant advances have been made in epilepsy research, convulsions in 30% of epileptics are inadequately controlled by standard drug therapy. Furthermore, compliance is often limited by adverse side effects most notably related to CNS exposure like diminished attention, executive function, intelligence, language skills, memory and processing speed. Thus the continued search for safer and more effective drugs is urgently necessary.

Previous researches from our laboratory have identified pyrrolidine-2,5-diones differently substituted at position-1 and -3 as targets for new antiepileptic drugs (AEDs). Many of them were effective in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) screens that are still recognized as the "gold standard" in the early stages of testing new drug candidates. The structure-activity relationships studies (SAR) demonstrated the potent and wide spectrum of anticonvulsant activity (MES, *sc*PTZ, 6-Hz tests), exclusively for the 2-(2,5-dioxopyrrolidin-1-yl)acetamides containing at the amide function phenylpiperazines with highly electronegative chlorine, fluorine or trifluoromethyl substituents.

Following these results in the current studies the library of new 2-(2,5-dioxopyrrolidin-1-yl)propionamides and 2-(2,5-dioxopyrrolidin-1-yl)butanamides was synthesized. These compounds were designed as analogs of previously described acetamides with additional methyl or ethyl group in the alkylamide linker. Furthermore, the proposed alkylamide function (propanamide or butanamide) enabled to approximate the structures of new molecules to levetiracetam which is one of the newest AEDs. The main modifications and the general structure of compounds designed are shown in Fig. 1.

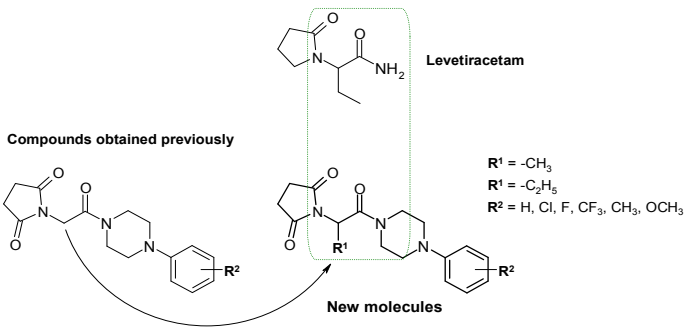

Fig. 1.

The anticonvulsant activity profile of final molecules was determined using maximal electroshock-induced seizure test (MES). This test is thought to be an experimental model of tonic-clonic seizures and of partial convulsions with or without secondary generalization in humans.

Acknowledgements

The studies were supported by the Jagiellonian University Medical College grant K/DSC/000792.

P30

Broadened pharmacological characterization of *N*-substituted-*N*-[ω-(ω-phenoxy-alkylpiperazin-1-yl)alkyl]guanidines as non-imidazole H_3 histamine receptor antagonists

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Histamine H_3 receptor (H_3R) is involved in the central and peripheral regulation of levels of histamine and other neurotransmitters (e.g. acetylcholine, noradrenaline, dopamine, serotonin, GABA, glutamate and substance P). Intensive pharmacological studies suggest the utility of H_3R antagonists/inverse agonists in CNS diseases (e.g. narcolepsy, ADHD, Alzheimer's disease, schizophrenia, epilepsy), allergic rhinitis, obesity and pain [1].

In recent years, many pharmaceutical companies and academic researchers have synthesized a large number of highly potent H_3R antagonists/inverse agonists, but still only few are being evaluated in clinical trials [2, 3]. Thus, there is a constant need for novel structures that could address histamine H_3 receptor as potential therapeutic target.

In present study we focused on series of *N*-substituted-*N*-[ω-(ω-phenoxy-alkylpiperazin-1-yl)alkyl]guanidines in search for novel histamine H_3 receptor ligands. Considered structures, previously characterized in electrically evoked contraction of the guinea-pig jejunum assay [4], were further evaluated for their antagonistic potential in cAMP accumulation assay with use of HEK cells stably expressing human recombinant histamine H_3 receptor. Moreover, basing on radioligand binding assay K_i values towards histamine H_3 receptor and off-target histamine H_4 receptor were obtained, allowing for determination compounds selectivity.

Performed assays confirmed antagonistic properties and good selectivity over histamine H_4 receptor for tested structures. Evaluated phenoxyalkylpiperazinyl-substituted guanidines showed affinity towards histamine H_3 in submicromolar concentration range. Thus, results obtained within the presented study would allow for selection of novel lead structure to further improve the activity at H_3 histamine receptor.

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[2] Łazewska D., Kieć-Kononowicz K. *Expert Opin. Ther. Pat.* 2014, 1: 89-111.
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Acknowledgements

We acknowledge the financial support of the Polish National Science Center grant, project Preludium I, No.: UMO-2011/01/N/NZ4/01126.

P31

New analogs of sildenafil; synthesis, X-ray analysis and biological activity

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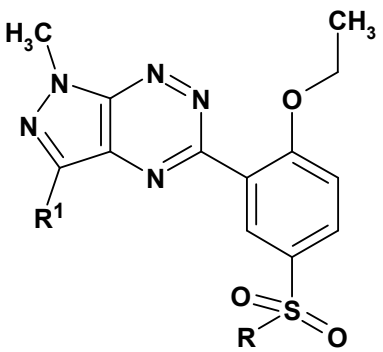
In the search for new biologically active chemotypes, several sildenafil analogs were prepared and characterized. The presence of the pyrazolo[4,3-*e*][1,2,4]triazine scaffold is thought to be of interest for the enzyme inhibitory activity of these compounds. The designed derivatives incorporating the sildenafil scaffold were assayed as carbonic anhydrase inhibitors. The new compounds were ineffective as CA I and CA II inhibitors, but were more effective against the tumor-associated isoforms CA IX and XII, with some compounds acting as low nanomolar inhibitors.



The X-ray analysis for model compound 3-(1,3-dimethyl-1*H*-pyrazolo[4,3-*e*][1,2,4]triazin-5-yl)-4-ethoxybenzenesulfonamide ($R^1 = CH_3$, $R = NH_2$) was performed and its crystal and molecular structure is described and compared with the structure of sildenafil.

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[3] Beavo, J.A., *Physiol. Rev*., 1995, *75*, 725.

P32

Synthesis, characterization and chelating properties of novel magnesium porphyrazine possessing peripheral bidentate ligands

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Porphyrazines (Pzs) are tetrapyrrolic macrocycles related to porphyrins and phthalocyanines, possessing meso nitrogen atoms in the macrocyclic core [1]. Potential usage of Pzs in medicine and technology depends on metal ions present in the macrocyclic core or their peripheral functionalization [2,3]. Many macrocycles possessing peripheral coordinating substituents have been considered as potential chelators or chemical sensors able to create complexes with diverse transition metal ions [4].

Chemical synthesis of target molecules consisted of sequential double-reductive alkylation of diaminomaleonitrile with 6-bromo-3-pyridinecarboxaldehyde and was subsequently accompanied by alkylation reaction with methyl iodide to give 2,3-bis[methyl(4-bromo-3-pyridylmethyl)amino]-2(Z)-butene-1,4-dinitrile. Novel maleonitrile derivative was utilized in the macrocyclization reaction in n-butanol with magnesium n-butanolate as a base to give the desired symmetrical magnesium porphyrazine. The chemical structure of novel macrocyclic compound possessing bidentate ligands at its periphery was confirmed by UV-Vis spectrometry, MALDI TOF, ^{1}H and ^{13}C NMR spectroscopy. The results of its chelating properties towards palladium metal cations in the UV-Vis will be presented.

Synthesis, characterization and evaluation of chelating sensor properties towards palladium ions of novel symmetrical magnesium porphyrazine were researched.

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Acknowledgements

This study was partly supported by PUMS grant No. 502-14-03313427-50623 and No. 502-01-03313427-08870 and the European Fund for Regional Development No. UDA-POIG.02.01.00-30-182/09.

P33

Potential aromatase inhibitors – synthesis, physicochemical and biological properties

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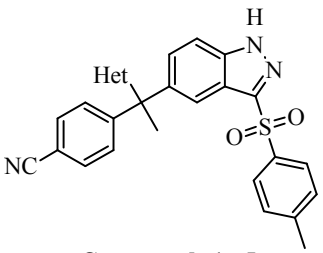
Aromatase inhibitors, e.g. *Letrozole* and *Anastrazole*, are considered to be first - line therapy in postmenopausal women with metastatic breast cancer who do not tolerate *Tamoxifen*. However, the current aromatase inhibitors are not devoid of side effects. The use of these anticancer agents is associated with high rates of fractures and myalgia, and an increase incidence of osteoporosis and hypercholesterolemia [1 - 3]. Thus, the search for new aromatase inhibitors is still very important for the breast cancer therapy.

We have developed a series of new heterocyclic anticancer agents **1** – **5** with potential aromatase inhibitory action. The six-step synthesis included, *inter alia*, vicarious nucleophilic substitution of hydrogen (*VNS*) and diazotization - cyclization reaction.

To analyze physicochemical and biological properties of compounds **1** – **5**, we used the ADMETPredictor program. The theoretical analysis have shown that some of the final products may have promising drug-like properties, such as ability to bind with the plasma proteins .Moreover, the toxicity of these compounds, including mutagenicity, a very important safety issues in drug discovery, seems to be relatively low.



Compounds 1 - 5

Heterocycles, Het :

1: 1,2,4 - triazole

2: 2 - methylimidazole

3: 3,5 - dimethylpyrazole

4: benzimidazole

5: 2 - trifluoromethylbenzimidazole

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Acknowledgements

Research was supported by Poznań University of Medical Sciences, grant no. 502-14-03308417-10167.

P34

The structure-selectivity relationship studies for hydantoin-derived 5-HT$_7$R ligands

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An achievement of high selectivity among GPCR ligands is a huge challenge. This problem especially concerns structures containing arylpiperazine moiety, which besides strong binding to desired protein, are also prone to interact with more than one receptor. This work is focused on searching for selective 5-HT$_7$R ligand among arylpiperazine hydantoin derivatives (Fig. 1). The choice of serotoninergic 5-HT$_7$R as a target is a consequence of recent studies which underline that regulation of this protein function may be essential in therapy of CNS disorders (e.g. depression, schizophrenia, anxiety).

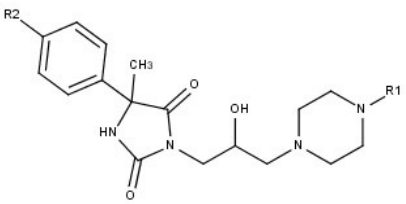

Fig. 1

To obtain the best results, organic synthesis was preceded by molecular modeling - selection of structures to prepare was performed by using following criteria: ligands position in docking rankings, potential selectivity evaluated in machine learning and synthesis difficulty level. As a consequence, the above-mentioned group of compounds which shows high activity to 5-HT$_7$R (3 nm<Ki<79nm) and also selectivity regards to 5-HT$_{1A}$R (23-71-fold) and D$_2$R (32-238-fold) has been synthesized.

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Acknowledgements

Partly supported by Polish program K/ZDS/003323.

P35

Docking of aryl-1,3,5-triazines to histamine H_4 receptor

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Latest addition to histamine receptor family - histamine H_4 receptor has been cloned in 2000 by four independent groups, on the basis of it's high sequence homology with histamine H_3 receptor [1]. Mainly expressed on the surface of leukocytes and mast cells, plays important role in immunological response [2] and constitutes an interesting target for drug development.

Amongst variety of chemical structures that show activity to histamine H_4 receptor, azines show prominent *in vitro* affinities [3]. Therefore, in our group we focus on obtaining 1,3,5-triazines with constant structural elements: amine group in position 2, *N*-methylpiperazine in position 4 and variable aryl substituents in position 6, with potential H_4R activity.

For this study we used histamine H_4 receptor homology model, based on recently published crystal structure of serotonin 5-HT_{1B} receptor [4] as a template. In order to determine protein-ligand interactions and it's possible influence on *in vitro* activity, a set of previously obtained in our group aryl-1,3,5-triazine derivatives [5], of known hH_4R *in vitro* affinity, as well as two reference compounds were docked using Schrödinger Suite 2012 [6].

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[4] Wang C., Jiang Y., Ma J. et. al; *Science 340* (2013) 610-614.
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Acknowledgements

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P36

Fragment-based drug discovery approaches in a search for novel anti HIV-1 chemotypes

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A concept of privileged structures (PS) was introduced by Evans [1]. The theory of PS evolved from a pragmatic tendency to simplify the complexity of drug design through fragmentation and has already been used in explaining the structure activity relationships in diverse groups of drugs. If PS prove to be true, then molecular motifs that enrich biological activity can be used when designing new drugs. Especially some molecular fragments, as quinoline, were claimed privileged among anticancer, antifungal, antimicrobial and also as HIV integrase inhibitors.

We attempted to test the attractiveness of the different azanaphthalene molecular fragments in chemical space [2]. Hence, we analyzed a number of the PubChem registered compounds having a given azanaphthalene structural motif. Quinoline appeared the most frequent hit. What is the origin of this popularity: practical applications, synthetic availability or else? To test the different possibilities, we considered two parameters: range of interest and b-value, representing respectively the number of compounds tested to all hits and active to tested ratio (b-value), which are the simplest measures of attractiveness and drug-likeness. The PS method can be seen as an offspring of fragonomics, which evolved from the experimental measurements of protein-ligand interactions. Recently, we developed a new concept (Fragmental Topology-Activity Landscapes, FragTAL) for encoding molecular descriptors for fragonomics into the framework of the molecular database [3].

In a search for new anti HIV-1 chemotypes we developed a multistep ligand-based virtual screening (VS) protocol combining machine learning (ML) methods with PS concept. In its learning step we based our VS protocol on the HIV integrase inhibitors fetched from ChEMBL database. The performance of various ML methods and PS weighting scheme was evaluated and applied as VS filtering criterion. Finally, using our multistep ligand-based VS cascade methodology, database of 1.5 million commercially available compounds was screened and 13 unique structures were indicated and bioactivity of these compounds was tested by measuring the inhibition of HIV replication in infected cells. This approach allowed us to discover two novel chemotypes with antiretroviral activity.

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[2] Polanski J, Kurczyk A, Bak A, et al. *Curr Med Chem* (2012), 1921–45.
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Acknowledgements

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P37

Synthesis and X-ray analysis of glycyrrhetic acid derivatives with increased lipophilicity

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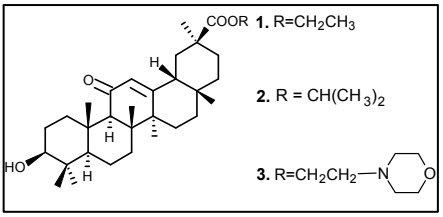
Licorice is a medicinal plant used in Europe and Asia since ancient times as a remedy for respiratory, gastrointestinal, cardiovascular or skin diseases [1]. Glycyrrhetinic acid (GE) is the metabolic product of glycyrrhizinic acid (GA), the main biologically active component isolated from licorice roots (*Glycyrrhizaglabra L., Leguminosae*) [2]. Remarkable therapeutic properties of GE as well as its chemically modified derivatives were studied over the last decades. Their effectiveness in viral infections, cancer, hepatitis, inflammation, allergy and peptic ulcer diseases has been reported in the experimental and clinical studies [3]. It has been shown that changes of lipophilic properties of GE, introduced through chemical modifications of carboxylic or hydroxyl functional groups influence the antitumor activity of GE derivatives [4].



The aim of our study was to synthesize of GE derivatives with increased lipophilicity and to examine its crystal structure in order to determine the influence of different substituents on the arrangement of the molecules in the solid state. The detailed information about supramolecular organization of GE derivatives may be helpful in designing prodrugs of GE with enhanced pharmacological properties e.g. with improved permeability. Ethyl (**1**), isopropyl (**2**) and ethylmorpholine (**3**) esters of GE were obtained according to slightly modified procedure published in the literature [5]. The products of the performed alkylation reactions were identified using mass spectroscopy. The crystal structures of **1**, **2**, and **3** were determined. **1**, **2** and **3** crystallize in $P2_12_12_1$ space group with one ester molecule in asymmetric part of the unit cell. As it was expected conformation of triterpenoid unit is conserved in the solid state. However, it is quite surprising that despite differences in the spatial requirements of ester groups introduced to the GE skeleton the arrangement of the molecules in all crystal structures is practically unchanged.

Ester molecules are assembled into helical chains through O-H···O hydrogen bonds. Only weak van der Waals forces are observed between these recurring 1-D structural motifs. Mutual orientation of chains allows the formation of the lipophilic niche, where ester substituents are directed. The size of this cavity can be adjusted to the spatial requirements of ester groups, therefore, despite introduction of more bulky substituents (crystals **2** and **3**), the overall crystal structures remain unchanged.

[1] Ożarnowski A. *Farmakodynamika Surowców Roślinnych*, WPLiS, Warszawa, (1960).
[2] Baltina L. A., et al. *Pharmaceutical Chemistry Journal*, 30 (1996) 47.
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P38

In vitro and *in silico* metabolic stability studies of H_3 receptor ligands

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The rapid and efficient determination of the ADME/Tox properties of new molecular entities (NMEs) at early stages in the drug discovery development has become very important during the last decade, due to the rapid rise in NMEs arising from combinatorial chemistry and high-throughput biological screening. *In vitro* and *in silico* studies of potential drugs allow to estimate drug-likeness at an early stage, which can greatly help plan the next steps of research. Time and funds saving, high-throughput screening and elimination of animals are just some of the many benefits which come from the using ADME/Tox *in vitro* and i*n silico* assays [1].

In the present work eight compounds with very high histamine H_3 receptor affinity (hH_3R K_i = 8.4 - 133 nM) were selected to determine very significant ADME/Tox parameter – metabolic stability (Fig. 1).

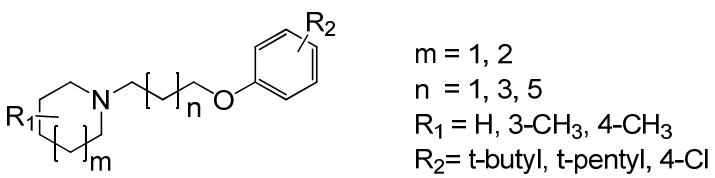

Fig.1 General structure of selected H_3R ligands

The compounds were incubated first with human liver microsomes (HLM) and next the LC-MS spectrometry was applied to determine the presence of obtained metabolites. By using *in silico* MetaSite computational method the routes of metabolic transformation were specified and the structural formulas of *in vitro* obtained metabolites were attempted to identify [2]. As the result, the most probably modifications of examined H_3R ligands were defined and included hydroxylation or the degradation of piperidine moiety followed by oxidation. Finally, the luminescent CYP3A4 P450-Glo™ Assay (Promega) was used for testing the effects of H_3R ligands on CYP3A4 activity [3]. Surprisingly, depending on the size of the compounds or the substituents positions either inhibition or activation of CYP3A4 were observed.

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[2] Boyer, D., et al. *Drug Met Disp*. 37(5), 999-1008, 2009
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Aknowledgements

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P39

Synthesis and physico–chemical properties of gadolinium porphyrazine as a potential contrast agent

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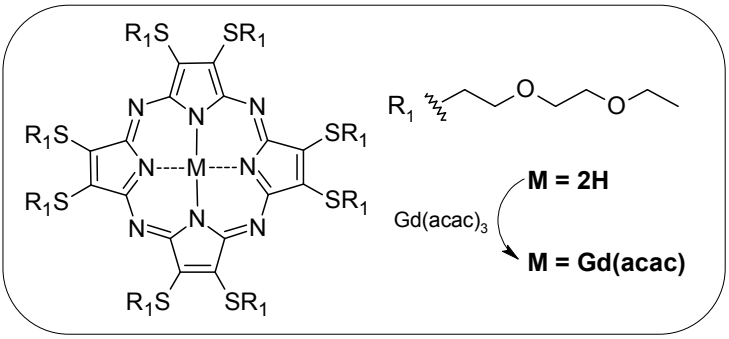
Porphyrazines constitute group of macrocyclic tetrapyrroles possessing many potential applications in industry, technology and medicine. Their medical applications embrace photodynamic therapy of cancer (PDT) and photodynamic diagnosis (PDD) [1].

Gadolinium compounds, due to their paramagnetic properties, can serve as Magnetic Resonance Imaging (MRI) contrast agents. To the commercially available MRI agents belong mainly amines and cyclic amine gadolinium complexes [2]. However, novel contrast agents, such as gadolinium–polylysine dendrimer complex Gadomer 17, possessing more desirable properties have been investigated [3]. Noteworthy, many porphyrinoid complexes with Gd ions have been considered as contrast agents [4].

Herein, we report synthesis of novel porphyrazine possessing gadolinium(III) ion in the center. This macrocycle was characterized using UV-Vis and MS. Moreover, electrochemical properties of obtained compound were studied using cyclic voltammetry and differential pulse voltammetry.



[1] Allison R.R., Sibata C.H., *Photodiag. Photodyn. Ther.* 7 (2010) 61–75.
[2] Laurent S., Vander Elst L., Muller R.N., *Contrast Med. Mol. Imaging* 1 (2006) 128–137.
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Acknowledgements

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P40

Sulfur porphyrazines possessing 4-bromobenzyl and 4-phenylbenzyl peripheral substituents – synthesis and physico-chemical properties

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Porphyrazines are aromatic macrocycles exhibiting interesting properties and numerous potential applications e.g. in medicine, in photodynamic therapy of cancer (PDT) and in photodynamic antimicrobial chemotherapy (PACT) that may represent an alternative treatment for drug resistant organisms [1]. Especially promising are porphyrazines possessing sulfur atoms in their periphery. Introduction of this heteroatom has proved to adjust desired properties such as an improved solubility in common solvents and singlet oxygen generation ability [2,3].

Two maleonitrile derivatives functionalized with 4–bromophenylmethylsulfanyl and 4-biphenylmethylsulfanyl groups were synthesized. Maleonitriles were applied in Linstead macrocyclization (magnesium butanolate in butanol) towards novel symmetrical sulfanylporphyrazines (Fig. 1). All compounds were characterized using MS, NMR and UV-Vis. Next photochemical properties of obtained porphyrazines were evaluated, including photostability and ability to generate singlet oxygen upon excitation with visible light.

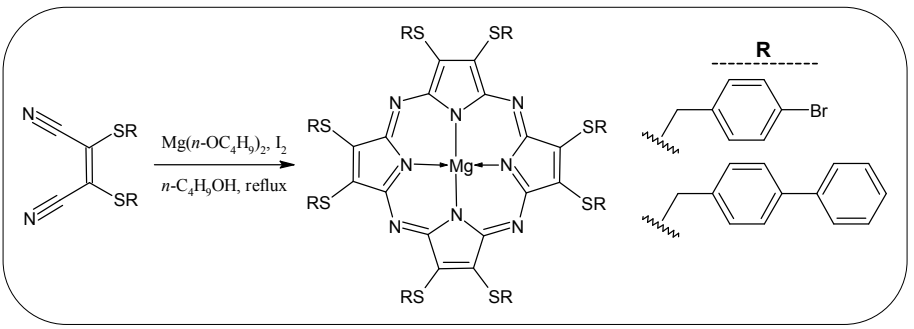

Fig. 1.

