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SCIENTIFIC PROGRAM

THURSDAY, 15.09.2016

15.00 – 17.00 Registration

17.00 – 17.10 Opening Ceremony

17.10 – 18.10 Inaugular Lecture (IL)

Diego Muñoz-Torrero, University of Barcelona, Spain

„Novel multitarget drug candidates that are effective in an animal model of Alzheimer's disease"

18.10 Concert

19.00 Welcome reception

FRIDAY, 16.09.2016

9.00 – 10.30 Lectures L1 & L2 (45')

Session moderators: Jadwiga Turło & Dariusz Matosiuk

L1

Jean-Marie Pages, UMR-MD1, Aix Marseille Université/IRBA, Marseille, France

„Membrane barrier and drug translocation: A permeability challenge to combat bacterial resistance"

L2

Maciej Dawidowski, (1) Helmholtz Zentrum München, Neuherberg, Germany, (2) Technische Universität München, Garching, Germany

„Disrupting the PEX14-PEX5 interaction by small molecules provides a novel therapeutic strategy for treatment of Trypanosoma infections"

10.30 – 11.30 Communications C1 – C3 (20')

Session moderators: Jadwiga Turło & Dariusz Matosiuk

C1

Przemysław Szafrański, Jagiellonian University, Cracow, Poland

„The application of Click chemistry methods in search of cannabinoid activity"

C2

Wojciech Płaziński, Polish Academy of Sciences, Cracow, Poland

„Ring inversion properties of hexopyranoses: from force field parametrization to the interplay between ring shape and glycosidic linkage conformation"

C3

Gáspár Pándy-Szekeres, University of Copenhagen, Denmark

„GPCRdb homology models – „Less model & more crystal""

11.30 – 12.10 Coffee break

12.10 – 13.50 Communications C4 – C8 (20')

Session moderators: Katarzyna Kieć-Kononowicz & Stanisław Ryng

C4

Jan Mazerski, Gdańsk University of Technology, Poland

„How to decribe physicochemical interactions between dsDNA and possibile chemotherapeutics"

C5

Jarosław Sączewski, Medical University of Gdańsk, Poland

„Synthesis and biological activity of fluorescently-labeled quinolones"

C6

Rafał Kurczab, Polish Academy of Sciences, Cracow, Poland

„The potential of halogen bonding in class A of GPCRs: application of XB hot spots for rational design of 5-HT_7R ligands"

C7

Anna Więckowska, Jagiellonian University, Cracow, Poland

„Novel multi-target-directed ligands for Alzheimer's disease: combining cholinesterase inhibitors and 5-HT_6 receptor antagonists. Design, synthesis and biological evaluation"

C8

Anna M. Waszkielewicz, Jagiellonian University, Cracow, Poland

„Anticonvulsant activity of some new N-[phenoxyalkyl]- and N-[2-(phenoxyethoxy)ethyl]aminoalkanols"

13.50 – 15.40 Lunch

15.40 – 16.25 Lecture L3 (45')

Session moderators: Agata Paneth & Jan Mazerski

L3

Krzysztof Kamiński, Jagiellonian University, Cracow, Poland

„New hybrid anticonvulsants derived from the pyrrolidine-2,5-dione scaffold with broad spectrum of activity in the preclinical studies"

16.25 – 17.05 Communications C9 & C10 (20')

Session moderators: Agata Paneth & Jan Mazerski

C9

Marta Struga, Medical University of Warsaw, Poland

„Biological activity of thiourea derivatives"

C10

Berenika Szczęśniak-Sięga, Wroclaw Medical University, Poland

„Synthesis and properties of new 1,2-benzothiazine derivatives as potential multitarget drugs with chemopreventive and analgesic activity"

17.05 – 17.40 Coffee break

17.40 – 19.10 Norwegian session NS1 – NS4

Session moderators: Jadwiga Handzlik & Andrzej Bojarski

NS1

Ingebrigt Sylte, UiT The Arctic University of Norway, Tromsø, Norway

„Screening for $GABA_B$ receptor compounds"

NS2

Adam S. Hogendorf, (1) Polish Academy of Sciences, Krakow, Poland

(2) Jagiellonian University, Krakow, Poland

„Low-basicity agonists of 5-HT_7 receptor synthesized by van Leusen multicomponent reaction"

NS3

Jarosław Walory, National Medicines Institute, Warszawa, Poland

„Neuroprotective and proapoptotic activity of serotonin transporter inhibitors and serotonin receptor ligands"

NS4

Rafał Kurczab, Polish Academy of Sciences, Krakow, Poland

„Development of a New methods for virtual screening protocol"

SATURDAY, 17.09.2015

9.00 – 9.45 Lecture L4 (45')

Session moderators: Barbara Malawska & Marek Cegła

L4

Marcin Kołaczkowski, (1) Jagiellonian University, Cracow, Poland, (2) Adamed Ltd., Pieńków, Poland

„New directions in the search for therapies of behavioral and psychological symptoms of dementia"

9.45 – 11.25 Communications C11 – C15 (20')

Session moderators: Barbara Malawska & Marek Cegła

C11

Katarzyna Gobis, Medical University of Gdańsk, Poland

„Synthesis and activity of the salicylic acid ester of bakuchiol in psoriasisform keratinocytes and skin substitutes"

C12

Joanna Śniecikowska, Jagiellonian University, Cracow, Poland

„Novel, selective 5-HT_{1A} biased agonists as new pharmacological tools in the development of treatment of psychiatric and neurodegenerative disorders"

C13

Zofia Mazerska, Gdańsk University of Technology, Poland

„Glucuronidation of antitumor agents: the way to drug resistance?"

C14

Marcin Wierzchowski, Poznan University of Medical Sciences, Poland

„Porphyrinoids functionalized with 1,4,7-trioxanonyl and imidazole moieties as promising photosensitizers"

C15

Aneta Pogorzelska, Medical University of Gdańsk, Poland

„Novel 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives - synthesis and QSAR studies of compounds with prominent anticancer activity"

11.25 – 12.00 Coffee break

12.00 – 12.45 Lecture L5 (45')

Session moderators: Anna Bielawska & Krzysztof Bielawski

L5

Jolanta Zawilska, Medical University of Lodz, Poland

„Novel psychoactive substances („dopalacze") – from structure to clinical symptoms

12.45 – 13.45 Communications (C16, C17)

Session moderators: Anna Bielawska & Krzysztof Bielawski

C16

Dorota G. Piotrowska, Medical University of Lodz, Poland

„β-Lactam- and isoxazolidine-conjugates of quinazoline-2,4-diones"

C17

Anna Bogucka-Kocka, Medical University of Lublin, Poland

„*Modulation of multidrug resistance genes expression by coumarin derivatives in multidrug resistance human leukemic cells*"

13.45 – 15.15 Lunch

15.15 – 16.00 Lecture L6 (45')

Session moderators: Zofia Mazerska & Jarosław Sławiński

L6

Jadwiga Turło, Medical University of Warsaw, Poland

„*Structural characterization of immunoactive polysaccharides - methodological problems*"

16.00 – 17.00 Communications C18 – C20 (20')

Session moderators: Zofia Mazerska & Jarosław Sławiński

C18

Beata Morak-Młodawska, Medical University of Silesia, Sosnowiec, Poland

„New derivatives of dipyridothiazines - synthesis, structural properties and anticancer activities"

C19

Paulina Koczurkiewicz, Jagiellonian University, Cracow, Poland

„Piperlongumine as a multifunctional agent modulating doxorubicin activity”

C20

Krzysztof Marciniec, Medical University of Silesia, Sosnowiec, Poland

„Synthesis and in vitro antiproliferative activity of acetylenic quinoline derivatives”

17.00 – 17.30 Coffee break

17.00 – 20.00 Poster session

17.30 – 20.00 Poster oral presentations PP1 – PP18 (7’)

Session moderators: Monika Wujec & Marcin Mączyński

PP1

Aneta Kaczor, Jagiellonian University, Cracow, Poland „Dimroth rearrangement in the search for new agents effective in the battle against MDR bacteria”

PP2

Marek Grosicki, Jagiellonian University, Cracow, Poland

„Histamine H4 receptor ligands - their in vitro study on eosinophils adhesion to endothelium”

PP3

Krzysztof Szafrański, Medical University of Gdańsk, Poland

„Novel 5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives: synthesis and in vitro anticancer studies”

PP4

Wojciech Szymanowski, Medical University of Bialystok, Poland

„Biological studies of novel piperazine derivatives in MCF-7 and MDA-MB-231 breast cancer cells”

PP5

Natalia Pawłowska, Medical University of Białystok, Poland

„The cytotoxic activity of novel isoquinoline derivatives in human breast cancer cells”

PP6

Anna Czajkowska, Medical University of Bialystok, Poland

„The cytotoxic efficacy of Nigella sativa seed extract in CRL-1739 human gastric adenocarcinoma cells”

PP7

Trimen Chemicals

PP8

Agata Tarczykowska, Collegium Medicum in Bydgoszcz, Poland

„Enantioselective acetylation of racemic atenolol with the use of lipases in native form”

PP9

Katarzyna Węgrzynowska-Drzymalska, Nicolaus Copernicus University in Toruń, Poland

„Synthesis of magnetic nanoparticles with surface modified with chitosan and poly(acrylic acid) blends for biomedical application”

PP10

Adam Sikora, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland

„The use of Candida rugosa lipase immobilized onto magnetic nanoparticles in enantioselective acetylation of (R,S)-atenolol"

PP11

Anita Płazińska, Medical University of Lublin, Poland

„Activation of the toggle switch and dynamic network of the β_2-adrenergic receptor"

PP12

Jakub Grynda, Gdańsk University of Technology, Poland

„Model free quantitative analysis of three component equilibria"

PP13

Tomasz Laskowski, Gdańsk University of Technology, Poland

„NMR and MD studies on the stereochemistry of the intercalation complexes of imidazoacridinone C-1311 (Symadex), a potential anticancer drug, with short fragments of dsDNA"

PP14

Vittorio Canale, Jagiellonian University, Cracow, Poland

„Towards metabolically stable arylsulfonamide derivatives of (aryloxy)ethyl piperidines as potent and selective 5-HT_7 receptor antagonists with antidepressant and anxiolytic properties"

PP15

Annamaria Lubelska, Jagiellonian University, Cracow, Poland

„Characterization of permeability and hepatotoxicity of the selective serotonin 5-HT_7 receptor ligands in vitro"

PP16

Monika Marciniak, Medical University of Warsaw, Poland

„Synthesis of new pyrolidine-2,5-dione derivatives with a long-chain arylpiperazine moiety, ligands of 5-HT_{1A} receptor"

PP17

Justyna Kalinowska-Tłuścik, Jagiellonian University, Cracow, Poland

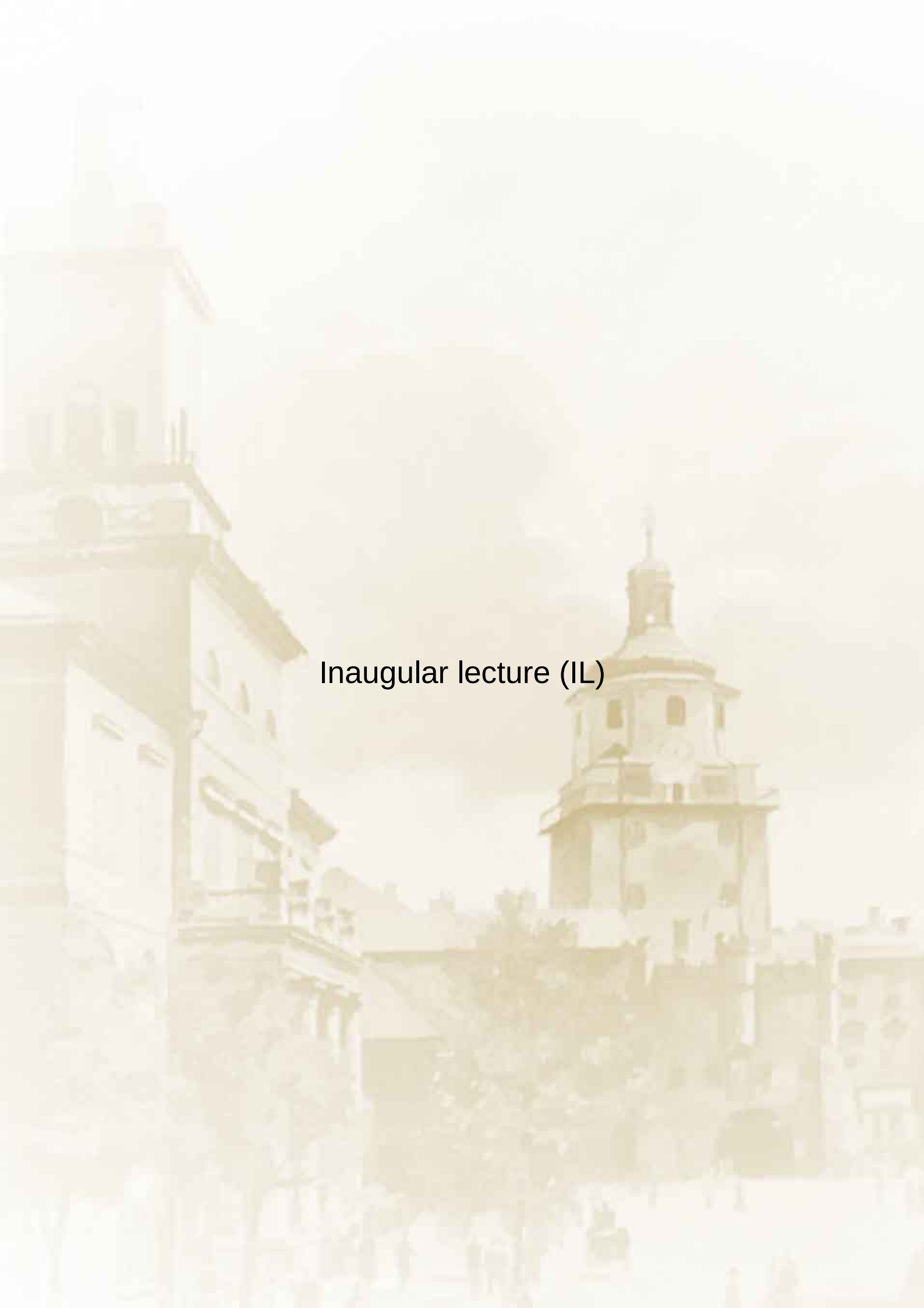
„Non-basic antagonists of the 5-HT6 receptor - a structure-activity relationship study"

PP18

Katarzyna Grychowska, Jagiellonian University, Cracow, Poland

„Novel 1H-pyrrolo[3,2-c]quinoline derivatives as 5-HT_6 receptor antagonists with procognitive properties"

20.30 – 24.00 Open air event



Inaugular lecture (IL)

IL

Novel multitarget drug candidates that are effective in an animal model of Alzheimer's disease

Diego Muñoz-Torrero

Laboratory of Pharmaceutical Chemistry (CSIC Associated Unit), Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028, Barcelona, Spain

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Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, is reaching epidemic proportions with population ageing, which aggravates its health and economic burden. Worryingly, the development of drugs that can cure, prevent, or delay this disease remains elusive. In the past decade, huge efforts have been made to develop drugs purported to tackle the underlying mechanisms of AD, by hitting mainly the formation or aggregation of β-amyloid peptide (Aβ), widely regarded as the main culprit of the neurodegenerative process of AD. Unfortunately, a number of clinically advanced Aβ-directed drug candidates failed to show efficacy in AD patients, which could be ascribed to the fact that AD pathology might not result from a straightforward process but from a complex pathological network where Aβ is not *the* cause but just *one* of the causes, together with protein tau hyperphosphorylation and aggregation, aberrant neuronal activity, synaptic dysfunction, or neuroinflammation, among others. In this context, modulation of a single target, even if it is Aβ, might result ineffective, whereas simultaneous modulation of several targets of the AD network would be a more realistic option to derive effective disease-modifying drugs.

Here, some recent work of our group on different structural classes of anti-AD compounds will be presented. After having demonstrated potent *in vitro* activities against multiple biological targets, chronic *in vivo* efficacy studies in APP/PS1 mice, a well-established mouse model of AD, have confirmed the ability of these compounds to rescue transgenic mice from amyloid pathology, Aβ-induced neuroinflammation, aberrant neuronal network activity, and cognitive deficits, thereby emerging as promising anti-Alzheimer drug candidates with potential to prevent, delay or halt the neurodegenerative process of this disease [1-3].

[1] Sola, I.; Aso, E.; Frattini, D.; López-González, I.; Espargaró, A.; Sabaté, R.; Di Pietro, O.; Luque, F. J.; Clos, M. V.; Ferrer, I.; Muñoz-Torrero, D. *J. Med. Chem.* 2015, *58*, 6018□6032.

[2] Viayna, E.; Sola, I.; Bartolini, M.; De Simone, A.; Tapia-Rojas, C.; Serrano, F. G.; Sabaté, R.; Juárez-Jiménez, J.; Pérez, B.; Luque, F. J.; Andrisano, V.; Clos, M. V.; Inestrosa, N. C.; Muñoz-Torrero, D. *J. Med. Chem.* 2014, *57*, 2549–2567.

[3] Serrano, F. G.; Tapia-Rojas, C.; Carvajal, F. J.; Cisternas, P.; Viayna, E.; Sola, I.; Muñoz-Torrero, D.; Inestrosa, N. C. *Curr. Alzheimer Res.* 2016, in press.

Lectures L1 – L6

L1

Membrane barrier and drug translocation: A permeability challenge to combat bacterial resistance

Jean-Marie Pagès

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With the continuing emergence of bacterial multidrug resistance, a molecular dissection of the membrane transport associated to cellular imaging analysis is needed to understand the uptake and the activity of antimicrobial agents in bacterial cells. This is particularly acute for Gram-negative bacteria that have two membranes, outer and inner membranes, controlling the transport and the intracellular accumulation of antibiotics.
The permeation process was followed within bacterial population and at single bacteria level to investigate the antibiotic concentration/location in multi-drug resistant isolates and derivative strains. In parallel, antibacterial activities were determined on same bacterial strains in order to correlate the intracellular accumulation of antibiotic to the bacterial susceptibility.
With new original methodologies the uptake/location of fluorescence antimicrobial agents can be followed and studied in bacterial population and individual bacterial cell. We can analyze the respective involvement of influx and efflux in the internal concentration of various molecules and in the bacterial susceptibility.
Combination of activity determination and image analyses open a new field of research in the molecular understanding of resistance mechanisms and in the rational approach of antibacterial chemotherapy.

L2

Disrupting the PEX14-PEX5 interaction by small molecules provides a novel therapeutic strategy for treatment of *Trypanosoma* infections

Maciej Dawidowski, Grzegorz Popowicz, Michael Sattler

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Trypanosoma infections remain the major parasitic burden in Latin America and in sub-Saharian Africa. The most frequent human diseases related to this genus are the sleeping sickness and the *Chagas* disease, caused by *Trypanosoma brucei spp*. and *Trypanosoma cruzi*, respectively. The chemotherapy approaches to control these parasitic infections remain unsatisfactory and there is a need for development of novel treatments[1,2]. *Trypanosoma* parasites couple glycolytic and detoxifying enzymatic processes in a single organelle, the glycosome. As this organelle completely lacks genetic information, all the lumen-active enzymes need to be translocated post-translationally. PEX14-PEX5 protein-protein interaction is postulated to play a crucial in this pathway[3]. Consequently, inhibiting this interaction has been proposed a potential way of disrupting glycosome function[4,5], leading to an accumulation of glycosomal enzymes in the cytosol, which in turn causes run-a-way phosphorylation of hexoses, ATP depletion and fatal metabolic catastrophe. In our multidisciplinary approach, we combined *in silico* screening, NMR, X-ray crystallography and medicinal chemistry to develop *first-in-class* PEX14-PEX5 inhibitors with nanomolar trypanocidal activities against clinically relevant *T. brucei* and *T. cruzi* species. In this lecture, a complete research workflow leading to a *proof-of-concept* is presented.

[1] Brun, R. et. al. *Lancet* 375 (2010) 148–159.
[2] Perry, B. et. al. *Science* 315 (2007) 333–334.
[3] Neufeld, C. et. al. *EMBO J.* 28 (2009) 745–754.
[4] Furuya, T. et. al. *PNAS* 99 (2002) 14177–14182.
[5] Choe, J. et. al. *Biochemistry* 42 (2003) 10915–10922.

Acknowledgements:
This work was supported by the Deutsche Forschungsgemeinschaft (FOR1905, ER 178/4-1), and by the EU Horizon 2020 (2014-2020) framework program under the Marie Skłodowska-Curie Grant Agreement No. 675555, AEGIS, and by the Helmholtz portfolio and cross-program activities “Drug discovery” and “Metabolic Dysfunction”.

L3

New hybrid anticonvulsants derived from the pyrrolidine-2,5-dione scaffold with broad spectrum of activity in the preclinical studies

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The multifunctional ligands approach is a novel strategy in the field of drug discovery for diseases with complex pathomechanism [1,2]. According to this approach different molecules with given biological properties are combined in a single chemical entity to provide complex and broad activity. To generate more efficacious antiepileptic drugs (AEDs), which will suppress different types of human seizures, we obtained new hybrid anticonvulsants derived from the pyrrolidine-2,5-dione scaffold. These hybrid molecules join the chemical fragments of clinically relevant AEDs (e.g., ethosuximide, levetiracetam, lacosamide). The hybridization process generated substances effective in three the most important animal seizure models, namely the maximal electroshock (MES) test, the subcutaneous pentylenetetrazole (*sc*PTZ) test, and the six-Hertz (6 Hz) model of pharmacoresistant limbic seizures in mice. These substances displayed wider spectrum of protection, more potent efficacy or/and better safety profile than aforementioned AEDs. Additionally, several compounds diminished the pain responses in the formalin model of tonic pain and notably in the neurogenic pain models (capsaicin-induced nociception and oxaliplatin-induced neuropathy) in mice. The *in vitro* binding studies showed that the most plausible molecular mechanism of anticonvulsant and antinociceptive action was the influence on the neuronal voltage-sensitive sodium and L-type calcium channels.

[1] Morphy, R. et al. *J. Med. Chem.* 48 (2005) 6523–6543.
[2] Bansal, Y. et al. *Eur. J. Med. Chem.* 76 (2014) 31–42.

Acknowledgments:
The studies were supported by the Polish National Scientific Centre grant DEC–2012/05/D/NZ7/02328.

L4

New directions in the search for therapies of behavioral and psychological symptoms of dementia

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Dementia affects 5% of people aged over 65, and half of those aged over 85 years old. Since the world's population is aging rapidly, it is predicted that by the year 2050, 115 million people will suffer from this condition. In the elderly, the most common cause of dementia is Alzheimer's disease. Dementia patients experience serious cognitive deficits but up to 90% of them also show behavioral and psychological symptoms (BPSD). The spectrum of BPSD is diverse and includes: psychosis, depression, anxiety, verbal and physical aggression, agitation, irritability, wandering etc. [1].
Antipsychotic drugs are a mainstay of psychopharmacological treatment of BPSD. However, these medications display only partial therapeutic efficacy and can also cause further cognitive impairment. In addition, long-term therapy with currently available antipsychotics can cause side effects including: weight gain and metabolic dysfunction, extrapyramidal syndrome (EPS), QTc interval prolongation, sedation and anticholinergic effects. Consequently, antipsychotic drugs are not currently approved for the treatment of BPSD and there is an urgent need to seek the effective and safe therapies for this condition [2].
The present lecture describes the complex approach to address the issue of BPSD at a preclinical drug discovery level. A problem of establishing an efficient screening process, developing novel models of a drug discovery relevance, as well as selecting suitable molecular targets is discussed. In that context, a relevance of a Designed Multitarget Ligands (DMLs) approach is analyzed. An example of such an approach is a "5-HT6 plus platform", collecting ligands being the 5-HT6 receptor antagonists and at the same time targeting other selected receptors/transporters, that could address both cognitive and non-cognitive symptoms of dementia. Development of two groups of lignads from the 5-HT6 plus platform, based on the arylsulfonamide scaffold is presented, from the computer-aided design to the advanced animal studies [3,4]. The achievements and limitations of the approach used are discussed in the context of novel drug discovery process.

[1] Petrovic, M. et. al. *Acta Clin. Belg.* 62 (2007) 426−432.
[2] Jeste, D.V. et al. *Neuropsychopharmacol*. 33 (2008) 957−970.
[3] Kolaczkowski, M. et. al. *J. Med. Chem.* 57 (2014) 4543−4557.
[4] Kolaczkowski, M. et. al. *Eur. J. Med. Chem* 92 (2015) 221−235.

Acknowledgements:
Supported by the National Centre for Research and Development grant nr KB/88/12655/IT1-C/U/08.

L5

Novel psychoactive substances („dopalacze") – from structure to clinical symptom

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Over the last decade, and particularly during the recent five years, a rapidly increasing number of novel psychoactive substances (NPS), often marketed as "designer drugs", "legal highs", "herbal highs", "research or intermediate chemicals", "laboratory reagents", and "dopalacze" in Poland, has appeared on the drug market in an effort to bypass controlled substance legislation. NPS encompass a wide range of compounds including, among others, tryptamines, phenethylamines, piperazines, pirrolidines, benzodiazepines, aminoindanes and benzofuranes.

Based on the spectrum of their actions on cognitive processes, mood, and behavior, 'NPS can be classified into six basis categories: amphetamine- and ecstasy-like stimulants, synthetic cannabinoids, hallucinogenic/dissociative, opioid-like compounds, sedative/anxiolytics, and others. NPS may, however, exhibit a combination of these actions due to their designed chemical structure.

The European Union Early Warning System notified the appearance of more than 550 new psychoactive substances during the period of May 2005 – December 2015. Importantly, two new substances were detected every week in 2014 and 2015. It is estimated that the number of NPS reported to the United Nations Office on Drugs and Crime was 2.3-times higher than the number of controlled psychoactive substances.

NPS are sold as "legal/herbal highs", "bath salts", "plant food", "insect repellents", "research chemicals", "air fresheners", "computer's or jewerly cleaners", with the disclaimer: "not for human consumption", "not tested for hazard and toxicity", "protect from children" or "for research purposes only". Despite of these warnings, however, NPS are advertised as compounds that mimic effects of narcotics.

Adverse effects of NPS include a vast array of symptoms. In addition to psychiatric (i.e. irritability, anxiety, panic attacks, lack of motivation, anhedonia, depression, agitation, dysphoria, paranoid delusion, visual and auditory hallucination, aggression that progressed sometimes to violent or even criminal behavior, self-destructive behavior, suicidal ideation and self-mutilation) and neurological (disturbed sleep patterns and nightmares, insomnia, tremors, seizures, hyperthermia, mydriasis, blurred vision, paresthesias, bruxism, motor automatisms, headache and dizziness) symptoms, these compounds could evoke sinus tachycardia, heart palpitations, chest pain, hypertension, S-T segmental changes, cardiac arrest, disseminated intravascular coagulation, rabdomyolysis, acute kidney and liver failure, and fatal multiorgan failure. Potent agitation and aggressive behavior often pose a serious treat not only for a person being under the drug's influence but also to other people.

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Supported by the grant No 2014/13/8/B/NZ7/02237 from the National Science Centre, Cracow, Poland.

L6

Structural characterization of immunoactive polysaccharides – methodological problems

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Several high molecular weight polysaccharides isolated from the natural sources (bacteria, fungi, plants) or obtained by biotechnological methods display anticancer activity. In most of these compounds it results from their immunostimulatory properties [1]. Based on the mechanism of their pharmacological activity, they are classified as biological response modifiers (BRM). Although the relationship between their activity and the chemical structure has been extensively investigated in the past three decades, results of these studies are often ambiguous [2,3]

Among the most commonly described factors affecting immunomodulatory activity of the polysaccharides are: monosaccharide composition, water solubility, polarity, molecular weight, degree of branching and triple-helical structure. Determination of the first three of these factors is relatively simple and gives conclusive results. Examination of the last three, however, causes considerable methodological problems and the acquired results may not be completely reliable.

The polysaccharide fractions obtained by biosynthesis are often subjected to structural modifications in order to improve their pharmacological activity or pharmacokinetic properties. Uncertainty of the data regarding the structure of the molecules considerably hinders the task.

This presentation covers our experiences in the field, resulting from long-term research on immunoactive polysaccharides.

[1] Leung M, Liu C, Koon J, Fung. Immunology Letters 105: 101–114 (2006)
[2] Zhang L, ***Li X,*** Xu ***X*****,** Zeng F. Carbohydr Res 340(8):1515–1521 (2005)
[3] Zhang M., Cui S, Cheung P, Wang Q. Food Sci. Technol. 18: 4–19 (2007)

Acknowledgement:
This work is supported by grant from the Polish National Science Centre DEC-2013/09/B/NZ7/03978

Communications C1 – C20

C1

The application of *Click chemistry* methods in search of cannabinoid activity

Przemysław Szafrański,[a] Anna Stasiewicz-Urban,[a] Irena Smaga,[b] Lucyna Pomierny-Chamioło[b] Małgorzata Filip,[b,c] Tapio Nevalainen,[d] Marek Cegła[a]

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The recent advancement in knowledge of the endocannabinoid system produces both, numerous answers and questions, as well as therapeutic opportunities[1,2]. The discoveries concerning allosteric CB receptor modulators[3] and peptide ligands[4] partially explain the diversity and complexity in the modes of action observed for different groups of cannabinoids, but they also support the interest in further search for novel cannabinoid ligands of various structures, which could serve as pharmacological tools or find therapeutic application.

The idea of *Click chemistry* proposed in 2001[5], has already anchored in the minds and laboratories of many pharmaceutical researchers, which produced numerous valuable results. It also brought the copper-catalyzed azide-alkyne cycloaddition as a useful chemical tool for compound synthesis and various types of biochemical research[6].

In this contribution, the synthesis of novel bicyclic 1,2,3-triazole derivatives is presented, and its optimization from a “classical” protocol similar to the one devised by Sharpless and Fokin[7] towards a cleaner and more efficient alternative developed in our laboratory[8]. Furthermore, the results of cannabinoid receptor affinity studies are presented and discussed.