[1] Allison R.R., Sibata C.H., *Photodiag. Photodyn. Ther.* 7 (2010) 61–75.

[2] Michel S.L.J., Hoffman B.M., Baum S.M., Barrett A.G.M., *Prog. Inorg. Chem.* 50 (2001) 473–590.

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Acknowledgements

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P41

New insights into biological activity and mode of action of thiosemicarbazones in cancer treatment

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Thiosemicarbazones are important class of compounds in biological applications. Their broad spectrum of biological properties includes antiviral, antifungal and antiproliferative activity. Due to the ability to chelate heavy metals, these compounds have been used extensively in cancer research to study their effects on the cell growth inhibition. Their mechanism of action includes generation of reactive oxygen species within cancer cells, iron chelation, inhibition of the enzyme ribonucleotide reductase (RR) necessary in the synthesis of DNA [1].

Iron is indispensable for proper cell proliferation. The essential character of elemental iron is due to its involvement in DNA synthesis, mitochondrial electron transport, and its participation in the Fenton reaction leading to reactive oxygen species (ROS) generation [1]. ROS together with antioxidants may also play a key role in anti-cancer therapies that exploit oxidative stress [2]. The generation of ROS by drugs is associated with loss of intracellular redox potential. Glutathione plays a central role in maintaining redox homeostasis. Decreases in cellular GSH levels leads to initiation of the Fas receptor activation or mitochondrial apoptosis pathway [3].

Moreover, another possible mechanism of action of thiosemicarbazones is the ability binding to topoisomerase II and DNA intercalation [4].

To understand the overall mechanism of action of these compounds we focused on their ability to induce oxidative stress, DNA damages and apoptosis. Obtained series of novel derivatives thiosemicarbazones, showing the highest antiproliferative activity against human colon carcinoma (HCT116 +/+) have been tested for effects on mitochondrial GSH. The next step was to determine, effects on activation of caspases 3/7 and their participation on the pathway by apoptosis. Furthermore, the binding ability of compounds in calf thymus DNA was studied by UV–Visible absorption.

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P42

Synthesis and evaluation of anticancer activity of some derivatives of quinolylthiomethyl-1,2,3-triazoles

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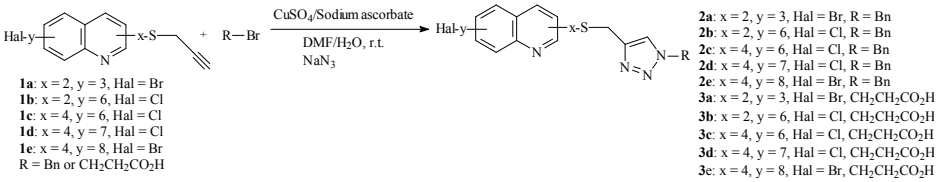
Triazole is one of the key structural units found in a large variety of bioactive molecules as anti-fungal, anti-bacterial and anti-allergic, anti-HIV, anti-tubercular and and anti-inflammatory agents. In recent years, people are increasingly focused on their anticancer activity [1].

On the other hand, quinoline have been attracting considerable interest because of their diverse activities. In the literature, quinoline derivatives have been described as anti-malarial, anti-fungal, anti-bacterial and 5-HT inhibitor [2,3]. Applications of the quinoline compounds in the treatment of cancer have also been explored. For example our group recently reported the synthesis of novel propargylthio- and propargylselenoquinolines exhibited good anticancer activity [4].

Inspired by the biological importance of 1,2,3-triazoles and quinoline compounds as anticancer agents, we herein reported the synthesis of novel 1,2,3-triazole-quinoline hybrids and their anticancer activity.



The title quinolylthiomethyl-1,2,3-triazoles **2a**-**e**, **3a**-**e** were synthesized using propargylthioquinolines **1a**-**e** as the starting compounds. The reactions were carried out in classical CuAAC conditions ($CuSO_4$, sodium ascorbate) in mixture of DMF and water. Yields in the range 79%–96% were obtained.

The ability of all of the synthesized compounds to inhibit the proliferation of the C-32 (human amelanotic melanoma), T-47D (human ductal carcinoma), and SNB-19 (human glioblastoma) cell lines was determined with the WST-1 assay. The normal fibroblast cell line (HFF-1) was used as a control. The cytotoxic properties of these new, modified quinolylthiomethyl-1,2,3-triazole derivatives were comparable to those of cisplatin.

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P43

Safety of new 8-methoxy-1,3-dimethyl-2,6-dioxo-purin-7-yl derivatives with anti-inflammatory and analgesic activity

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Preliminary studies reported by Chłoń-Rzepa *et al.* [1] demonstrated the anti-inflammatory and analgesic properties of novel 8-methoxy-1,3-dimethyl-2,6-dioxo-purin-7-yl derivatives. In order to determine their analgesic properties, they were evaluated in two pharmacological *in vivo* models: formalin test and writhing syndrome. In contrast, the anti-inflammatory potential of the tested compounds was determined in the zymosan-induced peritonitis and the carrageenan-induced edema models. Benzylamide and derivatives containing amide substituent showed very strong analgesic and anti-inflammatory (antiedematous) activity, even higher than acetylic acid. The purine-2-6-dione derivative with a carboxylic moiety showed the highest anti-inflammatory potential in the zymosan-induced peritonitis model. In addition, all these compounds significantly inhibited TNF-α in plasma of rats with endotoxemia [2]. These results proved the medicinal analgesic and anti-inflammatory potential of these derivatives. Therefore, their safety profile was evaluated through the determination of the mutagenic potential and the possible antimutagenic properties of tested compounds using the alternative *Vibrio harveyi* assay.

Vibrio harveyi test is a perfect alternative for a traditional methods of estimation anti/mutagenic properties of newly synthesized purine-2,6-dione derivatives. This assay is a microbiological method which utilizes four strains of *Vibrio harveyi*, a marine bacterium from Baltic sea: the wild type BB7 and his three genetic modifications: BB7X, BB7M, BB7XM. Each strain produces a different amount of neomycin-resistant colonies, fact connected with their different sensitivity to various mutagens [3]. Results were evaluated by comparison of the number of neomycin-resistant colony which have grown in the presence of the tested compounds and in presence of the standard mutagen (NQNO) [4].

Generally, experiment results from *Vibrio harveyi* assay show that the new derivatives of purine-2-6-dione does not possess mutagenic potential. Moreover, several of them have shown strong or moderate antimutagenic properties. Such chemopreventive activity may have potential importance in the development of newly synthesized derivatives. To validate the results obtained using this alternative methodology with *Vibrio harveyi*, parallel Ames tests with *Salmonella typhimurium* TA100 strain will be performed in the in the subsequent stages of the research.

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P44

The spatial structure of selected compounds showing tuberculostatic activacity

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In 2011 Poland reported 8478 cases of infection due to *Mycobacterium tuberculosis*. The cases in Poland are still high compared with other countries in the European Union [1, 2]. Lately the problem is the high rate of cases in the cities, and not in the countryside as before. The main problem in the treatment proves to be a quick acquisition by *Mycobacteria* a drug resistance. Specialized studies show that even the currently used modern BCG vaccine (**B**acillus **C**almette-**G**uérin) does not protect fully against infection and illness [3]. The need of the compilation of several drugs and a long list of side effects associated with their use forces the continuous search for new antituberculous agents.

For this purpose a series of compounds were synthesized in the Department of Organic Chemistry, Medical University of Gdansk and tested for tuberculostatic activity.

We present the molecular structures of the two active compounds (Figure 1.) and will discuss H atom allocations in main chain, which was unclear from spectroscopic data. In addition, we will speculate on the impact of the structure on the activity of the resulting compounds.

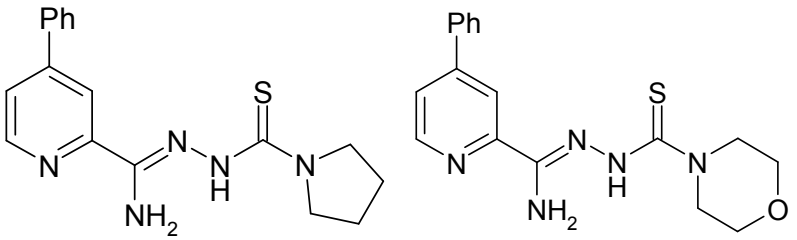

Figure. 1. Proposed structures of the studied compounds - potential antituberculous drugs

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P45

The influence of structural modification to immunomodulatory activity within isoxazole derivatives

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The key target of our research was design of a compounds with the higher activity than started compounds. First we synthesized a new series of substituted benzylamides of 5-amino-3-methylisoxazolo-4-carboxylic acid (MO). The compounds exhibited different, but generally immunosuppressive propertie in the applied tests. Determination of phytohemagglutinin A (PHA)-induced human peripheral blood mononuclear cell (PBMC) proliferation, cytokine production by human whole blood cell cultures, humoral immune response of mouse splenocytes *in vitro* to sheep erythrocytes (SRBC), cellular immune response in mice *in vivo* to ovalbumin (OVA) and inflammatory response to carrageenan in mice were tested. The next step was a definition, how the structural modification could impact immunological properties. We synthesized the new 5-substituted 3,6-dimethylisoxazolo[5,4-d]-pirymidin-4-one derivatives (MZ) in reaction of compounds MO with triethyl orthoacetate. This modification had not effect to immunological activity. In reaction compound MO with sodium nitrite we recived the new 5-substituted 3-methylisoxazolo[5,4-d]-1,2,3-triazin-4-one derivatives (MZA). This reaction caused an increase of immunosuppressing properties only within a few compounds.

Modification of position 5 of substituted benzylamides of 5-amino-3-methylisoxazolo-4-carboxylic acid (MO) caused to synthesis the new series (MZO). Also this reaction had influence on raising immunosuppressing activity only in a few compounds.

This study did not show, which modification of structures are valid to determine immunological activity and which part of compounds have influence to biological activity in described isoxazole derivatives.

Acknowledgements

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P46

Theoretical studies on mechanisms of energetic transitions in the molecule of 3,5-dimethylizoxazolo[5,4-*e*][1,2,4]-triazepin-4-one

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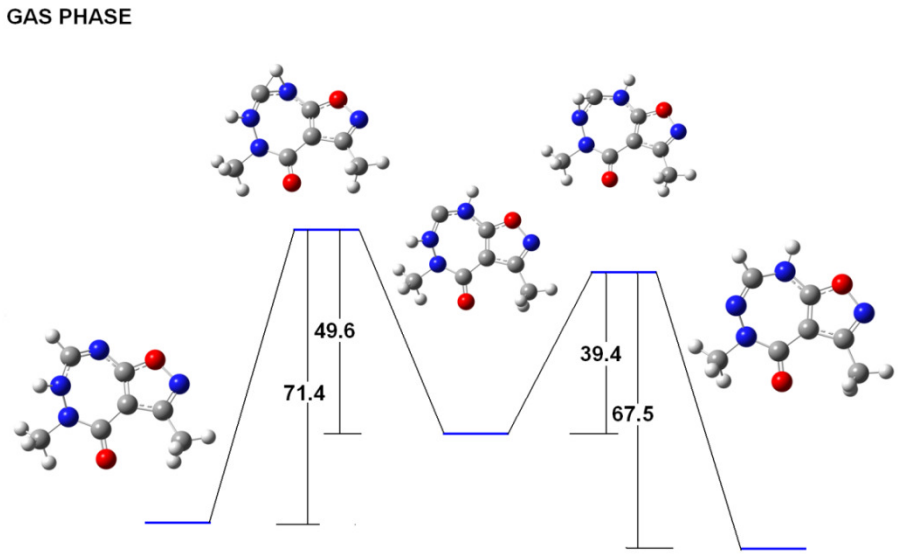
Quantum chemical calculations on the level B3LYP were performed to answer the question which of two isomers of 3,5-dimethylizoxazolo[5,4-*e*][1,2,4]-triazepin-4-one are more stable and what is the transition state between these two forms. Activation energy of transition was established. Gas phase calculations give 70 kcal/mol of activation energy. Calculations in water (PCM – model, Gaussian 0.9) give decrease of activation energy of the transitions. Δ Energy activation is on the level of 30kcal/mol.

These results show that only one of molecules obtained in the experiment are population in solution and biological activity is conned with the one form. Scheme of energy transitions is given below. Intermediate state was established which is a local minimum being about 30 kcal/mol above the stationary state. Additional calculations were made in which molecular interaction with one, two, three molecules of water are also considered.



P47

Computational study of quinoline derivatives with potential 5-HT_{1B} receptor agonistic

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Serotonin 5-HT_{1B} receptors are present in the CNS, where are involved in mood regulation, learning, memory, aggression and anxiety. Receptors located in vessels affect on pulmonary vasoconstriction, as well. 5-HT_{1B}Rs are presynaptic and are localized on axon terminals. [1] Mechanism of agonistic action of 5-HT_{1B}Rs is expressed on three levels: vascular, neurogenic and central. In this study we described a series of compound, which structure were inspired by the newest antidepressants Vortioxetine and Vilazodone. Designed compounds contain in their structure elements, which are important for activity to 5-HT_{1B}R. A high affinity for serotonin receptors is conditioned by two nitrogen atoms, one attached to an aromatic ring, a second fully aliphatic (II- or III-row). Probably, the diaromatic sulfide group limits the effect to serotonergic receptors only. [2]

As the protonation state plays a role in biological activity of serotonergic agents, we calculated the proton affinity and dissociation constant in water of studied compounds with B3LYP/6-311++G(d,p). Next, we determined physicochemical properties, for example logP, dipole moment. Global softness and dipole moment were proposed as the probable good predictors of the proton affinity of closely related compounds. [3] In the next step to estimate the binding affinity of the investigated compounds for 5-HT_{1B}R we applied molecular docking method. For computational studies Gaussian 09' and Accelrys Discovery Studio 2.5 were used.

The results of our study are useful to plan synthesis of compounds with high affinity for 5-HT_{1B}Rs and physicochemical properties affecting their good bioavailability.

Computational study leads to evaluate designed structures. It enables the straight of affinity of new compounds to receptor before their synthesis. This operation economises time and minimizes costs of research. Computational methods may reform searching for new antidepressant drugs, with novel mechanism of action.

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P48

Synthesis and characterization of novel sulfanyl tribenzoporphyrazines

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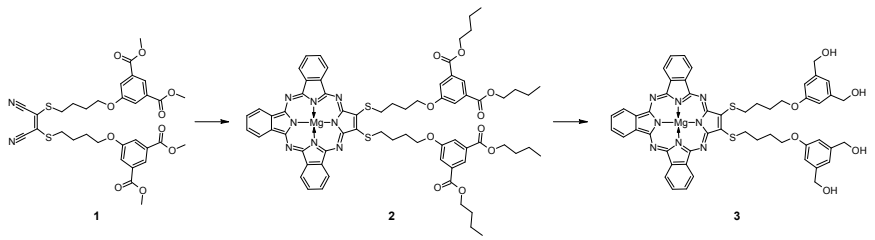
Porphyrazines (Pzs) have been widely studied for their unique spectroscopic, photochemical and electrochemical properties. These properties made them suitable for various potential applications in photodynamic therapy [1], diagnostics [2] or chemical catalysis [3]. Among Pzs, those bearing sulfanyl substituents have been found soluble in common solvents and exhibited high singlet oxygen generation quantum yield values [4].

Mixed Linstead macrocyclization reaction using maleonitrile derivative **1** was successfully applied to synthesize novel tribenzoporphyrazine bearing sulfanyl substituents **2**. Macrocycle **2** was isolated and purified by means of column chromatography. In addition, reduction reaction of tribezoporphyrazine **2** led to derivative **3**. Both compounds were characterized using NMR techniques, UV-Vis spectroscopy and HPLC.



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Acknowledgements

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P49

Relaxant effects of selected sildenafil analogues in the rat aorta

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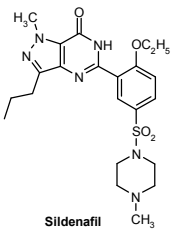
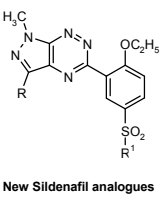
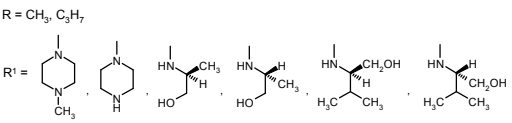
Sildenafil inhibits phosphodiesterase type 5 (PDE5), an enzyme that metabolizes cyclic guanosine monophosphate, thereby enhancing the cyclic guanosine monophosphate - mediated relaxation and growth inhibition of vascular smooth-muscle cells. Among all PDEs, PDE5 is widely expressed in a variety of tissues, such as brain, smooth muscle, lung, platelets and kidney [1]. Due to the PDE5 smooth muscle localization, inhibitors of this enzyme were initially developed for the treatment of erectile dysfunction (Viagra, Cialis, and Levitra) and subsequently pulmonary hypertension [2,3]. Recently, a number of studies have shown a growing interest in PDE5 as a promising target for the treatment of other diseases [4].

The aim of this work was to assess vasorelaxant properties of new structural analogues of sildenafil and explanation of their mechanism of action.







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P50

2D-SIFt – a matrix describing ligand-receptor interactions

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Structural Interaction Fingerprints (SIFts), as defined in [1], encode a ligand-receptor complex in form of a bit string depicting the detailed interactions of the receptor. The lack of recognition of pharmacophore features of the ligand is one of the major disadvantages of this methods. To address this issue, a modification of original SIFt methodology has been done, encapsulating interactions between the features of ligand and receptor in form of 6x9xN matrix (6 standard pharmacophore features, 9 types of interactions with amino acid [2], N – number of residues in described receptor). Matrix fields can take values greater than 1, there can be more than one separate pharmacophore feature of one type within ligand interacting with one residue (for instance three phenyl groups surrounding a phenylalanine).

Analogously to the previously demonstrated methodology, such matrices can be averaged to create profiles showing the most important interactions, thus being a hybrid between structure-based pharmacophore model and classical interaction fingerprint.

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Acknowledgements

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P51

The role of UDP-glucuronosyltransferases in metabolism of acridine antitumor agent C-1748

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The majority of xenobiotic compounds express lipophilic properties that make difficult their excretion and are responsible for their accumulation inside the organism. Enzymatic systems of living organisms allow to transform the lipophilic molecules to more hydrophilic metabolites. I and II phase of metabolic enzymes are involved in these processes and they result in detoxification and elimination of xenobiotics outside the cell and/or outside the whole body. Usually products of the I phase metabolism undergo the II phase transformation - conjugation with glucuronic acid, sulphate, glutathione or amino acids [1].

II Phase metabolism catalysed by UDP-glucuronosyltransferases (UGTs) is currently on the topic of interest in respect to antitumor therapy. Glucuronide metabolites are more polar than the native drugs what modifies their pharmacokinetics and pharmacodynamics. High UGT activity can result in better drug elimination and as a consequence in the resistance to the drug action. On the other hand, low level of UGT can be a reason of serious side effects induced by the drug. Glucuronidation usually takes place on aromatic hydroxyl and/or amino groups leading to O- or N-glucuronides [2].

In the present work we demonstrated the results of studies on glucuronidation of acridine antitumor agent, C-1748, which was effective in NCI panel of cell lines and in experimental antitumor therapy. It is selected last time to Phase I clinical studies. We showed that although it possesses only aliphatic hydroxyl group, the compound was metabolized to one product, glucuronide, observed after incubation with rat and human liver microsomes as well as with human intestine microsomes. The presence of glucuronide was confirmed by β-glucuronidase assay. Considering the possibility of drug-drug interactions in terms of UGTs' action we also investigated the impact of C-1748 on the UGTs' enzymatic activity. We demonstrated that the compound C-1748 inhibited the glucuronidation of SN-38 (7-ethyl-10-hydroxycamptothecin, the standard substrate for UGT1A1) and had no impact on the glucuronidation of TFK (7-hydroxy-4-(trifluoromethyl)-coumarin, the standard substrate for UGT1A subfamily except of UGT1A4) in rat liver microsomes. Moreover, the increase of the conversion rate of TFP (trifluoperazine, the standard substrate for UGT1A4) by human recombinant isoenzyme UGT1A4 in the presence of C-1748 was observed. The last result suggests that the compound is able to activate this isoenzyme. Summing up, the present results demonstrate that C-1748 can be a modulator of the UGTs' activity what indicates that drug-drug interactions should be considered in the combined therapy of this compound with other therapeutics.

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P52

Synthesis and antiproliferative activity in vitro of novel 2-arylideneaminobenzimidazole derivatives

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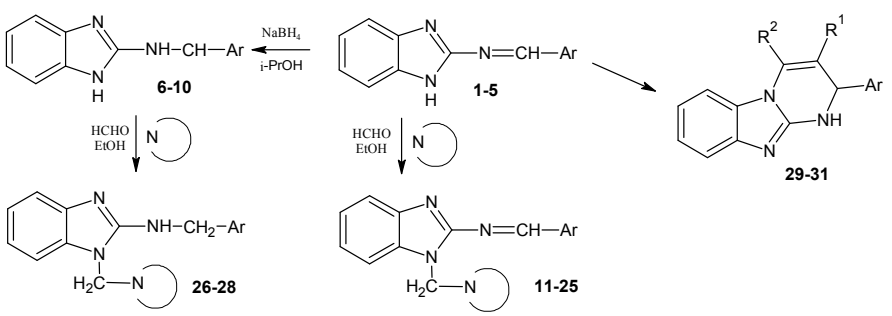
Imines exhibit various biological activity such as antidiabetic [1], anticancer [2], antioxidant [3] properties. Schiff bases are active against a wide range of microbes or protozoan [4]. Mannich bases, containing various heterocyclic systems, posses biological activities: anticancer [5], anticonvulsant [6] or antimalarial [7]. Chemical modification of various heterocyclic compounds containing azomethine bond and aminomethly group provides biological activity.

A new class of Mannich bases 11-28, derivatives of 2-amino-1H-benzimidazole, were obtained in the condensation of Schiff bases 1-5 or 2-benzylaminobenzimidazoles 6-10 with selected secondary amines: morpholine, piperidine, N-methyl-piperazine, N-phenylpiperazine, 1-(2-pyridyl)piperazine, 1(2-methoxyphenyl)piperazine, 1-(2-pyrimidinyl)piperazine and formaldehyde in ethanol. The pyrimido[1,2-*a*]benzimidazole derivatives 29-31 have been synthesized in the reactions of Schiff base 2 with selected compounds containing active methylene group: acetylacetone, benzoylacetone and malononitrile. The structures 1-31 were confirmed by the results of elementary analysis and their IR, ^{1}H-, ^{13}C-NMR spectra. All compounds were screened against the cells of MV4-11 human leukemia and then the most active of them 6, 8, 9, 11-18, 26-28, 30, 31 were tested towards human T47D breast and A549 lung cancer cells as well as normal mouse fibroblasts (BALB/3T3). The most active compounds against the cells of cancer cell lines were 2-(3-chlorobenzylamino)-1H-benzimidazole (9) and 4-amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-*a*]benzimidazole (31) showing in parallel very low cytotoxicity towards mouse fibroblasts.