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C2

Ring inversion properties of hexopyranoses: from force field parametrization to the interplay between ring shape and glycosidic linkage conformation

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Carbohydrates, including O- and N-glycans attached to protein and lipid structures, are increasingly being studied in cellular biology. However, the structural data related to carbohydrate in solution are often incomplete, leaving gaps that may be filled by molecular modeling techniques. Recently, we have developed a revised version 56A6$_{CARBO_R}$ of the GROMOS 56A6$_{CARBO}$ force field for hexopyranose-based carbohydrates [1]. This force field has been designed with a special emphasis put on correct reproducing the ring-inversion properties which creates an opportunity to consider this degree of freedom in the context of carbohydrate chains of arbitrary length, anomery and glycosidic linkage type.

The ring-inversion properties of the residue in a chain reflect the corresponding properties of an unfunctionalized monomer only in the case of chains with 1→6 linkages [2]. In such a case the systematic shift (positive for α- and negative for β-anomers) of the ring-inversion free energies is observed (in analogy with the findings for O_1-methylated hexopyranoses [1]) in comparison to the values for unfunctionalized monomers. In the case of remaining 1→2 and 1→3 linkages, inserting residue in a chain alters the ring flexibility in a non-systematic fashion (in analogy with 1→4-linked oligomers [1]). The type of the glycosidic bond has also a large effect on the correlation of the ring shape with the preferred orientation of the glycosidic linkages: the ring conformation has the littlest influence on the conformation of 1→6 linkages and much larger for 1→2 and 1→3 linkages. Contrary to the case of 1→4 linkages [3], the orientation of the glycosidic oxygen atoms and the related fluctuations of the residue-residue distance that occur upon ring inversion are not reflected by the changes in the conformation of 1→2, 1→3 and 1→6 linkages.

In general, out results suggest a significant extent of ring flexibility, *i.e.* small but often non-negligible equilibrium populations of inverted chairs (that may influence the overall shape of carbohydrate ring), and challenge the “textbook” picture of conformationally-locked carbohydrate rings.

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C3

GPCRDB homology models – “Less model & more crystal”

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Background
Although methods to determine protein 3D structures are improving, from the ~800 GPCRs we still only know the structure of 33 unique receptors. This prompts the need for receptor models that can capture the intricate structural characteristics of GPCRs. GPCRdb now introduces models of human non-olfactory GPCRs built on the principle of “less model & more crystal”. This employs a multi-template method that resulted in the best serotonin 5-HT_{1B} receptor model in the latest GPCR Dock assessment.

Materials and methods
The coverage from experimental structures is maximized, while *de novo* modeling is left as a last resort. Specifically, segments that are missing (e.g. loops, helix ends), non-representative (e.g. distorted, deleted or fused segments) or differ (e.g. TM helix bulges and constrictions) are replaced with more optimal local templates. Next, an in-house rotamer library is utilized that has been extracted from all GPCR structures and provides a specific rotamer for each position of the structure (by use of generic residue numbers). Finally, MODELLER is used to model regions where no template was available. The models are automatically updated in new GPCRdb releases, providing increased precision as new templates become available.

Results
Inactive models were built for 278 receptors from class A. All of them are accessible through the gpcrdb.org website along with information about the templates. Models for three recently determined structures were assessed along with corresponding models from other homology modeling services. Root-mean-square deviation calculations were used to compare the performance of the different services. Inactive models for classes B, C and F are in the works. Also, crystal structures were revised with the algorithm where distorted regions and segments with missing coordinates were modeled.

Conclusions
Our models exhibit a close approximation to the experimentally determined crystal structures mainly excelling in the 7TM RMSD comparison. The modeling of loops needs further improvement.

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C4

How to describe physicochemical interactions between dsDNA and possible chemotherapeutics

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One of the main paradigmat of medicinal chemistry assume that a good drug should interacts with its molecular target as strong as it is possible. There are several techniques to determine and quantitatively describe physicochemical reversible interactions between ligand (drug) and its molecular target. They are mainly dedicated to proteins as molecular target. However, in chemotherapy not only proteins but also nucleic acids are targets of the drugs. In such case scientists should solve several problems. Some of them will be presented in the lecture.

These problems could be divide to a few groups according to following questions: i) what is a model target of interaction, ii) sequence specific or total interactions, iii) structure of a complex or thermodynamics of its formation, iv) what is a source of signal that interaction occurs, v) screening of active ligands or determine properties of the known one.

There is no single technique that may be used to find even main set of information about particular ligand-DNA interaction. Thus, a proper scientific project on this field should *a priori* assumes precisely: questions answers for which are goals of the research, and ways (techniques) on which these answers might be obtained.

The lecture is based on literature data as well as on experience of our research group.

C5

Synthesis and biological activity of fluorescently-labeled quinolones

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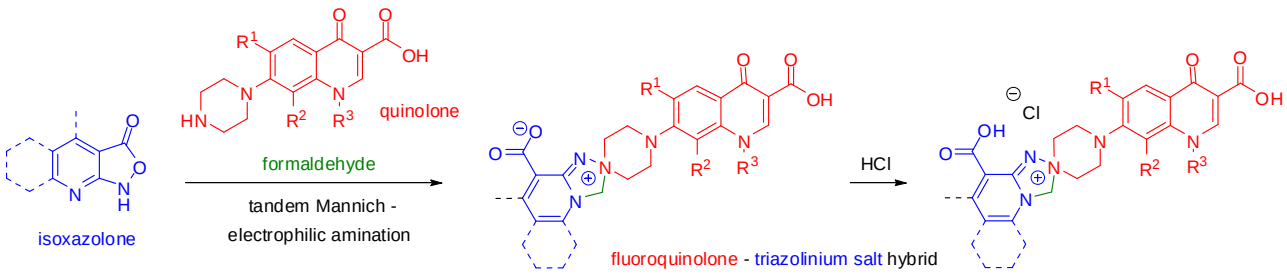
Antibiotic resistance is a particularly serious problem for patients whose immune systems are compromised, e.g. in post-transplant patients, people with AIDS and patients in critical care units. Persistent pathogens lead to higher health care costs because they often require more expensive drugs and extended hospital stays [1]. Fluoroquinolones are broad-spectrum antibiotics that play an important role in treatment of serious bacterial infections, especially hospital-acquired infections and others in which resistance to older antibacterial classes is suspected. Since the discovery of nalidixic acid over ten thousands analogues have been synthesized from which four generations of chemotherapeutics with broad spectrum of antibacterial activities have emerged [2]. Researchers in academia and pharmaceutical industry have pursued several approaches to identify new multitarget inhibitors. One of the most common strategies is dual action hybrid antibiotics.

The main objective of the project is to design and prepare a series new dual acting antibacterials incorporating a fluoroquinolone drug and a fluorescent triazolinium compound with proved antibacterial properties, aimed at evaluating the hypothesis that a new class of hybrid antibacterial agents can be obtained that exhibit an unique dual antimicrobial mechanism of action: (i) perturbation of the lipid bilayer of the bacterial cytoplasmic membrane and the outer membrane of Gram-negative bacteria due to presence of quaternary ammonium group, and (ii) inhibition of DNA gyrase / bacterial topoisomerase IV elicited by fluoroquinolone portion. Fluorescent properties of the hybrid agents should allow evaluation of their penetration into the bacterial cell and determination of antibiotic-bacteria binding efficiency.

We have modified a series of fluoroquinolones with use of isoxazolone profluorophores, as shown in scheme. MIC (Minimal Inhibitory Concentration) were determined using microbroth dilution method according to EUCAST and CLCI reference procedures.



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C6

The potential of halogen bonding in class A of GPCRs: application of XB hot spots for rational design of 5-HT$_7$R ligands

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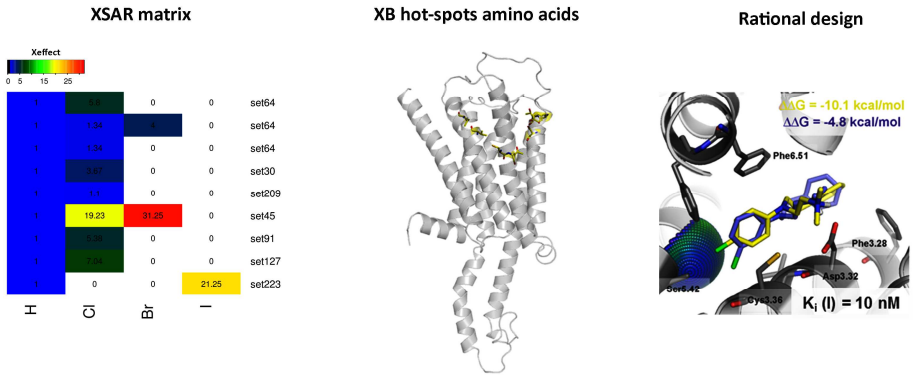
Since many years, halogen atoms have been regularly used in drug optimization processes, only recently their role in protein–ligand complexes has been attributed to the formation of a specific, direct interactions called halogen bonds. Incorporation of halogen atoms into molecule structure changes its steric (volumetric), electrostatic and conformational properties, lipophilicity (influencing membrane permeability and the oral absorption), and may lead to even 300-fold increase in the affinity for a given biological target [1, 2].



Herein we report on a systematic molecular modeling approach used to study the role of halogen atoms in the interaction of ligands with all crystallized receptors of family A GPCRs. The performed calculations distinguished several hot-spot amino acids, which were used to rational design/optimization of potent 5-HT$_7$R ligands.

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C7

Novel multi-target-directed ligands for Alzheimer's disease: combining cholinesterase inhibitors and 5-HT_6 receptor antagonists. Design, synthesis and biological evaluation

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Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by a progressive deterioration of memory and other cognitive functions. A majority of patients also experience behavioural and psychological symptoms of dementia, such as psychosis, depression, agitation and anxiety [1].
Such a wide range of symptoms results from a complex nature of the disease and is a real challenge for pharmacotherapy. Three out of four currently available drugs for AD are cholinesterase inhibitors (ChEIs), which diminish symptoms related to memory loss by increase of cholinergic transmission. These drugs are able only to retard the progression of memory loss with no improvement of the psychological symptoms.

Lack of effective therapy and increasing prevalence of dementia have prompted intensive search for novel drugs and new biological targets. In preclinical studies, 5-HT_6R antagonists proved to be effective in animal models of cognitive dysfunction [2], depression and anxiety [3]. Their therapeutic potential is now under investigation in several clinical trials. The most promising data were obtained in phase 2 clinical trials with the 5-HT_6 antagonist, (idalopirdine, Lu AE58054), which improved cognitive functions in patients with moderate AD treated with ChEI (donepezil) [4]. Combination therapy, however, is often burdened with high risk of adverse toxicological and pharmacokinetic interactions.

Considering potential advantages of a treatment with multi-target-directed ligands (MTDLs) over administration of a drug cocktail or a multicomponent drug [5] we aimed to obtain unique MTDLs combining cholinesterase inhibitory potency and 5-HT_6 receptor antagonism in a single molecule. The pro-cholinergic effect would in this case originate from the inhibitory potency towards acetylcholinesterase and butyrylcholinesterase and additionally from the 5-HT_6R-dependent release of acetylcholine. The 5-HT_6 receptor antagonism would also alleviate anxiety and depression. MTDLs with this combination of activities could significantly improve the quality of AD patients' life.

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C8

Anticonvulsant activity of some new *N*-[phenoxyalkyl]- and *N*-[2-(phenoxyethoxy)ethyl]aminoalkanols

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Epilepsy is a set of neurological disorders characterized by recurrent seizures. The disease concerns approximately 1% of human world population, and approximately 35% of seizures are considered pharmacoresistant, and this, when added to toxicity of antiepileptic drugs and their interactions, is premise for search of novel and safe therapies [1].

So far the most active in maximal electroshock seizures (MES, mice, *i.p.*) were (*S*)-(+)-2*N*-[(2,6-dimethylphenoxy)ethyl]aminobutan-1-ol hydrochloride (ED_{50}=7.57 mg/kg), [2] (*D,L*)-*trans*-2*N*-[(2,6-dimethylphenoxy)ethyl]aminocyclohexan-1-ol (ED_{50}=7.73 mg/kg), [3] (*R*)-2-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol (ED_{50}=5.34 mg/kg), [4]. (*R*)-2-[(2,6-dimethylphenoxy)ethoxyethyl]aminopropan-1-ol hydrochloride also proved activity (ED_{50}=12.92 mg/kg) [5].
The purpose of the present study was continuation of our former research, including effectiveness of *N*-[phenoxyalkyl]- and *N*-[2-(phenoxyethoxy)ethyl]aminoalkanols in relieving not only MES seizures (mice, *i.p.*) but also neurogenic pain. The most active compounds are 1-{2-[2-(2,3-dimethylphenoxy)ethoxy]ethyl}piperidin-3-ol hydrochloride (MES ED_{50}=68.71 mg/kg), *D,L-trans*-2-{2-[2-(2,3-dimethylphenoxy)ethoxy]ethyl}aminocyclohexan-1-ol (MES ED_{50}=32.87 mg/kg), as well as (*R,S*)-2-{2-[2-(2,4-dimethylphenoxy)ethoxy]ethyl}aminopropan-1-ol (MES ED_{50}=27.11 mg/kg). The compounds exhibit activity in phase II of the formalin test (mice, *i.p.*) and were evaluated for neurotoxicity in the chimney and in the rotarod tests. Influence on spontaneous activity (mice, *i.p.*) as well as maximal electroshock seizure threshold were also examined.

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C9

Biological activity of thiourea derivatives

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The simplest method of obtaining thiourea derivatives is reaction between amino group (NH_2) and isotiocyanate. Using this method we can obtain symmetrical and unsymmetrical 1,3-disubstitued thiourea. 1,3 –Disubstituted thiourea derivatives are associated with a broad spectrum of biological activities:

- antimicrobial (Gram-positive, Gram-negative bacteria, mycobacterium and *Candida* yeasts)
  - mechanism of activity
- inhibition of *S. epidermidis* biofilm formation
- cytotoxicity of thiourea derivatives
- activity on CNS
  - 5-HT_2 receptor affinity
  - docking studies
  - pharmacological evaluation
- disubstituted tiourea derivatives as a starting material to synthesis 1,3-thiazepine, tetrazole etc.
  - biological activity of heterocyclic derivatives of thiourea.

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C10

Synthesis and properties of new 1,2-benzothiazine derivatives as potential multitarget drugs with chemopreventive and analgesic activity

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Cancer is still a leading cause of death worldwide. Cancer treatment is more effective the sooner the disease is detected, but unfortunately most patients report to the doctor at an advanced stage of the disease. Recently there has been a noticeable increase in the level of resistance to cytostatics [1]. Taking all of this into account, it seems that the preventive approach (chemoprevention) would be a more effective solution in dealing with the curse of cancer.

Chemoprevention is the prevention of cancer and it is an action taken to reduce the risk of getting cancer. We have primary, secondary and tertiary prevention. The goal of primary prevention is to avoid the development of cancer. This includes maintaining a healthy lifestyle and avoiding exposure to known carcinogenic risks. The goal of secondary cancer prevention is to detect and treat precancerous conditions or early, asymptomatic cancer. Tertiary cancer prevention is addressed to people with a history of cancer and aims to prevent relapse [2].

The discovery in recent years of the relationship between cancer and inflammation has led to heightened interest in non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of cancer [3,4]. One of the NSAIDs, piroxicam, has demonstrated promising chemoprevention activity in the treatment of colon cancer. Unfortunately, it was withdrawn from further study because of its toxicity. It appears advisable to modify the structure of piroxicam to obtain safer drugs with chemopreventive and analgesic activity.

For this reason, we designed and synthesized a library of new 1,2-benzothiazine (piroxicam) derivatives. We examined their physico-chemical properties by means of spectral, chromatographic, crystallographic and thermal methods together with elemental analysis. Then we studied their analgesic and anti-inflammatory properties in an animal model (on mice and rats). A very important area of the study was to examine their toxicity, especially gastrotoxicity.

The most important were the chemopreventive tests. We examined cytotoxicity to cancer cells, sensitizing tumor cells to the antitumor drug doxorubicin, affecting the expression of proteins belonging to the ABC transporters and modulating the expression of apoptotic proteins for selected new 1,2-benzothiazine derivatives. A further element of the study was the examination of the ability of the 1,2-benzothiazines to inhibit the activity of COX-2. This is most important due to the fact that, in many cancers, we find the over-expression of COX-2, which correlates with poor prognosis.

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C11

Synthesis and activity of the salicylic acid ester of bakuchiol in psoriasisform keratinocytes and skin substitutes

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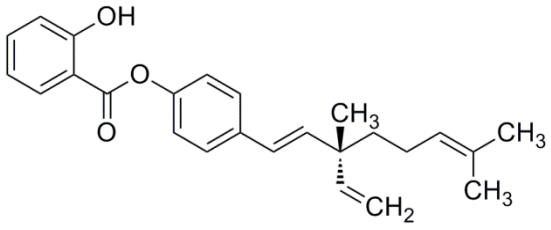
Topical retinoids are effective in retarding skin aging and restoring homeostasis in skin conditions, such as psoriasis [1]. However their adverse effects, which include irritation (retinoid dermatitis), photosensitivity and teratogenicity, limit their use level and patient compliance [2]. Development of a retinoid analogue without such limiting adverse effects would allow a broader and more compliant use. Here we report the synthesis of a novel retinoid functional analogue – bakuchiol salicylate (bakusylan). Bakuchiol salicylate was synthesized by the modified Steglich esterification method with DCC (dicyclohexylcarbodiimide) as condensing agent and DMAP (4-*N*,*N*-dimethylaminopyridine) as catalyst. The reaction was performed in DCM (dichloromethane) - a convenient solvent for Steglich esterification.



In vitro bioactivity of this molecule was explored using as reference materials 2 FDA psoriasis-approved retinoids (isotretinoin and tazarotene) as well as adapalene. Having a structure entirely different from existing retinoids, we hypothesized that at least a partial uncoupling of retinoid adverse effects from retinoid skin normalizing activity may be obtained with bakusylan. This hypothesis was tested in psoriasiform cultures of keratinocytes and organotypic skin substitutes. The results revealed the elimination of several components of the retinoid-like proinflammatory response and teratogenic signature without a substantial loss of normalizing potential, as determined by DNA microarrays and custom PCR arrays, among other techniques. A possible mechanism of action consisting in keratinocyte desensitization to cytokine signaling through the inhibition of the STAT1/3/interferon inflammatory signal transduction axis has also been identified.

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C12

Novel, selective 5-HT$_{1A}$ biased agonists as new pharmacological tools in the development of treatment of psychiatric and neurodegenerative disorders

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Serotonin 5-HT$_{1A}$ receptors are attractive targets for pharmacotherapy of pathologies associated with dysfunctional serotonergic neurotransmission, including anxiety, depression, Parkinson's disease, pain and schizophrenia. These receptors are expressed both as presynaptic autoreceptors on serotonergic cell bodies in the raphe nuclei and as postsynaptic heteroreceptors in multiple brain regions [1]. 5-HT$_{1A}$ receptor subpopulations in different brain regions are coupled to distinct intracellular signaling cascades and can be preferentially targeted by pharmacological agents [2,3]. The prototypical 5-HT$_{1A}$ biased agonists, have been shown to exhibit unique signal transduction and profiles in *in vivo* tests, unlike those of other 5-HT$_{1A}$ agonists. Notably, F15599 preferentially targets post-synaptic cortical 5-HT$_{1A}$ receptors leading to potent activity in models of mood deficit and improved cognitive function, while F13714 preferentially targets pre-synaptic 5-HT$_{1A}$ autoreceptors in raphe nuclei, displaying a robust anti-diskinesia effect [2,4]. Unfortunately, most of the existing substances activating 5-HT$_{1A}$ receptors are poorly adapted to probing the activity of receptor subpopulations. Our team, based on the structure of two known 5-HT$_{1A}$ biased agonists – F15599 and F13714 and molecular modeling, has designed and synthesized a series of novel strong and selective 5-HT$_{1A}$ agonists with potential functional selectivity. A series of novel compounds was tested for affinity for the 5-HT$_{1A}$ receptors as well as selectivity against basic antitargets (figure 1). The compounds with high affinity and selectivity were tested on three functional measures of agonist efficacy: ERK1/2 phosphorylation (pERK), adenylyl cyclase (cAMP) inhibition and calcium mobilization (aequorin test). Most of the compounds showed a very high affinity for the 5-HT$_{1A}$ receptor (pKi 8.8-10) and at least 1000-fold selectivity against the alpha1 and D$_2$ receptors (pK <6). Functional assays showed, that almost all of the tested compounds have significant agonistic activity (EC50 7-9, Emax 80-100%). Moreover, several compounds demonstrated different selectivity to intracellular signaling pathway. One of the new molecules (compound **1**) was tested in Porsolt test in rats and showed antidepressant activity at a very low dose (minimal effective dose 0.16 mg/kg p.o., ED50 = 0.3 mg/kg p.o.), exhibiting a very strong effect (total reduction of immobility in a dose of 2.5 mg/kg p.o.) (figure 1).

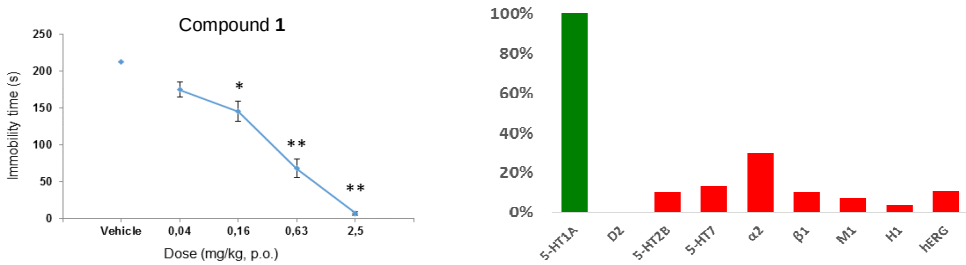

Figure 1. Antidepressant-like activity of the compound 1 in the Porsolt test in rats (on the left). Percent of binding for compound 1 to 5-HT$_{1A}$ receptors and antitargets in 1µM concentration (on the right).

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C13

Glucuronidation of antitumor agents: the way to drug resistance?

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Enzymatic system of II phase drug metabolism in the living organism is necessary to transform nonpolar exogenous compounds, including therapeutic agents, into their hydrophilic derivatives. By this way the products have been protected against the undesired accumulation in cells and tissues. Conjugation of uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) with the functional group of aglycone (hydroxyl, amine, carboxyl, sulfhydryl) catalysed by UDP-glucuronyltranferases (UGTs) are crucial among phase II metabolic pathways. The first biological function discovered for UGTs was deactivation of endogenous substrates (bilirubin, bile acids, lipid acids, steroid and thyroid hormones and lipid soluble vitamins) to maintain their balance in the organism. The main exogenous UGTs substrates are environmental pollutants, carcinogenic compounds but also the numerous groups of therapeutic agents. Being design as detoxification pathway glucuronidation is suspected to be the reason of antitumor drug resistance.

UGT-mediated drug resistance can be associated with: (i) inherent overexpression of the enzyme, named intrinsic drug resistance or (ii) induced expression of the enzyme, named acquired drug resistance observed when enzyme expression is induced by the drug or other factors, as food-derived compounds. This induction can be mediated by ligand binding receptors as AhR (aryl hydrocarbon receptor) PXR (pregnane X receptor), or other transcription factors. The effect of UGT dependent resistance may be strengthen by coordinate regulation of the expression of UGTs and ABC transporters. This coupling of UGT and multidrug resistance proteins has been intensively studied for antitumor drug action, because it is the case when this resistance is "improved" by differences in UGT expression between tumor and healthy tissue. The detoxification role of glucuronidation is well known for irinotecan, etoposide and epirubicin antitumor drugs, whereas tamoxifen, breast cancer therapeutic agent is activated by N-glucuronidation. The studies on acridinone antitumor agents developed in our group indicated that UGT isoenzymes could be involved in the observed resistance of cancer cells to these drugs. However, we also demonstrated the unexpected feature for acridinone C-1311 compound: its glucuronide expressed higher cytotoxicity than native compound.

Acknowledgements:
This study was supported by grant OPUS5, 2013/09/B/NZ3/00003 from the National Science Center, Poland

C14

Porphyrinoids functionalized with 1,4,7-trioxanonyl and imidazole moieties as promising photosensitizers

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Photodynamic therapy (PDT), photodynamic inactivation of bacteria and viruses (PACT) are a relatively new therapeutic approach that have been applied successfully against tumors and dermatological lesions [1], like psoriasis [2], and age-related macular degeneration [3]. The photodynamic reaction involves the cooperative action of three components: light, oxygen and the photosensitizer leading to the cytotoxic effects on subcellular organelles and molecules, and tumor vasculature, and to complex inflammatory and immune responses. Very promising group of photosensitizers are cyclic, aromatic compounds - porphyrinoids. Natural porphyrinoids are involved in oxygen transportation and photosynthesis (haem, chlorophyll). Artificial analogues such as phthalocyanines are applied in photodynamic diagnosis (PDD), energy production (solar cells), electronics (laser printers, LCD displays, switches, recordable CDs). The main objective of our work was modification of porphyrinoids resulting in improved solubility in water and retain solubility in lipids . This goal we achieve thanks introduction into structure of photosensitizer 1,4,7-trioxanonyl groups. Conjugation porphyrinoids with molecules of its own therapeutic activity gave a chance to improve activity. We decided to link photosensitizers with nitroimidazole compounds - applied in anaerobic bacteria and protozoa infections, in diagnostics of tissues in state of hypoxia and as radiosensitizers. In the course of work we obtain active photodynamic compounds such as [1,4,8,11,15,18,22,25-octakis(1,4,7-trioxanonyl)phthalocyanine]cooper(II) (**1**) and {1,8,18,25-Tetrakis[2-(2-methyl-5-nitro-1H-imidazol-1-yl)etoxy]phthalocyanine}magnesium(II) (**2**) (Fig.1). Compound **1** irradiated with light at λ_{max}= 735 nm reveal promising values of IC_{50} (line CEM 0.13±00.3 µM, HeLa 0.38±0.29 µM). Conjugate **2** encapsulated in (phosphatidylglycerol:1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) liposomes and irradiated with light at λ_{max}= shown in concentration 1.0 µM shown 100% photokilling of cultured cells (HSC-3).

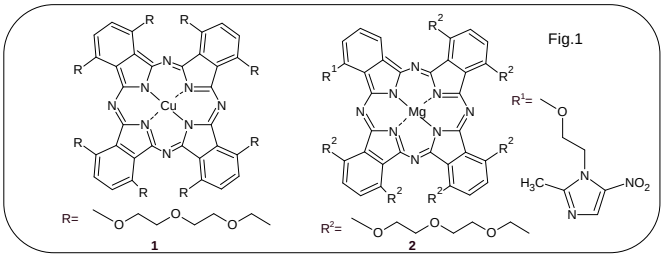

Fig.1

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Acknowledgements:
This study was supported by the grant Polish Ministry of Science and Higher Education/the National Scientific Centre (N N401 067238).

C15

Novel 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives - synthesis and QSAR studies of compounds with prominent anticancer activity

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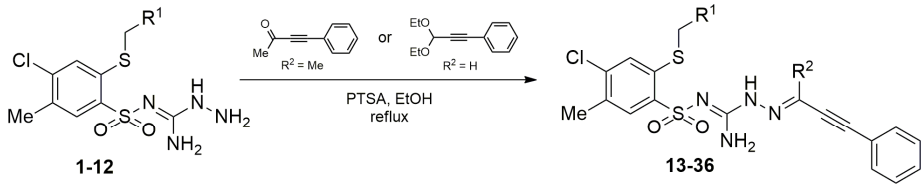
According to the search for new anticancer benzenesulfonamides [1-2], new 2-(2-alkylthiobenzenesulfonyl)-3-(3-phenylprop-2-ynylideneamino)guanidines **13**-**36** have been synthesized via reaction of 1-amino-2-(benzenesulfonyl)guanidine **1**-**12** with the appropriate carbonyl derivative.



14 R^1 = Ph, R^2 = Me
31 R^1 = 4-ClPh, R^2 = H
32 R^1 = 4-ClPh, R^2 = Me

The *in vitro* cytotoxic activity of the derivatives **13**-**36** was evaluated against human cancer cell lines HCT-116, MCF-7 and HeLa. The compounds **31** and **32** with 4-chlorobenzylthio as the R^1 moiety display the most prominent anticancer effects with IC_{50} values in the range of 6-18 μM for **31** and 8-14 μM for **32** SAR efforts in this study suggest that high electronegativity of substituent incorporated at methylenethio group (R^1) is mportant for their anticancer activity. Additionally, for prominent anticancer activity hydrogen atom is the preferable substituent attached at the imine carbon atom (R^2).

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C16

β-Lactam- and isoxazolidine-conjugates of quinazoline-2,4-diones

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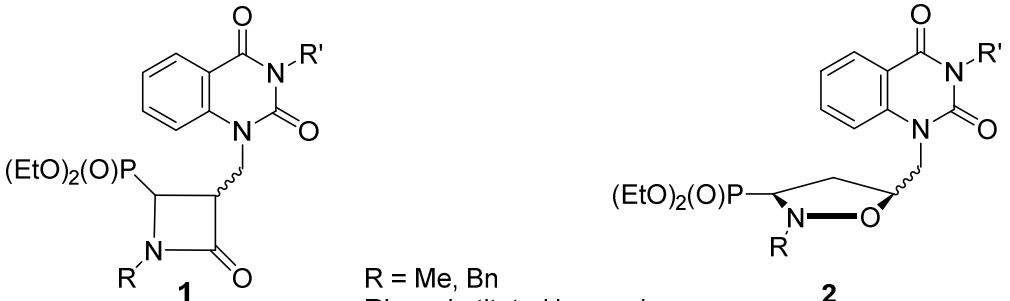
Herpes viruses are widespread among humans and may cause many diseases. Primary infection usually followed by a long-term latency of the virus and its reactivation is expected to occur especially during immunosuppresion. Infection with VZV results in varicella, usually with a mild course in children but more serious in adults. After latency in neural tissues, virus reactivates causing herpes zoster which is often accompanied by neuralgic pain and other complications [1,2]. Although four drugs are currently licensed for the treatment of VZV infections, namely acyclovir, valacyclovir, famciclovir and brivudine, immunosuppresed individuals often do not respond well to the therapy due to mutations of the virus. Therefore, extensive search for new anti-VZV agents is of high importance.
We designed new series of β-lactam- (**1**) and isoxazolidine-conjugates (**2**) of quinazoline-2,4-diones decorated at N3 of the quinazolinedione skeleton with either substituted benzoyl or benzyl residues and their antiviral activity was screened.