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[2] Sondhi S.M., Arya S., Rani R., Kumar N., Roy P. *Med. Chem. Res*. 21 (2012) 3620.

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[4] Anand P., Patil V.M., Sharma V.K., Khosa R.L., Masand N. *Int. J. Drug Design Discovery* 60 (2012) 851.
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P53

Synthesis and electrochemical properties of porphyrazines possessing isophthaloxybutylsulfanyl substituents and d-block metal ions in the center

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Porphyrazines (Pzs) are aromatic macrocycles exhibiting interesting properties and numerous potential applications in medicine, especially in photodynamic therapy of cancer (PDT) and in photodynamic antimicrobial chemotherapy (PACT) [1]. Especially promising are Pzs possessing peripheral sulfur atoms in their periphery [2,3]. The limitations of these macrocycles, such as poor solubility and low singlet oxygen generation ability, hindering their broader usage in medicine, may be overcome by peripheral modification with dendrimeric substituents.

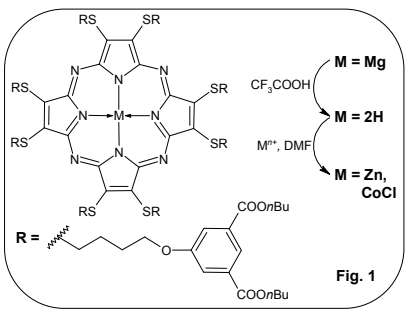

Fig. 1

Novel porphyrazine derivatives bearing [4-(3,5-dibutoxycarbonylphenoxy)butyl]sulfanyl groups in the periphery and zinc or cobalt ions in the core, were synthesized. Metalloporphyrazines were obtained starting from a demetallated porphyrazine following metallation reaction procedure (**Fig. 1**). The chemical structures of novel porphyrazines were characterized by MALDI MS spectrometry, UV-Vis and NMR spectroscopy, including ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC techniques. An oxidation-reduction potential of cobalt porphyrazine was assessed using electrochemical methods. Moreover, an ability to generate singlet oxygen upon excitation with visible light by obtained macrocycles was studied.

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Acknowledgements

This study was supported by the National Science Centre under grant No. 2012/05/E/NZ7/01204.

P54

Tricyclic xanthine derivatives – preliminary evaluation of anticancer activity

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The most common causes of death in Europe in last few years were cardiovascular and cancer diseases [1]. GLOBOCAN project inform, that since year 2002 incidence and mortality on account of all types of cancer in the World increased rapidly. In 2012 14.1 mln new cases and 8.2 mln deaths because of cancers (excluding non-melanoma skin cancer disease) were reported [2].

Cancer therapy encounters many difficulties. Most anti-cancer drugs affect not only tumor cells but also healthy, proliferating tissues. That condition leads to many side effects of anticancer therapy and decreases patients' quality of life. Moreover, multidrug resistance of cancer cells results in chemotherapy failure in many cases each year. Therefore, development of new anticancer agents and searching for novel methods of anticancer therapy are some of the most important fields of medicine and medicinal chemistry nowadays. [3]

Developmental Therapeutics Program (DTP) is the drug discovery and development arm of the National Cancer Institute (NCI) - the part of the United States National Institute of Health. DTP carries out anti-cancer compound screening program for identifying novel chemical leads and biological mechanisms of drugs actions [4].

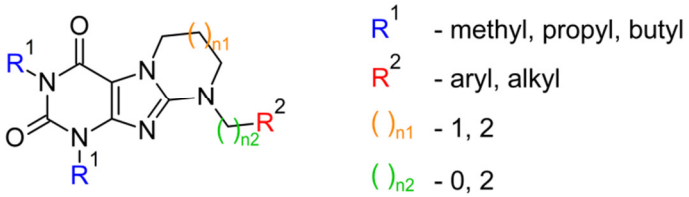

Fig. 1: Structure of the selected compounds

As the results of our cooperation with NCI, eight tricyclic xanthine derivatives (Fig.1) were accepted for a primary pharmacological screening in DTP program. Compounds were tested at 60 different human cancer cell lines: prostate, breast, ovarian, colon, renal, central nervous system and non-small cell lung cancer as well as melanoma and leukaemia. Evaluated structures exhibit low, moderate or high effect on cancer cells growth. The most active compound were selected for screening in cancer cell lines in dose-dependent manner.

Support of K/ZDS/004689 is kindly acknowledged

[1] http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Causes_of_death_statistics.
[2] http://globocan.iarc.fr/Default.aspx
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[4] http://dctd.cancer.gov/ProgramPages/dtp/

P55

Novel tetrahydroacridine and cyclopentaquinoline derivatives with fluorobenzoic acid inhibit human lung adenocarcinoma cell growth by inducing G1 phase cell cycle arrest.

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Lung cancer is not only the most commonly diagnosed cancers worldwide but is still the leading cause of cancer-related death. In general, the five-year survival rate for patients with lung cancer is less than 15%. Acridine derivatives are a known class of anticancer agents that display the ability to intercalate into DNA and inhibit topoisomerases, enzymes involved in replication and transcription.

In order to search for more effective anticancer compounds, we have evaluated the effect of new sixteen tetrahydroacridine and sixteen cyclopentaquinoline derivatives coupling with hydrazinonicotinic acid or fluorobenzoic acid on the viability and growth of human lung adenocarcinoma A549 cells. Interestingly, we found that anticancer activity of compounds containing fluorobenzoic acid correlated with the increased number of carbon atoms in aliphatic chain. Based on these results we selected the most effective compounds from each group for further biological evaluation.

The aim of this study was to investigate the effects of these novel compounds on cell cycle progression and apoptosis in lung cancer cells. The flow cytometry analysis showed that tetrahydroacridine and cyclopentaquinoline derivatives with fluorobenzoic acid moiety induced arrest of cell cycle progression through G1 into S phase in A549 cells. In contrast, tested compounds containing hydrazinonicotinic acid had no significant effect on cell cycle distribution. To determine the effects of tested compounds on apoptosis we evaluated the caspase 3/7 activity and apoptotic cells were also visualized by nuclear morphological changes in Hoechst-stained cells. Our results showed that all tested compounds induced cancer cell death by stimulating caspase 3/7-dependent apoptosis.These findings suggest that fluorobenzoic acid moiety and length of the aliphatic linker enhance anticancer activity and have impact on mode of action of tetrahydroacridine and cyclopentaquinoline derivatives. The results obtained in the present study demonstrate that novel derivatives with fluorobenzoic acid moiety are better candidates for treatment of lung cancer because they inhibit cancer cell growth by both mechanisms cell cycle arrest and induction of apoptosis.

Acknowledgements

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P56

Thiodipeptide esters - chance in the treatment of inflammatory and neoplastic diseases?

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Cathepsin C (EC 3.4.14.1) also known as dipeptidyl peptidase I (DPPI), dipeptidyl transferase, or cathepsin J is a lysosomal cysteine protease which sequentially removes dipeptides from the N - terminus of peptide and protein substrates. This enzyme is present in most mammalian cells, and the highest concentrations found in the spleen and kidney. The crystal structure of this enzyme has been determined and found being different from the other cysteine proteases. Cathepsin C is a tetramer composed of four identical subunits, each of which consists of a heavy chain, light chain and domains exclusion. Domain exclusion has no counterpart in the papain family and gives DPPI unique aminopeptidase activity. The catalytic center of the enzyme is the asparagine residue that interacts with an unblocked N-terminal residue of the protein substrate, and cysteine and histidine, which are responsible for the hydrolysis of a peptide bond. It was shown that human and bovine cathepsin C are characterized by an almost identical substrate specificity, suggesting that they have nearly identical catalytic center [1]. Binding center of DPPI is formed by two cavities S1 and S2 which differ in the depth and polarity, and thereby the preference for certain amino acid residues in substrates and inhibitors. The great similarity between human and bovine cathepsin C justify studies on inhibitory activirty of potential drugs using this enzyme. There is a growing evidence that cathepsin C plays a crucial role in many diseases such as: sepsis, asthma, Duchenne muscular dystrophy, rheumatoid arthritis, chronic obstructive pulmonary disease, sepsis, cystic fibrosis, and various inflammations. This enzyme is also involved in the activation of proenzyme serine proteases such as granzymes A and B, cathepsin G, chymase, and neutrophil elastase. Good knowledge of the crystal structure and architecture of catalytic center of cathepsin C governs the studies devoted to the design of inhibitors of this enzyme.

In order to study the requirements of S1 binding pocket of cathepsin C we have synthesized thiodipeptide esters by sulfa-Michael addition of structurally variable thiols (ethanethiol, thiophenol, 4-chlorothiophenol, 4-bromothiophenol, 4-fluorothiophenol, cycloheksanethiol, 1-adamantanethiol and cysteine) to the double bound of dehydrodipeptide methyl esters, namely BOC-Gly-ΔAla-OMe or BOC-(S)Phe-ΔAla-OMe. After deprotection of the amino group, which gives the thiodipeptide ester salts of *p*-toluenesulphonic acid. The compounds thus obtained were examined spectrophotometrically towards cathepsin C activity isolated from bovine spleen. Type of inhibition and inhibition constant K_i was determined by Dixon, Lineweaver-Burk (L-B), Hanes-Woolf and half-inhibitory concentration method. This enabled to study structure-activity relationship and to define the best susnstituent at P1 position of the inhibitor.

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Acknowledgements

Bartosz Oszywa and Paweł Lenartowicz are recipientes of a Ph.D. scholarship under a project funded by the European Social Fund. The poster was done in collaboration with Regional Hospital in Opole. „The research was supported by Wroclaw Research Center EIT+ under the project „Biotechnologies and advanced medical technologies – BioMed" (POIG 01.01.02-02-003/08-00 financed from the European Regional Development Fund (Operational Programme Innovative Economy, 1.1.2)".

P57

New cantharidin analogues with potent anticancer activity

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Introduction: In view of the ability of cancer cell to develop resistance against anticancer drugs, new treatments and new therapeutic approaches need to be introduced. Natural toxin - cantharidin was described as strong inhibitor of protein phosphatases - PP1 and PP2A, and cancer growth. Because of cantharidin nephrotoxicity there is interest in developing highly efficacy and safe its analogues.

Aim: The aim of this study was to evaluate the toxicity and antiproliferative potential of alifatic and aromatic new derivatives of cantharidin against human cancer liver cells (Hep3B cell line) in comparison with normal human liver stellate cells (LX-2 cell line).

Methods: Influence of studied substances on viability and proliferation of Hep3B and LX-2 cells was determined by MTT assay and flow cytometry. Cells of both Hep3B and LX-2 lines were incubated with different concentrations of norcantharidin and its derivatives (1-1000μM) for 24-48 hrs. We determined both EC_{50} and IC_{50} for respectively toxicity and proliferating inhibition dose. Additionally the immunofluorescence observation was performed to evaluate the presence of apoptotic cells.

Results and conclusions: We studied norcantharidin as reference substance, and its derivative. Among all chemicals *N*-substituted amides having 2-chlorophenyl and 4-bromophenyl groups induce cytotoxic effect similarly to norcantharidin, however without specific distinction between cancer and normal cells. This observation was confirmed by immunofluorescence staining with DAPI. None of tested substances inhibited cells proliferation similarly to reference substance. The general observation was that among substituted amides derivatives with aryl and substituted aryl groups was more effective than its aliphatic derivatives. On the other hand, the most interesting compound was *N*-isopentyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)-7-oxabicyclo-[2.2.1]-hept-2-carboxylic acid. This derivative displayed high toxicity against human cancer liver cells in comparison with small toxicity against normal human liver stellate cells.

P58

Molecular mechanisms of functional selectivity of fenoterol derivatives towards the β_2-adrenergic receptor

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Fenoterol is a long lasting β_2-adrenergic receptor (β_2-AR) selective agonist used in therapy as racemic mixture of (*R*,*R*')- and (*S*,*S*')-isomers. In our medicinal chemistry project we studied all four steroisomers of fenoterol and several its derivatives modified on aminoalkyl tail [1,2]. Our research showed that these compounds exhibit qualitatively different functional activities in various assays. Rat cardiomyocyte contractility studies using pertussis toxin indicate that (*R*,*R*')-isomers of fenoterol, 4−methoxyfenoterol and 4-aminofenoterol activate the β_2-AR to a form which couples selectively to G_s protein while the receptor activated by e.g. 1-naphtylfenoterol or 4-methoxy-1-naphtylfenoterol is able to couple uniformly both G_s and G_i proteins. Molecular modeling data suggest that hydrogen bond formation between *N*-alkyl moiety of a ligand with Y308 residue of β_2-AR is a key interaction distinguishing G_s selective derivatives of fenoterol from others, non selective derivatives.

To test this hypothesis in vitro, we examined the interaction of the series of fenoterol analogs with the Y308A β_2-AR mutant. Binding affinities for the mutant were determined using membranes from stably transfected HEK-293 cells, with [^{3}H]CGP-12177 as the marker ligand. The Y308A β_2-AR shows significantly reduced affinities for G_s selective derivatives of fenoterol as compared with the wild type-related data, while affinities of non selective analogs were not affected by the mutation.

Fenoterol derivatives appeared important drug candidates for therapy of congestive heart failure or certain brain tumors. Chemical modifications can be used to switch interactions with Y308 residue what seems to be interesting strategy for development of molecules specifically triggering desired cellular downstream effects [3].

[1] Jóźwiak K., Khalid C., Tanga M.J. et al. *J Med Chem.* 2007, 50(12), 2903-15.
[2] Jóźwiak K., Woo A., Tanga M.J. et. al. *Bioorg Med Chem.* 2010, 18(2), 728-36.
[3] Woo A.Y., Jóźwiak K., Toll L. et al. *J Biol Chem.* 2014;289(28), 19351-63.

Acknowledgements

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P59

New copper (II) complexes with N1-acylamidrazones and their antibacterial activity

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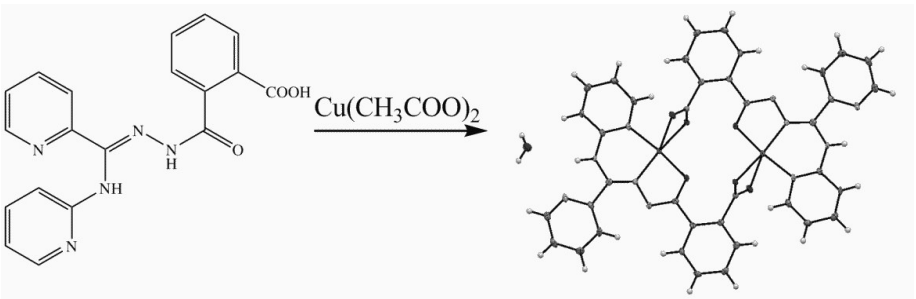
Four novel N1-acylamidrazones ligands were synthesized in reaction of N-(pyridine-2-yl)picolinohydrazonamide with cyclic anhydrides and transformed into copper (II) complexes. The structures of new compounds were confirmed by ^{1}H NMR and IR spectral analyses as well as by X-ray crystallography.



The antibacterial activity of obtained complexes was tested *in vitro* against Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Sarcina lutea, Mycobacterium smegmatis* ZFR 21, *Nocardia corralina*) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* O:3) bacterial strains. Preliminary tests showed week antibacterial activity of the ligands except one compound active against *M. smegmatis* and *S. aureus* (MIC=50μg/mL).

First copper complex showed good and selective activity against *M. smegmatis* while its ligand was devoid of significant antibacterial activity. The second complex showed the same activity as initial ligand against *M. smegmatis* and *S. aureus*. Third complex was less active against *M. smegmatis* and *Y. enterocolitica* (MIC=100μg/mL). Last complex was inactive due to its practically insolubility in most solvents.

Pharmacological tests revealed that the coordination effect could improve the antibacterial activity of amidrazone ligands especially against *Mycobacterium smegmatis* strain.

Acknowledgements

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P60

Binding sites of vinca alkaloid anticancer drugs in alpha1-acid glycoprotein. Molecular docking studies

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The binding of medicines to plasma proteins determines their pharmacological actions and side effects. A significant role in the drug disposition is played by human serum albumin (HSA) and alpha1-acid glycoprotein (AGP). In some pathological cases, such as rheumatism, myocardial infarction and malignant disorders, AGP can become the main drug binding protein, since its concentration grows when the concentration of HSA decreases. In the work we studied the interactions between AGP and two vinca alkaloid anticancer drugs (natural – vinblastine and semisynthetic – vinorelbine) by molecular docking simulation. Molecular docking experiment was carried out using following computer programs: Marvin Sketch [1], CLC Drug Discovery Worbench [2] and Discovery Studio [3]. The crystal structure of AGP required for computer simulation was downloaded from the Protein Data Bank (PDB ID: 3KQ0) [4]. The binding energy for each obtained complex was calculated by scoring function and presented in arbitrary units (a.u.).

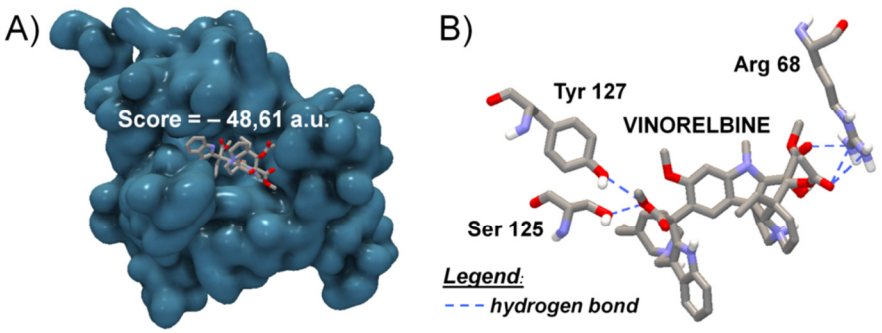

Figure 1. Visualization of vinorelbine binding site in native AGP macromolecule (A) and amino acid residues involved in the creation of hydrogen bonds with vinorelbine (B).

[1] Marvin 6.3.1, 2014, ChemAxon (http://www.chemaxon.com);
[2] CLC Drug Discovery Workbench 1.0.2, 2014, CLC bio, a QIAGEN Company;
[3] Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, San Diego: Accelrys Software Inc., 2013;
[4] RCSB Protein Data Bank (http://www.rcsb.org).

Acknowledgements

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P61

Investigation of tea tree oil safety – cellular model study

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[b]Department of Pharmacy, Friedrich-Wilhelms University of Bonn, Bonn, Germany

Tea tree oil is essential oil taken from the leaves of the Melaleuca alternifolia. Healing properties of this oil are used for centuries to treat wounds, as well as colds and headaches. Modern medicine uses of tea tree oil to treat skin diseases such as acne, dandruff, psoriasis, seborrheic dermatitis, fungal infections. This oil also combats the herpes virus, influenza virus, house dust mites, and also relieves insect bites. The cosmetics industry also uses it as a preservative. Despite the fact that this compound is so widely exploited, their cytotoxic activity was not investigated thoroughly.

The aim of the study was to investigate the cytotoxicity of tea tree oil against PNT2 – normal human prostate cell line, MCF10a – normal human breast cell line and HSF – human skin fibroblasts. Cytotoxicity test was performed by using trypan blue staining.

Our results show that increasing concentration of tea tree oil correspond with viability of the cells. Concentration which are usually used in cosmetology may be lethal for in vitro cells cultures.

Although tea tree oil has long been used as cosmetic ingredients, it seems reasonable to re-check the safety of their application using modern methods based on in vitro techniques. Preliminary results indicate that commonly used in many preparations, tea tree oil may affect toxic to cells, so it is necessary to conduct further research, leading to explain the observed changes.

P62

Structural studies of tiotropium salts by high-resolution solid-state NMR spectroscopy and GIPAW *ab initio* calculations

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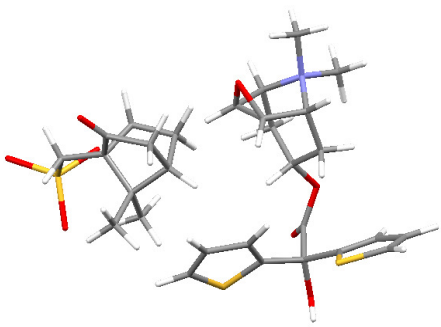
Tiotropium (1α, 2β, 4β, 5α, 7β)-7-[(hydroxy- di-2-thienylacetyl)oxy)-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.0]nonane) is a highly effective anticholinergic, used in the treatment of asthma and chronic obstructive pulmonary diseases (COPD) [1].



tiotropium with 1S-(+)-10-cmphorsulphonic acid

Solid state properties including polymorphism, solvate and salt formation can have a profound impact on two of the most important properties that are essential to the successful development of drug candidates: solubility and stability. There are several tiotropium salts [2], but only one of them - the bromide has been used in therapy.

Crystalline tiotropium bromide monohydrate is API of commercial pharmaceutical compositions: capsules containing a powder for inhalation (Spiriva®) and solution for inhalation (Spiriva Respimat®). This salt is susceptible to polymorphic transformation during manufacture and storage in inappropriate conditions. Therefore, scientists have been looking for new tiotropium salts, chemically and physically stable, highly pure, which can be obtained in high yield. Monohydrate of tiotropium with 1S-(+)-10-camphorsulphonic acid meets these requirements [3].

The solid state studies of the two polymorphs of tiotropium bromide anhydrate, tiotropium bromide monohydrate and monohydrate of tiotropium salt with 1S-(+)-10-camphorsulphonic acid were carried out using ^{13}C and ^{15}N CP/MAS NMR and ^{1}H MAS NMR spectroscopy, additionally supported by GIPAW calculations. The assignment of the solid-state NMR spectra was done by the comparison of chemical shifts between solid and liquid phases as well as by analysis of CP kinetics, dipolar-dephased spectra and by GIPAW calculations of chemical shielding. The experimental ^{13}C CP/MAS NMR results and the calculated isotropic chemical shifts were in the excellent agreement. Perhaps these studies will lead to the replacement of tiotropium bromide monohydrate with new monohydrate 1S-(+)-10-camphorsulfonate salt of tiotropium in the treatment of respiratory diseases.