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Acknowledgements:
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C17

Modulation of multidrug resistance genes expression by coumarin derivatives in multidrug resistance human leukemic cells

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The phenomenon of multidrug resistance caused by overexpression of ABC drug transporters in cancer cells confers cross-resistance to a multitude of drugs and presents significant obstacle limiting the effectiveness of cancer chemotherapy. In recent years, a number of natural, plant-derived compounds have been found to inhibit proliferation, induce apoptosis, suppress angiogenesis, retard metastasis and enhance chemotherapy, exhibiting anti-cancer potential both in vitro and in vivo. Many researchers pointed to the use of natural products as inhibitors of multi-drug resistance, often call them "fourth generation modulators".
The coumarins are secondary plant metabolites that are characterized by an enormous structural diversity. They have very diverse mechanisms of action. The biological activity is determined by their lactones' structure, whereas the pharmacological properties are determined by the structure of compounds.

These studies led to the total analysis of the impact of coumarins derivatives to reverse drug resistance in the five human leukemic cell lines via modulating of multidtug resistance genes expression. In a continuing search for potent and selective cytotoxic coumarin derivatives as antitumor agents, we analyzed 20 coumarin derivatives and evaluated their cytotoxic effects against human leukemic cells and the impact on *MDR1, MRP1, BCRP* and *LRP* genes expression.

C18

New derivatives of dipyridothiazines - synthesis, structural properties and anticancer activities

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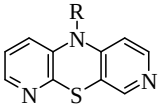
Phenothiazines are important class of heterocyclic compounds with wide spectrum of biological properties. Recent reports showed promising anticancer, antiplasmid, antibacterial, anti-inflammatory and immunosuppressive activities of classical and new phenothiazines [1,2]. Previously synthesized dipyridothiazine derivatives (1,6-, 1,8- and 2,7-diazaphenothiazines) were shown to possess interesting antiproliferative, anticancer, antioxidant and immunosuppressive activity correlating to some degree with their lipophilicity [3-5]. In continuation of our search we obtained new derivatives of dipyrido[1,4]thiazines being 3,6-diazaphenothiazines. As the thiazine ring formation may proceed through the Ullmann cyclization or Smiles rearrangement the crucial problem was the structure analysis of the products which was solved with the help of the 2D NMR experiments and X-ray analysis.



R = H, alkyl, aryl, heteroaryl, dialkylaminoalkyl

For those compounds, the anticancer action on selected tumor lines (MCF-7, SNB-19, C-32) was investigated. The compounds exhibited differential inhibitory activities but two compounds were more active (IC_{50} = 0.4 μg/mL) than cisplatin. For the most active compounds the expression of *H3, TP53, CDKN1A, BCL-2* and *BAX* genes was detected by the RT-QPCR method. The gene expression ratio BACL-2/BAX could suggest the mitochondrial pathway of apoptosis.

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C19

Piperlongumine as a multifunctional agent modulating doxorubicin activity

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Doxorubicin (DOX) is anthracycline antibiotic used in cancers therapy. Despite a great efficacy against broad spectrum of neoplasms, its application is limited by adverse effects – especially cardiotoxicity[1]. Mechanism of cardiotoxicity is still not fully understood, however it is associated with reduction of DOX to cardiotoxic metabolite – doxorubicinol[2]. This reaction is catalysed by enzyme **- carbonyl reductase 1 (CBR1).** Another clinical problem occured during DOX treatment is cancer cells resistance. It is caused by overexpression of membrane ABC transporters and gluthatione S-transferase π (GSTπ) in nucleus[3]. **Piperlongumine (PL)** is an amide alkaloid isolated from long piper - *Piper longum (Piperaceae).* Previous studies showed that PL work as inhibitor of CBR1, GSTπ and ABC transporters. What is more PL administered alone posses cytotoxic activity against cancer cells, but does not affect normal cells[4]. Above informations were encouragement to undertake studies on DOX and PL combination.

Analyses of DOX and PL combinations cytotoxicity have been performed using LDH assay in prostate cancer cell lines (DU 145). Combination Index has been used to determine type of interaction between drugs. Analyses of migration parameters (total length of trajectory, speed movement, rate of displacement) of individual cells were performed using videomicroscopy, at non-cytotoxic concentrations of DOX and PL. Influence of PL on DOX metabolism to doxorubicinol has been investigated in human S9 fraction using LC/MS/MS detection. DOX concentration at incubation mixture was 5 μM. Putative binding mode of piperlongumine (PL) in the active site of carbonyl reductase 1 (CBR1), has been elucidated throughout molecular docking to the protein target, represented by optimized crystallographic structure (1WMA). Preliminary studies of apoptotic activity of PL + DOX were also evaluated.

PL and DOX showed synergistic cytotoxic activity in DU 145 cell lines, with Combination Index less than 0.3, what indicates strong interaction. Cell motility parameters were significantly ($p < 0.05$) decreased by 50-70% in combination vs. only DOX or PL treatment. PL significantly ($p<0.05$) decreased formation of doxorubicinol, in concentration of 10 μM.

PL and DOX combination exerts synergism in both cytotoxic and anti-invasive activity. Moreover, PL has ability to decrease formation of cardiotoxic metabolite of DOX – doxorubicinol. Considering selectivity of PL, which is non-toxic to normal cells, such drugs combination, may leads to increase of treatment efficacy, with accompanying decrease of cardiotoxicity.

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C20

Synthesis and in vitro antiproliferative activity of acetylenic quinolone derivatives

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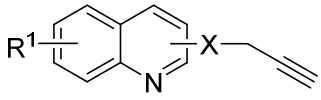
Acetylenic derivatives are an important class of compounds, since many of them have anticancer properties. This class of compounds includes both naturally occurring antitumor drugs, such as gummiferol, repandiol, and enediyne, as well as synthetic ones, such as erlotinib. Natural enediynes, such as calicheamicin, esperamicin, dynemicin, and namenamicin are the most potent anticancer agents discovered to date. Some members of this class are three orders of magnitude more potent than other anticancer drugs, but their clinical use has been limited due to their toxicity and modest selectivity for cancer cells. This has prompted several research groups to design, synthesize, and test new simplified acetylenic analogs, characterized by a similar mode of action. Several cyclic and acyclic derivatives, some including pyridine or quinoline units, have recently been developed.[1-4]



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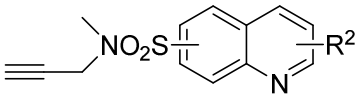

2

X = S, Se;
R^1 = Cl, Br, SCH_2CCH
R^2 = Cl, SCH_2CCH, $SeCH_2CCH$

As a continuation of our ongoing project concerning the synthesis of various acetylenic quinoline derivatives we designed new series propargylsulfanyl- and *N*-propargylsulfamoyl-quinolines of general formulae **1** and **2** and their antiproliferative properties were assayed.

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Norwegian session NS1 – NS5

NS1

Screening for $GABA_B$ receptor compounds

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γ-aminobutyric acid (GABA) is the main inhibtorial transmitter in the CNS. GABA exerts its function by binding to $GABA_A$, $GABA_B$ and $GABA_C$ receptors. $GABA_A$ and $GABA_C$ receptors are pentameric ligand gated ion channels, while the $GABA_B$ receptor is a family C GPCR. The $GABA_B$ receptor is implicated in a variety of psychiatric and neurological conditions including depression, anxiety, schizophrenia, epilepsy, addiction, pain and obsessive compulsive disorder, and is a functional heterodimer consisting of two subunits ($GABA_{B1}$ and $GABA_{B2}$). Each subunit consists of an N-terminal extracellular Venus flytrap (VFT) domain, a seven transmembrane (TM) helical domain and a C-terminal tail. The orthosteric binding site recognized by agonists (including GABA) and antagonists are located within the VFT domain of the $GABA_{B1}$ subunit, while an allosteric binding site is located within the 7TM of the $GABA_{B2}$ subunit. The structure of the orthosteric VFT domain is known, while the structures of the the $GABA_{B1}$ subunit and the allosteric $GABA_{B2}$ subunit are not known.

In the present study, we are using a combination of ligand based and structure based virtual screening to identify new compounds for the $GABA_B$ receptor. 2D fingerprints and pharmacophore models were generated based on known $GABA_B$ compounds, and used to screen available databases. Hits from the ligand based approach were used for docking. Homology modeling was used to construct models of the allosteric $GABA_{B2}$ subunit using structural templates from family A (rhodopsin, β2-adrenergic), family B (corticothropin releasing factor, glucagon receptor) and family C (mGlu1 and mGlu5). The different models were evaluated by docking of 74 known positive allosteric modulators and decoys, and the best performing models were used for docking hits from the ligand based approach. The most promising hits from the docking were purchased and tested experimentally. Preliminary experimental testing indicates that we have identified novel $GABA_B$ receptor compounds.

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NS2

Low-basicity agonists of 5-HT$_7$ receptor synthesized by van Leusen multicomponent reaction

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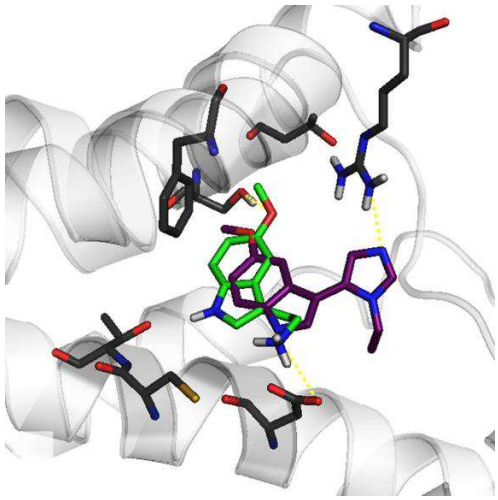
The term multicomponent reaction refers to any type of reaction in which at least three reagents are combined to produce an organic product of non trivial structure. MCR can refer to Mannich or Biginelli reactions, but is most often used when describing isocyanide chemistry.[1] In our study, we proved that the use of MCR protocols can boost GPCR ligands discovery projects.

We report a series of 3-(1-alkyl-1*H*-imidazol-5-yl)-1*H*-indoles, the first known examples of low-basicity 5-HT$_7$ receptor agonists and one of the very few low-basicity agonists of an aminergic receptor. The compounds were synthesized via three component van Leusen imidazole synthesis. Hit compounds within the 32-member series exhibit high affinity for 5-HT$_7$R, high intrinsic activity and metabolic stability, and very good calculated physicochemical parameters. The compounds are one of the most selective 5-HT$_7$R ligands. A prototypical synthetic scheme of a ^{11}C PET radioligand was designed and validated using 'cold' chemicals. The mechanism of binding of the discussed compounds was proposed based on homology modelling and SAR analysis of the series compared to analogous tryptamines. The possible binding mode for the compounds indicates an indole hydrogen bond with Asp3.32 and imidazole-Arg6.58 interaction. The exceptional selectivity of the compounds can be attributed to the fact, that Arg6.58 is a residue unique to 5-HT$_7$ receptor.



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Acknowledgements:
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NS3

Neuroprotective and proapoptotic activity of serotonin transporter inhibitors and serotonin receptor ligands

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According to the traditional two-state model of receptor theory, GPCRs were considered as operating in equilibrium between two functional conformations, an active (R*) and inactive (R) state. Recent data show that beside G proteins numerous other proteins, such as β-arrestins and kinases, may interact with GPCRs and activate intracellular signalling pathways. GPCR activation may therefore involve receptor desensitization, coupling to multiple G proteins, Gα or Gβγ signalling, and pathway activation that is independent of G proteins. This latter effect leads to agonist "functional selectivity" (also called ligand-directed receptor trafficking, stimulus trafficking, biased agonism, biased signalling). Serotonin exhibits multiple neural and non-neural functions. Several serotonin receptor ligands and serotonin reuptake inhibitors were reported to inhibit the growth of different tumor cell lines *in vitro*. It was shown that antidepressant drugs exhibit neuroprotective activity which could be connected to their antidepressant activity. On the other hand it was reported that certain antidepressant drugs may induce apoptosis in some cancer cell lines. Since such groups of antidepressants as SSRIs and TCAs (as well as 5-HT_{1A} receptor ligands) exhibit serotonergic activity in the present paper we examined proapoptotic activity of some serotonin transporter inhibitors and 5-HT_{1A} receptor ligands.

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 the Project PLATFORMex (Pol ‑ Nor/198887/73/2013).

NS4

Development of a new methods for virtual screening protocol

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Virtual screening (VS) is a very popular technique of selection of compounds with desired properties from, generally, large commercially/synthetically accessible chemical databases. Currently, a large variety of algorithms and software aiming at facilitating and automating the VS are available. However, due to increasing of computational power, the development of new VS tools is still required [1].

Herein we present the new algorithms and tools developed for VS, i.e. an approach for evaluation of docking results based on hybrid interaction fingerprint and machine learning methods and software for automatic generation of linear combination of pharmacophore models [2].

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Poster oral presentations PP1 – PP18

PP1

Dimroth rearrangement in the search for new agents effective in the battle against MDR bacteria

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Multidrug resistance is now a significant problem in the bacterial diseases. There are only few possibilities to increase the concentration of drug inside bacteria. In case of Staphylococcus aureus it is blocking the mechanism of MRSA resistance for example penicillinase. Finding a compound, which do not have an antibacterial activity, but increase the concentration of antibiotic inside bacteria is long term procedure comprise from steps like: (1) finding possible structures, (2) synthesis and (3) assay this compounds.
Firstly, based on the previous research [1] a possible structure and modifications were chosen. Then, using OSIRIS and Lazar Toxicity Predictions, we obtained results of drug score and toxicity in silico in the group of 5-arylideneimidazolone derivatives. The next step was chemical synthesis started from Knoevenagel condensation. Then, S-methylation was performed and the S-methyl compounds were used for the condensation with suitable aminealkylpiperazine derivatives to give three final compounds AK1 – AK3. During the last step, Dimroth rearrangement was observed that has giveen 2-amine-3-alkylamine derivatives of the arylidene-imidazolones. Crystallographic studies for compound AK1 were performed. Compounds AK1-AK3 were investigated on their anti-MDR properties in Gram positive bacterial strains (Staphylococcus aureus). Crystal structure X-ray analysis confirmed the product of Dimroth rearrangement. The most promising synergistic action with antibiotics was found for compounds AK1 and AK3, tested in the strain MM-0-21. The compounds displayed high Intrinsic MICs, and were able to improve efficacy of oxacillin even in 64- and 256-fold, respectively. Studies were partly supported by project K/ZDS/005593.

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PP2

Histamine H4 receptor ligands - their in vitro study on eosinophils adhesion to endothelium

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Histamine is a well known compound affecting many different aspects of human physiology and pathology. This broad histamine activity results from its' ability to react with four structurally and functionally different histamine receptors. As histamine receptors are involved in many different human clinical conditions, the selective histamine receptors ligands have become a potent drug candidates. Furthermore many of these have become blockbuster antihistamine drugs introduced into the clinics[1]. Newly discovered histamine H4 receptor has become an interesting new drug target, especially in the inflammatory conditions. However despite ongoing research no H4 selective drug has been approved yet, proving the need in developing new compounds with better drug-like properties.

The aim of this study was to screen the library of selective histamine H4 receptor ligands in order to determine their effect on histamine dependent eosinophils adhesion to endothelium.

For this study highly purified eosinophils have been isolated from the human peripheral blood, using immunomagnetic cell sorting methods. Cellular adhesion was evaluated during eosinophils co-culture with human Ea.hy.926 endothelium cell lines, under static conditions[2]. During the adhesion assays cells were exposed to: histamine, selective histamine H4 receptor ligand JNJ7777120, used as study control and newly synthesized compounds: KP-9D, TR-DL-49, MWJ-3, TR-18, JN-35, PHY-1 and JNJ10191584.

Histamine could significantly and in dose dependent manner upregulate the number of adherent eosinophils to endothelium. The reference compound JNJ7777120 (selective histamine H4 receptor antagonist) decreased the number of adherent cells in presence of 1μM histamine. Newly synthesized histamine H4 receptor ligands had different effect on eosinophils adhesion.
In conclusion we have proven that histamine is having a direct effect on human eosinophils adhesion to endothelium. We have screened the library of structurally different compounds with high affinity towards histamine H4 receptor. Among them we have selected the most potent one KP-9D, JN-35 and TR-DL-49, which will be used in future research.

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Acknowledgements:
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PP3

Novel 5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives: synthesis and *in vitro* anticancer studies

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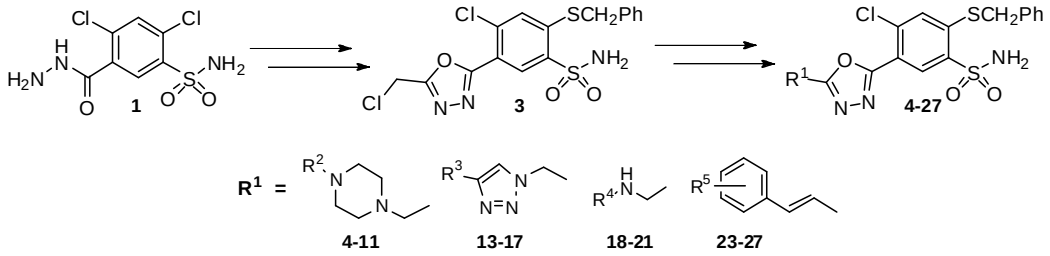
Arylsulfonamides constitute a class of compounds characterized by a broad spectrum of biological activity and clinical applications. Therefore the main object of our research are compounds based on the pharmacophoric scaffold of 2-mercaptobenezenesulfonamide (MBSA) having a proven antimicrobial, anticancer, or carbonic anhydrase inhibitory activity [1-3].Previously, we have shown that MBSA derivatives containing five-membered heteroaromatic ring attached to position 5 of benzene ring, exhibit promising *in vitro* anticancer activity [4, 5]. Thus, in presented studies we have undertaken synthesis of 2-benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives containing amine (**4**-**11, 18**-**21**), 1,2,3-triazole (**13**-**17**), or styryl (**23**-**27**) substituents at position 5 of 1,3,4-oxadiazole ring.



Anticancer *in vitro* screening of novel compounds was performed using an MTT assay on three human cancer cell lines: breast – MCF-7, colon – HCT-116 and cervical cancer – HeLa. Noteworthy profile of cytotoxic activity with IC_{50} value less than 20 µM against certain cell lines was observed for substantial part of tested compounds. Further studies involved determination of cytotoxicity against human keratinocytes, QSAR analysis based on OPLS method and determination of metabolic stability.

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PP4

Biological studies of novel piperazine derivatives in MCF-7 and MDA-MB-231 breast cancer cells

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Cancer is the second most common cause of death in the developed countries. Chemotherapy itself, or combined with surgery or/and radiotherapy is actually the most effective method in oncotherapy. The treatment includes the use of cytostatic compounds of such groups as e.g., alkylating agents, mitosis inhibitors, antimetabolites, kinase inhibitors or inhibitors of topoisomerase I and II [1].

DNA topoisomerases are nuclear enzymes that are involved in DNA replication, transcription, recombination, and chromosome condensation and decondensation. The topoisomerases fall into two major classes: the type I and type II enzymes. The type I enzymes cleave single strands of DNA, whereas the type II enzymes, such as topoisomerase II, generate a DNA double-strand break. In fact, topoisomerase II levels have been reported as possible prognostic markers in several cancers. Topoisomerase II inhibitors are categorized based on whether they stabilize the cleavable complex or catalytically inhibit enzymatic activity. Most of inhibitors of topoisomerase II have been in clinical use for many years. However, although these drugs are characterized by a very high cytotoxic efficiency, their use in chemotherapy is limited, because of severe side effects. This problem has become a source of inspiration for researchers around the world looking for new anticancer drugs [2, 3].

The aim of the present study was to investigate the effect of novel piperazine derivatives (MR-2 and MR-5) in MCF-7 and MDA-MB-231 breast cancer cells. Cytotoxicity was performed using MTT assay. The DNA biosynthesis was checked by incorporation of [^{3}H]-thymidine into DNA. Both, the viability of cells and [^{3}H]-thymidine incorporation were analyzed in human breast cancer cells after 24 hours of incubation with different concentrations of the tested compounds.

The results of this study show that both of the analyzed compounds exert inhibitory effects on the viability of MCF-7 and MDA-MB-231breast cancer cells.

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PP5

The cytotoxic activity of novel isoquinoline derivatives in human breast cancer cells

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Alkaloids are important class of chemical agents that serve as a rich reservoir for drug discovery. Several alkaloids isolated from natural herbs exhibit antiproliferative and antimetastatic effects on various types of cancers both *in vitro* and *in vivo* [1-3]. However, the high antitumor activity of these compounds is accompanied by a number of side effects. The need for alternatives to natural isoquinoline derivatives has consequently inspired further work towards the development of novel agents with improved and complementary properties. The aim of the present study was to investigate the influence of new synthesized isoquinoline derivatives on growth and viability of estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cells.

Evaluation of cytotoxic effect of novel synthesized compounds OM-71, OM-86II, OM-90 was performed using MTT assay. The DNA biosynthesis was checked by inhibition of [^{3}H]-thymidine incorporation into DNA in breast cancer cells. Viability of cells and DNA biosynthesis were analyzed in MCF-7 and MDA-MB-231 cells after 24 hours of incubation with different concentration of drugs. The cellular responses of human breast cancer cells to new isoquinoline derivatives has been studied using camptothecin as a reference. Moreover, to determine proapoptotic properties of the tested agents, we measured cell death by flow cytometric analysis after Annexin V-FITC and propidium iodide staining.

Our research proved that novel synthesized compounds OM-71, OM-86II and OM-90 had the cytotoxic effect in both estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 cell lines. Derivatives OM-86II and OM-90 were found to be significantly potent agents than camptothecin. The tested compounds also inhibited [^{3}H]-thymidine incorporation into DNA in human breast cancer cells after 24 hours of incubation with these agents. OM-86II and OM-90 led to higher antiproliferative effect in MCF-7 and MDA-MB-231 cells in comparison to the reference compound, whereas OM-71 had weaker potential than camptothecin. Moreover, our results suggested that the cytotoxic activity of OM-90 can be connected with its ability to induction of apoptosis.

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Acknowledgements:
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PP6

The cytotoxic efficacy of *Nigella sativa* seed extract in CRL-1739 human gastric adenocarcinoma cells

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In recent years, interest in naturally occurring compounds, which can suppress or prevent the process of carcinogenesis, greatly increased. Currently, one of the most extensively studied plants is a black seed herb (*Nigella sativa* L.). Its seeds and oil have been used for ages as a treatment of many human diseases and have a lot of traditional therapeutic applications worldwide. Recently, it has been shown that the active constituent of its volatile oil called thymoquinone plays important role for much of the *Nigella sativa's* biological effects. It is also known to exhibit antitumor activity against cancer in blood system, lung, kidney, liver, prostate, breast, cervix and skin [1].

In the present study, *Nigella sativa* seed extract was examined to evaluate its effect on viability, DNA biosynthesis and induction of apoptosis in CRL-1739 human gastric adenocarcinoma cells.
The cytotoxic activity of *Nigella sativa* seed extract was conducted using MTT assay. The impact of the oil on the DNA biosynthesis was checked by incorporation of [^{3}H]-thymidine into DNA. The effect on the induction of apoptosis was determined by flow cytometer using the Annexin V and propidium iodide. All of the tests were analyzed in CRL-1739 gastric adenocarcinoma cells after 24 hours of incubation with different concentrations of the tested extract.
Performed experiments showed cytotoxic effect of *Nigella sativa* seed extract in CRL-1739 gastric adenocarcinoma cells. The IC_{50} value was 0,48 mg/ml. The tested extract also inhibited DNA biosynthesis in CRL-1739 cells (IC_{50} = 0,46 mg/ml). Moreover, it had the ability to induce apoptosis in human gastric adenocarcinoma cells.

Our results showed that *Nigella sativa* seed extract is effective as cytotoxic agent in CRL-1739 gastric adenocarcinoma cells. In addition, it had antiproliferative properties and stimulated the process of apoptosis in the tested cell line. These observations show beneficial use of *Nigella sativa* as a therapeutic and chemopreventive agent against stomach cancer.

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PP7

TriMen Chemicals

PP8

Enantioselective acetylation of racemic atenolol with the use of lipases in native form

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Atenolol is a selective β1 receptor antagonist, belonging to the β-blockers group, which is known as one of the most relevant class of drugs widely used in treatment of cardiovascular disorders such as hypertension. Due to the fact β-blockers possess chirality center in their structure, they are presented in two enantiomeric forms, however only the *S*-enantiomer of these drugs demonstrates the desired therapeutic effect.

In this study authors developed the enzymatic method for the direct resolution of racemic atenolol and compared the catalytic activity of nine commercially available lipases. All enzymes were tested for the kinetic resolution of (*R*,*S*)-atenolol by enantioselective acetylation in different reaction mediums. Additionally, the influence of acetylating agent and its concentration were investigated.

The enantiomeric excesses of both substrate and product were determined by an UPLC-MS/MS system equipped with a chiral column. The highest parameters of conversion and enantioselectivity were achieved while using lipase from *Candida rugosa* (OF). However, from all tested reaction mediums toluene turned out to be the most effective, because its use allowed to obtain higher values of enantioselectivity and conversion compared to the results obtained with the use of different solvents. Finally, the use of *Candida rugose* OF lipase, toluene as reaction medium and isopropenyl acetate as acetylating agent allowed to obtain enantiomerically pure (*S*)-atenolol acetate with ee_p = 92.9%, whereas the conversion was c = 46.3% and the value of enantioselectivity E = 66.9.

The project was supported by a research grant from the National Science Centre, 2014/15/B/NZ7/00972.

PP9

Synthesis of magnetic nanoparticles with surface modified with chitosan and poly(acrylic acid) blends for biomedical application

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In recent years, the synthesis and characterization of nanoparticles have been the focus of intensive research. This type of nanoparticles have been highly employed in chemistry and biomedical applications such as magnetic hyperthermia, catalysis, diagnostic agent, and especially for biomolecule immobilization. The properties of the magnetic materials depends on the stabilizer type, which affect on covering surface. In this study the synthesis of new type of chitosan (CS) and poly (acrylic acid) (PAA) coated nanoparticles by photopolymerization is presented (Fig. 1) [1-3].

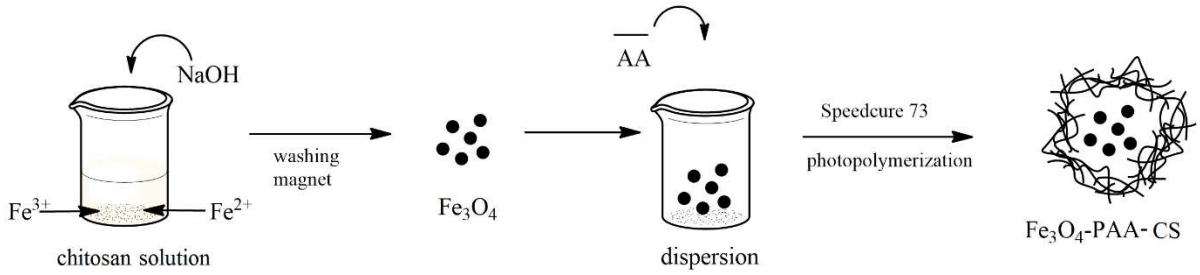

Fig. 1 The scheme of preparation of Fe_3O_4-PAA-CS nanoparticles.

Pure monomer of acrylic acid were subjected to photopolymerization, while the photopolymerization kinetics was followed by FTIR spectroscopy. The band corresponding to the C=C stretching vibrations has been selected for calculation of conversion degree of monomer. The structure of prepared magnetic material was processed by ATR-FTIR spectroscopy and XRD analysis. The morphology and size of the prepared nanoparticles were characterized by transmission electron microscopy. The amount of free amino groups on the surface of magnetic nanoparticles was estimated by the ninhydrin method. The activity, reusability, and the amount of immobilizer lipase were determined.

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PP10

The use of *Candida rugosa* lipase immobilized onto magnetic nanoparticles in enantioselective acetylation of (*R*,*S*)-atenolol

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Atenolol chemically knows as 2-(4-(2-hydroxy-3-(propan-2-ylamino)propoxy)phenyl)acetamide is one of the most important β-adrenolytic drugs widely used in treatment of hypertension and cardiovascular disorders. Due to the fact, β-blockers possess asymmetric carbon atom in their structure, they are presented in two enantiomeric forms. It was reported by many studies, only the *S*-enantiomers of these drugs have the desired therapeutic effect, whereas the administration of the racemate may cause dangerous side effects such as bronchoconstriction or diabetes. Nevertheless, β-blockers are still commercially available drugs mainly used in medicine as racemates.

The study is focused on the enzyme immobilization protocol as well as enzymatic method for the direct resolution of (*R*,*S*)-atenolol. The used magnetic enzyme carriers possess on theirs surface new-synthetized chitosan derivatives with free amine group distanced by ethyl or butyl chain. The catalytic activity of two lipases from *Candida rugosa* immobilized onto two types of magnetic nanoparticles were compared. The highest values of enantioselectivity (E = 66.9), enantiomeric excess of product (ee_p = 94.1%) and conversion (c = 41.84%) were obtained by using lipase from *Candida rugosa* OF immobilized onto Fe_3O_4-CS-EtNH_2. Additionally, the performed study confirmed, that even after 5 reaction cycles the immobilized lipase maintained its high catalytic activity.

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PP11

Activation of the toggle switch and dynamic network of the β_2-adrenergic receptor

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β_2-adrenergic receptor (β_2-AR) belongs to the superfamily of G-protein-coupled receptors (GPCRs) that mediate cellular responses to hormones, neurotransmitters, and the senses of sight, olfaction, and taste. The binding of an agonist to a GPCR leads to structural changes within the transmembrane regions allowing for activation of a G-protein. The most conserved residues across the GPCR superfamily are thought to play key roles in forming the active conformation of the receptor. One such regions is the CWxP motif within TM6, containing the conserved tryptophan residue (W286$^{6.48}$) originally reported to be involved in a rotamer toggle switch that is central to receptor activation. The molecular details of changes that occur in the W286 conformation during receptor activation process are still unknown. During the present study we have used both the experimental (time resolved fluorescence spectroscopy) and theoretical (molecular dynamics/metadynamics simulations) methods in order to assess the influence of functionally diverse ligands (agonists, antagonists and inverse agonists) on the W286 conformation. Multi Exponential analysis of fluorescence decay enabled assignment of the lifetime components to different tryptophan residues located in different segments of the protein, characterized by different polarity. Comparative analysis of amplitudes of the fluorescence lifetime components upon ligand binding confirmed localization of the active binding center in the protein. The results of molecular modeling studies show that W286 may exhibit diverse conformational states depending both on the type of the ligand present in the binding cavity and on the conformational state of the receptor associated with the ligand character or with the ligand absence ('active' vs. 'inactive'). The complexes containing agonist ligands exhibit the W286 orientation that promotes the open state of the water channel (i.e. the atomatic moiety of W286 is oriented parallel to TM6). The additional conformations existing in the presence of antagonists and inverse agonists correspond to the closed water channel. A very similar effect is observed in the context of aromatic residues present in the vicinity of W286, i.e. a notable changes in the preferred orientation of W286 occur upon point mutation of these residues (F289A, F290A, Y190A, Y308A).