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P63

Tacrine and melatonin cyclic derivative heterodimers – synthesis and evaluation of biological activity

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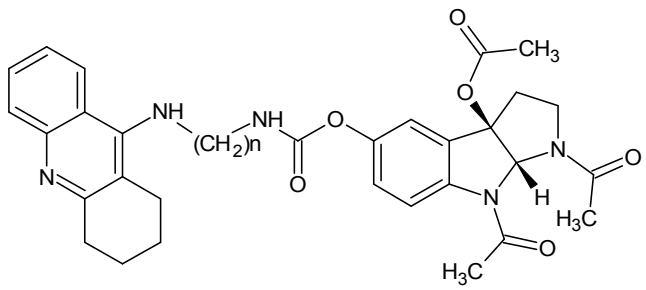
Alzheimer's Disease (AD) causes a number of pathologic changes in human brain, one of them is a decrease of cholinergic transmission. In order to improve acetylcholine transmission there are applied inhibitors of the acetylcholine enzyme acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Presented work is a continuation of our previous studies [1, 2].

We have synthesized heterodimeric molecules by combining tacrine, a known AChE inhibitor, with a cyclic melatonin derivative. Oxidation of a properly protected melatonin with a singlet oxygen produced *in situ,* was the deciding step in the process. By this oxidation we have obtained the cyclic derivative which is structurally similar to physostigmine - the secondary metabolites of melatonin which highly improve the neurotransmission of acetylcholine. The final compounds were yielded in a reaction of a diaminealkyl derivative of tacrine and an active ester of cyclic melatonin.



Inhibition activity for human AChE and BuChE of these new compounds was evaluated *in vitro* by the Ellman's colorimetric method.

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P64

Application of docking programs in search of the compounds with potential use in the treatment of alcoholism

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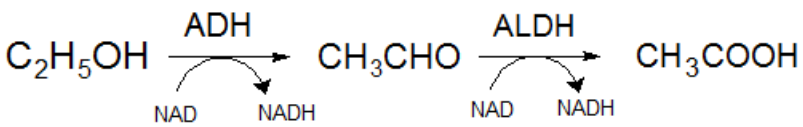
Docking is one of the basic tools used in the drug design as a method which enables to predict the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation may be used to predict the strength of association or binding affinity between two molecules. To assess the ligand binding affinity scoring functions are used and their effectiveness can be determined by comparing the calculation results with the values obtained experimentally.

Ethanol, as the recreational drug, has been used since ancient times. Even the consumption of a small amount of ethyl alcohol causes increased heart rate, agitation, euphoria, relaxation and talkativeness. When alcohol reaches the brain it has the ability to delay signals that are sent between nerve cells. This is done by interfering with ion channel function, increasing the fluidity of cell membranes of neurons, also acting directly on the NMDA, GABA, cholinergic and serotonin receptors.

Alcohol metabolism occurs mainly by oxidation to acetaldehyde, a reaction that is catalyzed by alcohol dehydrogenase (ADH) followed by aldehyde dehydrogenase (ALDH) to acetic acid. Naturally occurring isoflavonoid-daidzein inhibits ALDH-2 [1], and may be used in the treatment of alcohol dependence.

The aim of the study was to determine which other naturally occurring compounds from the group of flavonoids can potentially bind to the enzymes ADH and ALDH. The results are a contribution to further work on the design of new compounds with possible application in the treatment of alcoholism.



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P65

Synthesis and physical-chemical properties of novel porphyrazines possessing annulated styryldiazepine rings

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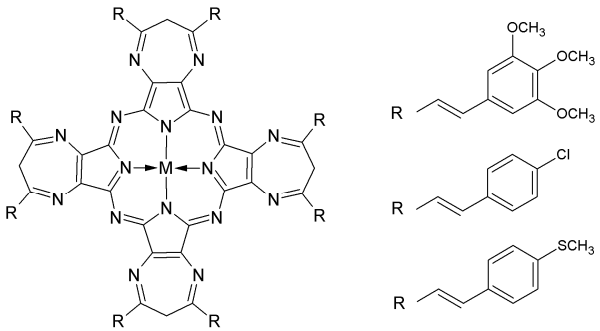
Porphyrazines are aromatic macrocyclic compounds consisting of four pyrrolic rings linked together with aza groups instead of methine bridges found in the naturally occurring porphyrins. In the recent years porphyrazines have been researched for many medical applications, including photodynamic therapy (PDT). PDT is a novel, anticancer treatment, which has been also used to cure cardiovascular, dermatological, ophthalmic diseases, as well as different microbial infections [1]. Porphyrazines have been also investigated as metal ion and gas sensors, precursors to optical data recording systems, electrochromic displays, magnetic, electronic, and conductive materials for nanotechnology [2].

A series of porphyrazines possessing annulated diazepine rings was synthesized and characterized using UV-Vis spectrophotometry, mass spectrometry and various techniques of Nuclear Magnetic Resonance (Figure). Novel macrocyclic compounds were subjected to physical-chemical studies concerning their absorption, emission and aggregation properties, photochemical stability, and ability to generate singlet oxygen.



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This study was supported by the National Science Centre under grant 2012/05/N/NZ7/00624. Jaroslaw Piskorz is a scholarship holder within the projects "Scholarship support for PhD students specializing in majors strategic for Wielkopolska's development". Sub-measure 8.2.2 Human Capital Operational Programme and the Polish National Science Centre "Etiuda" for PhD students under fellowship 2013/08/T/NZ7/00241.

P66

Determination of sensitivity of bacterial cells microaerophilic against some thiosemicarbazide derivatives containing pyridine ring

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Thiosemicarbazide derivatives are compounds of documented antibacterial [1,2], antifungal [3], antitumor [4] activity, and most importantly characterized by low cytotoxicity [3]. Bhat *et all* report that their chloride carbazide characterized minimal toxicity to normal human cells. It has been proved that their biological activity depends not only on the biological material (or strains) or cell lines but the chemical structure is also a key factor regulating their operation, in particular the nature of the ligand, such as Cl-, NCS-, CN-, substituted or benzene ring or quinazoline and their halogenation [5].

The aim of this study was evaluation of antimicrobial activity of novel synthesized 4-substituted 1-(pyridyn-2-yl)carbonylothiosemicarbazide derivatives (R=2-FC_6H_4, 2-ClC_6H_4, 2-morpholinoethyl, 2,4-$Cl_2C_6H_3$, 4-$CH_3SC_6H_4$) and to assess the cytotoxic activity of selected substances with antibacterial activity with respect to a normal human fibroblasts lines (BJ). In order to obtain information about potential antimicrobial activity of tested substances, the *in vitro* screening tests were made (using diffusion and liquid tests) against selected pathogenic strains*: Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus spp*, *Escherichia coli*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Lactobacillus acidophilus*.

Both the minimum inhibitory concentration MIC and cytotoxicity were determined for thiosemicarbazide derivatives and for reference standard. Novel thiosemicarbazides showed significant antibacterial activity, especially against specific pathogenic microaerophilic strains (MIC values from 7,81 µg/ml to 250 µg/ml depending on a strain). There is a relationship between the structure of thiosemicarbazide (ligand at the N-1 and N-4) and the strength of antibacterial and cytotoxic effect. Among the compounds tested, derivative MM1, MM3 and MM7 were found to be most potent. The toxicity of thiosemicarbazide derivatives was determined using MTT method on human fibroblasts cell lines (BJ). The compound MM20 derivative was not toxic up to a concentration 500 µg/ml. The compound MM20 also showed the highest value of the index of biocompatibility (BI), which is a measure of therapeutic efficacy and safety of the drug.

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P67

Search for mechanism(s) of cytotoxic activity of 2,5-disubstituted-1,3,4-thiadiazoles

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A series of 2,5-disubstituted-1,3,4-thiadiazoles (**1-6**) was synthesized using the respective thiosemicarbazide derivatives as precursors. The obtained compounds were subjected to a preliminary MTT assay in estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. The effect of the studied compounds on a viability of normal human skin fibroblasts was also evaluated. Inhibitory activities (IC_{50}) of compounds **1-6** on MCF-7 and MDA-MB-231 cells ranged from 70 to >200 μM (vs. 82 μM for etoposide). To determine the possible mechanism(s) by which 1,3,4-thiadiazole derivatives (**1-6**) decreases viability of the breast cancer cells, [^{3}H]thymidine incorporation and topoisomerase II relaxation assay were performed. According to the obtained results, some of the investigated compounds turned out to be potent inhibitors or poisons of human topoisomerase II (see Figure below).

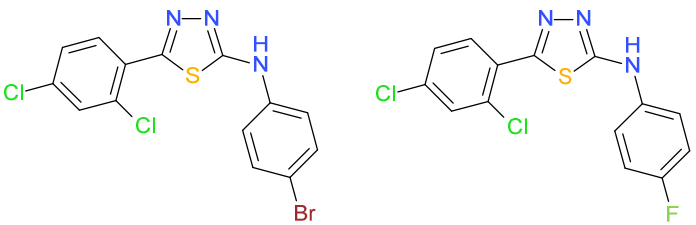

Figure 1. The chemical structures of 1,3,4-thiadiazole-based poisons of human topoisomerase II.

P68

Ligand association/dissociation to/from β_2-adrenergic receptor: the influence of the receptor conformation

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The recognition of the molecular basis of ligand binding is essential to understand the pharmacological properties of the β_2-adrenergic receptor (β_2-AR). Analysis of the ligand-binding region of β_2-AR using the recently solved high-resolution crystal structures revealed a number of highly conserved amino acids that might be involved in ligand binding. While much attention has been related to ligand binding and molecular mechanisms of receptor activation, the ligand dissociation/association process remain unresolved. We report the results of the computational study which provide insights into the agonist molecule dissociation/association process from/to β_2-AR both in its active and inactive state. In order to explore the configurational space of the ligand-receptor complex, and to surmount the various free energy barriers, we used the umbrella sampling technique. The chosen ligand was the agonist of the β_2-AR, fenoterol. For comparative purposes, the analogous study has been carried out for the inverse agonist, carazolol. The investigations were focused on estimating the free energy profiles (FEP) corresponding to the process of ligand binding/unbinding to/from β_2-AR and the subsequent interpretation of these profiles. The two different conformational forms of the receptor have been considered (based on the crystal structures PDB: 3P0G and 2RH1, referred further to as '*active*' and '*inactive*' states, respectively) which created an opportunity to compare the association/dissociation paths characteristic of these two states. The results indicate that the process of ligand binding is dependent both on the character of the ligand and on the conformational state of β_2-AR, **Fig. 1**. The fenoterol-related FEP is more rough, revealing a larger number of local minima and maxima of the free energy, indicated more complex binding mechanism when compared to FEP calculated for carazolol. The large local minimum present in the FEP corresponding to the fenoterol-'inactive' β_2-AR indicates the existence of the secondary binding cavity created by D113 (TM III), S203, S204 (TM V), N293, H296 (TM VI), Y308 (TM VII), T195 (ECL 2), N301 (ECL 3). Furthermore, we speculate on the ligand influence on the conformational state of the 'molecular switch' formed by the W286 (TM VI) residue and playing a role in the initial states of the β_2-AR activation process.

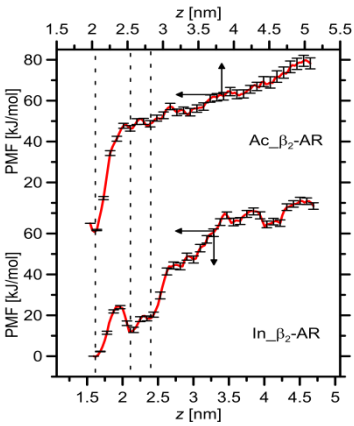

Fig. 1. The calculated free energy profiles characteristic for the process of binding/unbinding of the (*R,R*)-fenoterol molecule to/from the β_2-AR binding cavity. The profiles correspond to fenoterol created complexes with inactive (In_β_2-AR/fenoterol) and active state of β_2-AR (Ac_β_2-AR/fenoterol). The *z* coordinate scale is shifted to reflect the similarities between the fenoterol-related curves (e.g. local and global minima, marked by dashed lines). Error bars were calculated using the bootstrapping method.

Acknowledgments

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P69

Synthesis and antimicrobial properties of 5-substituted-1,3,4-thiadiazole-3(2*H*)-tione derivatives

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The 1,3,4-thiadiazoles are a class of small molecules that have received much interest in the fields of chemistry and biology due to their broad spectrum of activity. The 1,3,4-thiadiazole derivatives have been reported to be anticancer, antimicrobial, antitubercular, anticonvulsant, antiinflammatory, analgesic and antiviral agents. Some of the medicines with 1,3,4-thiadiazole scaffold such as Acetazolamide or Methazolamide are well-known for their therapeutic applications. Additionally the 1,3,4-thiadiazole scaffold is an interesting building block that has been used to synthesize a variety of useful bioactive compounds. For this type of derivatives, a different mechanism of action is assigned, depending on the type of modification of 1,3,4-thiadiazole ring.

Therefore this study presents the synthesis of a new series of 5-substituted 1,3,4-thiadiazole derivatives. New derivatives were obtained by the condensation reaction of 5-amino-1,3,4-thiadiazole-3(2*H*)-tiones with appropriate substituted aromatic aldehydes. The structure of synthesized compounds was confirmed by ^{1}H NMR and ^{13}C NMR spectra.

The examined compounds were *in vitro* screened for antibacterial and antifungal activities using the broth microdilution technique against a panel of reference strains of 20 microorganisms, including Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *S. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 49619, *S. mutans* ATCC 25175, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *M. luteus* ATCC 10240), Gram-negative bacteria (*E. coli* ATCC 3521, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. mirabilis* ATCC 12453, *B. bronchiseptica* ATCC 4617, *S. typhimurium* ATCC 14028, *P. aeruginosa* ATCC 9027) and fungi belonging to yeasts (*Candida* spp.).

On the basis of minimal inhibitory concentration values obtained by the broth microdilution method, it was shown that all of synthesized compounds had some antimicrobial activity. Three of obtained derivatives had the highest bactericidal or fungicidal effect against reference strains of Gram-positive bacteria, mainly opportunistic *S. epidermidis*, *M. luteus, Bacillus* spp., and yeast belonging to *Candida* spp.

P70

The influence of pyrazolo[4,3-e][1,2,4]triazine sulfonamides on cytotoxicity and biosynthesis DNA in human MCF-7 breast cancer cells

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The pyrazolo-1,2,4-triazines have received considerable attention due to their pharmacological activities as antiviral, antitumour, antifungal, analgesic, anti-inflammatory, and antipyretic agents. However, despite of the wide range of biological activity the pyrazolo[4,3-e][1,2,4]triazines are a less known class in the group of condensed pyrazolotriazines. In the past decades, the isolation and structural characterization of naturally occurring pyrazolo[4,3-e][1,2,4]triazines: pseudoiodinine, nostocine A and fluviols A–E, was reported. In addition, these compounds were reported to have cytotoxic activity on human cancer cell, such as A549 (lung adenocarcinoma).

Continuing our study on the synthesis of sildenafil analogues and aniline substituted pyrazolo[4,3-e][1,2,4]triazine sulfonamides we have prepared a series of new sulfonamides and for their anticancer activity against human MCF-7 breast cancer cell line. The tested sulfonamides were called: MM76, MM80 and MM85. Cytotoxic and cytostatic activities of new tested derivatives were checked after 24h incubation with MCF-7 breast cancer cells using four different concentrations: 50 μM, 100 μM, 150 μM and 200 μM. The measurement of the inhibition of biosynthesis DNA by incorporation of radioactive component into DNA of MCF-7 cells was performed.

Our studies showed that the most cytotoxic agent was MM76, it reduced the number of viable cells to 22% in concentration 200 μM. The less cytotoxic compounds were MM80 and MM85, which reduced the viability of MCF-7 cells to 70% and 71% after 24hour of incubation with concentration 200 μM.

All tested compounds inhibited the biosynthesis of DNA in MCF-7 breast cancer cells after 24hour of incubation with that agents. The strongest antiproliferative potential was showed by compound MM76. The agents MM80 and MM85 weaker inhibited the incorporation of radioactive component into DNA of breast cancer cells.Our researches proved that compound MM76 had the most cytotoxic and antiproliferative potential in MCF-7 breast cancer cells.

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Acknowledgements

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P71

The assessment of antimutagenic potential of azinesulfonamide analogs of aripiprazole using the Ames test

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To prevent genotoxic risk, it is vital to identify mutagens in order to minimize human exposure to them, as well as to enhance the exposure to antimutagenic agents [1]. Antimutagenic agents have attracted much interest with respect to their protective effects against free radical damage. ROS may play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation, which may be related to psychiatric disorders such as depression, anxiety and schizophrenia [2]. Multiple evidences suggest that depression is accompanied by an induction of oxidative/nitrosative stress pathways. Thus, the identification of antimutagenic compounds and the elucidation of their mechanism of action deserve particular attention, because of their possible role in ROS removal.

In this study we evaluated the *in vitro* mutagenic and antimutagenic properties of the new quinoline- and isoquinoline-sulfonamide analogs of aripiprazole using the Ames/*Salmonella* [3] bacterial reversion assay.

According to the results obtained, none of tested compounds showed mutagenic activity against *Salmonella typhimurium* TA100. Additionally, all compounds, exclude compound PZ387 exhibited moderate to strong antigenotoxic activity against 4-nitroquinoline-*N*-oxide (4-NQNO) induced mutagenesis. The inhibition rates ranged from 27.0% to 84.0%.

Based on the present study, it can be concluded that newly synthesized quinoline- and isoquinoline-sulfonamide analogs of aripiprazole do not display mutagenic potential in the Ames test. Moreover, some of the tested compounds like PZ549 and PZ380 which demonstrated antigenotoxic activity may be efficacious in preventing DNA damage induced by oxidative stress in neurological and psychiatric diseases.

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P72

2-Dimensional substructural fingerprints – a novel method of compound structure representation

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In silico methods are becoming more and more popular in drug screening efforts, allowing for a significant increase in the speed of the process, together with a major decrease of its costs. Many of those computational methods are based on the structure of the processed chemical compounds, which must be transformed from a 2-dimensional structural representation into a computer-readable form, preferably a bit string. In case of substructural fingerprints, each bit of such bit string represents the existence of a predefined substructure within the target compound [1]. There are many such representations [2,3], however they all share the same flaw: a complete loss of information about the connectivity between substructures and atoms. This often leads to curious cases, where two structurally diverse compounds are represented with identical bit string, hence rendering their differentiation impossible.

To address this issue, we created a prototype of a novel representation of chemical compounds – a 2-dimensional substructural fingerprint (2DFP). This method does not lose so much needed connectivity data, enabling a more complete depiction of the target compound, while retaining the form needed for computational purposes. The 2DFP methodology has been tested on a small set of 5-HT6R ligands, to determine its efficiency in discrimination between active and inactive compounds. The initial results show a major increase of MCC score of discrimination tests, compared to currently available substructural fingerprints.

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P73

Cytostatic emanines - derivatives of nitrogen mustard

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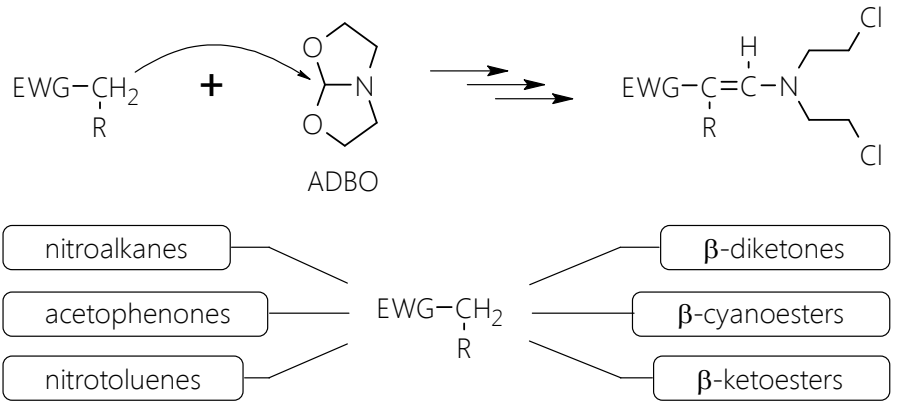
An efficient and versatile route for the preparation of the new cytostatic formamidines by incorporation of the nitrogen mustard moiety into the structure of different molecules containing an -NH_2 group (i.e. amines, sulfonamides, amides, aminoacids, hydrazides and hydrazines) was earlier presented [1].

We found that ADBO reacts also with compounds containing an active methylene group i.e. nitroalkanes, nitrotoluenes, aromatic ketones (acetophenones, propiophenones), β-diketones, β-cyanoesters, β-diesters, malononitrile, etc. One-pot reaction of such nucleophiles with bicyclic amidoacetal named 1-aza-4,6-dioxabicyclo[3.3.0]octane or ADBO [2] followed by chlorination or mesylation of obtained compounds leads to desired enamines characterized by strong but latent pharmacological activity.



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Acknowledgements

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P74

Genotoxicity of the benzo-bis-benzo group of polycyclic aromatic hydrocarbons based on the in vitro micronucleus test

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Although polycyclic aromatic sulfur heterocycles (PASHs) have been detected in the environment, the ability of these compounds to induce cellular and tissue responses remains poorly characterized. Lack of reference materials on PASHs (analytical standards) and limited information on the biological properties of these compounds makes a serious gap in our knowledge, which could be threatening to animal and human health related to environmental exposure. Since there are only a few PASH species that have been properly described, there is an urgent need to study the possible hazards associated with PASHs' presence in the environment. By building complex assays that cover different toxicological aspects (e.g. mutagenicity, clastogenicity, teratogenicity), and exploiting the throughput and the fidelity of the zebrafish model with modern tools of molecular biology, it should be feasible to define a predictive study that describes the adverse effects of PASHs in animals and their potential impact on the environment. In this study the *in vitro* culture of V79 cells of the Chinese hamster was subjected to tested PAHS: benzo[2,1-*b*:3,4-*b*]bis[1]benzotiophene, benzo[1,2-*b*:4,5-*b*]bis[1]benzotiophene, 5-hydroxybenzo-[1,2-*b*:4,5-*b'*]bis[1]benzotiophene, 1,2,3,4-tetrahydroxybenzo[2,1-*b*:3,4-*b'*]bis[1]benzotiophene, benzo-[1,2-*b*:4,3-*b'*]bis[1]benzotiophene. Then the micronuclei (MN) assay was applied in order to evaluate carcinogenic properties of the studied compounds. The results were compared to the results obtained for Ames test for these compounds.

P75

Application of molecular dynamics to investigate metabolites of fenoterol isomer

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Human cytosolic sulfotransferases (SULTs) transfer the sulfuryl moiety (-SO^3) from activated sulfate [3′-phosphoadenosine 5′-phosphosulfate (PAPS)] to the hydroxyls and primary amines of numerous metabolites, drugs, and xenobiotics [1]. Receipt of the sulfuryl group often radically alters acceptor–target interactions. How these enzymes select particular substrates from the hundreds of candidates in a complex cytosol remains an important question. Molecular dynamics has proven to be a valuable tool in SULTs selectivity recognition [2]. Hence, we employed this technique to investigate fenoterol isomer behavior in the active site of SULT1A3 isoform.