Acknowledgments:
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PP12

Model free quantitative analysis of three component equilibria

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Determining a type of interaction with molecular target and its thermodynamic parameters is one of the typical aim of biophysical research on novel potential therapeutical agent. The most crucial point in such research is to determine molar fraction of each component present in a mixture. For this task one of most used technique is UV-Vis titration with chemometric analysis of acquired spectra. Up to this day one of most popular attempt was so called hard analysis, which mean that at first mathematical model of interaction were chosen and then experimental data were fitted to this particular model [1,2].
In our presentation we will present a new chemometrical algorithm to determine molar fraction of three component mixture by using only experimental set of spectra and chemometric methods. After determine the molar fractions of each component the best fitted model of interaction can be chosen.
The usage of the algorithm will be illustrated on several simulated as well as experimental sets of spectra.

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PP13

NMR and MD studies on the stereochemistry of the intercalation complexes of imidazoacridinone C-1311 (Symadex), a potential anticancer drug, with short fragments of dsDNA

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5-Diethylaminoethylamino-8-hydroxyimidazoacridinone (Figure 1), codenamed C-1311 (Symadex) is a lead compound of novel class of imidazoacridinones, synthesized and studied in the Department of Pharmaceutical Technology and Biochemistry at Gdańsk University of Technology. C-1311 successfully made its way through the Phase I clinical trials and has been recommended for Phase II clinical trials for breast and colon cancers [1].

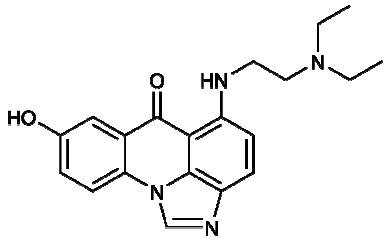

Figure 1. The structure of 5-diethylaminoethylamino-8-hydroxyimidazoacridinone, C-1311.

C-1311 was shown to accumulate in the cell nucleus and its cytotoxicity has been related to its ability to bind to duplex DNA by intercalation and to inhibit the catalytic activity of topoisomerase II [2]. When present in the nucleus, after formation of a relatively stable intercalation complex with DNA further steps of C-1311's mode of action involve peroxidase-mediated metabolic activation, which gives rise to C-1311 derivative species that might irreversibly, presumably covalently bind to DNA [3].
The $d(CGATCG)_2$:C-1311 [4] and $d(GAGGCCTC)_2$:C-1311 intercalation complexes have been studied by means of two-dimensional NMR spectroscopy, yielding a full assignment of the resonance lines observed in ^{1}H NMR spectra. The crude stereostructures of the complexes, resulting from NMR data, were then refined by means of molecular dynamics, using CHARMM force field and GROMACS software. Obtained results are in full agreement with biochemical data on the molecular mechanism of action of studied drug.

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PP14

Towards metabolically stable arylsulfonamide derivatives of (aryloxy)ethyl piperidines as potent and selective 5-HT$_7$ receptor antagonists with antidepressant and anxiolytic properties

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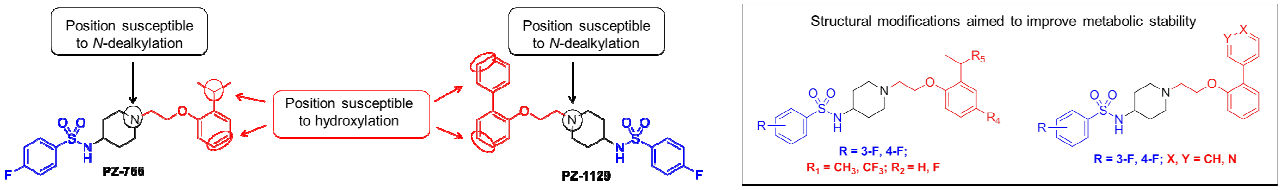
The type 7 Serotonin receptor (5-HT$_7$R) is the most recently identified member of serotonin family positively coupled with adenylyl cyclase through the stimulatory $G_{\alpha s}$ and $G_{\alpha 12}$ proteins.[1] Recent preclinical and clinical data support the hypothesis that 5-HT$_7$R antagonists may represent a valid alternative strategy for the treatment of affective disorders and neurodegenerative processes.[2]
We have recently developed a new class of potent and selective 5-HT$_7$R antagonists, namely arylsulfonamide derivatives of (aryloxy)ethyl alicyclic amines, identifying lead structures which displayed behavioral activities in animal model of depression, anxiety and cognitive impairment.[3,4]
In silico simulation and *in vitro* biotransformation studies on these derivatives revealed the potential sites susceptible to metabolic liability (i.e., enzymatic hydroxylation) which may lead to poor bioavailability. In an attempt to optimize the physicochemical properties with respect to the metabolic processes, we designed and synthesized metabolically stable arylsulfonamide derivatives of (aryloxy)ethyl piperidines as analogs of lead compounds PZ-766 and PZ-1129. To achieve this goal, structural modifications comprised the introduction of fluorine atom or trifluoromethyl moiety in the aryloxy fragment as well as the replacement of the *ortho* phenyl substituent with 3- or 4-pyridine moieties.



Synthetized compounds were evaluated in *in vitro* biotransformation studies displaying from moderate-to-low intrinsic clearance (Cl_{in} = 25–100 µg/mg/min) using rodent liver microsomes. Then, the most metabolically stable compounds were identified as highly potent 5-HT$_7$R antagonists (K_i < 50 nM, K_b = 1–40 nM) and selective over other monoaminergic 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$ and D$_2$Rs in *in vitro* cellular assays. Finally, tested compounds exerted antidepressant-like activity and anxiolytic properties (MED = 1.25 and 1 mg/kg, *i.p.*, respectively) in rodent models. Further studies would provide additional information regarding pharmacokinetic profile of these derivatives and their potential applications for the treatment of cognitive deficits.

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PP15

Characterization of permeability and hepatotoxicity of the selective serotonin 5-HT$_7$ receptor ligands *in vitro*

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5-HT$_7$ receptor (5-HT$_7$R) is the latest discovered member of the serotonin receptors (in 1993) by three different teams. The 5-HT$_7$R plays many important physiological roles in human body, for instance in hormonal regulation, circadian rhythm, thermoregulation, learning and memory processes. Lines of evidence indicate that selective antagonists of the serotonin receptor may be useful in treatment of several diseases of the central nervous system. Therefore, a series of phenylpiperazine derivatives of hydantoin with high selectivity and affinity against 5-HT$_7$R were designed and synthesized in our Department [1]. As continuation of the previous research, the main goal of this study is assessment of their hepatotoxicity and permeability through the biological membranes.

To estimate the permeability, PAMPA method was performed [2]. The assay imitates passive transport through the biological membranes for preliminary evaluation of bioavailability in the reference to the selected drugs with estimated high or low permeability. In the first step, the calibration curves of each compound and reference substances were prepared using Capillary Electrophoresis analytical system (CE). Next, after 5 hours of incubation, the concentrations in donor and acceptor compartments of the PAMPA plate were estimated using CE. As a result, compound KKB-15 showed the lowest permeability from the group of tested substances even in the reference to Norfloxacin, the control drug with low permeability. On the other hand, the lead compound MF-8 showed high permeability, similar to that of Verapamil, the high permeability control.

To assess the hepatotoxicity HepG2 hepatoma cell line and commercial, multiparametric Mitochondrial ToxGlo^{TM}Assay (Promega) were used. The compounds were examined in galactose - supplemented media in range 0.1 – 100 μM. The strong, known mito- (CCCP) and cytotoxin (Digitonin) were used as references [3]. In effect, none of 5-HT$_7$R ligands showed necrotic effect, even in the highest doses 100μM. Moreover, the significant decrease in ATP level showed only compound KKB-15 (in all examined doses) and compounds KKB-12, KKB-16 (only at the highest concentration 100 μM).

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Acknowledgements:
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PP16

Synthesis of new pyrolidine-2,5-dione derivatives with a long-chain arylpiperazine moiety, ligands of 5-HT_{1A} receptor

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Among 5-HT receptors, the 5-HT_{1A}R is involved in psychiatric disorders such as an anxiety, a depression and a memory loss. The most promising group of 5-HT_{1A} receptor ligands are Long-Chain Aryl-Piperazines (LCAPs) with several successfully developed drugs like buspirone, tandospirone or aripiprazole.

The aim of current research was the synthesis of series of pyrrolidine-2,5-dione derivatives with a long-chain arylpiperazine moiety. The structure of synthesized compounds was based on the structure of previously described pyrrolidine-2,5-dione derivatives with a confirmed dual high affinity for the 5-HT_{1A} receptor and SERT [1].
The series of 3-(1H-indol-3-yl)pyrrolidine-2,5-dione derivatives were synthesized. A number of modifications to the leading structure was designed focusing on one of main structural parts: the aryl group at N1 of the piperazine ring. The chemical structures of newly prepared compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-HRMS spectra.
The preliminary results obtained for the exanimated series of pyrrolidine-2,5-dione derivatives indicate considerable potential of this group of compounds as the ligands with dual binding to 5-HT_{1A} receptor and SERT. It allowed us to assess the influence of planned modifications on the binding of 5-HT_{1A} receptor, which in the future can contribute to the discovery of an efficient new drug.

[1] Wróbel, M. Z. et al. Synthesis and biological evaluation of novel pyrrolidine-2,5-dione derivatives as potential antidepressant agents. Part 1. Eur. J. Med. Chem. 63, (2013) 484–500.

Acknowledgments:
This study was supported by a grant 2013/09/B/NZ7/00748 from National Science Centre, Poland.

PP17

Non-basic antagonists of the 5-HT6 receptor - a structure-activity relationship study

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Antagonists of the 5-HT_6R are a promising class of biologically active compounds due to their procognitive and/or antiamnesic effects [1]. Until recently it was believed that the molecule of the aminergic receptor's ligand must consist of the basic nitrogen atom. This ionization center is responsible for crucial interaction with the highly conserved aspartic acid residue in the third transmembrane helix of the aminergic receptor [2]. However, in the last few years new, non-basic ligands of the 5-HT_6R have been discovered. This suggests possibility of the distinct mode of action of the non-basic ligands [3], nevertheless the binding mechanism of this new class of ligands is still unclear.

The aim of our study was to apply the X-ray crystal structure analysis in order to search for the structural features and geometrical parameters which could explain more selective binding of chosen non-basic ligands to the 5-HT_6R. Therefore, the consistent series of 1-(phenylsulfonyl)-1H-indole derivatives were synthesized and crystallized. For all obtained crystals the X-ray diffraction experiment was performed, followed by crystal structure solution and model refinement. For all determined structures, molecular conformations as well as the intra- and intermolecular interactions were compared. Results were correlated with binding affinities assessed in radioligand binding experiments.

According to our findings, the mutual orientation of the two aromatic fragments of the 1-(phenylsulfonyl)-1H-indole derivatives seems to be essential for better ligand-receptor recognition. Both aromatic moieties are in "facing" position. The indole π-electron system is less delocalised by the arylsulfonyl substituent due to the weaker electron withdrawing effect of the sulfonyl linker. It has been proven by the increased pyramidalisation of the N1 atom, which additionally allows more bent conformation of the investigated 5-HT_6R ligands.

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[3]Ivachtchenko, A. V.; Golovina, E. S.; Kadieva, M. G.; Kysil, V. M.; Mitkin, O. D.; Tkachenko, S. E.; Okun, I. M. J. Med. Chem. 2011, 54, 8161.

Acknowledgements:
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PP18

Novel 1*H*-pyrrolo[3,2-*c*]quinoline derivatives as 5-HT$_6$ receptor antagonists with procognitive properties

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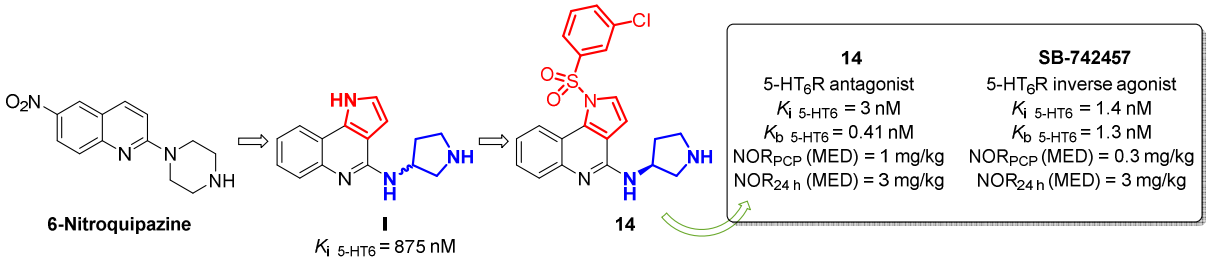
Alzheimer's disease, an irreversible neurodegenerative disorder, constitutes one of the most frequent forms of dementia worldwide. AD is characterized by progressive detoriation of cognitive functions, including memory and thinking. In the recent years 5-HT$_6$ receptor (5-HT$_6$R) has emerged as a promising molecular target for the treatment of cognitive deficits in AD.[1]

Herein we present the design, synthesis and pharmacological evaluation of novel class of 5-HT$_6$R antagonists based on 1*H*-pyrrolo[3,2-*c*]quinoline core. The study allowed for identification of compound **14** (*S*)-1-[(3-chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1*H*-pyrrolo[3,2-*c*]quinoline (K_i = 3nM and K_b = 0.41 nM), a more selective and potent 5-HT$_6$R antagonist than the reference compound SB-742457. Further evaluation of the 5-HT$_6$Rs constitutive activity at Gs signaling revealed that **14** behaved as a neutral antagonist, while SB-742457 was classified as an inverse agonist.[2]



Compounds **14** and SB-742457 reversed phencyclidine memory deficits and displayed procognitive properties in cognitively unimpaired animals (3 mg/kg) in NOR tasks. Additionally, compound **14** has demonstrated a higher anxiolytic effect (MED = 3 mg/kg) than SB-742457 in the Vogel test and showed similar antidepressant-like properties in 3-fold higher dose (MED = 10 mg/kg) than SB-742457 (MED = 3 mg/kg) in FST.
These results support the therapeutic potential of 5-HT$_6$R antagonists and inverse agonists in the treatment of cognitive decline and other symptoms associated with AD. More detailed biochemical studies would provide additional information about the action of 5-HT$_6$R antagonists and inverse agonists.

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Posters P1 – P115

P1

Novel benzisoxazolepropylpyrrolidine derivatives as multifunctional ligands with potential antipsychotic and procognitive activity

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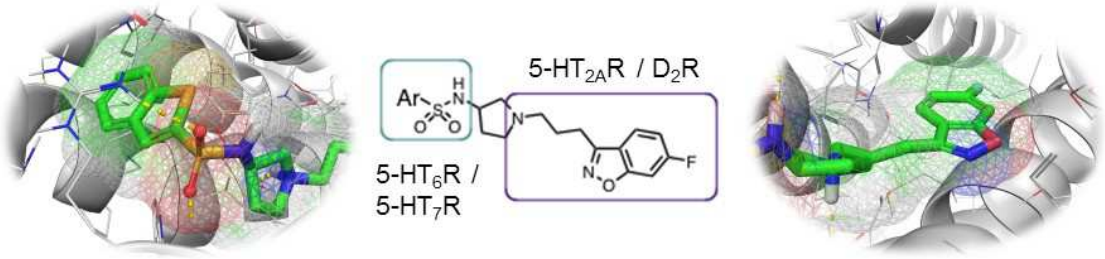
Dementia patients experience serious cognitive deficits and 90% of them show other non-cognitive behavioral and psychological symptoms of dementia (BPSD). The spectrum of BPSD includes: verbal and physical aggression, agitation, irritability, wandering, depression, anxiety and psychosis. Antipsychotic drugs have been commonly used off-label to control the burdensome symptoms. As nonselective molecules, antipsychotics cause serious side effects due to affinity to off-targets. Moreover, they cause cognitive impairment, which is particularly undesirable regarding dementia patients. In view of lack of targeted pharmacotherapies for BPSD, development of innovative antipsychotic suitable for elderly patients, causing no cognitive impairment, side effects and mortal risk, remains unmet clinical need [1,2].

A designed multitarget ligand, acting on serotonin 5-HT_{2A}, 5-HT_6, 5-HT_7 and dopamine D_2 receptors (R) could represent a promising strategy for treating symptoms of BPSD. In our research, we obtained a series of compounds by combining arylsulfonamide moiety with fluorobenzizoxazole linked via pyrrolidine ring, resulting in the multitarget ligands of high affinity for the targets of interest. Since binding to antitargets, such as M_3 receptors or hERG channel was substantially lower, the compounds are expected to have significantly reduced risk of major side effects [3].



Our study showed that arylsulfonamide fragment is crucial regarding interaction with 5-HT_6R and 5-HT_7R binding sites, which contribute to the potential pro-cognitive, antidepressant and anxiolytic activity, whereas benzizoxazolepropylpyrrolidyne moiety blocks 5-HT_{2A}R and D_2R, exerting antipsychotic-like activity. One of the most interesting examples, compound ADN-1459, potently blocked the 5HT_6R (K_i = 3 nM) and 5-HT_7R (K_i = 4.8 nM) and showed substantial affinity for the 5-HT_{2A}R (K_i = 0.4 nM) and D_2R (K_i = 0.8 nM). Functional studies revealed its antagonist efficacy (K_B = 0.6-38 nM). No significant interactions with the antitargets were observed. ADN-1459 showed a promising profile of antipsychotic-like activity in MK-801 induced hyperlocomotion in mice (MED = 2.5 mg/kg). Notably, the compound did not affect spontaneous locomotor activity (MED > 5 mg/kg), nor induced catalepsy (MED = 30mg/kg) in mice in active dose, which proved its benign safety profile.

References:

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P2

Synthesis of novel cycloalkylamino-thiosemicarbazides with tuberculostatic activity

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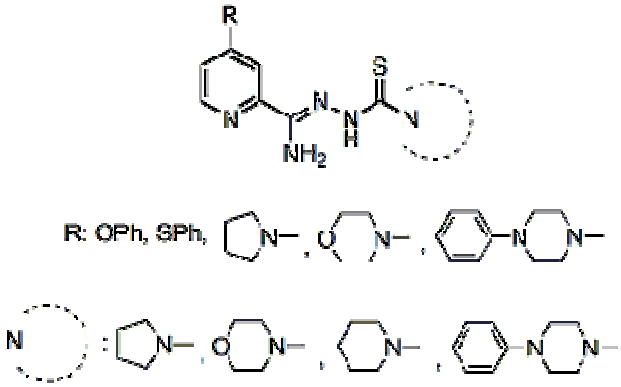
Tuberculosis is a second cause of death among infectious disease. Although the greatest number of new cases occur in Africa, South Asia and Oceania, increased mobility and migration of people to Europe may change this situation quickly. We also observe the increase in incidence of MDR-TB in the world. Currently applicable drugs become ineffective[1]. Therefore, new compounds with antituberculosis activity are demanded.

Previously we obtained derivatives of 4-methylipicolinonitrile and 4-phenylpicolinonitrile. Obtained compounds showed various tuberculostatic activity. The most potent were compounds with the thiosemicarbazide structure[2].Promising results of biological activity prompted us to the synthesis of 4-chloropicolinonitriles derivatives with thiosemicarbazide structure.The first step of the synthesis was the nucleophilic substitution of chlorine atom in 4-chloropicolinonitrile by morpholine, pyrrolidine, phenol, thiophenol or *N*-phenylpiperazine. Subsequently 4-substituted picolinonitriles were conducted into 4-substituted picolinimidates in the presence of methanol and catalytic amounts of DBU. In the last step picolinonitriles reacted with cycloalkylamino-1-carbothiohydrazides. As a result of the synthesis 20 novel derivatives of 4-chloropicolinonitrile have been obtained. All compounds were characterized by IR, ^{1}H NMR spectra and elemental analysis.



They have been tested for tuberculostatic activity in vitro against *M. tuberculosis* strains: H_{37}Rv and Spec. 210. The highest activity exhibited compounds that contained a morpholine ring in the thiosemicarbaizide moiety (<3,1 µg/mL). The cytotoxic activity was also tested on mouse melanoma cell line B16-F10 and human dermal fibroblasts HDF. The cytotoxicity assays have been extended by microscopic observations. The findings indicate that the supplied derivatives are good leading structure for new tuberculostatic agent discovery.

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P3

Dendrimeric phthalocyanine and pyrazinoporphyrazine derivatives as potential photosensitizers for photodynamic therapy (PDT)

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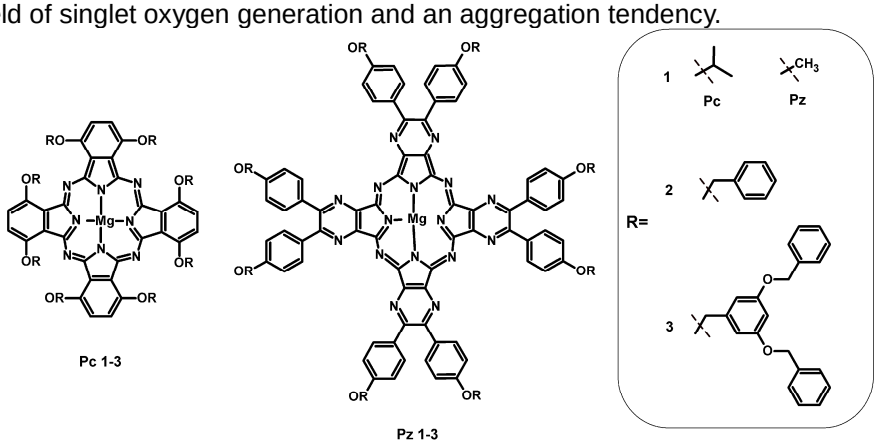
Phthalocyanines (Pc) and porphyrazines (Pz) are synthetic macrocycles, which are analogues of naturally occurring porphyrinoids like heme or chlorophyll. Pyrrole rings found in the studied structures are bound together by aza bridges, in contrast to methine groups found in porphyrins. Synthetic analogues of porphyrinoids have been for many years a subject of interest both for technical as well as biomedical sciences. Some of the recent applications of Pcs and Pzs in medicine include: photodynamic therapy (PDT), boron-neutron capture therapy (BNCT), radiation therapy and bioimaging [1]. The aim of this study was to explore the novel structures for their possible use in PDT. In this medical therapeutic approach, photosensitizer is delivered to the tumor tissue, where upon irradiation with light of an appropriate wavelength, generates reactive oxygen species, including singlet oxygen. These have the ability to kill tumor cells leading to necrotic or apoptotic cell death. Currently used photosensitizers are still far from being ideal. Improving their biophysical properties, while maintaining high singlet oxygen generation yield is a subject of ongoing research. One of the main factors, hampering biological activity of porphyrinoids is their poor solubility in water and the tendency to form aggregates. Noteworthy is that aggregation of the macrocycles can lead to significant decrease of their photosensitizing efficacy [2].

In presented work, six novel phthalocyanine and tetrapyrazinoporphyrazine macrocycles have been synthesized. The periphery of the compounds was expanded with moieties of varying size, including polyaryl ether dendrimers. The potential application of novel macrocycles in photodynamic therapy was evaluated by examining the yield of singlet oxygen generation and an aggregation tendency.



[1] Vicente, M.G. *Curr. Med. Chem.-Anti-Cancer Agents* 1 (2001) 175–194.
[2] Jaung, J-Y. *Dyes Pigm.* 75 (2007) 420–425.

Acknowledgements:
This study was supported by the National Science Centre under grant No 2012/05/E/NZ7/01204. PL and SJ acknowledge financial support from the National Centre for Research and Development under grant No PBS1/A9/13/2012.

P4

Binding mode analysis of a series of novel 5-HT_{1A} ligands

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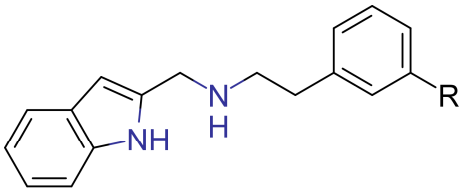
Halogens, especially the lighter fluorine and chlorine, are widely used substituents in medicinal chemistry. Until recently, they were merely perceived as hydrophobic moieties and Lewis bases in accordance with their electronegativities. It has to be stressed however, that compounds containing chlorine, bromine, or iodine can also form directed close contacts of the type R−X···Y−R', where the halogen X acts as a Lewis acid and Y can be any electron donor moiety. This interaction, referred to as “halogen bonding” since 1978, is driven by the σ-hole, a positively charged region on the hind side of X along the R−X bond axis that is caused by an anisotropy of electron density on the halogen.[1,2,3]
Within the arylmethyl-(2-phenylethyl)amines developed in our lab as serotonin receptor ligands, [(1*H*-indol-2-yl)methyl](2-phenylethyl)amines emerged as potent and selective 5-HT_{1A} receptor ligands. Introduction of halogen atoms to the benzene ring resulted in a significant increase in affinity. Notably, the -chloro (K_i = 19 nM) and -bromo (K_i = 34 nM) derivatives outperform both unsubstituted (K_i = 216 nM) and -fluoro (K_i = 160 nM), -methyl (K_i = 52 nM) and -trifluoromethyl (K_i = 81 nM) substituted compounds indicating the hydrophobicity of the substituent to be of secondary importance. The -methoxy substituted derivative exhibited K_i = 47 nM. The compounds structures were docked to 5-HT_{1A} receptor homology models using QM/MM protocol to investigate the possibility and nature of halogen bonding.



R = H, Cl, Br, F, Me, MeO, CF_3

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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 the Project PLATFORMex (Pol-Nor/198887/73/2013) and by the National Science Center Grant No DEC-2014/15/D/NZ7/01782.

P5

Effect of the new 1,3-dimethylpurine-2,6-dione derivatives with hydrazide or amide moieties on the LPS-induced TNF-α production

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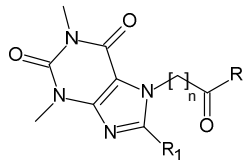
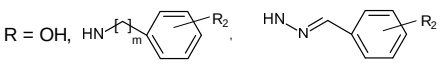
Autoimmune diseases (AID) such as multiple sclerosis, rheumatoid arthritis, Alzheimer's disease are characterized by cytokine overproduction, e.g. tumor necrosis factor alpha (TNF-α) and interleukins: IL-12, IL-23 and IL-17, with resultant inflammation and fibrosis. It is well known, that an elevation of cAMP level decreases the TNF-α production and produces immunosuppressant and anti-inflammatory effects. Among 11 known families of PDEs that hydrolyze the second messenger molecules (cAMP and cGMP) special interest has been focused on PDE7 and/or PDE4 isoenzymes that are expressed in immune cells and specifically control intracellular levels of cAMP. Followed by these findings the PDE7 and PDE4 inhibitors, that cover many heterocyclic systems, have been discovered as potential anti-inflammatory and immunosuppressant agents [1].
In this study we designed and synthesized a new series of compounds, chemically based on the purine scaffold, containing carboxyl, amide or hydrazide groups in position 7 of purine-2,6-dione core as dual PDE7/4 inhibitors having the ability to decrease TNF-α production.





R_1 = alkoxyl, amine, arylalkylamine
R_2 = OH, OCH_3
n = 1, 3; m = 0, 1

The inhibiting activity of the new compounds for PDE7A and PDE4B was tested *in vitro* using PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE7A and PDE4B expressed in Sf9 cells. The results of this study indicated that some of the tested compounds inhibited PDE7A and/or PDE4B at concentration close to that of BRL 50481 and rolipram, respectively. For the most active compounds the effect on the LPS-induced TNF-α production in rat whole blood has been determined *in vitro*. Additionally, to identify the molecular determinants responsible for the ligand binding to PDE7 and PDE4, computational modelling was performed for the selected compounds.

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Acknowledgements:
This study was supported by the National Science Centre, Poland, grant No 2014/15/B/NZ7/00885.

P6

In vitro, *in silico* and *in vivo* studies of a novel dopamine D_2 receptor antagonist as a potential antipsychotic

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The modern approach to drug design and discovery for the treatment of complex diseases, like neurodegenerative diseases, cancer and many psychiatric disorders, involves searching for medicinal substances which fulfil criteria of several pharmacophores, instead of acting on a single molecular target. Indeed, in complex psychiatric illnesses, including schizophrenia, selective single-target drugs have been to a great extent a failure. The pharmacological profile of clozapine reflects the molecular pathogenesis of schizophrenia, which involves cross-talk of many neurotransmitter systems (especially dopaminergic, serotonergic, adrenergic and glutamatergic). The new paradigm in drug design and discovery is to search for compounds which modulate the activity of several molecular targets simultaneously. To achieve this, it is necessary to identify structural features that link important classes of drug targets, which will enable the design of drugs with the desired selectivity profiles.

We identified a novel dopamine D_2 receptor antagonist, D2AAK1, with K_i of 58 nM using structure-based virtual screening [1]. D2AAK1 possesses additional nanomolar or low micromolar affinity to D_1, D_3, 5-HT_{1A} and 5-HT_{2A} receptors, making it an ideal candidate for a multi-target drug [2]. Here we present homology modeling, molecular docking and molecular dynamics of D2AAK1 and its molecular targets and animal studies of D2AAK1 as a potential antipsychotic. The main contact of D2AAK1 and all the receptors studied is the electrostatic interaction between the protonatable nitrogen atom of the ligand and the conserved Asp(3.32) as typical for orthosteric ligands of aminergic GPCRs. We confirmed antagonistic/partial agonistic properties of D2AAK1 towards all the receptors in *in vitro* essays and in *in silico* studies as the ligand stabilizes the ionic lock interaction. We also demonstrated neuroleptic, anxiolytic and, importantly, procognitive properties of D2AAK1 in mouse models.

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[2] Kaczor, A. A. et al. *Neurochem. Int.* 96 (2016) 84-99.

P7

Synthesis and cytotoxic activity of 30-substituted derivatives of 3,28-*O*,*O′*-diacetylbetulin

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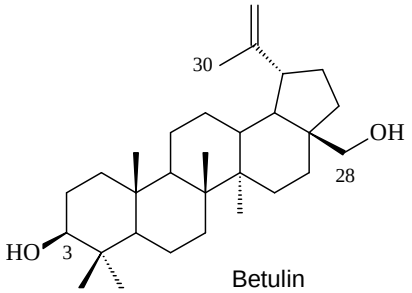
Betulin is a pentacyclic triterpene of the lupane type, which can be modified at C-3, C-28 and C-30 positions into new semisynthetic compounds possess various pharmacological properties, including antitumor, antibacterial, antiviral and antimalarial activity [1,2].



Betulin

Additionally, derivatives of betulin were showed significant antitumor activity towards colon cancer cells such as DLD-1, HT-29, Col-2 and SW-707 [3-5]. In the present work, was described the synthesis 30-substituted derivatives of 3,28-*O*,*O*'-diacetylbetulin. All new obtained compounds were tested for their anticancer activity against the colon cancer cell lines such as HT-29 and Caco-2. Moreover, the pharmacokinetic study of the synthesized derivatives was performed by determination of lipophilicity (cLogP), molecular mass (M), topological polar surface area (tPSA), hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) using the ACD/Labs software.

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P8

Monoamine oxidase B activity of novel tricyclic xanthine derivatives

Agnieszka Olejarz, Urszula Cichoń, Anna Drabczyńska, Jakub Schabikowski, Michał Załuski, Martin Hagan, Tadeusz Karcz, Katarzyna Kieć-Kononowicz

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In drug development for Parkinson's disease (PD) it became more popular to approach multiple target than single target. Two proposed dual-targets for PD drugs are: adenosine receptor A_{2A}/A_1 antagonists and adenosine A_{2A} antagonists/ MAO-B inhibitors.[1] Monoamine oxidase B (MAO-B) is known for its crucial role in neurodegenerative diseases. MAO-B inhibitors, such as selegiline and rasagiline are drugs registered in the treatment of Parkinson's disease. We investigated new compounds from the group of tricyclic xanthine derivatives as potential MAO-B inhibitors which can also exhibit adenosine receptor A_1 and A_{2A} antagonistic activity.[2]

Compounds were investigated for inhibition of human recombinant MAO-B. The Amplex Red® Monoamine Oxidase kit was used. Inhibition activity was measured in presence of the reference substrate, p-tyramine (200µM).
Data were calculated in GraphPad Prism 5 free trial. Instant JChem was used for structure database management, search and prediction.

We investigated 32 new compounds for their activity towards MAO-B in one concentration (1µM). Compounds, which exhibited more than 50% of the maximum inhibition activity (presented by pargyline in 10µM conc.) were chosen for further investigation. For 13 compounds IC_{50} and K_i values were experimentally calculated. IC_{50} values ranged between 82nM and 21 940nM.

From the large group of investigated compounds we managed to find xanthine derivatives that exhibit inhibition activity towards MAO-B. The structure-activity relationship can be helpful in drug development in this group of compounds.

References:
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[2] Koch, P. et al. Bioorg. Med. Chem. 21 (2013), 7435–52.

Acknowledgements:
Support of K/ZDS/004689 and Polish National Science Center grant (decision no. NCN-DEC-2012/04/M/NZ4/00219) are kindly acknowledged.

P9

Effects of antitumor-active acridinones, C-1311 and C-1305, on liver cytosolic glutathione S-transferase activity

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C-1311 and C-1305 are representative antitumor-active imidazo- (IA) and triazoloacridinone (TA) derivatives, respectively, developed in our laboratory. Our previous studies on molecular mechanisms of their metabolic transformations were concentrated on the phase I drug metabolism systems (liver microsomes, human recombinant cytochromes P450 and flavin-containing monooxygenases). These studies let define the direction of C-1311 and C-1305 conversions and enzymes involved in. However, we still know very little about their phase II biotransformation.

Glutathione S-transferases (GSTs), the crucial phase II metabolizing enzymes, catalyze the inactivation of various electrophilic drugs and other xenobiotics as well as endogenous substrates via conjugation to the tripeptide glutathione (GSH). Therefore, the alteration of GST activity may change the final therapeutic effect of the drug action. In fact, GST-mediated metabolism of some antitumor drugs is related to several drug resistance phenomena and adverse toxicity effects. Moreover, the overexpression of some GSTs has been linked to both natural and acquired resistance to various structurally unrelated antitumor drugs. Tumor overexpression of these proteins has provided a rationale for the search of GST inhibitors and GST activated cytotoxic prodrugs.

The present study aimed to investigate the *in vitro* effects of C-1311 and C-1305 on the activities of cytosolic GSTs in human liver cytosol and HepG2 cells with normal and overexpressed level of GSTM1 isoform, which plays a crucial role in the metabolic detoxification. The reaction progress was followed by reversed-phase high-performance liquid chromatography (RP-HPLC) in liver cytosolic fractions. Both C-1311 and C-1305 GSH conjugates were not detected by RP-HPLC. In order to identify and characterize the potential modulatory activity of C-1311 (C-1305), we measured GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) with GSH. The values obtained were corrected for the non-enzymatic reaction rates. The results showed that C-1311 and C-1305 were able to slightly decrease (by 15 – 20% when compared to controls) the activity of human liver cytosolic GSTs as well as recombinant human GSTM1 isoenzyme.

We suppose that C-1311 and C-1305 might bind to the active site of the enzyme, but their position prevents the GSH conjugation. Thus, we conclude that antitumor compounds, synthesized in our laboratory, may be promising prototype for new GST inhibitors pharmacologically useful in the treatment of tumors and the resistance of cancer to chemotherapy. The obtained results suggest the possibility of interactions of C-1311 (C-1305) with drugs related to phase II enzyme inhibition.

Acknowledgements:
Financial support by the National Science Centre (Poland) (project No 2012/07/D/NZ7/03395) is gratefully acknowledged.

P10

Synthesis and pharmacological characterization of structurally diverse chemical analogues of compound PQ-10

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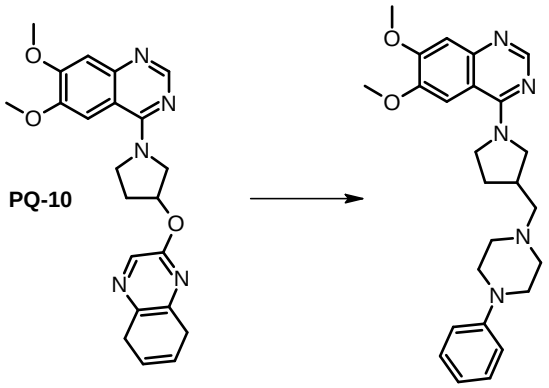
Localization, duration of action and concentration of cyclic nucleotides within specific subcellular domains convey signal strength and specificity. Any change within these parameters may contribute to alterations in receptors function. The significance of cyclic nucleotides in recent years caused by extended pharmacological, biochemical and molecular studies led to clarify the role of the phosphodiesterases (PDEs) – enzymes which degrade cAMP and/or cGMP by hydrolysis of phosphodiester bonds. Understanding of the enzyme localization has led to the pursuit of PDE10 inhibitors as a potential target for mood and cognitive disorders. Connecting the classic monoaminergic hypothesis of depression, anxiety, and schizophrenia with the results of the studies on proliferation/neuroplasticity related evidence, gave us the starting point to design of novel series of potential multi-target ligands based on inhibitor of PDE10A.

Novel series of potential multi-target ligands was designed using computer-aided molecular modeling tools. Structurally diverse novel chemical entities are based on compound PQ-10, which is a potent inhibitor of PDE10A (IC_{50} 6 nM). Primarily, modifications of the original structure comprised in the introduction of basic nitrogen atom, necessary for serotonin or dopamine receptor ligands and practically absent in the structure of PDE10A inhibitors. The preliminary studies suggested possibility of introducing the above mentioned modifications, while maintaining PDE10A inhibition activity.



Some of the compounds showed to be effective inhibitors of PDE10A compared to papaverine. The selected compounds were tested in for their affinity and their functional activity towards 5-HT_{1A}, 5-HT_7 and D_2 receptor. Our studies proved that it is possible to join the multiple PDE10A/serotonin receptor activity in one active molecule. The results of above described studies gave us the starting point to design novel analogues characterized by improved affinity for both targets of interest enzyme and receptors.

Acknowledgements:
The studies were supported by Jagiellonian University Medical College grant K/ZDS/004654

P11

Studies on 5-HT_7 /α_1 -AR/ D_2 -dopamine receptors discrimination for novel (hydroxy)propylpiperazine derivatives of 5,5-dimethylhydantoin

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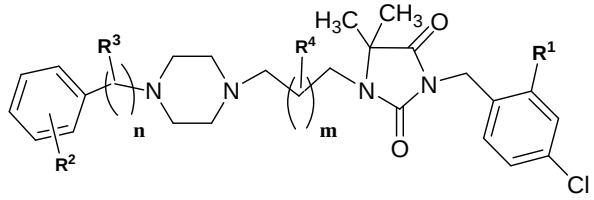
The 5-HT_7, α_1 -AR/, D_2 -dopamine receptors are important GPCRs biological targets involving in various diseases of peripheral or central nervous systems. The dopamine D_2 and 5-HT_7 receptors play an important role in neurodegenerative diseases like schizophrenia and Parkinson's disease as well as they can be used in depression and insomnia. The selective α_1-adrenergic receptor antagonists have important therapeutic perspectives as they are able to improve the urodynamic parameters and reduces the symptoms of benign prostatic hypertrophy. In this context, the search for GPCRs has been, and still is, an important topic in medicinal chemistry.

In the previous studies we investigated a number of phenylpiperazine derivatives of 5,5-diphenyllhydantoin [1], which possessed two additional aromatic fragments at position 5. The compounds were selective in respect to dopaminergic and serotonin receptor 5-HT_7 but their affinity for α_1-AR was moderate only. Thus, we decided to reduce the number of aromatic moieties and moved it from position 5 into position 3 of hydantoin [2]. The current study is concentrated on design and synthesis of new arylpiperazine derivatives of 3-benzyl-5,5-dimethylhydantoin (Fig.1).



1-24

R^1 = H; Cl
R^2 = H, 2-MeO, 2-CN, 2,4-diF, 2-F, 4-F, 2,5-diMe, 3-MeO, 3,4-diMe, 2,3-diCl, 3,4-diCl,
R^3 = Ph
R^4 = H, OH
n = 0,1
m= 3,4

Fig. 1

The final products were obtained within three-step synthesis, using two-phase alkylation processes. The new compounds were tested on their affinity for 5-HT_7 receptor in comparison to other closely related GPCRs: α_1-AR and dopamine D_2 receptors. SAR analysis indicates that the chemical modifications significantly improved the affinity for α_1-AR comparing to that of 5,5-diphenylhydantoin analogues. The best activity was found for the 2-fluorophenylpiperazine derivative with hexyl linker. 4-chlorobenzyl derivative with 2-metoxy substituent at phenylpiperazine phenyl ring and pentyl linker have shown high affinity for 5-HT_7 and D_2 receptors. Compounds were evaluated on their "drugability" and toxic effects using OSIRIS program.

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Partly supported by project K/ZDS/005593.

P12

Synthesis and antimicrobial activity of pyrrole N-arylhydrazone derivatives

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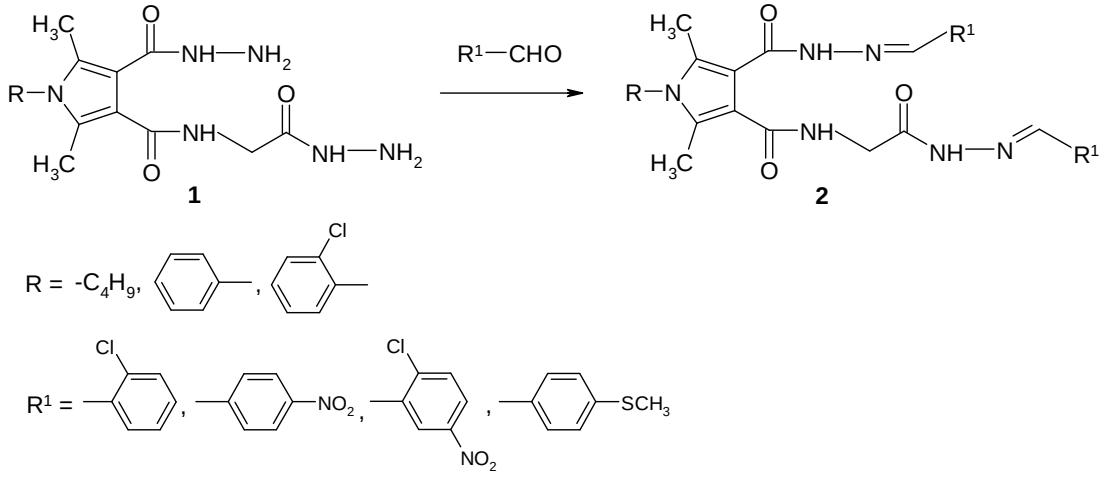
Hydrazones, related to ketones and aldehydes belong to a class of organic compounds possess diverse biological and pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, cardioprotective, antihelmintic, antiprotozoal, anti-trypanosomal, antischistosomiasis [1,2].

A series of new bis-arylhydrazones derivatives **2** were synthesized and studied for their antimicrobial activity. These compounds were prepared by condensation of hydrazide **1** with corresponding aldehydes.



The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR and CHN analyses. All the compounds were evaluated for their in vitro antimicrobial activity against Pseudomonas aeruginosa (Gram negative strain), Staphylococcus aureus (Gram positive strain) and also against fungi Candida albicans. The reduction or increase of microbial load in result of compounds activity was measured spectrometrically using wavelength of 580nm. No significant reduction was observed for any of strains investigated.

[1] Verma G. et.al. *J Pharm Bioallied Sci* 6(2) (2014) 69–80.
[2] Kumar N., Chauhan L.S. *International Journal of Pharmaceutical and Clinical Research* 7 (2015) 154–161.

P13

Synthesis of some new 1,3-oxazole derivatives and their biological activity

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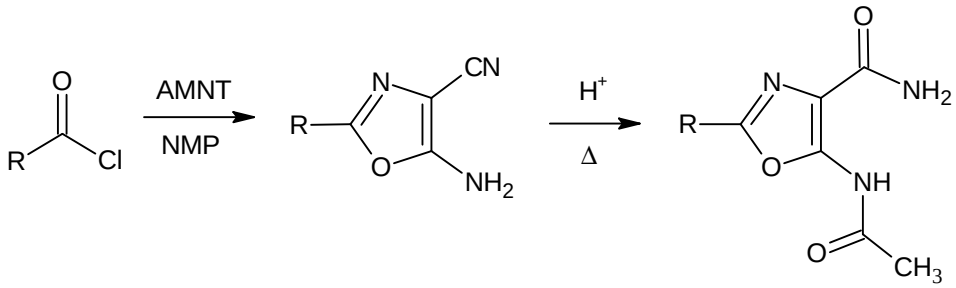
1,3-Oxazole and isoxazole derivatives are heterocyclic ring systems present in a number of natural or synthetic products with interesting pharmacological properties [1,2]. 1,3-Oxazoles display various biological activities, such as anti-cancer [3], anti-HIV/AIDS [3] and anti-inflammatory [4]. 1,2-Oxazoles exhibit immunostimulatory and immunosuppressive activities, which have previously been reported by our research group [5-7]. The subject of the present study was synthesis of 2-substitued, 5-amino-4-cyano-1,3-oxazoles and 5-acetamido-1,3-oxazole-4-carboxamides with potential immunomodulatory activities.



The biological studies showed the *in vitro* effects of the obtained compounds on the phytohemagglutinin (PHA)-stimulated proliferation of human mononuclear peripheral blood cells (PBMC) and lipopolysaccharide (LPS)-stimulated release of tumor necrosis alpha (TNF-α). The results indicated a relatively low cytotoxicity of the tested compounds and inhibition of the immune functions regarding PBMC proliferation and cytokine production.

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P14

Magnetic nanoparticles for ketoprofen adsorption

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During the recent years, research on nanotechnology, in particular, engineered nanomaterials, has received considerable attention. Engineered nanomaterials such as magnetic nanoparticles are among the promising materials because of their very specific properties which can be changed by external magnetic fields. Depending on the size and subsequent change in magnetic properties, the magnetic nanoparticles are useful for wide range of applications in areas of biology, pharmacy, medicine, and diagnostic.[1].
In the present study, magnetic nanoparticles are used as adsorbent materials to interact with non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs inhibit the metabolism of arachidonic acid to proinflammatory prostaglandins (PGs) by cyclooxygenase COX-1 and COX-2. Non-steroidal anti-inflammatory drugs provide important analgesic and anti-inflammatory benefits to millions of patients, but in Poland are available over the counter which cause increasing occurrence of the pharmaceuticals in aquatic environment. Therefore it can constitute an ecotoxity danger to aquatic animals and human being. The interactions between prepared nanomaterials and NSAIDs take place on the way of physisorption or/and chemisorption what allows to apply these materials for the removal of these drugs and their metabolites from water. [2].

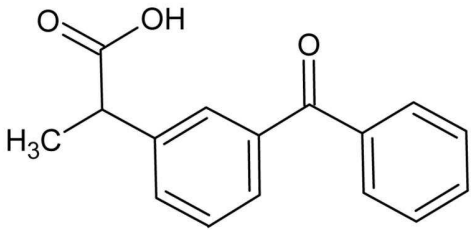

Fig.1. Structure of ketoprofen – example of NSAID.

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The project was supported by research grant: National Science Centre 2014/13/B/ST8/04342.

P15

New acyclic analogues of nucleotides

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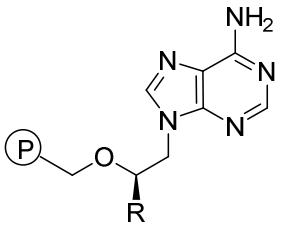
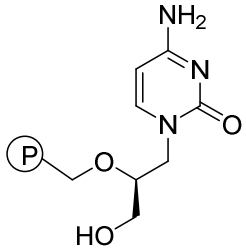
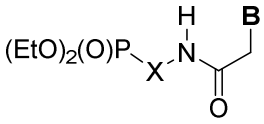
Acyclic phosphonate analogues of nucleosides (ANPs), for example adefovir **1** [1,2], tenofovir **2** [3] and cidofovir **3** [4], belong to the important group of drugs used for treatment of viral infections. However, their low selectivity, drug resistance and long-term toxicity in connection with permanent mutation of viruses stimulates many research groups to design new, more potent molecules within an ANPs group.
We proposed new structural analogues of ANPs **4** in which a methylene ether [–**O–CHR**–] group is replaced by the amide fragment [–**HN–C(O)**–]. We expect the latter modification will significantly influence the biological activity of new analogues by increasing specific donor-acceptor interactions by incorporation of a rigid amide bond into the acyclic part of ANPs.



1 R=H Adefovir
2 R=CH_3 Tenofovir



3 Cidofovir



B = nucleobase
X = CH_2, $(CH_2)_2$, $(CH_2)_3$, $CH_2OCH_2H_2$, $CH(OH)CH_2$, $CH(OH)CH_2CH_2$, $CH_2CH(OH)CH_2$

4

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Acknowledgements:
This project is supported by the National Centre under Decision DEC-2013/09/B/NZ7/00729.

P16

Synthesis, anticonvulsant, and antinociceptive activity of novel N-Mannich bases derived from 5,5-disubstituted imidazolide-2,4-diones

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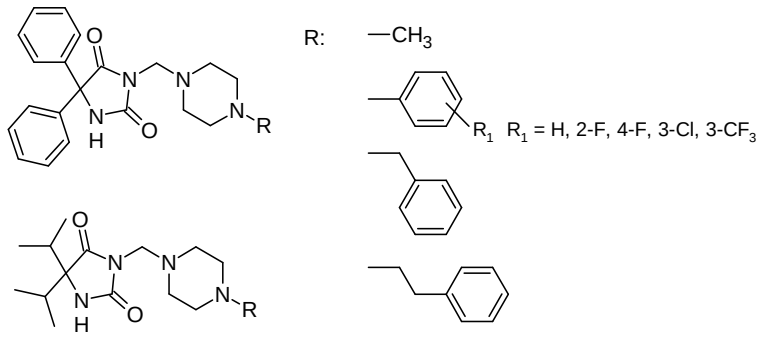
Epilepsy is a chronic neurological disorder, characterized by recurrent seizures, which are result of excessive electrical discharges of neurons in different parts of the brain. The disease affects more than 50 million people worldwide, but only 75% of them respond to the implemented treatment. In view of the fact that 25% of patients are refractory to the available treatment, there is still need to search for new, more effective anticonvulsants [1].
One of the ways of designing new antiepileptic compounds is to modify pre-existing drugs [2] in order to increase their efficacy and reduce a number of occurring side effects. Herein we present a continuation of our research in a group of phenytoin derivatives. Phenytoin is a well-known and widely used anticonvulsant with the hydantoin core. We modified its structure by substitution of N3 atom with variety of alkyl- and arylpiperazines (N-methylpiperazine, phenylpiperazine, benzylpiperazine, phenylethylpiperazine) and by changing phenyl rings at position 5 for isopropyl substituents.



The designed compounds were evaluated for their anticonvulsant activity (MES, scPZT, and 6 Hz tests) in Antiepileptic Drug Development Program (ADD) developed by the National Institute of Health (Rockville). Beyond epilepsy, anticonvulsant drugs are used for treating neuropathic pain [1]. Therefore, the most active compound of this series, was chosen for the assessment of their antinociceptive activity in the formalin test and in an oxaliplatin-induced pain model.

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Acknowledgements:
This study was financially supported by Funds for Statutory Activity of Jagiellonian University Medical College (No K/ZDS/006208) and National Science Centre grant No DEC-2013/11/B/NZ7/02081.

P17

Synthesis and antiproliferative activity *in vitro* of fluoro derivatives of 2-amino-1H-benzimidazole

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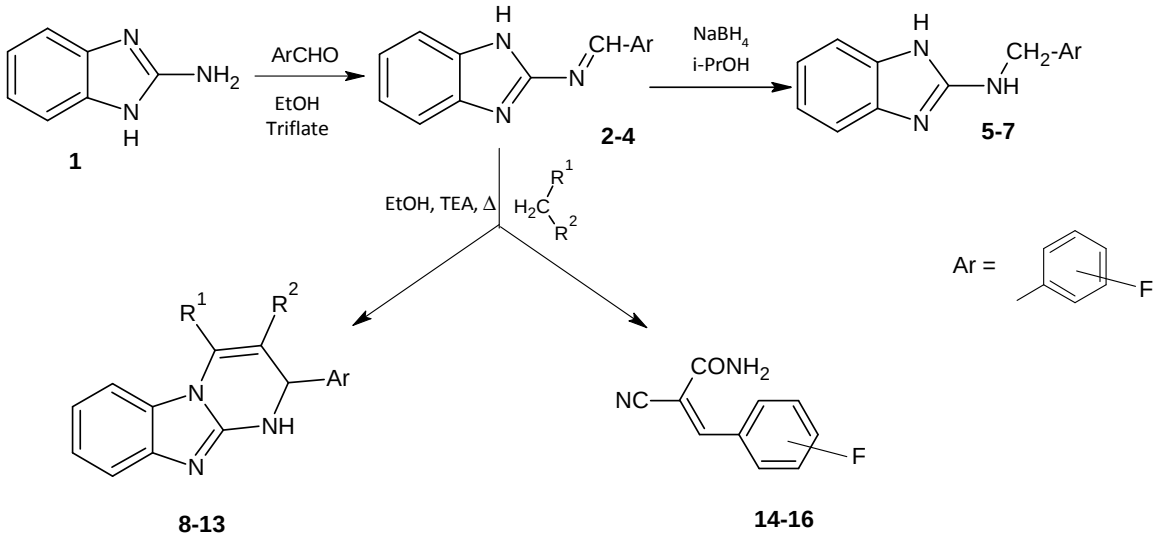
2-Aminobenzimidazole derivatives possessing diversified biological applications such as antitubercular, anticancer, antibacterial, anti-inflammatory, antifungal herbicidal, anthelmintic, antiproliferative, anticonvulsant and antiparasitic activities. Chemical modification of Schiff bases, derivatives of various heterocyclic compounds provides biological activity.

The purpose of this work was to synthesize and determinie the chemical structure of new derivatives of 2-amino-1H-benzimidazole. The substrates of synthesis were 2-amino-1H-benzimidazole obtained in Leonards reaction and 2-fluoro, 3-fluoro- and 4-fluorobenzaldehydes, that formed the Schiff bases **2-4**. Schiff bases were a subject of chemical modifications: reduction and reactions with selected compounds having active methylene group: acetylacetone and nitriles: malononitrile, cyanoacetic amide.
In the reactions of Schiff bases **2-4** with selected compounds containing active methylene group: the products **8-16** of various chemical structure pyrimido[1,2-a] benzimidazole **8-13** and α-cyanocinnamic acid derivatives **14-16** were obtained.
The structures **2-16** were confirmed by the results of elementary analysis and their IR, ^{1}H-, ^{13}C-NMR spectra. All compounds of different chemical structures, were examined for their antiproliferative activity *in vitro* against the cells of human cancer cell lines and normal mouse fibroblasts.



P18

In vitro antibacterial evaluation of some new derivatives of semicarbazide and 1,2,4-triazole-5-one

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Derivatives of semicarbazide and 1,2,4-triazole-5-one are heterocyclic compounds with wide spectrum of biological activities. Such compounds possess antifungal, antimicrobial and antitubercular activity. There are also drugs containing 1,2,4-triazole system. Among them are Flutrox, Nefazodone, Trazodone, Letrazole, Vorozole, Voriconazole and Posaconazole. Many drugs have lost their effectiveness due to frequent use. This fact is related to the appearance of drug resistance and its growth. So it is very important reason to search and synthesize novel compounds as potential drug candidate.

Our research concentrated on the antimicrobial activity of new derivatives of semicarbazide and 1,2,4-triazole-5-one. Among of examined compounds one semicarbazide **6** and five triazoles **11**, **15-18** indicated some antibacterial activity only against reference Gram-positive bacteria. The substance **6** had a spectrum of activity towards all these bacteria. This compound showed moderate effect against *M. luteus* ATTC 10240, *B. subtilis* ATTC 6633, *B. cereus* ATTC 10876 and *S. aureus* ATTC 43300 with MIC = 500 µg/ml and MBC ≥ 1000 µg/ml. The remaining staphylococci were less susceptible to **6** (MIC = 1000 µg/ml and MBC ≥ 1000 µg/ml). The substance **15** possesed also moderate activity towards *M. luteus* ATTC 10240, *B. subtilis* ATTC 6633 and *B. cereus* ATTC 10876 with MIC = 250 – 500 µg/ml and MBC ≥ 1000 µg/ml and mild effect towards staphylococci (except S. aureus ATTC 25923) with MIC = 1000 µg/ml and MBC > 1000 µg/ml). The compound **6** and **15** showed bactericidal or bacteriostatic activity. The other examined compounds, **11** and **16-18** indicated mainly mild bioactivity (MIC = 1000 µg/ml and MBC > 1000 µg/ml) or had no any effect towards these bacteria.
In turn, compounds **1-5**, **7-9**, **12-14** and **19-20** were inactive against reference microorganisms.
In conclusion, our data showed that some of the newly synthesized compounds indicated mild or moderate antimicrobial activity only against reference Gram-positive bacteria.

P19

GABAergic component enhances antidepressant activity of a new 5-HT6 antagonist, MM-165

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Despite remarkable advances in the treatment of depression, only approximately 50% of patients respond to the available medications [1]. The inefficacy of antidepressant treatment still results from the delayed onset of action and certain side-effects. Therefore, development of novel antidepressant agents that use different mechanisms of action has become one of the main focuses in the current drug discovery. Several *in vivo* studies with structurally different serotonin 5-HT6 receptor ligands (both agonists and antagonists) revealed their antidepressant activity [2]. Moreover, available data points that GABAergic dysfunction is associated with the pathophysiology of depression [3,4] and the functional interaction between GABAergic system and 5-HT6 receptors have been confirmed in behavioral and neurochemical studies [5].

The aim of this study was to evaluate antidepressant-like activity of the new compound MM-165, combines in its structure GABA and a defined 5HT6 antagonist (MM-198), in the modified forced swim test in rats. To compare the above-obtained data with the effect of GABA itself and MM-198 itself, being ethanolpiperazine derivative of 1-phenylsulfonylindole, the additional experiments have been conducted.

Both compounds MM-165 and MM-198 significantly shortened the immobility time and increased the climbing time of rats. MM-165 was active in relatively low doses (0.1mg/kg, 0.3mg/kg and 1mg/kg) in that test, while MM-198 was active in a 100-fold higher dose of 10mg/kg. No activity of MM-198 was observed at lower doses, i.e. 1mg/kg and 3mg/kg. GABA injected at a dosage range of 0.1-10mg/kg was not active in that paradigm. Antidepressant-like activity of both compounds seems to be specific, since MM-165 and MM-198 did not affect rats' locomotor activity measured in the open field paradigm.

The obtained results strongly suggest that the combination of GABAergic activity and 5-HT6 antagonism results in enhanced antidepressant-like activity, leading to improved therapeutic potential as compared to pharmacological properties of 5HT6 antagonist itself and the potential utility of such combination as possible antidepressant treatment.