Wilson *et al* reported that SULT1A3 isoform selectively catalyzed the sulfoconjugation of the (*R*,*R*')-enantiomer (eutomer) at the 4-hydroxyphenyl-position, whereas the mono-sulfoconjugation of the 3,5-*bis*-hydroxyphenyl moiety was selective for the (*S*,*S*')-enantiomer. We have built an *in silico* system after reaction consisting of SULT1A3 isoform, cofactor and sulfated fenoterol. Results will inspect which interactions govern dissociation from the enzyme active site of fenoterol molecule.

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Acknowledgements

The authors kindly acknowledge Professor Lennart Nilsson and his lab memebers from Karolinska Institutet, Sweden for providing computing resources. The work is supported by the found from Foundation for Polish Science (TEAM Programme 2009-4/5).

P76

Antiproliferative activity of new netropsin analogues containing in the structure lysine derivatives

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Carbocyclic analogues of netropsin and other minor groove binders are readily available, can be modified easily, and are stable under most experimental conditions. Generally available nitro benzene derivatives could be used as starting materials for syntheses. These compounds after reduction, in reaction of acylation with using the appropriate acid chloride,leads to create an amide bond. A molecular mechanics and molecular dynamics approach confirmed that carbocyclic derivatives have structures appropriate to bind to the minor groove B-DNA [1]. Distamycin analogues containing benzene, a pyridine or a thiazole ring, obtained by conventional synthesis, inhibited the proliferation of breast cancer cells MCF-7 and MDA-MB-231, bound to DNA and inhibited the action of DNA topoisomerases [2, 3]. Introduction to the synthetic DNA ligands structure of an amino acid fragment should broaden the scope of action of these compounds and cause an increase in their antitumor activity.

Newly obtained netropsin analogues containing in the structure lysine derivatives (Fig. 1) were tested in vitro on breast cancer cell line MCF-7 in order to determine cell survival and determine the value of IC_{50}.

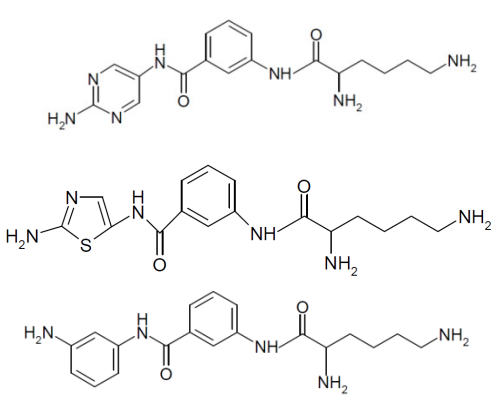

Fig. 1. Some structures of Lys-netropsin analogues

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P77

Pharmacological characterization of zinc interaction with 5-HT$_7$

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Zinc, as an essential trace element in living organisms, has many functions, including participation in various processes within the central nervous system [1]. The role of zinc in depression and its therapy is emphasized by numerous preclinical and clinical studies, however, the exact mechanism of its action is still not fully understood [3]. Our interests are focused on its effects mediated by serotonin receptors, which are key players in the etiology of anxiety and mood disorders [2].

The main objective of this study was to investigate the effect of zinc on the serotonin receptor 5-HT7 using in vitro methods [4,5]. At first, saturation binding assays were performed in a presence of various zinc concentrations in order to determine whether the shift in radioligand affinity reflects an allosteric mode of action. Two different radioligands (of agonistic and antagonistic activity) have been used, as allosteric regulation is highly sensitive to the type of orthosteric ligand (probe-dependence). Next, kinetic effects on radioligand dissociation rate (Koff) were measured to quantify allosteric effects of zinc ions.

Results of both types of in vitro experiments indicated allosteric mechanisms mediated by zinc during 5-HT7 receptors regulation.

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Acknowledgements

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P78

Synthesis of a new series of N^4-substituted 4-(2-aminoethyl)benzenesulfonamides and their inhibitory effect on human carbonic anhydrase cytosolic isozymes I and II and transmembrane tumor–associated isozymes IX and XII

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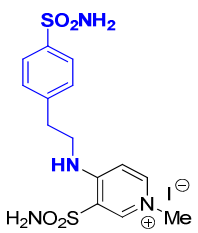
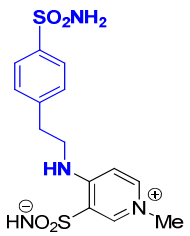
Lack of selectivity of the classical sulfonamide inhibitors toward CA IX and CA XII caused the progress in the design of new inhibitors targeting tumor-associated isozymes without side effects associated with inhibition of physiologically dominant isozymes such as CA I or CA II. Herein, we have developed methods for the synthesis of novel series N^4-substituted 4-(2-aminoethyl)-benzenesulfonamides 5-17. The thirteen compounds have been assayed for the inhibition of four physiological CA isozymes, such as hCA I, II, IX and XII. A relatively weak inhibitory activity against the human CA I was observed for all investigated compounds with K_I values from 96.3 to 3520 nM. Toward the second physiological isoform hCA II, all tested compounds 5-17 showed lower activity (K_I: 18.1 - 2055 nM) in comparison with reference AAZ, MZA, EZA, IND (K_I: 8 - 15 nM). Against hCA IX the new compounds showed inhibition constants in the range of 5.9 - 419 nM. It was shown that inhibitor activity toward hCA IX increased for compounds 11-17 (K_I: 5.9 - 38.5 nM) containing single aromatic ring in substituent R. Moreover, compounds 11-15 and 17 (K_I: 5.9 - 10.7 nM) were better hCA IX inhibitors when compared to the reference sulfonamides AAZ-IND (K_I : 25 - 50 nM). In the case of hCA XII the inhibition constants were in the range of 4.0 - 414 nM. The compounds 5-7 and 11-17 showed promising inhibitory activity (K_I: 4.0 - 33.1 nM) comparable with the reference sulfonamides AAZ-IND (K_I: 3.4 - 50 nM). The research revealed that the most potent inhibitors of both hCA IX and XII were the cationic compounds 11 and 12 (K_I: 5.9 and 6.2 nM for hCA IX and 4.3 and 4.0 nM for hCA XII, respectively). These derivatives presented meaningful affinity to the hCA IX and XII than to the hCA I and II with the selectivity ratios hCA IX versus hCA II, and hCA XII versus hCA II in the range of 10 – 15, respectively. This result confirms that the positively charged sulfonamides may be used in the design of new inhibitors targeting tumor-associated isozymes.



hCA I K_I = 923 nM
hCA II K_I = 61.3 nM
hCA IX K_I = 4.3 nM
hCA XII K_I = 5.9 nM

11



hCA I K_I = 887 nM
hCA II K_I = 60.1 nM
hCA IX K_I = 4.0 nM
hCA XII K_I = 6.2 nM

12

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P79

Mutagenic and antimutagenic evaluation of selected active aminoalkanol derivatives with the Ames test

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Mutagenic activity is one of the most important endpoints for risk assessment of chemical compounds including drug substances and drug candidates.

The aim of the present study was to investigate the mutagenic and antimutagenic potential of five (I-V) aminoalkanol derivatives presenting anticonvulsant activity with the Ames test. Test compounds were synthesized in the Department of Bioorganic Chemistry Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College. The mutagenicity assay was performed by a preincubation method with TA100 strain of Salmonella typhimurium. Sodium azide (SA) was used as a positive control. The antimutagenic properties of compounds I-V were assayed with a modified method of Maron and Ames. The antimutagenic effect was defined weak (inhibition up to 25%), moderate (25–40% inhibition) or strong (40% or more inhibition).

The result of the present study showed that compounds I-V did not cause a doubling in the number of colonies over the spontaneous number. Thus, present compounds were not mutagenic to Salmonella typhimurium strain TA100. Additionally, all of tested compounds reduced SA mutagenicity in S. typhimurium strain TA100. The strongest antimutagenic effect was observed for compound I, which at the highest and the lowest concentration reduced highly SA mutagenicity. The same compound exhibited moderate antimutagenicity for the remaining three concentrations. Compounds II-V demonstrated small to high antimutagenic potency depending on the concentration.

To sum up, the results of the study demonstrated that compounds I-V presenting anticonvulsant activity showed no mutagenic effects on Salmonella typhimurium TA100 strain. Additionally, tested compounds demonstrated the ability to reduce mutagenicity of a standard mutagen - sodium azide in the same tester strain.

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[2] Eastmond D.A., Hartwig A., Anderson D. et. al. *Mutagenesis.* 24 (2009) 341-349.
[3] Maron D., Ames B. *Mutation Research*. 113 (1983) 173-215.
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P80

Combination of structural interaction profiles as a method for optimization of its application in docking results analysis

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Prediction of spatial orientation of a molecule in a binding pocket of a given receptor and inferring on its basis about the potential activity of a particular compound still constitutes a very challenging task for computational part of drug design campaigns [1]. There are some approaches that enable automation of the procedure of ligand-protein complexes analysis, among which there is a combination of Structural Interaction Fingerprints with machine learning algorithms [2]. However, there still remains the problem of selection of proper set of models for docking studies – should it be just one receptor providing the best discrimination between actives and inactives or maybe using ensemble approach is better, as it is in case of ALiBERO [3].

The primary objective of the study was to optimize the number of homology models used for SIFTs profiles calculations on the basis of ligand-beta2 adrenergic receptor complexes. The results obtained for homology models of the receptor constructed on 9 different templates were also compared with docking performed with the use of crystal structures of the protein. The docking outcome was represented by Structural Interaction Fingerprint (SIFt) and for each ligand such representation was averaged over various models used for particular analysis (the number of models taken into account ranged from 3 to 20). Such data was then examined with the use of the Support Vector Machine algorithm to distinguish profiles belonging to active molecules from those that were characterizing inactive compounds. The analysis enabled determination of the optimal number of models that are recommended for use in this kind of study.

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Acknowledgements

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P81

Interactions of azaphenothiazine derivatives with DNA: the experiment and prediction by means of molecular docking

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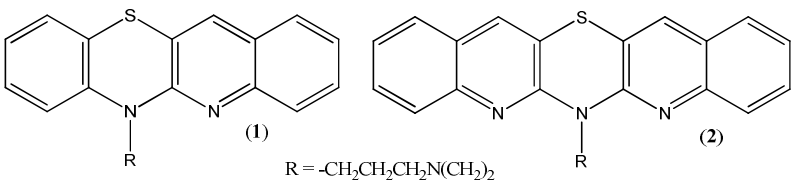
Phenothiazines are an important class of heterocyclic compounds possessing not only widely recognized neuroleptic activity, but also antihistaminic, antitussive and antiemetic activities. Typical neuroleptic phenothiazines and new phenothiazine derivatives have also anticancer activity. In an effort to develop active pyridine and quinoline derivatives, the phenothiazine structures were modified with either a pyridine or quinoline ring to form three new types of azaphenothiazines. Some of them exhibited potent anticancer activities [1, 2].

In this work, the interaction of DNA with two azaphenothiazines [**1**, **2**] with the dialkylaminoalkyl groups as the substituents at the thiazine nitrogen atom was studied by absorption spectroscopy and supplemented with computational method for molecular docking.



In the experiment, the solutions with a fixed concentration of compound **1** or **2** were titrated by successive addition of nucleic acid solution and the UV-Vis absorption spectra were recorded in the wavelength range of 280–600 nm. Molecular docking was performed using the Molegro Virtual Docker (MVD) computer program. The 3D structure of dsDNA was downloaded from the Nucleic Acid Database (PDB ID:1N8C). The base pair sequence was CGGTCACGAGG : GCCAGTGCTCC.

The obtained UV absorption spectra for compounds **1** and **2** in the presence of an excess of dsDNA were characterized by batochromic and hypsochromic shifts in reference to the UV absorption spectra of the unbound compounds. The isosbestic points suggested the presence of two species (free and bound compound **1** or **2**). The molecular docking results indicated that the modes of interactions between DNA helix and compounds **1** and **2** can be considered as intercalative binding.

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P82

Structural studies of two polymorphs of ethenzamide co-crystals with gentisic acid

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Ethenzamide (2-etoxybenzamide, **EA**) is a nonsteroidal anti-inflammatory drug used in combination with other ingredients for the treatment of mild to moderate pain. **EA** is a poorly water soluble drug with low bioavailability, which makes many scientists look forward to finding better soluble modifications of **EA** [1]. Most formulation strategies for poorly water-soluble drugs seek to improve their dissolution rate and/or solubility *in vivo* by achieving different polymorphic forms, solvates, or hydrates, amorphous forms, complexes, co-crystals or solid dispersions of drugs in hydrophilic polymers [2]. Interest in pharmaceutical co-crystals and polymorphism in co-crystals is increasingly arousing.

In this work, we report a 1 : 1 co-crystal of **EA**, with gentisic acid (2,5-dihydroxybenzoic acid) **EGN** which exists in three polymorphic forms. Both polymorphs were characterized by ^{13}C and ^{15}N CP/MAS NMR and FT-IR spectroscopy. The NMR assignments were supported by GIPAW calculations of chemical shielding, performed using X-ray determined geometry [3].

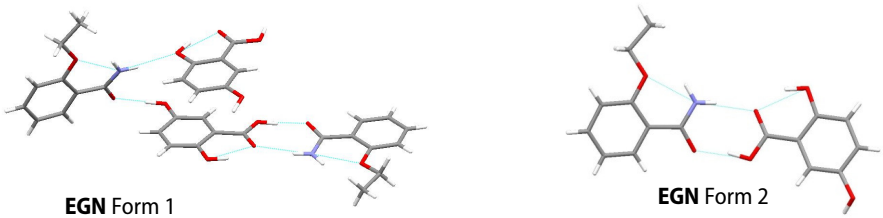

Fig. 1 Two different structures of **EGN.**

The experimental ^{13}C CP/MAS NMR results and the calculated isotropic chemical shifts were in excellent agreement. It has been shown that in the case of **EGN** co-crystals combining solid-state NMR with FT-IR spectroscopy is a good method of molecular structure confirmation.

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[3] Aitipamula S., Pui Shan Chow, Tan R.B.H., Cryst Eng Comm., 11 (2009), 1823.

P83

Determination of isoflavones content in different varietes of Glycine max L.

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Isoflavones are derivatives of 3-phenyl-chromen-4-one. They are found in many plants and plant-derived foods in both as aglycon forms and as β-glucosides, acetyl- or malonyl-esters. Red clover (*Trifolium pratense* L.), kudzu (*Pueraria lobata*, Willd.) and soy (*Glycine max* L.) are the rich sources of these compounds. Genistein and daidzein belonging to isoflavones possess estrogenic activity thus they are used alleviating menopausal symptoms in women (1,2). Additionally, they stimulate osteoblastic bone resorption and protect against osteoporosis (3).

In our investigation, the content of genistein and daidzein in different varieties of soy was determined with use HPLC-PAD method on RP-18 column. The chromatographic conditions were chosen experimentally. Mixture of acetonitrile and water containing 0.025% trifluoroacetic acid was used as a mobile phase during gradient elution. The significant variation of investigated compounds content between varieties was observed.

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P84

The biological activity of new series of styryl quinoline analogues

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Quinoline moiety is present in many classes of biologically active compounds [1-3]. A group of styrylquinoline derivatives were synthesized and tested for their antiproliferative activity. Most of the compounds were obtained with use of microwaved assisted synthesis. Antiproliferative activity of the synthesized compounds were assessed by the MTS assay against human colon carcinoma (Hct116) cell lines with wild type p53 (p53 +/+) and with a p53 deletion (p53-/-), and murine melanoma cell line (B16-F10). All tested compounds were also examined for their cytotoxicity effects against the normal human dermal fibroblasts (NHDF). Some compounds of the quinoline series showed promising antiproliferative potency. Additionally we investigated fluorescence properties of the compounds. Several quinoline derivatives can be successfully used as fluorescent dyes.

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Acknowledgements

Ewelina Spaczyńska appreciates the support of the DoktoRIS studentship.

P85

Synthesis and characteristics of novel rhodanine derivatives

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The rhodanine based compounds belong to a huge group of chemical moieties which are intensively investigated because of their biological particularly anticancer, antidiabetic, antimalarial, antibiotic and antifungal activity [1-5]. Despite the fact that some of them are already used in therapy (eg. Eparlestat in the treatment of diabetic neuropathy), there is still a need for obtaining new xenobiotics which are characterized by a broad spectrum of pharmacological activity and in parallel low toxicity.

In this work we present the synthesis pathways of two rhodanine derivatives (fig. 1) and the example crystalographical structure of one of them, obtained by x-ray diffraction analysis. In the studied structure the crystal network is built of homosynthons. Two molecules of the investigated compound are connected into a dimer by strong hydrogen bonds O-H···O formed by the carboxyl groups.

Additionally the interaction between rhodanine derivatives and human serum albumin (HSA), the most abundant protein in the blood plasma, was carried out. Human serum albumin is the major macromolecule contributing to the osmotic blood pressure. It is also responsible for small molecules as well as drugs binding and transport. In this context it is extremely important to examine the interactions of tested rhodanine derivatives with the human serum albumin. The binding ability of rhodanine compounds to HSA was tested by emission and absorption spectroscopy. A comparison of the results obtained for both moieties will be presented.

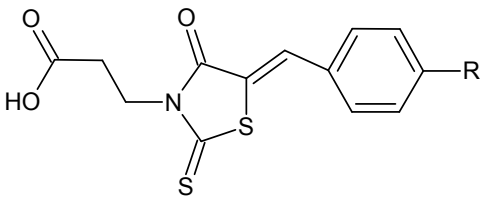

Figure 1. The studied rhodanine derivatives, R=$N(CH_3)_2$, $N(C_2H_5)_2$).

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P86

Design, synthesis and biological evaluation of novel combretastatin A-4 derivatives – potential antimitotic agents

Tomasz Stefański [a], Rafał Kurczab [b], Zbigniew Dudtkiewicz [a], Renata Mikstacka [a], Michał Cichocki [c], Marek Murias [d], Agnieszka Gielara-Korzańska [a], Stanisław Sobiak [e]

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Microtubules are key components of the cytoskeleton consisting of αβ-tubulin heterodimers and are involved in a wide range of various cellular functions, such as cell division, where they are responsible for mitotic spindle formation and proper chromosomal separation [1]. The biological importance of microtubules in mitosis and cell division makes them an interesting target for the development of anticancer agents: many of them are already in clinical use (epothilone, paclitaxel) or in clinical trials such as combretastatin A-4 (3′-hydroxy-3,4,4′,5-tetramethoxy-*cis*-stilbene, CA-4), however the search of new potent agents is still continued [2].

A series of sixteen CA-4 thioanalogs was prepared as a part of our on-going search for novel tubulin inhibitors. They were designed using parallel virtual screening protocol of reaction based combinatorial library. Antitubulin properties of the obtained compounds were studied *in vitro* with the use of tubulin polymerization assay kit (Cytoskeleton, USA). Cytotoxic activity was estimated against a panel of eight cancer and normal human cell lines with the use of MTT test.

In a series of oxazole CA-4 thioanalogs two potent inhibitors of tubulin polymerization were found: 4-(3,5-dimethoxy-4-methylthiophenyl)-5-(3-hydroxy-4-methoxyphenyl)oxazole (KomOx3) and 4-(3-bromo-4,5-dimethoxyphenyl)-5-(4-methoxy-3-methylthiophenyl)oxazole (KomOx7) with IC_{50} values of 1.05 and 0.80 μM, respectively, showing a stronger inhibition of tubulin polymerization than CA-4 (IC_{50} = 2.5 μM). Potency of antitubulin activity of studied compounds correlated well with their cytotoxic action on cell lines.

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P87

Porphyrazines possessing isophthaloxyalkylsulfanyl substituents - photochemical study

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Porphyrazine macrocycles are synthetic analogs of porphyrins possessing four pyrrolic rings linked together with azamethine groups [1,2]. They revealed potential for technology (nonlinear optical materials, chemical sensors, energy conversion, optical data storage, molecular semi-conductors, liquid crystals, infrared dyes for laser technology) and as photosensitizers for photodynamic therapy (PDT) [3,4]. PDT is a novel treatment of cancer and non-cancerous diseases, such as age-related macular degeneration, actinic keratosis or psoriasis [5].

The aim of our study was to characterize novel porphyrazines with isophthaloxyalkylsulfanyl groups in the periphery. Photochemical properties of those compounds were investigated in two solvents: DMSO and DMF at an ambient temperature. The evaluation of the singlet oxygen generation, photodegradation assessment and the emission properties of compounds were measured.

The Uv-Vis absorption spectra of analyzed porphyrazines consist of two bands: Soret band between 300-400 nm and Q band in the range of 600-800 nm. The singlet oxygen quantum yields were investigated using the relative method with zinc phthalocyanine as a reference. 1,3-Diphenylisobenzofuran was applied as a chemical quencher of singlet oxygen. The quantum yields of all compounds were found to be lower than that measured for zinc phthalocyanine. In addition, changes in the absorption spectra during photochemical stability examination were observed, therefore kinetic parameters were determined. A decrease in the Q band absorption intensities was not accompanied by the appearance of new bands. Thus, all the investigated compounds were found to undergo the photobleaching process. Moreover, emission properties of novel macrocycles were assessed. Fluorescence quantum yields and emission spectra, which were slightly red-shifted in comparison to the absorption spectra, were researched.

The results indicate, that the novel S-*seco*-porphyrazine reveals the best photosensitizing properties. It turned out to be the most efficient singlet oxygen generator within a series of compounds tested, with quantum yield values of 0.27 and 0.26 in DMF and DMSO, respectively.

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Acknowledgements

This study was supported by the National Science Centre under grant No. 2012/05/E/NZ7/01204.

P88

Synthesis, antiviral activity and molecular modeling of N-substituted derivatives of 1-arylimidazolidyn-2-ylideneurea

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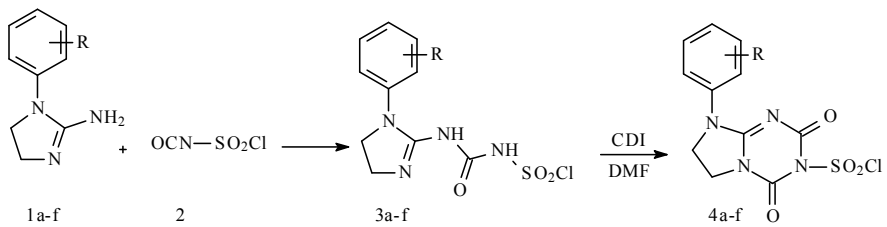
Viral infections are a permanent health problem of mankind. Human herpesviruses cause mild or asymptomatic infections in patients with properly functioning immunological system. The illness is more serious in patients with deteriorated cellular immunity, e.g. human immunodeficiency virus (HIV) infected patients who must receive chronic therapy with antiviral agents, favouring the selection of resistant variants. Searching for novel antiviral compounds, we designed N-substituted derivatives of 1-arylimidazolidyn-2-ylideneurea. New derivatives 3a-3f were obtained in the reaction of 1-aryl-4,5-dihydro-1*H*-imidazol-2-amies with chlorosulfonic isocyanate in the dichloromethane solution and under nitrogen atmosphere. The second series of compounds 4a-4f was obtained from the respective 1-(1-aryl-2-amine-4,5-dihydro-1H-imidazolin)-3-chlorsulfonylureas 3a-3f and 1.1′-carbonylodiimidazole (CDI) in DMF solution.



R = H, 4-CH_3, 2-OCH_3, 4-OCH_3, 3-Cl, 4-Cl

The studies of antiviral activity showed that derivative 3d in concentration of 800 µg/mL inhibited HSV-1 replication with 2.43 log (38.1%). Derivative 4a exhibited antiviral activity in all the tested concentrations (500, 625, 800 µg/mL), inhiiting HSV-1 replication with 2.05 and 2.18 log (32.1% and 32.6%), respectively. Derivatives 3c and 4b–4d did not have antiviral activity. Molecular modeling was used to address structure-activity relationship of novel compounds. Input conformations of the investigated compounds were prepared using the LigPrep protocol from the Schrodinger Suite. To sample different protonation states of ligands in physiological pH Epik module was used. Next, we calculated structural, electronic and ADMET parameters (volume, surface area, polar surface area, ovality, dipole moment, HOMO and LUMO energies, polarizability, molar refractivity, lipophilicity, the charges on the heteroatoms, aqueous solubility, and blood-brain barrier permeation parameter) and combined them with pharmacological activity. These parameters were calculated with Gaussian09, VegaZZ, Schrodinger, PREADMET and Discovery Studio software.

P89

Synthesis, antiviral activity and molecular modeling of 1-(1,3-disubstutedimidazolidyn-2-ylidene)-3-ethoxycarbonylmethylurea derivatives

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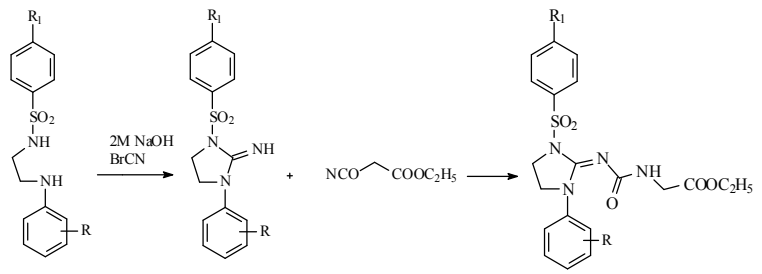
Viral infections caused by the viruses from *Herpesviridae* cause mild or asymptomatic infections in patients with properly functioning immunological system. They constitute, however, a much serious problem at patients with a malfunctioned immunological system. A number of nucleoside analogues, especially the guanosine analogue acyclovir, have been developed as antiherpetic agents. The therapeutic limitation of these nucleoside analogues is that drug resistant strains develop readily through mutations in viral genes for thymidine kinase and polymerase. There are many drugs active against herpesviruses now, but despite their high selectivity they have many defects, like numerous side-effects. Thus, novel drugs against *Herpes simplex* virus are highly needed. Searching for such drugs we designed 4a-4g. These derivatives were obtained in the reaction of 1-aryl-3-(arylsulfonyl)imidazolidyn-2-imin 2a-2g and ethyl isocyanatoacetate.



Derivatives 4a-4g were studied for their antiviral activity against *Herpes simplex* virus. Molecular modeling was used to address structure-activity relationship of novel compounds. Input conformations of the investigated compounds were prepared using the LigPrep protocol from the Schrodinger Suite. To sample different protonation states of ligands in physiological pH Epik module was used. Next, we calculated structural, electronic and ADMET parameters (volume, surface area, polar surface area, ovality, dipole moment, HOMO and LUMO energies, polarizaibility, molar refractivity, lipophilicity, the charges on the heteroatoms, aqueous solubility, and blood-brain barrier permeation parameter) and combined them with pharmacological activity. These parameters were calculated with Gaussian09, VegaZZ, Schrodinger, PREADMET and Discovery Studio software.

P90

Stability analysis and decomposition products of JCC1-45, a micromolar CB1 ligand

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The peroxide moiety is one of the most common oxidants in biological systems. It is the source of reactive oxygen species, which are the key element of both, oxidative stress phenomena and the oxidative metabolic processes (degradation of lipids and phase I metabolism). To mimic oxidative transformations of organic molecules, hydrogen peroxide and tert-butyl peroxide can be used as simple and reliable chemical models. Hydrogen peroxide is the simplest of the peroxide systems, common in the living organism. In the cell, it can be generated as a product of oxidoreductase activity or through dismutation of the superoxide anion radical. Tert-butyl hydroperoxide structurally resembles the peroxide species which are generated during lipid peroxidation [1]. It is commonly used as a model peroxide in oxidative stress and lipid degradation research [2,3].

These model systems have been used to investigate the chemical stability and decomposition products of JCC1-45 (fig. 1), a triazole CB1 receptor ligand, similar to the non-classical cannabinoids, displaying low-micromolar affinity.[4] Prior to the oxidative degradation studies, high chemical stability of JCC1-45 had been proven at various pH and elevated temperatures (up to 100°C).

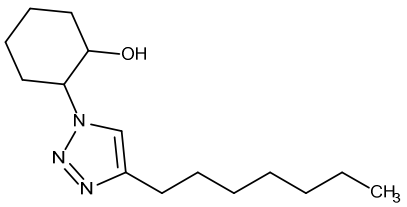

Fig.1: The structure of JCC1-45, a triazole CB1 ligand

The oxidative degradation studies of JCC1-45 have been performed in dilute (0,1-10%) and concentrated aqueous solutions (30% and 70%, respectively) of hydrogen peroxide and tert-butyl hydroperoxide, at various temperatures (25-100°C). In dilute solutions, the process was monitored by UV-VIS spectroscopy. In concentrated oxidative media, samples were taken at discrete time intervals and subject to HPLC and HPLC-MS analysis. Based on the HPLC-MS results analysis, decomposition product structures have been suggested.

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P91

Synthesis and lipophilicity of series of novel piperazine 4-*N*-substituted derivatives as histamine H_3 receptor ligands

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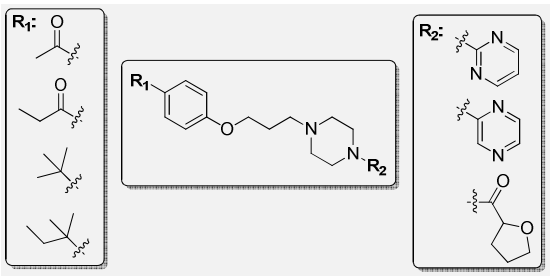
Histamine H_3 receptors (H_3R) are constitutively active G-protein coupled receptors mostly expressed in CNS, described as presynaptically located autoreceptors as well as heteroreceptors. Interaction with these receptors results in modulation of histamine levels as well as that of other neurotransmitters such as ACh, NA, 5-HT etc. Therefore blockade of these receptors could be useful in the treatment of different CNS disorders [1,2].



The aim of this work was to obtain *N*-alkylpiperazine ether derivatives with expected H_3R affinity. Novel compounds were designed basing on our previous results as well as of those described in literature, according to a blueprint pharmacophore proposed for H_3R antagonists (Figure 1) [3].

The novel compounds were evaluated for H_3R in vitro affinity in displacement assay at histamine hH_3R receptor stably expressed in HEK-293 cells.

In the aim to evaluate their physicochemical properties their lipophilicity was evaluated expressed by R_{M0} values using planar RP-TLC method. The theoretical partition coefficient parameters (logP) were also calculated using computer programs: Marvin and QikProp for Schrödinger [4]. The influence of the lipophilicity parameters (R_{M0}, logP) on their activity (K_i) for selected compounds was also discussed.

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Acknowledgements

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P92

Polimorphism of a new isoniazid derivate

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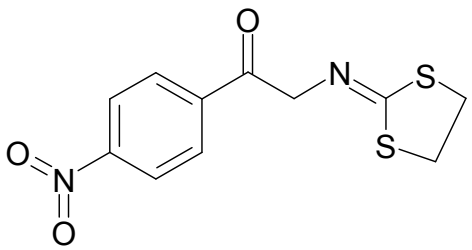
Each substance can exist in more than one crystalline state. The different crystalline form are called polymorphs [1,2]. In this study, we present four new polymorphic forms (**1**-**4**) and one pseudopolymorph (**5**) (Table) of 2-([1,3]dithiolan-2-ylideneamino)-1-(4-nitro-phenyl)-ethanone (Scheme). The compound shows tuberculostatic activity similar to that of isoniazid and pyrazinamide, being common drugs used in tuberculosis treatment.



Main differences In the found polymorphs are intermolecular C-H…O hydrogen bonds and conformations.

Table. Basic crystallographic parameters of new polymorphs

No.	Space group	Unit cell [Å,°]	R1 [%]
1	$P2_1/c$	7.8582(6) 6.1277(4) 24.4149(17) 98.206(2)	2.8
2	P-1	5.0506(2) 8.0441(3) 14.1953(5) 96.878(1) 95.133(2) 96.306(1)	2.9
3	$P2_1/c$	15.0609(2) 5.0408(1) 16.0954(2) 112.7200	2.4
4	$P2_1/c$	7.8582(6) 6.1277(4) 24.4149(17) 98.206(2)	3.5
5	$P2_1/n$	7.3654(3) 6.4550(2) 25.8911(9) 90.808(1)	2.8

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P93

Synthesis, characterization and biological properties of water soluble phthalocyanine possessing morpholinethoxy groups

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INTRODUCTION

For the last 30 years, porphyrazines and phthalocyanines substituted at their peripheral positions with alkyl, aryl, nitrogen, oxygen or sulphur residues have been synthesized and subjected to various physicochemical as well as biological studies [1]. Many novel phthalocyanines have been considered as potential photosensitizers in photodynamic therapy and photodynamic antimicrobial chemotherapy – a new promising approach to treat bacterial infections [2].

RESULTS

Alkylation reaction of 2,3-dicyanohydrochinone with 4-(2-chlorethyl)morpholine hydrochloride led to phthalonitrile derivative. It was utilized in macrocyclization reaction following Linstead conditions with magnesium n-butanolate in n-butanol. The desired water-soluble symmetrical magnesium phthalocyanine was subsequently subjected to alkylation reaction with methyl iodide in chloroform to give quaternary salt. All novel macrocyclic products were characterized using various analytical techniques – MALDI MS, UV-Vis, ^{1}H and ^{13}C NMR. Moreover, novel magnesium phthalocyanine and its quaternary salt were subjected to antimicrobial photocytotoxicity studies.

CONCLUSIONS

Synthesis, analytical characterization and potential applications in photodynamic antimicrobial chemotherapy of novel phthalocyanine derivatives were studied.

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Acknowledgements

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P94

Synthesis, analysis and pharmacological evaluation of some xanthone derivatives

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Cardiovascular diseases including coronary heart disease, cerebrovascular disease, hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure are caused by disorders of the heart and blood vessels. Even though there are many modern and effective drugs, cardiovascular diseases still remain the main cause of death, being responsible for 30 % of all global deaths [1].

Xanthone itself was proved to posses vasorelaxating properties in thoracic aorta isolated from rats [2]. Other research on a series of aminoalkanolic xanthone derivatives confirmed hypotensive activity of the tested compounds. The most potent seemed to be 3-(3*N*-*iso*-propylamine-2-hydroxypropoxy)-9*H*-xanthen-9-one (xanthonolol), which lowered systolic blood pressure by about 32%-7% in dependence of dosage (from 5 to 0.1 mg/kg) [3]. Xanthonolol possess typical, β-blocker moiety (3-amine-2-hydroxypropan-1-yloxy) characteristic for cardiovascular drugs such as propranolol and carvedilol.

Our Laboratory of Bioorganic Chemistry, Chair of Organic Chemistry has also documented experience in searching for hypotensive, aminoalkanolic xanthone derivatives. The strongest hypotensive effects were observed for compounds containing piperazine moiety [4].

Basing on literature survey, herein we report *in vitro* and *in vivo* cardiovascular activity of some new isomeric structures. Structures were designed to combine xanthone and piperazine rings. These compounds possessed typical β-blocker moiety (3-amine-2-hydroxypropan-1-yloxy) and contained methoxyphenylpiperazine – structural element of urapidil. These derivatives were optical isomers of previously described, active structure [5].

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P95

Cytotoxic effect of the cosmeceutical products components on selected cell lines – Argireline® case study

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Cosmeceuticals are innovative products used in cosmetic market that combine both cosmetic and pharmaceutical properties [1]. Argireline® is an example of that products, produced by company Lipotec S.A In cosmetic formulations such as emulsions, gels or sera, 0,05% argireline® stock solution is used as active ingredient. This acetyl hexapeptide-3 prevents formation of skin lines and wrinkles in a very similar way as botulinum toxin (Botox). This peptide is competing with SNAP-25 protein in the SNARE complex, resulting in attenuation of skin muscle contraction [2]. In our previous studies we determined that 0,05% argireline® solution characterized with antiproliferative effect at argireline® concentration in solution above 20 μM [3]. In that study however we could not determine whether observed effects were caused by active ingredient itself or formulation components present in argireline® solution. The aim of presented study was to determine which component of argireline® solution was responsible for observed antiproliferative effect. For that we analyzed argireline® solution using LC/MS method, which showed the presence of additional compound identified as the caprylyl glycol. Next we performed antiproliferative test (EZ4U assay), against three preselected cell lines (*human embryonic kidney 293 cells* (HEK-293), *human neuroblastoma cell line* (IMR-32) and *human 30 years old male skin fibroblasts* (HSF)) using purified acetyl hexapeptide-3. Obtained results were compared with antiproliferative effect of doxorubicin against mentioned cell lines and examined previously 0,05% argireline® solution. As a result, argireline® peptide in purified form had no effect on proliferation rate of examined cell types, even at concentration above 2 mM. In conclusion it was determined that argireline® peptide had no cytotoxic effect on examined cell lines. We identified additional components of argireline® solution, including caprylyl glycol acting as a cosmetic formulation stabilizer and viscosity regulator. This compound might be responsible for antiproliferative effect observed when cells were treated with argireline® solution. That however require further investigation. Presented study clearly shows that cytotoxic properties of cosmetic's active ingredients need to be examined very cautiously, as they determine the safely of cosmeceutical products.

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Acknowledgements

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P96

Studies on phenylalanine-based AMPA/KA receptor ligands

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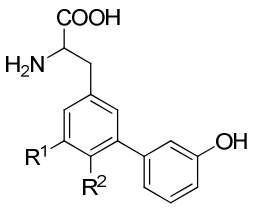
Glutamate- and GABA-releasing neurons form two basic, excitatory and inhibitory systems responsible for neurotransmission in the mammalian central nervous system. Fast excitatory synaptic transmission in the CNS relies almost entirely on the neurotransmitter glutamate and its family of ion ligand-gated channel receptors (iGluRs). The family of iGluRs is divided into three functionally distinct subclasses: NMDA, AMPA and kainate receptors. Structurally, AMPA-receptors are cation-selective tetrameric heterooligomers formed by combinations of the highly homologous subunits GluA1-4, while kainate receptors are tetrameric assemblies of GluK1-5 subunits.

The present project is a continuation of earlier studies on potent and selective competitive AMPA and/or KA receptors ligands among phenylalanine derivatives [1]. In the process of molecular modelling and docking to known X-ray structures of the glutamate ionotropic receptors binding sites, a new group of compounds were designed on the basis of previously described results [1]. Candidates contained in their structure either small substituents in 3rd and 4th position of the phenyl ring (R^1, R^2), or larger but flat fragments (phenyl, tiophen) in the 3rd position (R^1). A series of the most promising compounds with the structure shown on the Figure below was synthesized and pharmacologically characterized on native NMDA, AMPA, KA receptors. The results of design, synthesis and pharmacological tests are described.



R^1 = H, Cl, NO_2, 5- or 6-membered aromatic heterocyclic rings
R^2 = H, Cl, NO_2, NH_2, OH, OMe

[1] Szymańska, E.; Pickering, D.S.; Nielsen, B.; Johansen, T.N. 3-Substituted phenylalanines as selective AMPA- and kainate receptor ligands. *Bioorg Med Chem* 17, 6390, **2009**.

Acknowledgements

This work was partly supported by Jagiellonian University funds, program K/ZDS/003324.

P97

Synthesis and fluorescence properties of new ester derivatives of isothiazolo[5,4-*b*]pyridine

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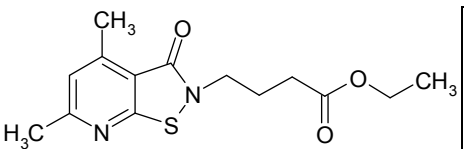
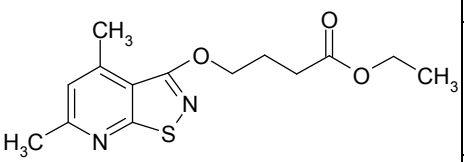
The interest in the fluorescent molecules has steadily increasing in recent years. The fluorescent dyes bound to receptors can show their location and provide a better understanding of their function and regulation.

Here we report the synthesis and fluorescence properties of novel ethyl 4-(2*H*-4,6-dimethyl-3-oxo-2,3-dihydroisothiazolo[5,4-*b*]pyridin-2-yl)butanoate **1** and its 3-O-substituted isomer **2** with potential bioactivity. Early studies demonstrated that some of isotiazolopyridines substituted differently into isothiazole ring exhibited interesting biological properties, which include analgesic [1], antimycobacterial [2], antiagregative [3] activities. Based on those findings, we decided to obtain a new set of isothiazolo[5,4-*b*]pyridine.



1



2

Compound	UV-vis spectra λ_{max}(nm)		Fluorescence spectra λ_{max}(nm)	
	ethanol	n-hexane	ethanol	n-hexane
1	exp. 320 cal. 336	exp. 320 cal. 309	exp. 430 cal. 425	exp. 407 cal. 403
2	exp. 305 cal. 291	exp. 305 cal. 294	exp. 380 cal. 362	exp. 360 cal. 360

Table 1

Structures of new compound were confirmed by FTIR, 1H NMR, elemental analysis techniques. Their optical properties were studied in ethanol and n-hexane by UV-vis absorption and fluorescence spectroscopy. The ground-state and excited-state properties were investigated using the density functional theory (DFT) and the time-dependent density functional theory (TDDFT) methods. The results obtained clearly show differences in optical properties of N- and O-isomer (Table 1).

[1] Malinka W., Sieklucka-Dziuba M., Rajtar G. et. al., *Pharmazie* 55 (2000) 416.

[2] Malinka W., Świątek P., Śliwińska M. et. al. *Bioorg. Med. Chem*. 21 (2013) 5282.

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P98

Pyridoisothiazolones as inhibitors of histone acetyltransferases (HATs)

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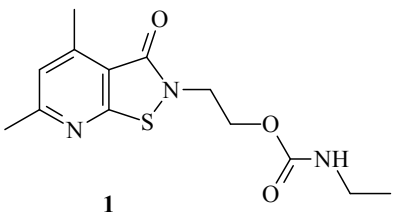
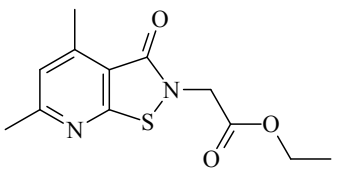
Histone acetyltransferases (HATs) are interesting targets for the treatment of cancer and HIV infections but reports on selective inhibitors are very limited.

Here we report structure activity studies of pyridoisothiazolones **1**, **2**, **3** in the in vitro inhibition of four histone acetyltransferases, namely PCAF, CBP, Gcn5 and p300 using a heterogeneous assay with antibody mediated quantitation of the acetylation of a peptidic substrate. Dependent on the chemical structure distinct subtype selectivity profiles can be obtained. The best inhibitor **1** show micromolar inhibition of CBP (IC_{50}=1.67±0,46 μM). Interestingly, **1** are highly potent on CBP with pronounced selectivity over p300 despite the high similarity of the two enzymes.



1



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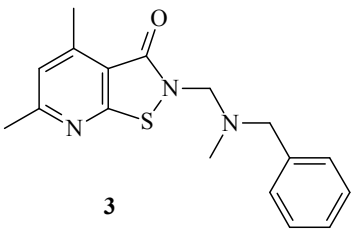

3

P99

Synthesis of new derivatives of quinoline with affinity to serotonin receptors

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Here we report our studies that cover synthesis and molecular modelling of serotonin receptors and serotonin transporter ligands. The compounds synthesized are derivatives of 8-(phenylsulfanyl)-2-(piperazin-1-yl)quinoline and their salts.

Designed by us chemical compounds contain in their structure the most important pharmacophoric elements of so far explored structure. The presence of two nitrogen atoms, one attached to an aromatic ring, a second fully aliphatic (II-or III-row) appear to be determinants for a high affinity to serotonin receptors [1]. Moreover, the presence of the quinoline system in the molecule enhances the activity. The role of diaromatic sulfide group is probably to limit the effect only to serotonergic receptors and SERT.

The molecular modelling studies so far are focused on determination of conformation, and protonation sites of piperazine ring. Here three protonated forms are likely to exist: two distinct monocations and dication. The site of protonation is crucial, because interaction with serotonin receptors is believed to occur with positively charged side chain of the ligand.

Those compounds are new chemical entities obtained in multistep reaction pathway. Two last steps of the reaction pathway are newly explored stages: halogen exchange by aromatic Fienkelstain reaction [2] and sulphide synthesis by Buchwald reaction [3]. All compounds were purified by column chromatography, and their structures were determined by the ^{1}H, ^{13}C NMR spectra and MS.

Preliminary receptors study was also performed and the results are quite satisfactory. They confirm thesis about importance of the nature, number and position of substituent in benzene ring.

[1] Timo Heinrich, et al.,, Journal of Medicinal Chemistry, 47, 9, 4684-4692, 2004
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Acknowledgements

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P100

In vivo activity of 3-furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of the α7 nicotinic receptor

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Emerging evidence points out that the α7 nicotinic acetylcholine receptor (nAChR) is an important target for the development of drugs for the treatment of several neurological disorders, including cognitive impairments, depression, and schizophrenia. The aim of our study was to determine whether 3-furan-2-yl-N-p-tolyl-acrylamide (PAM-2), a positive allosteric modulator of the human α7 nAChRs [1], mediates in vivo activity in mice.

In this regard, passive avoidance tests, forced swim tests, and elevated plus maze tests were conducted to evaluate PAM-2 influence on memory and cognitive function, depression-like behavior and anxiety, respectively. Our findings show that PAM-2 improves memory consolidation after acute and chronic (three weeks) treatments and memory acquisition after acute administration in male mice.