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Acknowledgements:
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P20

Selected structural modification of azaheterocyclic fragrant compounds

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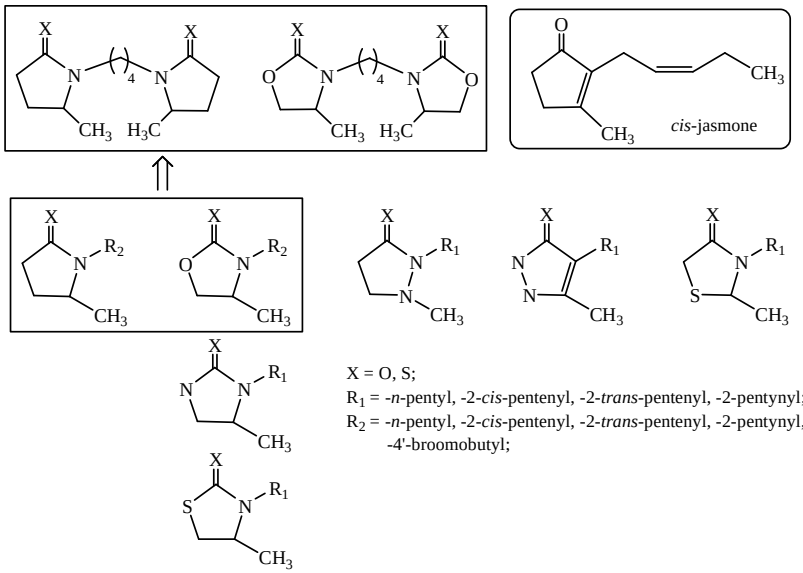
Jasmone is a well-known 2,3-disubstituted five-membered carbocyclic ketone which is component of plant volatiles and is an important substance determining the odor of jasmine flower. We have reported microwave synthesis and fragrant properties of some jasmone azaheterocyclic analogues containing heterocyclic five-membered ring such as: 1-pyrrolidin-2-one, 1,3-oxazolidin-2-one, 1,2-pyrazolidin-3-one, 1,2-pyrazol-3-one, 1,3-thiazolidin-4-one and their thiocarbonyl derivatives. It has been found that the most of heteroanalogues mentioned above exhibited interesting fragrant properties, both from the point of odor as well as odor durability. Taking the rising interest in jasmone structure based fragrant compounds into account it has been decided to take up an attempt to synthesize the succeeding new heterocyclic derivatives of this compound. The possibility of its structure modification are as follows: modifications in the side chains, modifications in the ring, modifications within the carbonyl group and modifications in the alpha position of the carbonyl group. Because one of the most interesting jasmone fragrant heteranalogue is 1,3-oxazolidin-2-one system it was decided to prepared succeeding 1,3-heterocyclic-2-ketones and 2-thioketones based on imidazolidinone and thiazolidinone systems. In addition, bromine atom was introduced into the terminal position of alkyl side chain and dimeric derivatives were obtained also.



Ecofriendly microwave assisted organic syntheses have been used to obtain the most of compounds. Odor evaluation and relationships between their structure and fragrant properties have been studied.

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P21

SAR determination and preliminary modelling studies for a new mGluR4 positive allosteric modulators

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Metabotropic glutamate receptors (mGluRs) are members of the group C family of GPCRs and play important roles in a broad range of central nervous system functions having therapeutic potential in a variety of neurological and psychiatric disorders [1]. Due to the lack of receptor subtype selectivity and physiochemical properties of mGluR orthosteric ligands (poor bioavailability and low potential of blood-brain barrier penetration) a significant effort has been made to identify compounds that can act as allosteric modulators which potentiate the response of endogenous glutamate [2]. A number of reviews are available summarizing recent progress in developing new allosteric ligands of mGluRs [3]. Among all, the group III subtypes: mGluR4, mGluR7 and mGluR8 still remains the least explored but with mighty potential for future development of clinical drugs [4].
Herein structures of a recently discovered mGluR4 PAM's are disclosed together with the results of in vitro and in vivo experiments. The developed mGluR4 homology models were used to rationalize the observed structure-activity relationships.

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Sciences.

P22

Synthesis of new 6-phenyl-pyrrolo[3,4-*c*]pyridine derivatives with potential antitumor and antimicrobial activity

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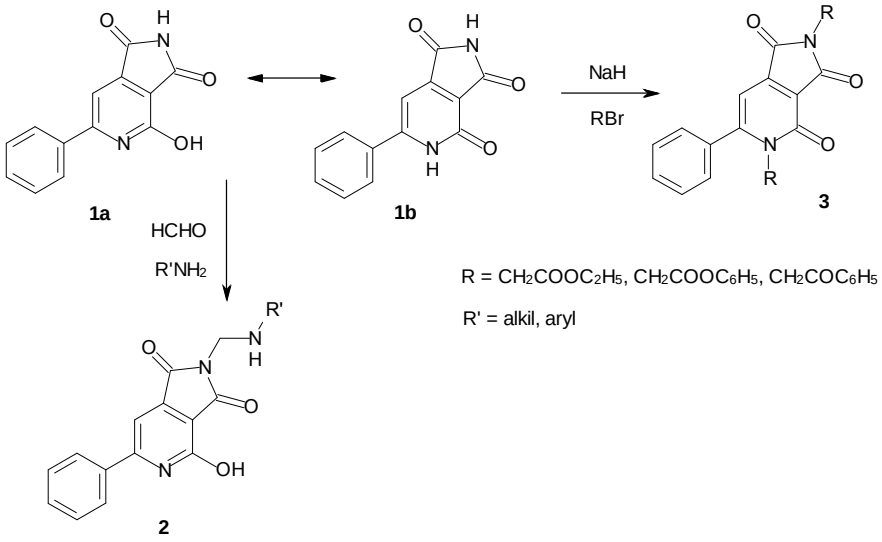
Pyrrolo[3,4-*c*]pyridine derivatives show anticancer and antimicrobial activity or have been reported as analgesic and sedative agents [1]. The broad spectrum of biological activity of pyrrolo[3,4-*c*]pyridine derivatives is the main of reason for the preparation of new compounds containing this scaffold.

In our previous work [2] we determined a way of synthesizing pyrrolo[3,4-c]pyridine derivative in two tautomeric form: 4-hydroxy-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione **1a** and 6-phenyl-5*H*-pyrrolo[3,4-*c*]pyridine-1,3,4-trione **1b**. As a continuation of our research, two series of new 6-phenyl-pyrrolo[3,4-*c*]pyridine derivatives have been synthesized. The Mannich bases **2** were prepared via the condensation of 4-hydroxy-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione **1a** with selected, pharmacophore amines and formaldehyde. 6-Phenyl-5*H*-pyrrolo[3,4-*c*]pyridine-1,3,4-trione **1b** was alkylated to corresponding *N*-alkil derivatives. The antiproliferative and antimicrobial activity *in vitro* of the new pyrrolo[3,4-*c*]pyridine derivatives are evaluated.



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P23

Computational studies of etoposide plasma protein binding

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Nowadays computational methods are becoming increasingly important in medicinal chemistry, because they allow for reduction of working time and cost at the initial stages of preclinical research. Molecular docking provide a fresh look at the effect of pharmaceuticals on receptors, nucleic acids and carrier proteins. Human serum albumin (HSA) and alpha-1-acid glycoprotein (AGP) are acute phase proteins responsible for binding and distribution of pharmaceuticals in human blood [1, 2]. Therefore, changes in the concentration of HSA and AGP in plasma can influence on the pharmacological actions and side effects of drugs [3].

The aim of the present study was to investigate the binding of etoposide to plasma protein (HSA and AGP) by means of molecular docking. Etoposide is topoisomerase inhibitor widely used as a basic anticancer drug in the treatment of non-small cell lung cancer, acute lymphoblastic leukaemia and various lymphomas. The crystal structures of HSA (PDB ID: 1AO6) and AGP (PDB ID: 3KQ0) were downloaded from the Protein Data Bank (PDB) database. The conformation of etoposide was energy-minimized using the Austin Model 1 (AM1) semi-empirical method. Computational experiments were performed using the CLC Drug Discovery Workbench computer program (version 2.5) and results were elaborated with Accelrys Discovery Studio software (version 4.1). Our computational results have been compared with the results of X-ray study of Wang et. al. [4].

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Acknowledgements:
This work was supported by grants KNW-2-O15/N/6/K and KNW-1-034/K/6/0 from Medical University of Silesia, Katowice, Poland. Calculations have been carried out using resources provided by Wroclaw Centre for Networking and Supercomputing (http://wcss.pl), grant No. 382.

P24

Lipophilicity of new anticancer 3,6-diazaphenothiazines

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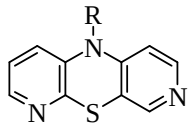
Lipophilicity has been long considered as a vital component of drug discovery and development. It is crucial physicochemical parameter which is important for *in vivo* distribution of organic compounds by influencing their solubility, cell uptake, blood-brain penetration and rationalizing a number of biological events as membrane penetration and permeability [1,2]. A review of the literature demonstrates that compounds that display lipophilicity (log*P*) between one and three appear to be optimal for achieving appropriate physicochemical characteristic to ensure downstream drug success [3]. For this reason, a quantitative assessment of lipophilicity is an important tool in quantitative structure–activity relationship studies [4].

In the continuation of the search of bioactive azaphenothiazines, the synthesis, analysis of structure and biological activities of new 3,6-diazaphenothiazines were reported [5].



R = H, alkyl, aryl, heteroaryl, dialkylaminoalkyl

Some of these compounds exhibit promising and significant *in vitro* antiproliferative acivity against the human cancer cell lines: glioblastoma SNB-19, melanoma C-32 and breast cancer MCF-7.
The aim of this project was examination of the lipophilicity of new series of 10-substituted 3,6-diazaphenothiazines determined experimentally by RP-TLC and calculated with computer programs, as well as search for relationships between their lipophilicity, structure and biological activity.
Furthermore, the lipophilicity was studied in hope of providing a deeper insight into the differences of biological activity.

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P25

Synthesis of new olivacine derivatives with potential anticancer properties

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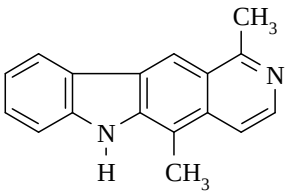
The alkaloid olivacine 1 and some analogues demonstrated a strong cytostatic activity [1, 2]. In previous papers we have described the synthesis and cytostatic activity of 1-phenylsubstituted pyrido[4,3-*b*]carbazole derivatives [3]. Now, we have decided to modify the structure of main ring system and we obtained 1-substituted [4,3-*b*]carbazole derivatives. The present study described the synthesis of new olivacine derivatives 2.



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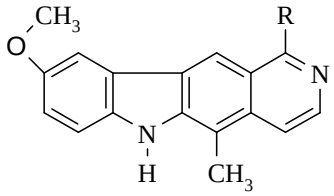

2

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P26

Apoptosis-inducing activities and metabolic stability of 5-substituted 2-(arylmethylthio)-4-chloro-*N*-(5-aryl-1,2,4-triazin-3-yl)benzenesulfonamides as potential anticancer agents

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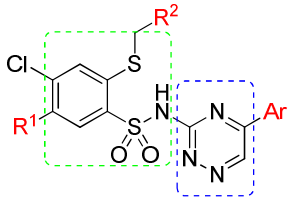
Among the heterocycles, the 1,2,4-triazines have occupied a prominent position in medicinal chemistry with a wide range of biological profile including anticancer activity [1-4]. Searching for innovative low-molecular chemotherapeutics we designed and synthesized a series of new 5-substituted 2-(arylmethylthio)-4-chloro-*N*-(5-aryl-1,2,4-triazin-3-yl)benzenesulfonamides, bearing the molecular skeleton of 3,5-disubstituted 1,2,4-triazine and 2-mercaptobenzenesulfonamide moiety, as potential anticancer agents.



The anticancer activities of compounds were evaluated *in vitro* on MCF-7, HCT-116 and HeLa cell lines by MTT assay. It was found that for the most active analogues, the 1-naphthyl (R^2) and 4-CF_3-C_6H_4 (Ar) moieties contributed significantly to the antitumor activity in MCF-7, HCT-116 and HeLa cell lines. The apoptotic potential of the most active compounds was analyzed through various assays: cells' morphology, DNA fragmentation, mitochondrial potential disruption, phosphatidylserine translocation and caspase activation.

The compounds with outstanding activities were submitted to metabolic stability study in order to assess their ability to remain unchanged in the presence of human metabolic enzymes. Metabolic stability study was performed using pooled human liver microsomes and NADPH. Several tested compounds exhibited resistance for human metabolism, both R^2 = 4-CF_3-C_6H_4 and Ar = 4-CF_3-C_6H_4 substituents of 2-(R^2-methylthio)-*N*-(5-aryl-1,2,4-triazin-3-yl)benzenesulfonamides simultaneously increased metabolic stability.

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P27

The evaluation of the antioxidant activity of xanthine derivatives and preliminary study on the neuroprotection against 6-hydroxydopamine neurotoxicity

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In previous studies it was found that the series obtained in our Department xanthine derivatives are active in three ways: they inhibit monoamine oxidase B (MAO-B) and block both A_2 adenosine (A_{2A}AR) and A_1 adenosine receptors (A_1AR) [1]. These activities lead to a synergistic effect which may be useful in the treatment of Parkinson's or Alzheimer's diseases. Oxidative stress also seems to play an important role in neurodegenerative diseases. The imbalance of reactive oxygen species (ROS) and antioxidants could be responsible for the destruction of the cells. Thus, antioxidant activity also means protection of nerve cells from ROS, a further advantage of the xanthine derivatives.
The antioxidant activity was tested with two different *in vitro* methods: oxygen radical absorbance capacity fluorescent assay (ORAC-FL) and ferric reducing antioxidant power assay (FRAP). In both methods all compounds of this series showed antioxidant activity. Especially compound KD-403 showing strong antioxidant properties. Moreover, SAR study showed that the bicyclic compounds seem to be more active than the three-cyclic compounds in ORAC. It was also found that the order from highest to lowest antioxidant activity in the examined series of compounds differs from ORAC-FL and FRAP. However, ORAC-FL seems to be more reliable and preferable due to the presence of radical species in this method.
Additionally, we performed multiparametric *in vitro* assay for evaluation of the neuroprotection against neurotoxin 6-hydroxydopamine (6-OHDA). 6-OHDA is a neurotoxin for catecholaminergic neurons and neuroblasts. It was shown that cell death induced by 6-OHDA is thought to be caused by reactive oxygen species (ROS) derived from 6-OHDA autooxidation and also by a possible direct effect of 6-OHDA on the mitochondrial respiratory [2]. For this study we used a IMR-32 neuroblastoma cell line. It expresses most of the proteins of cholinergic neurons and is appropriate to study oxidative stress related cell death, suggested to occur in Alzheimer disease [3]. The chosen compound for this assay KD-461 showed statistically significant (P<0.05) neuroprotection from the toxic activity of 6-OHDA against cell's mitochondria. Further neuroprotection study are planned using dopaminergic, related to Parkinsonism SH-SY5Y cell line.

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P28

QSAR analysis of tetrahydroacridine derivatives as against methicillin resistant staphylococci

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Though many traditional antibiotics are available on the market now, resistance to many of them (including even vancomycyn) reveals an urgent need in development of new more effective therapies as an alternative. [1] On the other side, derivatives of tacrine showed different biological activity against wide range of targets associated with various diseases including cancer, malaria, viruses, inflammation and much more. [2,3]

We synthesized derivatives of tacrine and cyclopentaquinoline and tested their biological activity using 8 strains (one strain of methicillin-resistant *S. aurerus* as well as *Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus chromogenes, Staphylococcus epidermidis, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus epidermidis)*. After that, we created databases of the 2-D molecular structures of the ligands and their experimental activities. ADMEWORKS ModelBuilder software [6] was used for SAR analysis of each database. For the analysis all the descriptors were calculated and the most independent were chosen for the modeling. Interactive qualitative multiple linear regression model was created for chosen set of the descriptors, antimicrobial activity and database. T-statistic values were calculated and compared for each parameter together with R^2 values for all of the models. Though the most important descriptors in different models are also different, some of them present in several models simultaneously: dipole moments (DIP), minimum electron density value (EDMN), maximum free valence value (FVMX), mass weighted length/width (GEOMWL), lowest unoccupied molecular orbital (LUMO), OPREA rule, shadow areas (SHDW; the areas of three projections of a molecule), maximum nucleophilic superdelocalizability (SNMX) and strain energy (STRA5). According to estimated t2-values, the most representative descriptors for estimation of antibacterial activity were SHDW, SNMX, DIP and STRA5. Our observations also revealed correlation between the activity and the carbon chain length of the compounds (parameter that is also described by STRA5 and SHDW descriptors): activity is positively related to the chain length.

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P29

Complex allosteric events at human mu opioid receptor

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Allostery is one of the most important features of proteins, that greatly contributes to the complexity of life. Allosteric modulators can alter the response of a protein to its ligand – e.g. they can modulate response of a receptor to its agonist, or rate of a reaction catalyzed by an enzyme. Moreover, they present some unique properties, e.g. ceiling effect or probe dependence. The latter, which allows modulators to make proteins respond differently to diverse ligands, is one of their most important and interesting features. It allows for unprecedented selectivity, with different both intensity and quality of modulated response to different ligands. This enables design of a very complex pharmacological action. Unfortunately, allosteric mechanisms are difficult to investigate experimentally.

One of the modulators exerting pronounced probe dependence is BMS986122. The compound is a recently discovered positive allosteric modulator of human mu opioid receptor (MOR). It elicits different influence on number of MOR agonists, depending not on their structure, but on their nature - full agonists are affected in a different way than partial agonists [1]. The exact mechanism of this effect remains unknown.

In the presented work, we used all-atom molecular dynamics simulations of the active-state human mu opioid receptor (MOR) in complex with G protein and in a native-like environment, in order to investigate diverse effects of BMS986122 on action of full (R-methadone) and partial (buprenorphine) agonist. Principal component analysis (PCA) was used to sift all relevant information. This approach have proven to be useful in investigation of allosteric events during our previous studies [2].

Analysis of the results revealed, that the modulator makes the MOR's 7th transmembrane helix (TM VII) act as if the full agonist was bound, even if the orthosteric binding pocket is occupied by the partial agonist. The most affected residues were Y7.53 and Q7.31. There were also noticeable changes in TM VII bending and rotation. Moreover, influence of the modulator on conformation of the 6th transmembrane domain (TM VI) was observed.

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P30

Dendrimeric tribenzoporphyrazines as promising photosensitizers for photodynamic therapy

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Photodynamic therapy (PDT) is a novel non-invasive method of cancer treatment. It involves a photosensitizer, that upon irradiation with UV or visible light generates reactive oxygen species, especially singlet oxygen. Singlet oxygen is known to be a highly oxidative and destructive agent for cells and tissues. Porphyrazines and phthalocyanines exhibit suitable light absorption spectra in the UV-vis range which allows to consider them as potential PDT photosensitizers. Dendrimers are highly branched uniform polymers that have gained a lot of attention due to their applications in drug delivery.

Our goal was to obtain unsymmetrical porphyrazines – tribenzoporphyrazines, substituted in their periphery with dendrons and assess their biological activity. Thus, sulfanyl and pyrazine porphyrazine derivatives decorated with bulky G_0 and G_1 generation aryloxydendrons were synthesized and characterized with special emphasis on their singlet oxygen generation abilities. Next, sulfanyl tribenzoporphyrazines were evaluated in terms of their activity against squamous carcinoma cell lines. All the macrocycles were insoluble in water. Therefore, selected tribenzoporphyrazines were encapsulated in liposomic formulations and subjected to anticancer photocytotoxicity study.

This study was supported by the National Science Centre under grant No 2012/05/E/NZ7/01204. LP and SJ acknowledge financial support from the National Centre for Research and Development under grant No PBS1/A9/13/2012. KK and ND acknowledge funds from the University of the Pacific, Arthur A. Dugoni School of Dentistry.

P31

Synthesis, spectroscopic properties and anticancer activity of new derivatives of 5-chloro-2-methyl-4-nitroimidazole and 4-chloro-2-methyl-5-nitroimidazole

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Nitroimidazoles are very important group of drugs with a wide range of applications in medicine. This compounds are applied in anaerobic bacteria and protozoa infections, in diagnostics of tissues in state of hypoxia and as radiosensitizers. There are reports of anticancer activity of nitroimidazoles. Sobiak and coworkers describe active group of halogenophenacyl derivatives of 5-bromo-2-methyl-4-nitroimidazole and 5-bromo-2-methyl-5-nitroimidazole.

In our work 18 starting derivatives of 5-bromo-2-methyl-4-nitroimidazole and 5-bromo-2-methyl-5-nitroimidazole were converted into proper products **A** and **B** (Fig. 1) by halogen exchange reaction. Exchange process was carried out in concentrated hydrochloric acid. Yield and time of reaction depends on position of bromine in heterocyclic ring (easier reacts 4-bromo-5-nitro compounds) and type of halogen in phenacyl substituent. All new compounds were characterized with 1D and 2D MNR techniques and mass spectrometry. Anticancer activity of new 5-chloro-2-methyl-4-nitroimidazole and 4-chloro-2-methyl-5-nitroimidazole derivatives was examined on 4 cell lines (HeLa, KB, MCF-7, A-549). Generally, highest biological activity was observed for *ortho*- substituted compounds in phenyl ring. Most favorable values of IC_{50} were observed for 5-chloro-1-(2-fluorophenacyl)-2-methyl-4-nitro-*1H*-imidazole – structure **C** in Fig. 1 (HeLA 0.35±0.03 µg/ml, KB 0.35±0.59 µg/ml, MCF-7 0.67±0.02 µg/ml and A-549 0.41±1.07 µg/ml).

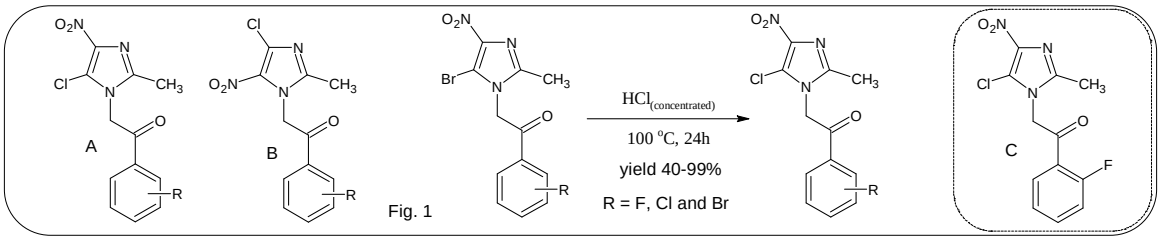

Fig. 1

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P32

Design, synthesis and biological evaluation of hydroxyethylamine derivatives as potential multifunctional anti-Alzheimer's agents targeting cholinesterases and beta-secretase

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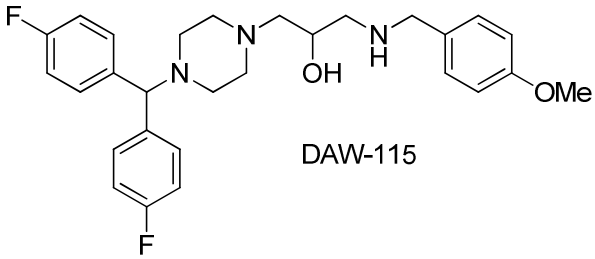
Although more than 100 years have passed since doctor Alois Alzheimer described the most common neurodegenerative disorder, the etiopathogenesis of the Alzheimer's disease (AD) has still not been fully understood. Currently used pharmacological therapy is ineffective and only focuses on treating symptoms, not the causes of the disease. Therefore the development of novel drugs with disease-modifying properties is a great challenge in the modern medicine. Currently, the most recognized theory, that explain the disease process is "β-amyloid cascade hypothesis", which 1emphasize the key-role of β-secretase (BACE-1). [1].

In view of the multifactorial nature of AD, we decided to apply a multi-target-directed-ligand strategy, in order to search for new effective compounds as potential anti-AD agents. In this research, we present the development, synthesis and biological evaluation of compounds, focused on three molecular targets: acetylcholinesterase (AChE), butyrylcholinoesterase (BuChE) and β-secretase. We chose cholinesterases as valid, symptomatic targets still used in pharmacotherapy, and BACE-1 as a disease-modified target. For the design, we used as a lead structure compound NVP-BXD552, which is described as BACE-1 inhibitor [2] and applied a molecular modelling techniques. We estimated the basic physicochemical parameters, characteristic for drug-like compounds using suitable, available computer programs. The results of these studies were used to select the best compounds for synthesis. Several series of N-arylalkyl or alkyl piperazine derivatives, with hydroxyethylamine core and 4-methoxybenzylamine moiety were synthesized. Among the compounds obtained, we found mixed AChE/BuChE and selective BuChE inhibitors with moderate potency also endowed moderate inhibitory activity against BACE1. As the most promising multifunctional ligand, compound DAW-115 was selected.



DAW-115

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P33

Application of linear combination of pharmacophore models in modeling and screening of UDP-N-acetylmuramoylalanine glutamate ligase inhibitors

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UDP-N-acetylmuramoylalanine glutamate ligase (MurD) is one of the most promising biological targets in the development of next-generation anti-bacterial compounds. Along with other members of the amide ligases (MurC–F), MurD inhibits the synthesis of peptidoglycan (PG) – a key bacterial metabolite, crucial for bacterial growth. Anti-bacterial action of MurC–F is indeed very promiscuous, given that this metabolic pathway is common for bacteria and inhibitors of a single enzyme may be multipotent antibacterial agents.
A set of 87 MurD ligase inhibitors available in ChEMBL database (Feb, 2016) and in the literature enables application of *in silico* aproaches for the discovery of new ligands. Here, we applied a linear combination of pharmacophore models as described previously [1]. All MurD inhibitors were hierarchically clustered using Canvas software [2] with some manual refinements ensuring appropriate chemical classification. Next, for each cluster multiple hypotheses were generated. After initial evaluation on DUD-like test set [3], one hpothesis per cluster, with the best statistical parameters, was selected to form the linear combination of pharmacophore models, i.e. the first, general pharmacophore hypothesis of MurD ligase inhibitors.
This collection was used as part of a multistep virtual screening protocol for narrowing down of ca. 8M compounds from commercial databases as well as 100K compounds from combinatorial library of easy-to-synthesize compounds.

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P34

Aggregation of molecules in crystals of glycyrrhizic acid and its hydrophobic analogues

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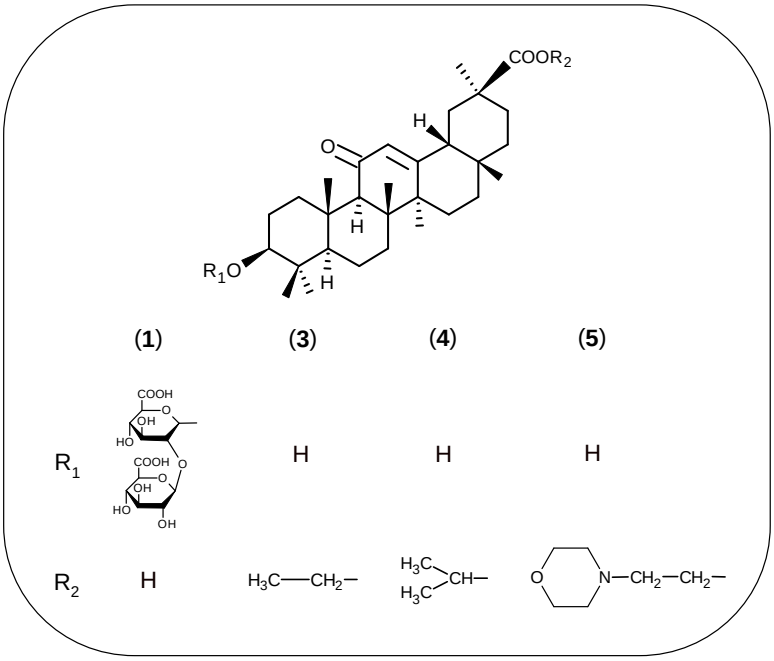
Understanding the nature of intermolecular interaction has a strong impact on designing of novel drug delivery systems (DDS). Glycyrrhizic acid (GA), the triterpene saponin (**1**) found in the licorice roots (*Glycyrrhiza glabra* L. *Leguminosae*) [1] is the natural amphiphilic molecule of great interest to the pharmacy. Both GA and its hydrophobic aglycone – glycyrrhetinic acid (GE) as well as their derivatives are known for the broad range of pharmacological properties [2, 3]. Their application in DDS [4] and the synergistic impact on the therapeutic activity of many drugs have been evaluated in many *in vitro* and *in vivo* studies [5, 6]. However, the mechanism of this effect is not well recognized.



The aim of presented studies was to analyze the supramolecular architectures of biologically active ester derivatives of GE (**2**-**4**) differing by the size of ester substituents and to compare their solid state structures with the structures of GA and its hydrophobic triterpene analogues found in the Crystallographic Data Base. Examination of factors influencing the aggregation modes of GA and its related compounds may be helpful in a better understanding of intermolecular interactions of these amphiphilic and hydrophobic molecules in solution. This knowledge can contribute to a rational design of DDS based on GA molecules.

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P35

Magnetite nanoparticles coated with aminated chitosan for lipase immobilization

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Over the last decade the magnetic nanoparticles research has rapidly increased. Magnetic nanoparticles are an important class of engineered nanomaterials that can be manipulated by external magnetic fields. This type of nanoparticles has been highly employed in chemistry and biomedical applications such as catalysis, hyperthermia, magnetic resonance imaging (MRI), bioseparation, and drug delivery [1-2].
In the present study, a three types of novel multifunctional composite nanoparticles based on magnetite and coated with chemically modified chitosan have been prepared. The chemical modification of chitosan have been performed to give magnetic materials with surface rich of free amino groups distanced from the polymer chain (Fig.1.). The amount of free primary amino groups available on the obtained nanoparticles was estimated by ninhydrin method. The structure of each new synthesized material was fully characterized by XRD, ATR-IR, TEM, BET, and thermal analysis. Prepared nanoparticles were applied for lipase from *Candida rugose* immobilization [2].

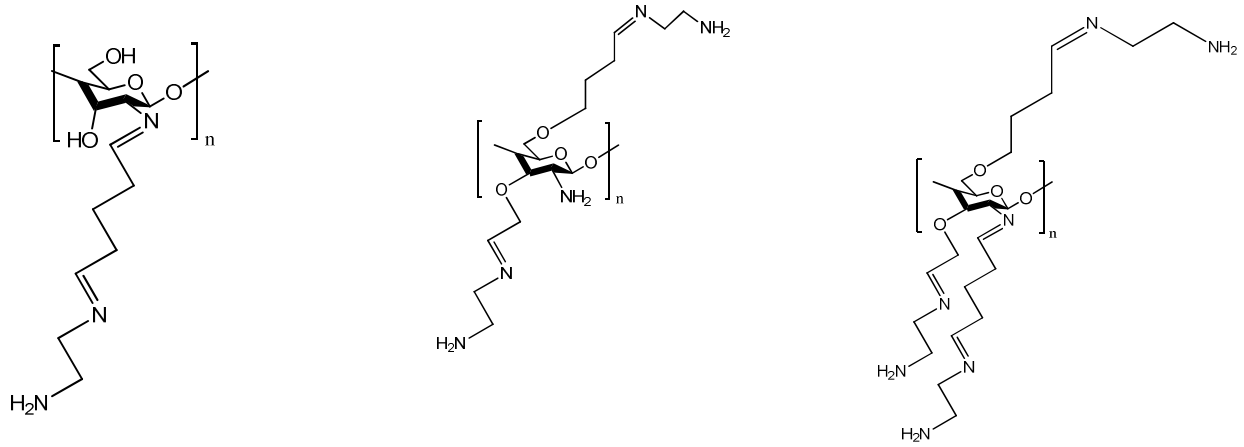

Fig.1. Structure of the modified chitosans

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Acknowledgements:
The project was supported by research grant: National Science Centre 2014/15/D/NZ7/01805.