We also found that the memory impairment elicited by scopolamine is reversed by acute administration of PAM-2. In addition, we found that PAM-2 induces antidepressant-like effects in male or female mice after one (subchronic) and two (chronic) weeks, whereas it does not induce acute antidepressant-like effects [2]. Interestingly, the residual antidepressant-like activity of PAM-2 after one week withdrawal is observed only in female mice, indicating gender-selectivity [2]. Finally, our results indicate that acute treatment with PAM-2 mediates anxiety-like behavior in male, but not female, mice, and this activity lasted for one more day after injection. Moreover, chronic treatment (three weeks) with PAM-2 produces higher anxiolytic activity compared to acute treatment, whereas there is no residual activity after one week of treatment cessation.

Our findings clearly demonstrate that PAM-2 has pro-cognitive, antidepressant-like, and anxiolytic activities. Thus, PAM-2 is a promising candidate for the treatment of Alzheimer's disease (AD) patients with depression, which correspond to approximately 40% of the AD population. Since PAM-2 enhances agonist-activated α7 nAChRs, we hypothesize that PAM-2 enhances the agonistic activity of the endogenous neurotransmitters ACh and/or choline, finally producing the observed behavioral effects.

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[2] Targowska-Duda K.M., Jozwiak K., Arias H.R. Neuroscience Letters 553 (2014) 186-190.

Acknowledgements

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within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion. The research was supported from the Polish National Science Center (SONATA funding, UMO-2013/09/D/NZ7/04549).

P101

Microwave assisted condensation of the derivatives of thiazolidine-4-ones with aldehydes

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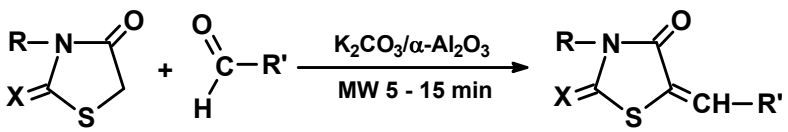
Compounds containing a core of thiazolidine-4-one are widely studied because of their biological (antibacterial, antiviral, anti-inflammatory and antifungal) activity [1]. New synthetic methods allowing to receive these derivatives with a higher yield are still searching. According to literature, one of the first research into using microwave radiation to conduct aldol condensation reaction between aromatic aldehydes and rhodanine was the investigation described by Villemin and Alloum [2]. They carried out the reaction of 3-methylorhodanine with aromatic aldehydes in the presence of potassium fluoride on alumina. In organic synthesis alumina activated with potassium carbonate is also used to catalytic purposes [3,4]. Based on these studies, we decided to carry out the condensation reaction of three thiazolidine-4-one derivatives with aliphatic and aromatic aldehydes. The following compounds were used as substrates: 3-phenylorhodanine, 3-phenyl-2-phenyliminothiazolidine-4-one and 3-phenylothiazolidine-2,4-diones. Potassium carbonate on α-Al_2O_3 was used as a catalyst. It was obtained via modification of the method described by Wu et al. [4]. The reactions were carried out in solvent-free conditions. The mixture of catalyst, aldehyde and the corresponding thiazolidine-4-one derivative was exposed to microwave irradiation for 5-15 minutes.



X = O, S or R-N
R' = alkyl or aryl

The course of the reactions with aromatic aldehyde are very satisfactory. In case of aromatic aldehydes reaction time is shorter and its yield is higher than in the case of aliphatic aldehydes.

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P102

Pyrazine-2,3-dicarbonitrile derivatives and their reactivity in macrocyclization reactions towards pyrazinoporphyrazines

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[d]Department of Macromolecular Physics, Faculty of Physics, Adam Mickiewicz University,Umultowska 85, 61-614 Poznan, Poland

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Porphyrazines are analogues of naturally occurring porphyrins. Porphyrazine macrocycle revealed potential for medicine, especially photodynamic therapy and technology. Novel porphyrazines containing peripherally annulated pyrazine rings have revealed interesting optical and electrochemical properties[1,2].

The aim of our study was to obtain the tetrapyrazinoporphyrazines possessing peripheral aryl- and aryloxy substituents. Known compounds, 5,6-disubstituted pyrazine-2,3-dicarbonitrile derivatives, 4-methoxyphenyl- and 4-(benzyloxy)phenyl-, were synthesized and converted to porphyrazines. However, novel macrocyclic compounds revealed high affinity to form aggregates what hampered their purification and physico-chemical characterization. In addition, novel 5,6-bis(3,5-dimethoxyphenoxy)pyrazine-2,3-dicarbonitrile was synthesized and subjected to macrocyclization reaction. However,this molecule did not undergo tetramerization reaction towards the porphyrazine product using the classical Linstead macrocyclization conditions. The desired product was obtained by 5,6-bis(3,5-dimethoxyphenoxy)pyrazine-2,3-dicarbonitrile melting with complex zinc salt – $Zn(pyridine)_2Cl_2$. Novel porphyrazine was characterized and subjected to physico-chemical study.

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Acknowledgements

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P103

Synthesis 4-(chlorophenyl)thiosemicarbazone derivatives of [(2,4-dioxothiazolidin-5-yl(ylidene))acetoxy]benzaldehydes as potential antimicrobial agents

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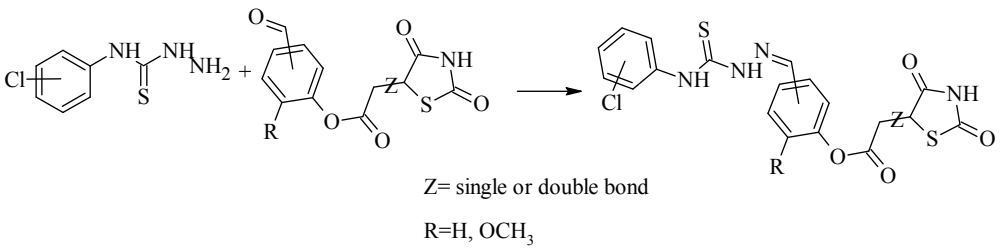
2,4-Dioxothiazolidine and their derivatives are very important class of compounds in biological applications. They exhibit antidiabetic (as PPAR receptor agonists), anti-inflammatory (COX-2/5-LOX as inhibitors or phospholipase A2), antibacterial (as inhibitors UDP-MurNAc/L-Ala-ligase), anti-tumor (as inhibitors of JSP-1) activities. Modifying the 2,4-dioxothiazolidine derivatives in position 5 can lead to new potential biological activity compounds.

As a starting materials we used (2,4-dioxothiazolidin-5-yl)acetic acid and (2,4-dioxothiazolidin-5-ylidene)acetic acid. By the reaction with $SOCl_2$ the corresponding acid chlorides were obtained. Then, the obtained chloride by the modified Schotten-Baumann reaction with vanillin, p-hydroxybenzaldehyde, m-hydroxybenzaldehyde and salicylaldehyde have led to formation of the corresponding formylphenyl acetate derivatives. Afterwards, by the condensation reactions of 4-(chlorophenyl)thiosemicarbazide with formylphenyl acetate derivatives with 2,4-dioxothiazolidine ring new corresponding thiosemicarbazone were synthezised (Scheme 1.).



Scheme 1. Synthesis 4-(chlorophenyl)thiosemicarbazone derivatives of [(2,4-dioxothiazolidin-5-yl(ylidene))acetoxy]benzaldehydes

The structures of newly obtained compounds were confirmed using ^{1}H NMR and ^{13}C NMR methods. The newly synthesized compounds were tested for their antimicrobial activity.

P104

New polyamine derivatives with potential antiproliferative activity

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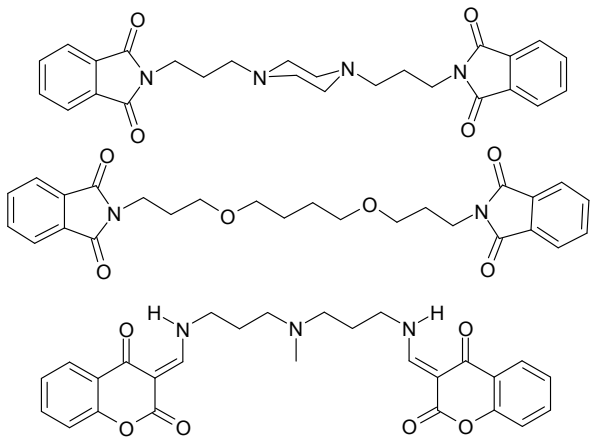
Treatment of cancer is a major challenge for the contemporary medicine. A large percentage of antineoplastic drugs are small molecules that intercalate into DNA. These non-covalent interactions describe the process of inserting planar, polyaromatic molecules between adjacent base pairs of the double helix [1]. A series of new bisintercalators were synthesized in Medical University of Lodz [1, 2] and crystal structures of their three representatives were determined by X-ray diffraction in Lodz University of Technology. Compound **1** crystallizes in the space group P1, with a = 5.4930(5), b = 8.4029(8), c = 12.4350(11), α = 78.377(2), β = 78.566(2), γ = 88.595(2), compound **2** in $P2_1/n$, with a = 7.0501(4), b = 4.5328(3), c = 35,031(2), β = 93,376(2), and compound **3** in $P2_1/c$, with a = 9.0896(4), b = 18.2905(8), c = 14.5418(6), β = 104.9590(10). As expected, π-π stacking of the aromatic fragments of the molecules was observed in crystal structures determined in this study as well as in similar compounds found in CSD [3].



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[3] Allen F. H., *Acta Cryst.,* B58(2002), 380.

Acknowledgements

Congress attendance of AT was supported by RKN Lodz University of Technology.

P105

Prediction of the biological properties of new acetylcholinesterase inhibitors

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Alzheimer's disease is a progressive neurodegenerative disease, which is the most common cause of dementia in people over 60 years old and so far it is not known cause of its occurrence. Currently approved by FDA are four reversible acetylcholinesterase inhibitors, such as tacrine, galantamine, rivastigmine and donepezil and memantine, which is N-methyl-D-aspartate receptor antagonism. Studies also show a protective effect caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs), HMG-CoA reductase inhibitors (Statins), some vitamins and antioxidants. There are several theories regarding the mechanism of Alzheimer's disease, they include: the oldest is cholinergic hypothesis, the next amyloid cascade hypothesis and the hypothesis associated with tau pathology. Unfortunately, despite intensive research has not developed effective drugs without serious adverse events. It is possible only symptomatic treatment, which does not reverse or even halt the progression of the disease. [1, 2]

In the Laboratory of Radiopharmacy, Department of Pharmaceutical Chemistry, Drug Analyses and Radiopharmacy of Medical University in Lodz conducts research on new drugs that inhibit acetylcholinesterase. The purpose of this research is to develop innovative compounds that can ensure the diagnosis of deficiency of acetylcholine in the CNS and effective treatment of Alzheimer's disease.

The idea of the present study was to obtain new derivatives of N-(2,3-Dihydro-1H-cyclopenta[b]quinolin-9-yl)- ω-1,4-diamine. The purpose of research was to combine the diamine derivatives of cyclopentaquinoline which potentially have an affinity for AChE with derivatives of benzoic acid and nicotinic acid. Next, were performer computer simulations of basic parameters ADME, such as: protein binding, penetration of the blood-brain barrier or toxicity. Based on the obtained results were selected derivative with the best pharmacodynamic properties.

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P106

The influence of novel amine derivatives of 5-arylideneimidazolone on P-glycoprotein activity and proliferation of cultured cancer cells

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Multidrug resistance (MDR) is one of the major concerns in the treatment of cancer and one of the major causes of therapy failure. The mechanism of the cancer MDR is mainly connected with overexpression of ATP-dependent drug-efflux pumps; those pumps act as drug transporters reducing the intracellular accumulation of anti-cancer agents. P-glycoprotein (P-gp, ABCB-1) seems to be a crucial factor responsible for cancer MDR and for this reason the successful inhibition of its activity during chemotherapy is a major goal of medicinal science [1, 2].

In present work a series of novel amine derivatives of 5-arylideneimidazolone, exhibiting high potency to inhibit P-gp activity (as judged by preliminary studies) were further characterized in the context of their direct influence on P-gp ATP-ase activity and ability to inhibit growth of prostate cancer cell line.

Direct influence of examined structures on P-gp's ATP-ase activity was determined by measuring ATP hydrolysis rate in the presence of tested compounds using microplate luminescence assay and commercially available recombinant P-gp. Verapamil and Na_3VO_4 were chosen as model stimulator and inhibitor of P-gp's ATP-ase activity, respectively.

Antiproliferative and cytotoxic effect of considered structures was tested at PC-3 prostate cancer cell line. Inhibition of cell proliferation was determined by performing MTS assay after 72 h incubation with a reference cytostatic drug – doxorubicine – or with tested compounds. Additionally, short term cytotoxic activity was evaluated with use of lactate dehydrogenase activity assay.

Investigated compounds represented low to moderate influence on ATPase activity of P-gp. Among tested compounds 5-(4-chlorobenzylidene)-2-(3-hydroxypropylamino)-1H-imidazol-4-one (DS-4) occurred to be the most active; DS-4 exhibited 74% of verapamil activity when used in the same concentration. Moreover, only one compound: 5-(2,4-dichloro-benzylidene)-2-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-imidazol-4-one (BM20) showed significant antiproliferative activity on PC-3 prostate cancer cells.

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Acknowledgements

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P107

Searching for new derivatives of indole alkaloid-olivacine

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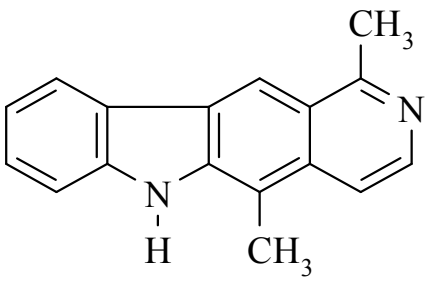
Olivacine 1 is one of indole alkaloids which was firstly isolated from *Aspidosperma olivaceum Müll. Arg*. [1].

Some olivacine analogues demonstrated a strong cytostatic activity [2].

1-Phenyl-6H-pyrido[4,3-*b*]carbazole derivatives were described in our earlier paper [3], now are presented some new pyrido[4,3-*b*]carbazole derivatives, substituted with pyrazoles or imidazoles moieties at position 1.



1

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[2] Jasztold-Howorko, R., et al., J. Med. Chem. 1994, 37, 2445-2452.
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Acknowledgements

Supported by statutory funds of the Wrocław Medical University No ST-768.

P108

Lessons learned from analysis of bioisosteric substitution in ligands of a serotonin receptor family

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A bioisosteric replacement transforms an active compound into another one by exchanging a group of atoms with broadly similar (in physicochemical properties) groups. Implementations of this technique are aimed on increase of affinity, improvement of pharmacokinetic properties or exploration of new, unknown scaffolds.

For compounds with determined affinity for any serotonin receptor stored in the ChEMBL [1] database (version 16 May 2013) all possible bioisosteres were generated in Pipeline Pilot [2]. Analysis of this collection, consisting of more than 1 million structures, showed that in average 31% of known ligands of a particular target are mutual bioisosteres.

Data exploration revealed the most frequent and the most efficient replacements in modulating ligands activity for different subtypes of serotonin receptors. Statistical analysis shows the most appropriate fragments for increasing the ligands affinity for particular targets, providing a collection of tips for synthetic chemists how to modify existing ligands to obtain more potent compounds. Moreover, principles of modifying ligands of one target to create compounds acting on another one are also given.

[1] Gaulton, A., Bellis, L. J., Bento, A. et al. *Nucleic Acids Research*. 40 (2011), D1100-D1107.
[2] Pipeline Pilot, version 6.0, Accelrys, Inc., San Diego, CA, USA.

Acknowledgements

The study was partially supported by the Polish-Norwegian Research Programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014 in the frame of Project PLATFORMex (Pol-Nor/198887/73/2013).

P109

X-ray analysis of 2-methyl-4-nitroimidazole derivatives

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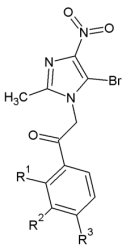
The group of nitroimidazole compounds is known not only for its antimicrobial properties but also for their tendency to accumulate in hypoxic cells [1-2]. Examination of this group of compounds may lead to development of more effective anticancer therapy.

Since arrangement of the drug molecule in the solid state may influence its pharmacological profile, crystal structures of new 2-methyl-4-nitroimidazole derivatives (Scheme 1) were determined in course of our research.



1. R^1=Cl, R^2=H, R^3=H
2. R^1=Br, R^2=H, R^3=H
3. R^1=I, R^2=H, R^3=H

4. R^1=H, R^2=F, R^3=H
5. R^1=H, R^2=Cl, R^3=H
6. R^1=H, R^2=Br, R^3=H
7. R^1=H, R^2=H, R^3=Cl

Scheme 1

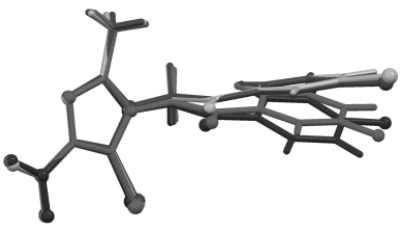


Figure 1 Superposition of molecules from crystals 4-7

Molecules of nitroimidazole derivatives in crystals **1**-**7** (Scheme 1) are assembled into 1-D chains through C-H···O hydrogen bonds. The crystal structures can be divided into two main groups depending on organization of this 1-D motif.

Crystals **1**-**3** from *Cc* space group belong to one group [3]. In these crystal structures, chains are arranged into noncentrosymmetric layers through halogen bonds. No specific interactions are observed between the layers. The association of the molecules in crystals **1**-**3** is almost identical, thus we can specify them as isostructural.

Second group is composed of crystals **4**-**7** that crystallized in the $P2_1/c$ space group. In these cases $\pi\cdots\pi$ interactions are observed between the chains what results in formation of centrosymmetric layers. Differences occurring in conformation of molecules in crystals **4**-**7** (Figure 1) are reflected in small dissimilarity of their arrangement in the crystal structures. Only crystals **5** and **6** that contain nitroimidazole molecules in almost identical conformation (Figure 1) are fully isostructural.

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Acknowledgements

This study was supported by fund No. 502-01-03313427-08870.

P110

Potential substrates for diazepinoporphyrazines - synthesis and characterization of novel 5,7-disubstituted 1,4-diazepine-2,3-dicarbonitriles

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Porphyrazines carring peripherally anullated diazepine rings modified with spherically branched dendrones constitute a relatively novel subject of research with potential applications in medicine and bionanotechnology. Novel compounds may be considered as potential antitumor agents in Photodynamic Therapy. Study embracing dendrimeric porphyrines has been found very encouraging. Irradiation of these compound localized at the affected site has caused generation of singlet oxygen by conversion of light energy into chemical energy and led to chemical destruction of tissues or pathogens [1].

The aim of our study was to synthesize novel 1,4-diazepine-2,3-dicarbonitrile derivatives, equipped with phenyl substituents at 5th and 7th position, and assess their reactivity in Linstead macrocyclization reactions towards diazepinoporphyrazines. 2,3-Dicyano-5,7-dimethyl-6H-1,4-diazepine was obtained in a two-step synthesis and used subsequently in condensation reactions with a series of aryl aldehydes to give bis[(3,5-dibromophenyl)ethenyl]-6H-1,4diazepine-2,3-dicarbonitrile, bis[(4-tert-butylphenyl)ethenyl]-6H-1,4-diazepine-2,3-dicarbonitrile and bis[(3,5-dibenzyloxyphenyl)ethenyl]-6H-1,4-diazepine-2,3-dicarbonitrile. Novel products were purified by both crystallization and flash column chromatography. Maleonitrile derivatives were characterized by various Nuclear Magnetic Resonance techniques: correlation spectroscopy, heteronuclear multiple-bond correlation spectroscopy, heteronuclear single-quantum correlation spectroscopy. Diazepine dicarbonitriles revealed promising reactivity in macrocyclization reactions in butanol and magnesium butanolate as a base. The stability of novel diazepinoporphyrazines seem to depend on the electron-donating or withdrawing properties of styryl substituents in the 5th and 7th positions of the diazepine ring.

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This study was supported by the National Science Centre under grant No 2012/05/E/NZ7/01204 and by the National Centre for Research and Development under grant No PBS1/A9/13/2012.

P111

Is the free amine group in the s-triazine core necessary for the histamine H_4 receptor affinity?

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The histamine H_4 receptor (H_4R), the newest member of the histamine receptor family, was first identified at the turn of the millennium. Its expression pattern, together with postulated involvement in a wide variety of immunological and inflammatory processes make H_4R an interesting target for drug development. H_4R antagonists may have therapeutic utility in allergic rhinitis, asthma, rheumatoid arthritis, pruritus and pain. Up to now there are many well-known selective and potent histamine H4R ligands. None of them have been introduced to the market but some of H4R antagonists have entered clinical development. [1]

Our investigations deal with the search for H_4R antagonists in the group of 1,3,5-triazine derivatives. So far we examined compounds in which the fixed parts were: free amine group in position 2 and 4-methylpiperazine moiety in the position 4 of triazine ring. In the present work we investigated the influence of the free amine group on the affinity at human H_4R. For this research the most active compound in our database, the 4-(4-methylpiperazin-1-yl)-6-(4-chlorophenyl)-1,3,5-triazin-2-amine, was chosen as a lead structure. In this compound the amine group was removed or changed to phenylamine, amide or substituted urea moiety.

The results indicate that the presence of free amine group or its lack is tolerated, but its substitution significantly reduces the H_4R affinity.

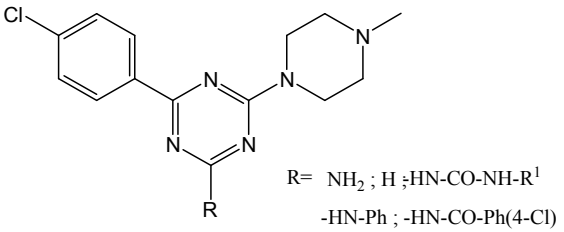

Fig. General structure of investigated compounds

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Acknowledgements

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P112

Enhancement of the oxacillin efficacy in *S.aureus* strains by tellurium/selenium salts: an innovative approach to the fight against resistant bacteria

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The unique properties showed by tellur- and selenocompounds convert them in interesting candidates for drug development. These molecules have a strong antiperoxidation activity. Thus, they are able to inhibit different proteins and enzymes, kill various microorganisms including some species of bacteria, fungi and plasmodia as well as selectively induce apoptosis in certain cancer cells [1]. Remarkably, tellurium compounds were used to treat certain bacterial infections prior to discovery of antibiotics [2].