P36

Evaluation of tuberculostatic activity – structure dependence among isomeric bicyclic nitroimidazoles

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The threat of tuberculosis (TB) is still a reality for millions of people worldwide. What is more, TB accounts the loss of two million lives annually as a result of e.g. presence of multidrug-resistant (MDR) strains [1]. In the last decade, there has been growing interest in developing new substances efficient against *M. tuberculosis*. Nowadays, some bicyclic nitroimidazoles (PA-824, CGI-17341, OPC-67683) (Figure 1) undergoing clinical trials as promising tuberculostatic agents. In our study bicyclic 7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazoles, isomeric with CGI-17341 and 3-hydroxy-8-nitroimidazo[5,1-*b*]-1,4,5,6-tetrahydro-pyrimidines, which are structural isomers of the structure of PA-824 were synthesized and tested for physicochemical properties. It has turned out that there are interesting differences between bicyclic isomers. In order to explain the experimental disparities in biological activity of tested compounds and referenced nitroimidazooxazine PA-824, it was decided to carry out a molecular docking simulations. On the base of the data known from the literature [2], the potential target binding site has been identified – Deazaflavin-dependent nitroreductase (Ddn) from *M. tuberculosis* involved in bioreductive activation of PA-824. Also, it is known, that the most potent aminoacids within of Ddn structure able to interact with PA-824 are: Tyr-130, Tyr-136, Ser-78, and Trp-20. In this analysis, nine conformers has been generated. It was found that only in four cases overlapping of PA-824 and tested 8-nitroimidazotetrahydropyrimidine is observed (Figure 2).

Figure 1.

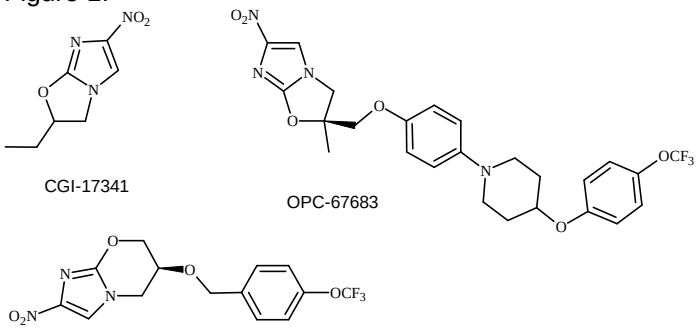
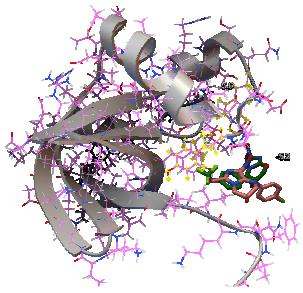


Figure 2.



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P37

In vitro and *in silico* metabolism and microbial transformation of biologically active xanthone derivative (-)-MH3

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(-)-MH-3 is a xantone derivative with typical β-blocker moiety (3-amine-2-hydroxypropan-1-yloxy) showing potent cardiovascular (antiarrhythmic and hypotensive) effect in animal model [1]. What is more this structure was reported as the first compound with virtually the same potency at the low- and high-affinity site (or conformation) of β1-adrenoceptors revealed in *in vivo* studies [2].
In this study, we employed the fungi *Cunninghamella echinulata* NRRL 1384, *Cunninghamella elegans* DSM1908 and rat hepatic microsomes as CYP-P450 models to investigate the *in vitro* metabolism of (-)-MH3. We used MetaSite software to facilitate identification of metabolites produced.
Unchanged (-)-MH3 and 5 metabolites (M1-M5) were profiled and tentatively identified on the basis of UPLC-MS/MS data. The metabolic pathways for (-)-MH3 were proposed. The two major metabolic pathways that are common for rat and microbial systems are: *O*-demethylation (pathway A) and hydroxylation (B). Pathway A formed O-desmethyl - MH3 that is major metabolite in rats while produced in small amounts in both species of fungi. Pathway B formed hydroxy-MH3 (M2 and M3), that that are major metabolites in fungi, and minor metabolites in rats. *Cunninghamella* produced dihydroxy-derivative of MH3 (M4) that was not detected in rats. Prolongation of incubation time with fungi resulted with metabolite M5 – formed in reaction of N-dearylation of MH3, that was also not found in rodent *in vitro* mode. Differences in rate of biotransformation between rat and fungi models, as well as between two species of *Cunninghamella*, were observed. *C. elegans* transformed (-)-MH3 most effectively.
As a conclusion: (-)-MH-3 is substantially metabolized by rat hepatic microsomes fraction and fungi.

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Acknowledgements:
This study was supported by Jagiellonian University Medical College grants: K/ZDS/006161, K/ZDS/005487 and K/DSC/001954.

P38

Antioxidant activity of selenium-containing LC-33 polysaccharide fraction from mycelial culture of *Lentinula edodes* (Berk.)

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Lentinula edodes (Berk.) Pegler, known also as shiitake mushroom, is a great source of bioactive polysaccharides from which the most pharmacologically important is LC-33 (lentinan). According to recent papers, β-glucans can act not only as immunomodulators, but antioxidants as well [1]. The similar mode of action of both polysaccharides and selenium leads to a conclusion that a molecule formed by the incorporation of the microelement into the polymer chain may exert a pronounced biological activity.

The aim of the research was to examine whether the antioxidant activity of the LC-33 polysaccharide fraction might be enhanced by the combination of the molecule with selenium atoms. Selenium-cointaining LC-33 was obtained by the cultivation of *Lentinula edodes* in a liquid medium enriched with 30 ppm of Na_2SeO_3, and its further isolation according to the method described by Chihara and co-authors [2]. We compared antioxidant properties of the polysaccharide obtained from the selenated mycelium with the reference one without selenium, as well as we examined the relationship between the structure of both fractions and their ability to scavenge free radicals.

The structural studies showed that both polysaccharides are protein-free glucans with signals in the IR spectra typical for beta conformation (860-900 cm^{-1}). Structural similarities between the compounds were also manifested in their molecular weight values, as well as in the monosaccharide composition. Both of them consisted of two homologous fractions – the high-molecular weight fraction with the value of above 1000 kDa and the smaller one being in the range of 4.3 to 6.7 kDa. The major monomeric units were glucose (ca. 80%), followed by mannose, and glucuronic acid. HPLC analysis revealed that the content of selenium in the polysaccharide obtained from the mycelium cultivated with the addition of this micronutrient was 23 µg per g of polysaccharide. Based on the results of antioxidant activity assays, we found that either selenium-containing LC-33 or its Se-free analogue did not show reducing power for a ferricyanide compound into ferrocyanide. The scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals was much lower than that of any of commonly known antioxidants (ascorbic acid, α-tocopherol, BHA and BHT). In the test of the ability of inhibiting the peroxidation of linoleic acid selenium-containing LC-33 exhibited a stronger effect compared to the reference polysaccharide [3].

Taking into account the lack of significant differences between the structure of the two polysaccharides, it can be assumed that an increase in antioxidant activity is associated with the presence of selenium incorporated into a polysaccharide molecule. Therefore, for our further research the biosynthesis of polysaccharides with a higher content of selenium needs to be considered.

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P39

Investigation of new, effective and safe substances with photoprotective activity in the group of cinnamic acid and imidazolidine-2,4-dione derivatives

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Sunscreen products protect the skin against detrimental UV radiation. They may counteract sunburn, hyperpigmentation, photoaging and skin cancer formation. The most commonly used chemical UV filters comprise a conjugated bond system that covers a wide range of wavelengths from the UV region. Among the currently available UV filters we can distinguished among others 4-aminobenzoic acid and its esters and benzophenone, cinnamic acid, and salicylic acid derivatives. Unfortunately, most of them posses a number of adverse toxic effects including: neurotoxicity, hepatotoxicity and estrogenic activity. Some of these compounds can be accumulated in different organs, which contributes to the occurrence of unpredictable toxicity. Thus, the need to search for a new effective chemical filters with a favorable safety profile is of great importance.

The aim of the study was to determine UV radiation absorbing ability of new, potential sunscreens (compounds **A-94** and **KM-560)**. Additionally, both compounds lipophilicity and biological properties such as neurotoxicity, hepatotoxicity and estrogenic activity as well as metabolic stability were investigated.
In order to determine the neurotoxicity and hepatotoxicity, MTT cytotoxicity assay was performed against the SH-SY5Y and HepG2 cell lines. Estrogenic activity was determined using estrogen-dependent breast cancer cell line MCF-7, whereas *in vitro* metabolic stability was tested in mouse liver microsomes.
Test compounds which demonstrated UV light absorbing ability showed no neurotoxicity and hepatotoxicity, even at high concentrations, and did not affect the proliferation of estrogen-dependent MCF-7, suggesting a lack of estrogenic activity. In addition, **A-94** was more metabolically stable than **KM-560** in mouse liver microsomal system as evidenced by its longest half time and slowest intrinsic clearance values.
To sum up, test compounds **A-94** and **KM-560** absorb UV light in the desired wavelength range. Both substances demonstrated *in vitro* no adverse toxic effects. Moreover, the metabolic stability and lipophilic nature of these compounds may suggest that both of them will not be accumulated in the body tissues. The obtained preliminary results indicate that test compounds have favorable physicochemical and biological properties and encourage further research on their potency and photoprotective activity.

P40

New 1-(1,4-disubstituted-4,5-dihydro-1*H*-imidazo)-3-arylurea derivatives-synthesis and pharmacological evaluation

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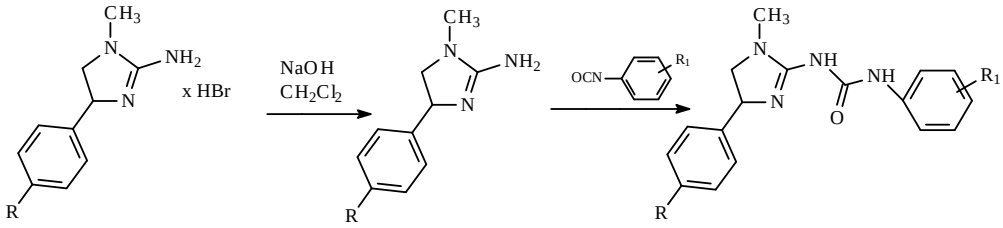
Despite the great progress in the treatment of pain, depression, anxiety, and other central nervous system (CNS) disorders, the used drugs do not fulfil all expectations. And there is still a need to search for new, more effective therapies as well as need to know the mechanisms of action of existing drugs. The tested compounds are a group of urea derivatives with expected potential central activity in the CNS.

Urea derivatives show a broad spectrum of biological activities, such as analgesic, antimicrobial, anticancer and central properties [1-3]. What is more, several behavioural reports indicated that urea [1-2] derivatives demonstrated a potential anticonvulsant activity in different behavioural tests.
Progress can be achieved not only by using novel mechanisms, but also by exploiting established targets by new structures. In this context, new derivatives of 1-(1-alkyl-4-aryl-4,5-dihydro-1*H*-imidazo)-3-substituted urea derivatives, that are generally hoped to possess fewer side effects, were synthesized as attractive therapeutic sources for the development of new relevant drugs for the management of pain.

The results of the pharmacological studies showed that new compounds exerted substantial impact on the CNS in mice. The most important seems to be their influence on the transmission of serotonin (activity in the head-twitch test, changes in body temperature) as well as antinociceptive effects, probably connected with endogenous opioid system. Novel compounds were synthesized from appropriate 1-aryl-4,5-dihydro-1*H*-imidazo-2-amines and appropriate isocyanate in dichloromethane (Scheme 1).



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P41

Possibilities of using techniques of hydrophilic interaction chromatography (HILIC) in the analysis of natural compounds

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Hydrophilic Interaction Liquid Chromatography (HILIC) was defined by Andrew Alpert in 1990, however the first time this technique was used was in the 70s, when Linden proposed separation of sugars on a column filled with silica, modified with amino groups with mobile phase of acetonitrile-water (75:25) [1,2].
HILIC allows the determination of polar, water soluble compounds. It is an alternative to reverse phase chromatography (RP) way of separating organic polar compounds. It can be used in the determination of carbohydrates, amino acids, proteins, vitamins, medicines [3].
The order of elution of the substance is generally opposite to the normal phase. Hydrophilic compounds have greater retention than hydrophobic compounds. Increase in organic component of the eluent increases retention - contrary to chromatography in reverse phase systems [4].
The mechanism of retention in HILIC is complex and not completely understood. It is believed that the water part of mobile phase is adsorbed on the surface of the polar stationary phase in form of a thin layer, where division of the test sample between the phase and the organic part occurs [5].
Also, depending on the content of organic solvent in the eluent, combination of mechanisms characteristic of chromatography in regular and reverse phase systems, has an impact on the total retention system [3]. Large impact on the retention mechanism, particularly with low water content eluents, may also have absorption interactions of hydrogen bonding, hydrophobic interactions as well as electrostatic interactions [3].
Due to the possibility of using modified stationary phases with functional groups with diverse characteristics, it becomes possible to solve complex separation problems, separation and determination of monosaccharides and disaccharides in the process of control of fermentation processes or food processing and analysis of flavonoids in natural samples.

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P42

Search on phenylalanine-based AMPA receptor ligands

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Kainate (KA) receptors are cation-selective ligand-gated ion channels that belong to the family of ionotropic glutamate receptors together with α-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. They are widely expressed in the mammalian central nervous system, particularly in the hippocampus, where they act principally as the modulators of synaptic transmission and neuronal excitability. Functional KA receptors are tetrameric structures formed by the combination of subunits GluK1–5, which are assembled into two dimers of two homo- or two heteromeric subunits.

In recent years, dynamic growth has been reported in the understanding of the biophysical properties and function of KA receptors in the brain. A particular problem in the studies on this type of receptors is the relative lack of specific pharmacological tools, especially for the GluK3 receptor. Most known KA receptors agonists and antagonists, including AMPA and kainate themselves, act on both AMPA and KA receptors.[4] GluK1 antagonists have been shown to be highly effective in animal models of epilepsy, neurodegeneration, neuropathic pain and migraine. More compelling evidence supports a role for KA receptors in Huntington's disease and fear memory.

The present project is a continuation of earlier studies on potent and selective competitive ligands of kainate receptors among phenylalanine derivatives. A new series of carboxyaryl-substituted phenylalanines was designed, synthesized and pharmacologically characterized *in vitro* at native rat ionotropic glutamate receptors as well as at cloned homomeric kainate receptors GluK1-GluK3. Among them, compounds binding selectively to GluK1 receptor subtypes with high affinity have been identified. A structure-activity relationship (SAR) for the obtained series, focused mainly on the pharmacological effect of structural modifications in the 4- and 5-position of the phenylalanine ring, was established.

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P43

Crystal structures and evaluation of 2[*1H*]-pyrimidinethione/selenone derivatives for antimicrobial activities

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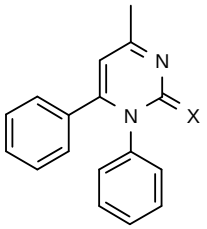
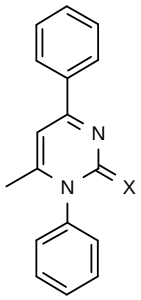
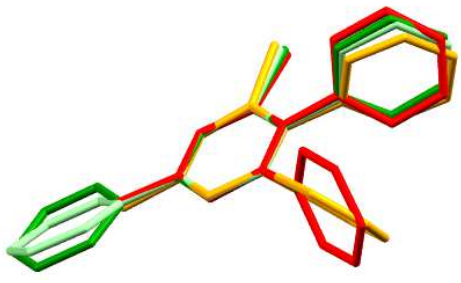
Nowadays, the design of new scaffolds with antibacterial and antifungal activities has become one of the most important areas of medicinal chemistry. Despite continuous search for new antimicrobial agents, there is still a need to find new compounds due to resistance development of microorganisms (bacteria, fungi, viruses and parasites) to antimicrobial drugs [1]. In the group of antimicrobial drugs there are very often imidazole, triazole, thiazole and other compounds with nitrogen atom or atoms in six-membered ring e.g. ciclopirox, flucytosine. Our interest focused especially on pyrimidinone derivatives, which sulfur and selenium analogues have been synthesized in our laboratory.

In order to find new antimicrobial compounds, sulfur and selenium analogues of pyrimidin-2[*1H*]-one derivatives were evaluated for their antibacterial and antifungal activities. The investigated compounds were synthesized as described previously [2]. Preliminary investigations have identified a highly potent antibacterial and antifungal compound.



X = S, Se





The molecular structures of investigated compounds were determined by single crystal X-ray diffraction method. The investigated compounds possess different orientation of phenyl groups relative to the pyrimidine plane.

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P44

In vitro evaluation of metabolic activity of GPR18 ligands

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The endocannabinoid system plays a crucial role in impacting human health and diseases. This system with its several endogenous and exogenous modulators seem to have a therapeutic potential which encompass metabolic disorders, cardiovascular disorders, pain management or inflammation. Recent reports showed that GPR18 which is G protein-coupled receptor (GPCR) is the site of action of several natural and synthetic cannabinoids and has been recognized as a putative cannabinoid receptor [1,2]. GPR18 with yet unknown biological function may be considered as a potential novel drug target. To understand the physiology and pharmacology of GPR18 is vital to examine its association with the well characterized cannabinoid receptors and ligands.

Chemical synthesis can provide a variety of compounds which exert their action via binding to receptor and led to changes in general activity within cells. According to the recent research for new potent and selective GPR18 antagonists [3], new compounds were synthesized and investigated to confirm the lack of their certain toxic properties. We studied CB-5, CB-27, MZ-1415, MZ1440 in metabolic activity of several human cancer cell lines: endometrial carcinoma (HEC-1B), lymphoma (HuT 102), melanoma (M10, BLM) hepatocarcinoma (HepG2), neuroblastoma (SH-SY5Y) and cancer-like cells: embryonic kidney (HEK-293). We confirmed cell proliferation by MTS assay, which detects mitochondrial enzyme activity correlating with the numbers of living cells. We found that in the presence of MZ1415 (10µM and 1µM) and MZ1440 (10µM and 1µM) the metabolism of all cell lines was decreased. Surprisingly, phase-contrast microscopy of several cell lines in the presence of 10µM MZ1415 and MZ1440 showed large cytoplasmic vacuoles whereas the plasma membrane was unaffected. In contrast, no such difference between the action of CB-5 and CB-27 in all cell lines was observed. In addition, Δ^9-tetrahydrocannabinol (Δ^9-THC) – an ogonist of GPR18 [2] - had no significant effect on cell proliferation except for lymphoma and neuroblastoma cells.

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P45

Aminoporphyrazines as potential photosensitizers for photodynamic therapy

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Photodynamic Therapy (PDT) is based on the irradiation of photosensitizer localized at the affected site of the tissue, which causes generation of the singlet oxygen. It is known that conversion of light energy into chemical energy leads to chemical destruction of tissues or pathogens. Porphyrazines (Pzs) possess interesting optical properties, especially fluorescence and an ability to generate singlet oxygen. Therefore Pzs can be considered as potential agents for Photodynamic Therapy (PDT) [1]. The UV–vis spectra of these macrocycles have a broad Q-band, which in most of the solvents, is divided into two sub-bands located in the range of 630–640 nm and 660–680 nm. The presence of long Q-band absorption wavelengths observed for diazepinoporphyrazines suggests that these macrocycles can be considered as potential photosensitizers for PDT, as light of longer wavelength is capable of deeper penetration of irradiated tissue.

In our study we present synthesis of novel diazepineporphyrazine with peripheral 4-methoxyphenyl and 3,5-bisbenzyloxybenzyl substituents. The synthesis of the expected porphyrazines was performed following a multistep procedure starting from commercially available diaminomaleonitrile. The dimainomaleonitrile derivatives were subjected to the macrocyclization reaction with magnesium *n*-butanolate in *n*-butanol to give novel magnesium porphyrazines. Macrocyclic products were carefully purified *via* flash colum chromatography and characterized using various analytical techniques, especially NMR (1D and 2D). Pzs possessing peripherally annulated diazepine rings have been subjected to advanced physical–chemical studies. The potential photosensitizing activites of the obtained porphyrazines were evaluated by measuring their abilities for singlet oxygen production, which is the result of an interaction between an activated photosensitizer and oxygen.

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Acknowledgements:
This study was supported by the National Science Centre under grant No 2012/05/E/NZ7/01204. PL and SJ acknowledge financial support from the National Centre for Research and Development under grant No PBS1/A9/13/2012.

P46

AMPA/KA receptors antagonists with potent antioxidative activity

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The recent study have demonstrated the direct association of mitochondrial dysfunction and oxidative stress with epileptogenesis, acquired chronic epilepsy as well as with the Alzheimer's and Parkinson's diseases [1-2]. In this context, the new multi-target acting compounds are desired in the treatment of neurodegenerative diseases which would act not only *via* relevant receptors but also possessing neuroprotective activity preventing from reactive oxygen species (ROS), for instance antioxidants which could directly scavenging ROS and/or compounds triggering protective mechanisms inside the cell, resulting in an improved defense against ROS.

One of the effective ways to preventing glutamate-induced neurotoxicity is to target over-activation of ionotropic glutamate receptors (iGluRs), especially AMPA and kainate (KA) receptors. Competitive antagonists of this family of receptors have been demonstrated to possess a broad-spectrum of neuroprotective and anticonvulsant activity. In our Department the series of new class of competitive and potent AMPA/KA receptor antagonists, based on the structure of phenylalanine, have been successfully obtained [3].

During this study twenty eight most active aryl-substituted phenylalanine derivatives were screened for their antioxidative activity. The antioxidant properties were tested with two different methods: oxygen radical absorbance capacity fluorescent assay (ORAC-FL) and colorimetric-based ferric reducing antioxidant power assay (FRAP). The results were compared to the antioxidative effect of the references: Trolox - the analog of vitamin E, and ascorbic acid. The results were expressed as Trolox equivalents (TE) in ORAC-FL method and % of ascorbic acid activity (%AAA) in FRAP.

Surprisingly, the order from highest to lowest antioxidant activity in the examined series of compounds differs from ORAC-FL and FRAP. However, ORAC-FL seems to be more reliable and preferable due to the presence of radical species in this method. Based on calculations we emerged the best antioxidant from each method: ES 13-19 for ORAC-FL (TE=6.97) and ES 13-25 (AAA%= 748.49) for FRAP. Moreover, the preliminary SAR study was also conducted. Further study on their neuroprotective activity using cholinergic IMR-32 and dopaminergic SH-SY5Y cell lines are planned.

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P47

Synthesis of new 4-aryl-pyrido[1,2-C]pyrimidine derivatives with dual SERT/5-HT$_{1A}$ activity

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Earlier research on synthesis and biological evaluation of pyrido[1,2-c]pyrimidine derivatives carried out in the Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw described a series of compounds with 3-(piperidin-4-yl)-1H-indole residue in the pharmacophore element. They possessed a high affinity to both molecular targets – 5HT$_{1A}$-R and SERT - as well as a suitable functional profile with 5-HT$_{1A}$ receptor binding (pre- and postsynaptic agonism) [1]. The aim of current research was to implement 3-(piperidin-3-yl)-1H-indole structure in place of 3-(piperidin-4-yl)-1H-indole residue in order to achieve more serotonine-like pharmacophore element.
The concept of combining SSRI activity with 5-HT$_{1A}$ agonism was proposed and extensively studied in recent years as a promising strategy for potential new antidepressants development [2-4]. The validity of this approach was confirmed by the registration of vilazodone (Viibryd) and vortioxetine (Brintellix), which entered the USA market in 2011 and 2013 respectively as dual acting antidepressants. Moreover, in clinical trials vilazodone proved to be well tolerated, with a low discontinuation level and lack of severe, life-threatening adverse effects [5,6].
Novel 4-aryl-pyrido[1,2-c]pyrimidine derivatives are obtained in a way of a multi-step chemical synthesis and subjected to analytical studies, using the methods of 1H NMR and 13C NMR spectroscopy as well as HRMS. The pharmacological profile of the obtained compounds was assessed in radioligands binding assays (5-HT$_{1A}$, SERT). In vivo functional studies will be conducted in the Institute of Pharmacology, Polish Academy of Sciences and Department of Pharmacobiology, Jagiellonian University Medical College in Kraków; metabolic stability evaluation in the Department of Pharmaceutical Chemistry, Medical University of Gdańsk. The results of in vitro and in vivo studies will allow to draw conclusions regarding structure-activity relationship in the tested group of compounds and to select compounds for further pre-clinical evaluation.

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P48

Phosphonylated naphthalimide derivatives

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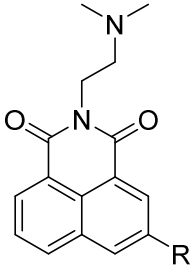
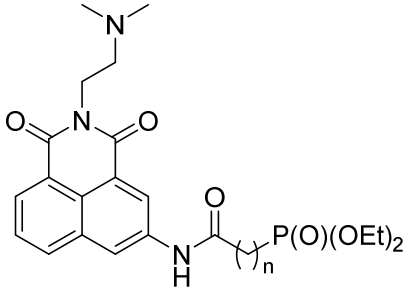
Pharmacologically important compounds having various heterocyclic systems, including powerful pharmacophores such as naphthalimides, are of special interest. Naphthalimide derivatives, namely amonafide **1** [1,2] and mitonafide **2** [3], exhibit the intercalating properties, however their clinical use have been limited due to low therapeutic index as well as poor water-solubility [4]. In order to improve therapeutic properties of naphthalimides many efforts have been undertaken to obtain new compounds with higher activity and lower toxicity.

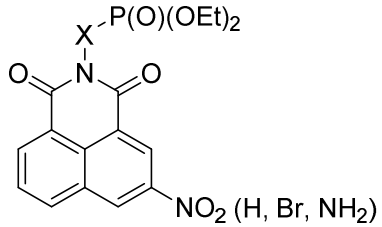
As a continuation of our ongoing project directed towards the synthesis of various naphthalimide derivatives we designed new series of compounds of general formulae **3** and **4** and their antiviral and anticancer properties were assayed.



$R=NH_2$ amonafide **1**
$R=NO_2$ mitonafide **2**



3 n=1, 2



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P49

Spectral characteristic and preliminary anticancer activity in vitro of selected rhodanine derivatives

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Rhodanine (2-thioxo-4-thiazolidinone) derivatives possess a broad spectrum of various biological activities, among which are anticancer, antifungal, antimicrobial, antidiabetic, antiapoptotic and pesticidal ones.[1-6] Although some of the already known compounds are used clinically, there is still a need for new xenobiotics, which are characterized by a broad spectrum of pharmacological activity and low toxicity.

Herein we present the characteristic of interactions of selected rhodanine derivatives, see Figure 1, with Human Serum Albumin (HSA), the most abundant protein in the blood plasma. HSA is one of the macromolecules responsible for the drugs binding and transport. Therefore, it is particularly important to examine the interactions of tested rhodanine compounds with the protein.

The obtained spectroscopic results allow us to determine the influence of the length of the linker between the carboxyl group and the nitrogen atom at N3 position in the 2-thioxo-4-thiazolidinone ring on the interactions between the protein and the proper derivatives. Furthermore, based on the bimolecular quencher constant k_q values and the independence of the rhodanine derivatives concentration on the average fluorescence lifetime of the protein, the mechanism of quenching has been proposed. Additionally, the anticancer activity of the tested compounds was shown in human ovarian adenocarcinoma (A2780) and A2780 resistant to cis-platinum (A2780cisR) cell lines.

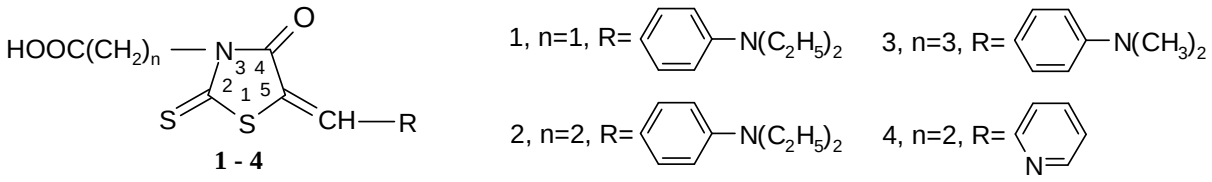

Figure 1. The chemical structures of studied rhodanine derivatives.

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P50

Methylpiperazine derivatives with 1,3,5-traizine scaffold - a novel group of ligands for serotonin receptors 5-HT$_6$

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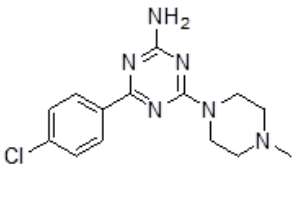
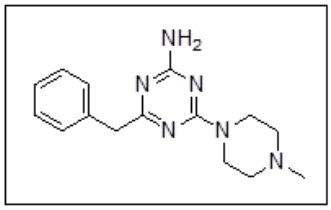
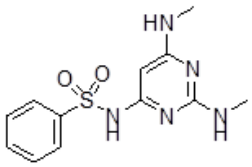
The 5-HT$_6$ receptor is the most recently identified member of the 5-HT receptor superfamily. Intensive preclinical studies have shown that 5-HT$_6$R antagonists could be a promising drug with cognitive improvement in psychiatric (*e.g.* schizophrenia, depression) or neurodegenerative diseases (*e.g.* Alzheimer's disease), and for obesity treatment [1]. Not only antagonists but also agonists have potency for the treatment of obesity or cognitive disfunctions [2]. In this context, the search for new ligands of the 5-HT$_6$R is a current topic in medicinal chemistry. Recently, 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives have been described as histamine H$_4$ receptor ligands [3], i.e. **TR7** and **TR20** (Fig. 1). As first known selective 5-HT$_6$ ligands, e.g. **RO046790** (Fig. 1), contain some structural similarities to 2,4,6-trisubstituted 1,3,5-triazines, we decided to investigate this interesting chemical group on its potency toward 5-HT$_6$ receptors.