The methicillin-resistant *S.aureus* (MRSA) is one of the most frequently reported human pathogen in nosocomial infections in the world as it causes around the 40-60% of *S. aureus* infections [3]. Accordingly, our group has evaluated the effect of selenium or tellurium salts such as Na_2TeO_3, Na_2SeO_3, Na_2SeO_4 over the two clinical strains of *Staphylococcus aureus*: MRSA HEMSA 5 and MRSA HEMSA 5M and the reference strain *S. aureus* ATTC 25923. Although neither selenium nor tellurium tested compounds do not exhibit antimicrobial activity against the aforementioned staphylococci, the tellurite salt (Na_2TeO_3) strengthen the bactericidal effect of oxacillin 16- and 1024-fold against multidrug resistant MRSA HEMSA 5 and MRSA HEMSA 5M, respectively. Simultaneously, it did not show any synergistic activity with respect to the reference strain *S. aureus* ATTC 25923. Abovementioned results are in accordance to previous lines of evidence indicating that K_2TeO_3 alters the membrane integrity of *Rhodobacter capsulatus* [5], as well as enhances the action of antibiotics in clinical strains of *Escherichia coli* [4]. In conclusion, using tellurite based compounds as antibiotic adjuvants is a novel and potent strategy that could have clinical applications in the treatment of infections caused by multidrug-resistant *S.aureus* pathogen. The work was partly supported by UJ CM grant K/DSC/001407, K/PBM/000197 and K/ZDS/ 003323.

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P113

Lipophilicity determination of novel coumarins by means of RP-HPTLC

Agata Włodarczyk[a], Kamil Wojnicki[a], Dariusz Matosiuk[a]

[a]Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Faculty of Pharmacy with Medical Analytics Division, Medical University in Lublin

Drug lipophilicity plays an important role in their biological activity. Chromatographic techniques are suitable to predict LogP parameter. The LogP investigation of novel compounds were performed using RP-HPTLC. The linear relationship between known LogP values of chosen standards and their experimental R_{M0} parameter had been used for calculating LogP values for coumarins. Series of standards were chosen powered by structures similaritys. Thin-Layer Chromatography offer practical advantages, including speed, reproducibility, insensitivity to impurities or degradation products, online detection and reduced sample sizes[1].

Many coumarins derivatives have a broad spectrum of biological properties. Anti-tumor, anti-microbial, anti-viral and anti-inflammatory effects are the main examples of their role in medicine[2].

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Acknowledgements

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P114

Analysis of triterpenes with aniinflamatory activity in *Symphytum officinale* L.

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Departament of Chemistry, Medicinal University in Lublin, Chodźki 4a, 20-093 Lublin

Symphytum officinale L. (*Boraginaceae*) is widely used in folk medicine as externally remedy to promote wound healing, to reduce of inflammation, in the treatment of broken bones and tendon damages, and also in rheumatic and arthritic diseases.

The therapeutic activity of plant is related to a presence of biologically active substances such as: allantoin (0.6-2%), phenolic acids (e.g, caffeic, chlorogenic, rozmarinic acid), triterpenic saponides, proteins, tannins (2.4%), carotene (0.63%) and others [1].

In our research, the methanol extract from root of *S. officinale* was analyzed for presence of oleanolic and ursolic acid. Both compounds widely exist in food, medicinal herbs and plants and have anti-inflammatory property. [2,3].

The identification and quantification of triterpenic acids was performed using of high performance liquid chromatography with DAD detection. The separation was achieved on RP 18 column at 1 mL/min flow rate and at temperature of 10°C. Acetonitrile, water and 1% phosphoric acid (80:20:0.5 v/v/v) was used as a mobile phase. The established calibration curves and the other validation parameters: correlation coefficient and precision expressed as relative standard deviation were found to be satisfactory for the proposed method. The determined contents of oleanolic and ursolic acid in *S. officinale* were 18.8 µg/g and 15.2 µg/g of dry plant material, respectively.

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P115

Synthesis and antiproliferative activity *in vitro* of novel pyrrolo[3,4-c]pyridine-1,3-dione derivatives

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Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

Wydział Farmaceutyczny z Odziałem Analityki Medycznej

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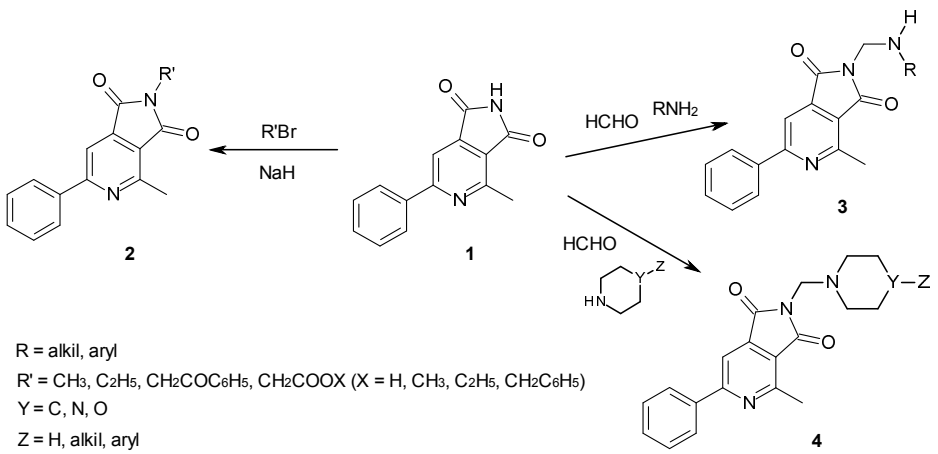
Biological investigations have shown that compounds containing the pyrrolo[3,4-*c*]pyridine scaffold have a wide spectrum of actions. Most of them have been studied as sedative and analgesic agents. Pyrrolo[3,4-*c*]pyridine derivatives can be used in the treatment of the nervous and immune system diseases. Antitumor, antituberculostatic, and antiviral activities also have been found [1].

The various biological properties encourage the synthesis of new pyrrolo[3,4-*c*]pyridine derivatives. The substrate in this study was obtained by us before 4-methyl-6-phenyl-1H-pyrrolo[3,4-c]pyridine-1,3-dione **1** [2]. Two series of novel pyrrolo[3,4-c]pyridine-1,3-dione derivatives have been synthesized.

In the first step the carboxyimide **1** was alkylated to the corresponding N-alkil-4-methyl-6-phenyl-1H-pyrrolo[3,4-c]pyridine-1,3-dione derivatives **2**. Next, the series of Mannich bases **3-4** were synthesized by treating pyrrolo[3,4-c]pyridine-1,3-dione **1** with appropriate amines and formaldehyde.

The newly synthesized compounds were tested for their antiproliferative activity *in vitro*.



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P116

Novel nitrophenyl urea gsk-3 inhibitors with antiproliferative properties

Tomasz Wrobel[a], Michal Kielbus[a], Agnieszka Kaczor[a], Monika Aletańska-Kozak[a],Sylwia Krol[a], Andrzej Fruzinski[b], Zbigniew Karczmarzyk[c], Vladimir Krystof[d], Andrzej Stepulak[a], Dariusz Matosiuk[a]*

[a] *Department of Synthesis and Chemical Technology of Medicinal Substances, Medical University of Lublin, ul. Chodzki 4A, 20-153 Lublin, Poland*

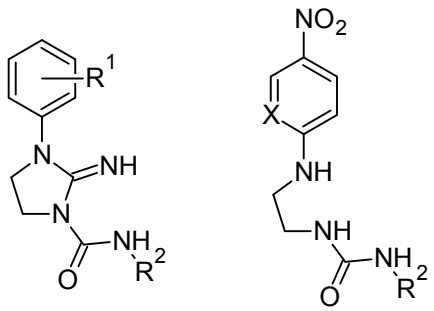
[b] *Institute of General and Ecological Chemistry, Technical University, ul. Żeromskiego115, 90-924 Łódź, Poland*

[c] *Department of Chemistry, Siedlce University, ul. 3 Maja 54, 08-110 Siedlce, Poland*

[d] *Laboratory of Growth Regulators, Faculty of Science, Palacký University, Šlechtitelů 11, 78371 Olomouc, Czech Republic*

GSK-3 has become recently an attractive drug target in medicinal chemistry. Initially discovered in 1980 to be responsible for regulation of glycogen synthase[1] now it remains fully established target implicated in cancer and a variety of neurological disorders like Alzheimer's, bipolar disorder or Huntington disease[2].

In this work we present a synthesis of novel compounds together with biological data. Some of the obtained compounds showed activity in GSK-3 assays and closely related CDK. Together with data from apoptosis and viability studies on two cancer cell lines these newly designed compounds are promising structures that might lead to incorporation of new scaffolds into kinase inhibitors research.



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P117

Synthesis and X-ray crystallographic studies of arylhydrazinecarboxamide derivatives with potential microbiological activity

Waldemar Wysocki [a], *Monika Aletańska-Kozak* [b], *Zbigniew Karczmarzyk* [a], *Dariusz Matosiuk* [b], *Przemysław Kalicki* [c] *and Zofia Urbańczyk-Lipkowska* [c]

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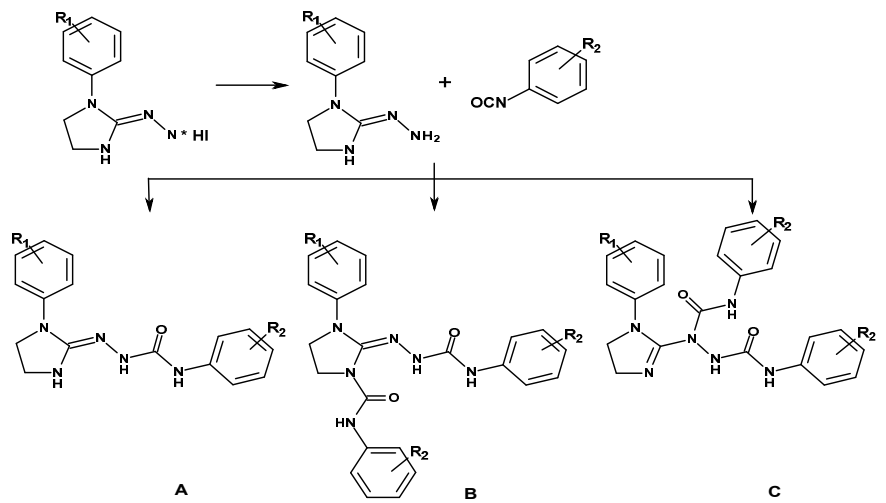
[b] *Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University of Lublin, Chodźki 4A, 20-093 Lublin, Poland*

[c] *Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*

In this communication we presented synthesis and crystallographic structures of new arylhydrazinecarboxamide derivatives which were obtained as substances with potential microbiological activity. For this purpose, 1-aryl-2-hydrazineylideneimidazolidines derived from hydroiodides reacted with the appropriate isocyanates giving N-aryl-2-[1-arylimidazolidin-2-ylidene]hydrazinecarboxamides (**A**), N-aryl-2-(2-arylcarbamoylhydrazinylidene)-3-arylimidazolidine-1-carboxamides (**B**) and N,N'-bisaryl-1-(1-aryl-4,5-dihydro-1*H*-imidazol-2-yl)hydrazine-1,2-dicarboxamides (**C**) [1].



R_1 = H; 4-Cl; 2,3-CH_3; 4-OCH_3
R_2 = H; 3-Cl; 4-Cl; 3,4-Cl; 4-OCH_3

The structures of obtained compounds were characterized on the basis elemental analysis, ^{1}H and ^{13}C NMR spectra. The crystal and molecular structure of 1-(4-chlorophenyl)-3-{[1-(4-chlorophenyl)imidazolidin-2-ylidene]amino}urea (**A;** R_1=4-Cl, R_2=4-Cl) and (2*Z*)-*N*-(3-chlorophenyl)-2-

[(3-chlorophenyl)carbamoyl-hydrazono]-3-(2,3-dimethylphenyl)-imidazolidine-1-carboxamide (**B**; R_1 = 2,3-CH_3, R_2 = 3-Cl) were determined using X-ray analysis.

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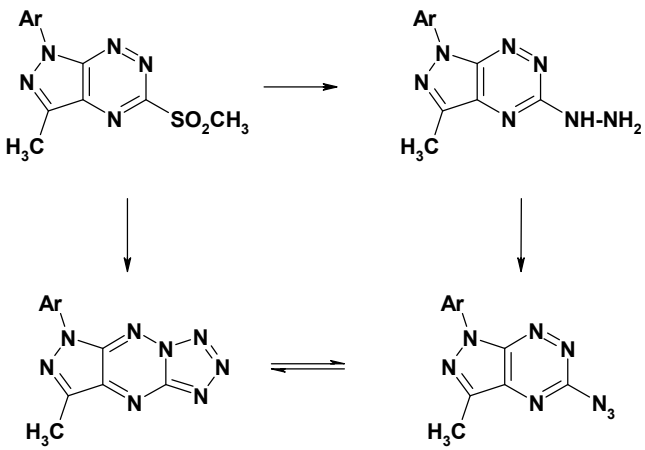
P118

Synthesis and structural characterization of new pyrazolo[4,3-*e*]tetrazole[4,5-*b*][1,2,4]triazines with potential anticancer activity

Waldemar Wysocki [a], *Mariusz Mojzych* [a], *Zbigniew Karczmarzyk* [a], *Arkadiusz Wielgosz* [a]
Przemysław Kalicki [b] *and Zofia Urbańczyk-Lipkowska* [b]

[a] *Department of Chemistry, Siedlce University, 3 Maja 54, 08-110 Siedlce, Poland*
[b] *Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*

Annulated 1,2,4-triazine derivatives are present as important core structures in many biologically active compounds, both naturally occurring and synthetic [1,2]. As a part of an ongoing research program into synthesis of heteroaromatic analogues of this system, we were interested in the formation of new pyrazolo[4,3-*e*][1,2,4]triazines fused with tetrazole ring with potential anticancer activity [3].



Ar = 4-CH_3-Ph, 4-CH_3O-Ph, 4-Cl-Ph

The ^{1}H NMR spectra reveal tautomeric equilibrium azido and tricyclic linear or angular forms of these compounds in chloroform solution. The X-ray investigation of 4-metoxyphenyl derivative shows that this compound exists as the linear tricyclic pyrazolo[4,3-*e*]tetrazolo[4,5-*b*][1,2,4]triazine tautomeric form in the crystalline state. In order to determine tautomeric equilibrium in the gaseous phase and solutions the theoretical calculations at DFT/B3LYP/6-311++G(d,p) level were undertaken.

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P119

Arylsulfonamide analogs of PZ-766 as potent 5-HT$_7$ receptor antagonists

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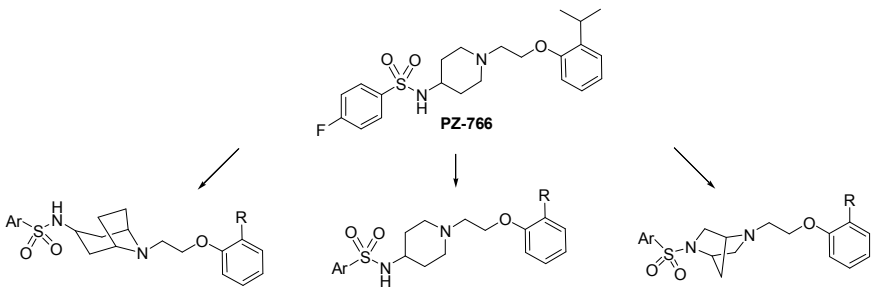
A growing body of preclinical and clinical data supports the hypothesis that 5-HT$_7$ receptor (5-HT$_7$R) antagonists may be regarded as an alternative for currently available drugs for the treatment of depression and/or anxiety as well as for the treatment of memory dysfunction in cognitive disorders (Alzheimer's disease, age-related decline) [1, 2]. Aiming at development of selective 5-HT$_7$R antagonists, our research group has recently designed and synthesized a library of arylsulfonamide derivatives of 3-amino-pyrrolidines, 4-amino-piperidines and 4-aminomethyl-piperidines. The study allowed us to identify compound PZ-766 as potent 5-HT$_7$R ligand ($K_i = 0.3$ nM) with strong antagonist properties ($K_b = 1$ nM) and a 1450-fold selectivity over 5-HT$_{1A}$ subtypes [3, 4].



In the present study we synthesized a focused library of new arylsulfonamide derivatives of alicyclic amines, as close analogs of PZ-766. Structural modifications comprised the replacement of the piperidine fragment with the steric hindered azabicyclo-[3.2.1]-octane and diazabicyclo-[2.2.1]-heptane as well as the introduction of phenyl substituents in *ortho* position at the aryloxy moiety. All library members displayed high affinity for 5-HT$_7$R and were classified as potent 5-HT$_7$R antagonists in *in vitro* functional assays. The most potent compounds were further investigated for *in vivo* studies towards potential antidepressant activity in force swim test (FST) in mice. Results showed that compounds PZ-1130 given in a dose of 5 and 10 mg/kg produced a distinct antidepressant-like effect similar to that exerted by PZ-766 (5 mg/kg). These preliminary results are promising to provide further detailed studies aimed at the developing of 5HT$_7$R agents for the treatment of depression.

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Alkyl- and cykloalkylamide substituted derivatives of 1,3-dialkylpyrimido[2,1-*f*]purinediones as ligands of the adenosine receptors

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Adenosine is a purine nucleoside that plays important role in many biological functions through interaction with four subtypes of G-protein-coupled adenosine receptors: A_1, A_{2A}, A_{2B}, A_3. They present different biochemical, pharmacological properties and distribution in tissues. Previous studies showed potential role of adenosine receptors in many therapeutic areas such as: inflammation, pulmonary, cardiovascular and CNS disorders. Therefore adenosine receptor modulators are interesting targets for the treatment of the wide range of diseases.[1,2]

Previous research in the group of annelated xanthines confirmed their activity toward adenosine receptors. We made the efforts to study a new groups of alkyl- and cykloalkylamide derivatives of 1,3-dialkylpyrimido(2,1-*f*)purinediones as new ligands of adenosine receptors. The designed compounds were synthesized according to the previously described procedures [3,4]. Firstly xanthine core was obtained by using Traube's method. Subsequently third ring was built on to the xanthine core by condensation with tyramine. In the next step ester derivative was obtained and then it was conjugated with amine moiety.

We received the small library of compounds that differ in the length of the substituent in position 1 and 3 (propyl-, butyl-) and attached amine moiety. The synthesized compounds were tested in the radioligand binding studies. Affinity to A_1 was tested on rat brain cortical membranes, A_{2A} on rat brain stratial membranes, A_{2B} and A_3 on CHO cell membrans. Structure activity relationship analysis has shown better affinity to A_1, A_{2B} A_3 receptors in the group with butyl- substituents . The most potent A_1 ligand had Ki value 24.8 nM. The Ki value for the most potent A_{2B} receptor ligand was 66.3 nM. The most potent A_3 receptor ligand was 204 nM. Compounds with propyl substituents presented higher affinity to A_{2A} receptors comparing to butyl- derivatives. The most potent A_{2A} ligand belongs to propyl – derivatives which Ki value was 303 nM.

Drug-like properties (logP, logS, toxicity, drug score) of the obtained compounds were evaluated by using OSIRIS program [5]. The research has confirmed that alkyl- and cykloalkylamide derivatives of the annelated xanthines present high affinity to adenosine receptors. Furthermore, our investigation has shown the significant effect of the length of substituents in the 1 and 3 position on the affinity to the adenosine receptor subtypes. There is an urgent need for further studies to investigate the impact of 1,3 variously substituted derivatives of annelated xanthines on their selectivity and affinity to the different adenosine receptor subtypes.

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Acknowledgments

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P121

Metabolic soft spot identification of selected new biologically active compounds by MetaSite in comparison to *in vitro* studies

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CONCEPT

Metabolite identification study plays a key role in determining the sites of metabolic liability of new chemical entities in the early stages of the drug discovery process. It is important factor in the evaluation of safety and efficacy of drug candidates. In this studies we used two alternative methods of predicting metabolic pathway: *in silico* (MetaSite software) and *in vitro* (*Cunninghamella* species) The use of microorganisms in the mimicking of the mammalian metabolism of many molecules of pharmacological importance is well documented [1]. The other approach to identify possible soft spot of compounds related to cytochrome-mediated reactions in phase I metabolism is using *in silico* tool, such as MetaSite software. This software package can assist with predicting or even with identification of the structure of possible metabolites [2].

RESULTS

A dataset of 11 newly synthesized compounds were analyzed. Eight compounds underwent biotransformation by three species of *Cunninghamella* giving at least one metabolite. Comparing data from mass spectra analyses after *in vitro* studies with those from *in silico* studies helped us identify site of metabolism. The difference in metabolism between enantiomers was observed. For three compounds we did not observe biotransformation in *in vitro* studies. It was probably determined by their poor solubility in media.

This approach to modulating the metabolic stability of a drug-like compound is widely applicable and can be used to address metabolic issues of otherwise good lead compounds in drug development.

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Acknowledgements

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Crystal and molecular structure of the phenylpiperazine derivative of 5-methyl-5-phenylhydantoin with activities towards GPCRs: α_1-adrenergic/serotonin 5-HT_{1A} and 5-HT_7

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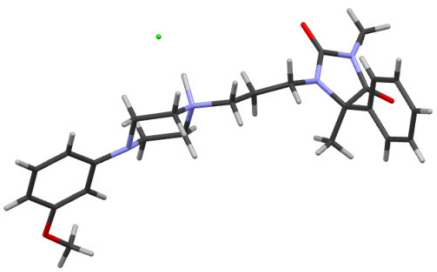
Recent studies have indicated a usage of α_1-adrenergic receptors (α_1-AR) antagonists in the treatment of arrhythmia, asthma or diabetes. The adrenergic receptors are the earliest identified of the G-protein coupled receptors (GPCRs).The serotonin 5-HT receptors are another group of GPCRs that participate in various physiological and pathophysiological processes within brain. 5-HT_{1A} receptor belongs to the largest class of 5-HT_1 receptors which are the earliest investigated group in the treatment of anxiety and depression. The 5-HT_7 receptor is involved in thermoregulation, circadian rhythm, learning, memory and sleep. This receptor may be a useful target in the treatment of depression [1].

Phenylpiperazine derivatives of hydantoin were previously identified as antiarrhythmic agents with moderate activity and low selectivity at α_1- and α_2-adrenoceptors. The interest in phenylpiperazine derivatives has increased for last two decades due to their interactions with α_1-adrenergic and serotonin 5-HT_{1A} and 5-HT_7 receptors, therefore their selectivity is a topic question.

In this report, we present results of the crystal structure analysis of 5-methyl-5-phenyl-1-(3′-(4-(3-methoxy-phenyl)piperazine)-propyl)-hydantoin. Crystals were obtained for hydrochloride of this compound. The nitrogen atom of piperazine is protonated and forms the hydrogen bond with chloride anion. The molecule of investigated compound adopts extended conformation. The crystal network in the studied structure can be characterized by C-H···O and C-H···N intermolecular interactions.



The geometry of this molecule was studied in the aspect of pharmacophore models in analysis of interactions with GPCRs: the Barbaro's model for phenylpiperazine antagonist of α_1-AR [2], the Lepalieur's model for an antagonist of 5-HT_{1A} [3] and model of 5-HT_7 antagonists elaborated by Bojarski's

research group [4]. The results of the theoretical studies of pharmacophores were compared to that of crystallographic data and experimental activity data for this compound and for other derivatives.

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Indeks wystąpień:

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