TR7
hH$_4$R K$_I$ = 203 nM



TR20 (lead)
hH$_4$R K$_I$ = 7499 nM



RO046790
h5-HT$_6$ K$_i$ = 55 nM

Fig. 1

Docking studies and radioligand binding assays for compounds **TR7** and **TR20** indicated significant 5-HT$_6$ affinity for compound **TR20,** whereas the **TR7** was almost inactive. Thus, the compound **TR20** was selected as a lead structure for further modifications to give a series of 14 benzyl-triazine derivatives of methylpiperazine. Synthesis, molecular modelling and radioligand binding assays have been performed. Nine new compounds displayed higher affinity for 5-HT$_6$ than **TR20**. The docking-based SAR studies indicated a crucial role of substituent at *m*-position of benzyl ring for the considered 5-HT$_6$ affinity.

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Acknowledgements:
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P51

Synthesis of novel oxicam derivatives as potent colorectal cancer chemopreventive agents and their interaction with model phospholipid membranes

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Oxicams (e.g. piroxicam, meloxicam) are a class of nonsteroidal anti-inflammatory drugs (NSAIDs), which are widely used to treat pain, inflammation and fever. One of molecular targets of NSAIDs is cyclooxygenase-2 (COX-2), which is also overexpressed by most solid tumours. The trials of aspirin and COX-2 inhibitors showed that those medicines reduce the risk of colorectal cancer (CRC), although, unfortunately, neither former nor latter may be used in chemoprevention because of an increased risk of vascular events (COX-2 inhibitors), or the greater risk of bleeding complications (aspirin) [1]. This is the reasoning behind the examination of other NSAIDs as potential inhibitors of CRC. We designed new oxicams derivatives as potential multitarget drugs which would be an analgesic, anti-inflammatory and, at the same time, chemopreventive in cancer. The former preliminary experiments carried out on the few newly synthesized oxicams derivatives revealed that they reduce the level of the expression of COX-2, Bcl-2 protein and ABCG2 multidrug transporter in the human colorectal adenocarcinoma cell line LoVo [2].
Furthermore, all COX enzymes – the primary target of NSAIDs – reside in biological membranes, which is why, the drug–membrane interactions play a crucial role in enabling the drug– enzyme connection, and also affect the NSAIDs selectivity [3]. It is accepted that NSAIDs can induce changes in the fluidity, permeability, and biophysical properties of cell membranes.
On the basis of this previous work, and having read several articles on the subject, we decided to extend our research into the areas of synthesis and the effects of three novel benzothiazine 1,2 derivatives.
In the present work, we describe the synthesis and the effects of 3 novel 1,2-benzothiazine derivatives on model lipid membranes using calorimetric and fluorescence spectroscopic experiments. We examined the influence of these new compounds on the phase behaviour of phospholipid bilayers and their membrane location by studying the incorporation of different fluorescent probes (laurdan and prodan).

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P52

Synthesis, QSAR studies and metabolic stability of novel 2-mercapto-*N*-(5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)benzene-sulfonamide derivatives with potential anticancer activity

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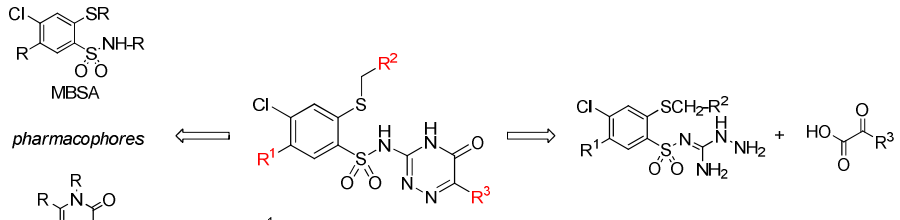
Recently, we reported on the significant anticancer activity of a series of 2-mercapto-N-(1,2,4-triazin-3-yl)benzenesulfonamides bearing phenyl or substituted phenyl attached to the 1,2,4-triazine moiety simultaneously at positions 5 and 6 [1]. In the present study we elaborated the synthesis, anticancer evaluations and QSAR studies of novel N-(6-substituted 5-oxo-4,5-dihydro- 1,2,4-triazin-3-yl)benzenesulfonamide derivatives as shown in Scheme below.The synthesis of the desired N-(5-oxo-4,5-dihydro-1,2,4-triazin-3-yl)benzenesulfonamide derivatives were achieved by reacting of the corresponding 3-amino-2-(benzenesulfonyl)guanidines [2,3] with suitable alpha-oxo acids in refluxing glacial acetic acid for 4-5 h.



Anticancer *in vitro* screening was performed at the Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG with using three cell lines of breast (MCF-7), colon (HCT-116) and cervix cancer (HeLa) and at the NCI (Bethesda MD, USA) using 60 cell lines derived from 9 types of human tumors. QSAR analysis was performed to correlate the cytotoxic activity of 1,2,4-triazin-5-one derivatives with their chemical structures. The constructed QSAR models for HeLa, HCT-116 and MCF-7 cell lines possesses good predicative performance in predicting cytotoxic activity with high statistical significances.

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P53

Synthesis and properties of thiazole derivatives incorporating 1,3,5-triazine moiety

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In recent years an incidence of fungal diseases has increased dramatically [1]. Similarly, multidrug resistance reaches alarming level which makes the treatment extremely difficult, very costly and associated with high mortality [2]. To minimize this process there is an emergency need for discovering innovative classes of medications [3].
In new drug development studies, a combination of various pharmacophores in the same molecule might result in enlarged spectrum of activity, reduced potential for spontaneous mutations and reduction of toxicity of therapy [4,5].

Considering these facts, the present research was aimed at synthesis of twelve thiazole derivatives of 1,3,5-triazine as potential antimicrobial and anticancer agents. The final products contain in their structure two pharmacophores: the thiazole ring and the 4,6-diamino-1,3,5-triazine, which allows formation of hydrogen bonds with the DNA [6]. Thiazole and triazine derivatives have become the subject of intensive studies because of their wide range of biological activities and interesting chemical and structural properties [7-10].
The compounds were obtained in a three-step synthesis with a good yield and high chemical purity. In the first step the 4,6-diamino-1,3,5-triazine was combined with the corresponding aminoacetophenone. Next, obtained ketone was converted into thiosemicarbazone in the presence of hydrochloric acid. In the final step Hantzsch cyclization reaction of thiosemicarbazone with appropriate *para*-substituted bromo- or chloroacetophenones afforded the corresponding triazine-thiazoles. Profiles of biological activity of the synthesized substances and the simulations of their carefully selected physicochemical properties were also provided by using computer programs.

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P54

Synthesis and physicochemical properties of new intercalating-alkylating compounds

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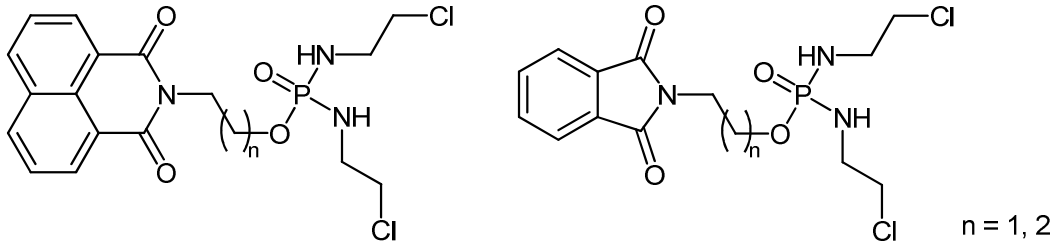
The problem of cancer diseases every year becomes more and more challenging for all of humanity.[1] In the face of the emergence of newer and newer, more malignant tumors, localized in difficult to reach places, modern scientists are forced to continuously search for new treatments. Unfortunately, despite efforts to obtain effective “weapon” against cancer remains an open question.

Modern cancer therapy is based on chemotherapy, radiotherapy, hormonal therapy, immunotherapy, targeted therapy and surgery. Chemotherapy drugs affect on the tumor cells in a different way: the impact on the DNA synthesis process, transcription or different phase of the cell cycle. Chemotherapeutic agents damage the genetic material interacting with the double helix of DNA by alkylation, intercalation, binding to a large or a small groove or mixed non-specific interaction.

According to recent studies DNA alkylating agents are promising in the cancer chemotherapy. In our research we design such compounds which contain a potentially intercalating unit, derivatives of naphthalimide and phthalimide, coupled with isophosphoramide mustard, a DNA alkylating agent, which is active, cytotoxic metabolite of ifosfamide (IF), a widely used anticancer alkylating drug.[2].



Our research concentrated on the synthesis, the selected chemical properties, compliance with the Rule of Five, expected antitumor activity and impact of synthesized compounds with DNA.

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P55

Conformational analysis of *N*-[4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl]phthalimide as 5-HT_{1A} ligand

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Our studies have shown that in the group of ligands with 1-(2,3-dichlorophenyl)piperazine moiety the chain length significantly impact on the affinity activity for the 5-HT_{1A} receptor. In the case of ligands, which have in terminal part an amide or a sulfonamide group, the elongation of the alkyl chain increases the affinity activity for the 5-HT_{1A} receptor. However, in the case of the ligands having a phthalimide moiety, the highest activity shows ligand **1a** which contains four carbon atoms linker [K_i = 9 nM]. Elongation of the chain by one CH_2 group results in a decrease in affinity for the 5-HT_{1A} receptor [**1b**, K_i = 35 nM]. However, further extension for the hexyl connector causes an increase in activity [**1c**, K_i = 15 nM]. Interestingly, in the group of the corresponding derivatives containing 1-(2-methoxyphenyl) piperazine moiety the affinity for the 5-HT_{1A} decrease with increasing alkyl chain (**2a**, K_i = 0.6 nM [1], **2b**, K_i = 7.2 nM; **2c**, K_i = 22 nM [2]).
Considering the above, an assessment of the bioactive conformation of the ligand **1a** was performed conducted. Within the framework of the research work, we evaluated the conformation of the ligand in the 5-HT_{1A} receptor binding site, which was compared with the conformation in the crystal and obtained from DFT calculations (b3lyp/6-311+G**). in vacuo with fully relaxed geometry.

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P56

Synthesis and evaluation of anticonvulsant properties of new amides derived from 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid

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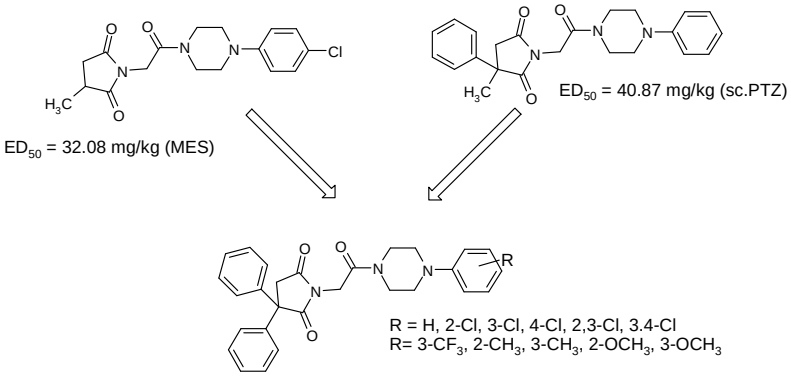
Recent studies on the structure-activity relationship (SAR) demonstrated the potent anticonvulsant activity of the amides of 3-methyl- and 3-methyl-3-phenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acids among which the most active were 1-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (**I**) with an ED_{50} = 32.08 mg/kg in the MES-test and 3-methyl-1-[(2-oxo2-(4-phenylpiperazin-1-yl)-ethyl]-3-phenyl pyrrolidine-2,5-dione with an ED_{50} = 40.87 mg/kg in the *sc*.PTZ test.[1,2]

Considering the after mentioned results, as part of our efforts to designed new anticonvulsant agents in the present study we have synthesized a series of analogs in which we introduced two phenyl substituents at position-3 of imide ring. The proposed structural modifications enable to assess the influence of aromatic groups on anticonvulsant activity in this series of amides. With the aim of ensuring the reliable SAR study as an amine function, variously substituted 4-phenyl-piperazines have been introduced.



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The target compounds were prepared in a coupling reaction of 3,3-diphenyl-succinic acid with appropriate substituted 4-arylpiperazines in the presence of carbonyldiimidazole reagent.
The anticonvulsant activity was determined in the maximal electroshock (MES), subcutanous pentylenetetrazole (scPTZ) and in the 6-Hz psychomotor seizure tests in mice after ip administration of doses 100 and 300 mg/kg. The acute neurological toxicity was determined in the minimal motor impairment rotorod screen [3].

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P57

Homology modeling of adenosine A_3 receptor

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Adenosine receptor (AR) ligands are currently being developed as promising agents for CNS disorders (Parkinson's, Alzheimer's diseases, ischaemia) [1]. Moreover, the adenosine A_{2A} receptor has been co-crystallized with several ligands, agonists as well as antagonists. It can thus serve as an optimal template with a well-described orthosteric ligand binding region for AR.
As not all subtypes have been crystallized yet, and in order to investigate the usability of homology models in this context, multiple adenosine A_1 receptor (A_1R) homology models have been previously obtained and a library of lead-like compounds has been docked [2]. As a result, a number of potent and one selective ligand toward the intended target were found. However, in *in vitro* experimental verification studies, many ligands also bound to the A_{2A}AR and the A_3AR. Therefore, the aim of this work was to build an A_3AR homology model in order to see whether the experimentally obtained binding profiles [2] can be reproduced *in silico*.

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P58

A novel mGluR7 negative allosteric modulators

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The metabotropic glutamate receptors (mGluR) represent class C GPCRs and play significant neuromodulatory roles throughout the brain. The metabotropic glutamate receptor 7 (mGluR7) is a member of group III family that binds to protein G and inhibits the adenylate cyclase [1]. The mGluR7 has the highest CNS dentisity of all group III mGluR subtypes due to widely distribution and presence at broad range of synapses [2,3]. Many studies have shown that mGluR7 receptor is an important target for therapeutic intervention in a number of neurological and psychiatric disorders including anxiety, post-traumatic stress disorder, depression, autism, drug abuse, and schizophrenia [1-4]. In terms of discovery of new selective ligands the mGluR7 receptor is one of the most challenging of the all mGluR subtypes [4]. So far only few compounds which influence on the mGluR7 receptor are known: positive modulator - AMN082 [5], negative allosteric modulators - MDIP, MMPIP [1] and ADX71743 [6]. Therefore the discovery of highly selective mGluR7 ligands which can be used in clinical trials seems to be still the most significant challenge.
The development of novel chemical scaffold possessing activity towards mGluR7 receptor is the aim of present study. So far a variety of chemotypes were synthesized and examined *in vitro*, followed by the primary *in vivo* evaluations. The studies have shown new quinazolinone derivatives as promising mGluR7 negative allosteric modulators. Primary pharmacokinetics results demonstrated that concentration of ALX-171 in plasma as well as in brain is higher than for the reference ADX-71743 compound

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P59

Modifications of phenyl ring of 5-methyl-5-phenylhydantoin derivatives in search for selective 5-HT$_7$ agents with antidepressant action

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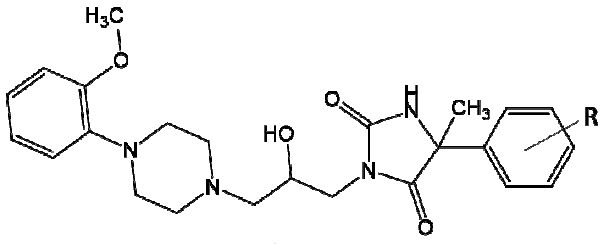
According to recent literature, serotoninergic 5-HT$_7$ receptors seem to be reasonable therapeutic target for treatment of CNS disorders such as schizophrenia, depression, migraine [1]. Our previous studies were focused on selection of the best substituent on piperazine moiety (Fig. 1) and resulted with synthesis of 14 novel 5-HT$_7$ antagonists with affinity (*Ki*) range from 3 nM to 79 nM [1,2].



R= H, F, Cl, Br, CH_3, OCH_3
Fig. 1

The second step was to find the most profitable substituent and its position on phenyl ring in position 5 of hydantoin (Fig. 1), which is presented within this work. Crystallographic analysis has been performed for one representative compound from the series (KKB23). Synthesized derivatives showed high to moderate affinity to 5-HT$_7$ (6nM<*K*i<94nm) in radioligand binding assays. Two of them (compounds KKB24 and KKB28) were evaluated in behavioral tests to check potential antidepressant activity. Both compounds reduce immobility time in forced swim test on mice.

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P60

Synthesis and properties of novel quinuclidone-based thiazoles

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Epilepsy is a disorder expressed by recurrent seizures of cerebral origin. Seizures are revealed by convulsions, impaired sensory and movement disorder. Convulsions often, but not always are a symptom of epilepsy. They might be caused by various conditions such as: fever, infections, hypoxia, usage of narcotic drugs, withdrawal syndrome, head injury or brain tumors. In some cases their origin remains unknown. The reason of these disorders is excessive excitability of neurons in the brain stem and in the motor cortex. About 0.5 to 1% of the population suffer from chronic epilepsy, with almost 30% of patients being resistant to available drugs. The search for new effective anticonvulsants is thus very important [1].

Strong correlation has been shown between infections of central nervous system caused by bacteria, fungi, viruses or parasites and various neurological disorders [4].

Thiazole derivatives due to their various pharmacological properties are subject of increasing interest among researchers in recent years. Potential anticonvulsant and antimicrobial activities are investigated [1].

The purpose of this work was to design and synthesize the series of 2,4-disubstituted-1,3-thiazoles being derivatives of quinuclidone. The products contain in their structure different substituents including electron-donating and electron-withdrawing or sterically extended adamantyl and chromene groups. As a result of the synthesis eleven novel compounds were obtained. The synthesis of each compound was a two-step process. In the first step the thiosemicarbazone was obtained from thiosemicabazide and ketone in the anhydrous ethyl alcohol. In the second step, the expected products were obtained in the reaction of thiosemicarbazone with various bromoacetophenones with 28 to 78% yields.

Lipophilicity, hydrogen bond donors and hydrogen bond acceptors, and the number of rotary bindings were calculated for the obtained products. Expected molecular polar surface area was also defined. Performed computational analysis showed that all of the synthesized compounds have the proper bioavailability.

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P61

Synthesis and anticonvulsant activity of new phenoxyalkyl derivatives of aminoalkanols

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Introduction of aminoalkanol scaffold constitutes an interesting structural modification in design of new anticonvulsants, as aminoalkanols are structural components of known antiarrhythmic drugs exhibiting anticonvulsant activity (*e.g.* propranolol, mexiletine) [1, 2].
We previously achieved phenoxyalkyl derivatives of aminoalkanols exhibiting anticonvulsant activity in animal models (*e.g.* (*S*)-(+)-2*N*-[(2,6-dimethylphenoxy)ethyl]aminobutan-1-ol hydrochloride, ED_{50} in maximum electroshock (MES) test equal to 7.57 mg/kg, mice, *i.p.*) [3]. Positive results of our previous studies became the basis for the development of new potentially active compounds.
Eighteen new phenoxyalkyl derivatives of aminoalkanols have been obtained *via* multistep chemical synthesis, according to formerly published procedures [3, 4, 5]. In the first step, substituted phenoxyalkyl halides have been obtained from the appropriate phenols. The products were subsequently used in *N*-alkylation of the appropriate aminoalkanols. Two of synthesized compounds were converted into hydrochlorides. The purity and identity of all final products were confirmed by means of LCMS and ^{1}H NMR.
All achieved compounds were evaluated for their anticonvulsant activity in MES and their neurotoxicity was determined with the use of rotarod test. Among tested compounds *D,L-trans*-2-[2-(2,4-dimethylphenoxy)ethyl]aminocyclohexan-1-ol and 1-[3-(2,4,6-trimethylphenoxy)propyl]piperidin-4-ol exhibited the highest protective index value (PI equal to 3.93 and 2.41, respectively). They were additionally screened for anticonvulsant activity in maximum electroshock seizure threshold (MEST) and pentylenetetrazole (PTZ) tests, as well as for neurotoxicity in chimney test.

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P62

Pharmacomodulation of oleanolic skeleton by linker-mode method

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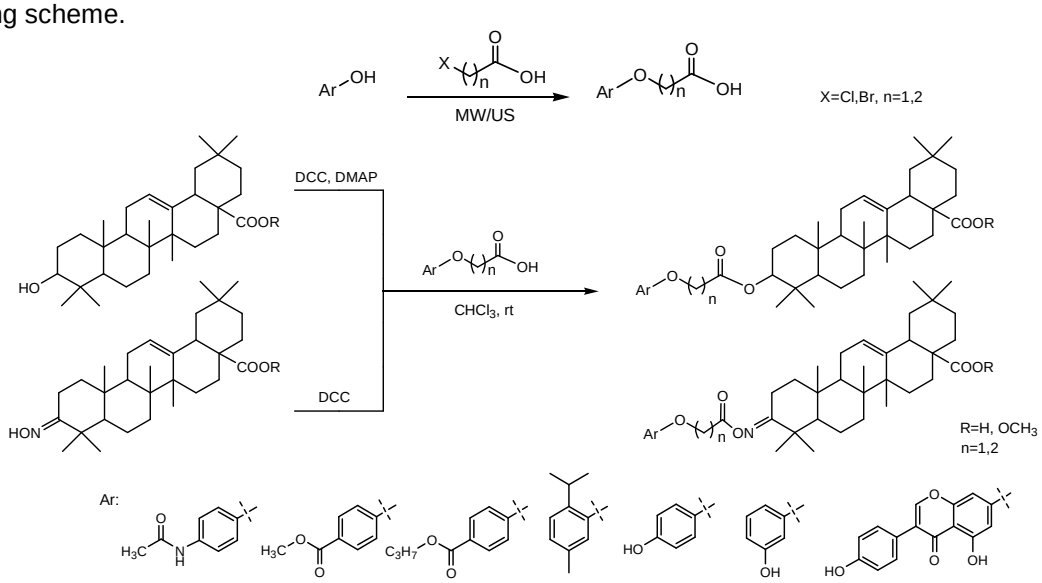
The “chemical hybridization” aims at combining two drugs acting by different mechanisms. By taking into account the knowledge about the pharmacological, structural and molecular interaction profiles of drugs, hybrid molecules also called multifunctional or conjugated drugs wherein two or more drugs having different activities can be co-formulated by connection through a stable or metabolizable linker, allowing simultaneous delivery. According to recent research, such compounds derived from different bioactive molecules are often characterized by a synergy of their individual component activities and may have potential application in the fields of pharmaceuticals and medicine.

In our study synthesis of several hybrid individuals combining natural oleanolic acid skeleton and phenol moieties were examined. In this work, structural modifications of the oleanolic structure by the use of reactivity of hydroxyl or hydroxyimino groups at C-3 position of triterpenoid skeleton with hydroxyl function of phenols using intermediate methods involving the association of components by suitably selected reactive difunctional linkers, acting as intermolecular couplings between the segments. As difunctional linkers 2-chloroacetic acid, 2-bromoacetic acid and 3-bromopropionic acid were choosen. The novel ester and iminoester type derivatives of oleanolic unit with the different phenols such as nipagine M, nipagine P, thymol, paracetamol, resorcinol, hydrochinone and genistein were obtained and characterized.

The type of structure designed with the use of linker fragments planned to receive were illustrated in the following scheme.



The synthesis of planned compounds was performed using classical methods of organic synthesis as well as by using (MW), and ultrasounds (US) factors and their mutual combination SMUI (Simultaneous Microwave and Ultrasound Irradiation) as environmentally-friendly methods of synthesizing new compounds in accordance with rational and sustainable chemistry.

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P63

Pharmacological properties of series of novel histamine H_3 receptor ligands

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Histamine H_3 receptors (H_3R) are constitutively active G-protein coupled receptors expressed mostly in CNS, described as presynaptically located autoreceptors and/or 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].
For this study, five new histamine H_3 receptor ligands were chosen. A preliminary assessment of binding to H_3R, their impact on body weight in rats and the amount of calories taken in the model of excessive overeating was conducted. In addition, spontaneous activity in chronic studies in the same animals and the effect on blood glucose and plasma triglycerides were determined.
One of the test compounds (KSK3), through the reduction of calories intake, significantly reduced body weight gain of test animals compared to the weight gain of the control animals. Moreover it did not show the sedative effect. Other test compounds also reduced calories intake, but the statistically significant weight loss of the test dose has not been specified.
In summary, the selected and tested histamine H_3 receptor ligands show potential anorectic effects in the model of excessive overeating. However tests at higher doses and prolonged administration time period are performed.

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P64

3-furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of α7 nicotinic acetylcholine receptor improves memory and modulates ERK1/2 phosphorylation in mice

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Nicotinic acetylcholine receptors (nAChRs) are promising targets for the treatment of many neurological disorders, including depression, schizophrenia, Alzheimer's disease (AD), and drug addiction. In our study we focused on 3-furan-2-yl-N-p-tolylacrylamide (PAM-2), a novel positive allosteric modulator (PAM) of human α7 nAChR [1], to evaluate weather PAM-2 attenuates drug-induced memory impairment, the role of α7 nAChRs in the PAM-2 promnesic activity as well as possible intracellular signaling pathways involved in this process.

Our findings indicate that: (A) PAM-2 improves memory acquisition/consolidation processes after acute treatment as well as memory consolidation after chronic treatment by using the passive avoidance (PA) test in male mice [2]; (B) PAM-2 activity is blocked by methyllycaconitine (MLA) (an α7-selective antagonist), confirming the role of α7 nAChRs in the PAM-2 promnesic activity [2]; (C) the synergistic effect between inactive doses of PAM-2 and DMXBA (a selective α7-agonist); (D) PAM-2 recovers the memory impairment in animals treated with the muscarinic antagonist scopolamine [2]. Finally, we found that (E) PAM-2 did not affect the α7 nAChR expression after acute and chronic treatment, whereas increased the extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation in the hippocampus and prefrontal cortex in mice after 21 consecutive days of treatment [2].

Based on our data we demonstrated that PAM-2 may constitute a promising therapeutic agent for the treatment of memory impairment in neurological conditions such as Alzheimer's disease and schizophrenia where the cholinergic tone is altered.

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P65

The effect of pentoxifylline and lisofylline on TGF-β induced fibroblasts to myofibroblasts transition in bronchial asthma

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Bronchial asthma is one of the most common chronic lung diseases. It is considered to be an inflammatory disease, but it is widely known that chronic inflammation leads to irreversible changes in the structure of the bronchi, named bronchial wall remodeling. This complex phenomenon involves many cell types and growth factors including transforming growth factor β (TGF-β) induced fibroblast-to-myofibroblast transition (FMT).

Methylxanthines are one of the groups of bronchodilating and anti-inflammatory drugs used in the treatment of asthma. In the recent years there are reports indicating that theophylline (THEO) and pentoxifylline (PTX) may act as an anti-fibrotic agents. Following these facts, the aim of this study was to examine whether selected methylxanthines can affect TGF-β-induced FMT. In the study PTX active metabolite - lisofylline (LSF) has also been used.

All experiments were carried out in human bronchial fibroblasts (HBFs) derived from patients with diagnosed asthma. THEO, PTX and LSF were able to decrease TGF-β-induced HBFs transition into myofibroblasts in a dose dependent manner. PTX proved to be the most potent inhibitory methylxanthine. The number of differentiated myofibroblasts after PTX, LSF and THEO administration was reduced at least twofold. The observed limitation of myofibroblast phenotype in each cell population was accompanied by impaired p-Smad-2 translocation to the nucleus [1].

The obtained data opens a new perspective in the searching for new properties and applications of the compounds with already defined activity. Studies of methylxanthines derivatives may be important in the development of new anti-fibrotic strategies in asthma therapy.

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P66

Influence of glycoxydation on methylparaben – human serum albumin interaction

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Human serum albumin (HSA) is a protein of blood plasma, which plays a main role in the binding and transport of substances in the circulatory system. HSA tertiary structure may be posttranslational modified by oxidative stress or/and glycation. These modifications are characteristic for *in vivo* pathological states. Advanced glycoxidation end products are implicated in the complications of diabetes and aging. Glycation is a non-enzymatic process of proteins' nucleophilic groups modified by reactive carbonyl compounds. Glucose is the most significant glycation reagent in human body. Glycated albumins (gHSA) are typical for diabetics, while oxydated albumins (oHSA) are characteristic for aging organism. The major players of oxidation process are oxidative stress and reactive oxygene species (ROS). During the oxidation the amino acid residues of proteins, especially cysteine and methionine, are modified. Both, oHSA and gHSA properties are changed in comparison to native protein. Moreover, oxidative stress is thought to be accompanying in diabetes. If glycation is associated with the generation of ROS and oxidative stress, this process is called glycoxidation. The glycoxidation products increase in diabetics proteins and during normal aging. Methylparaben (MP), methyl ester of p-hydroxybenzoic acid is one of the most abundant preservatives in cosmetics as well as in food and drugs. It is known that some amount of MP easily penetrates the skin and gets into circulatory system. In the blood plasma, MP is bound to HSA in the specific binding sites.

The aim of this study was to determine binding sites, binding affinity and the types of interaction between methylparaben and human serum albumin: native (HSA), oxidated (oHSA) and glycoxydated (goHSA) using fluorescence spectroscopy. The binding constants obtained for MP-HSA, MP-oHSA, MP-goHSA complexes as well as the number of class of binding sites were determined by the use of the Scatchard, Stern-Volmer, modified Stern-Volmer, Hill and Klotz methods.

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Acknowledgements:
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P67

Synthesis and antitumor activity of novel diisoquinoline derivatives

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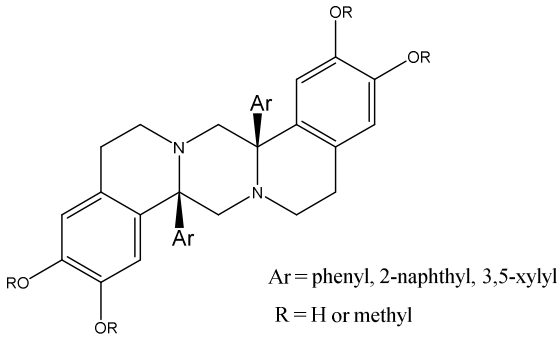
Recently, our group reported a new strategy suitable for producing numerous new octahydropyrazin[2,1-a:5,4-a']diisoquinoline derivatives (Fig. 1) [1]. These compounds demonstrate a unique profile of cytotoxicity showing comparatively more activity in breast cancer cells compared to etoposide and camptothecin [2]. It is probable that deregulation of DNA replication and transcription by inhibition of topoisomerases activity contribute significantly to the cytotoxicity of these compounds [2].



These compounds bind to the minor groove of duplex DNA in A/T-rich regions, where they are thought to exert their biological activity through the inhibition of DNA-associated enzymes such as DNA topoisomerases I and/or II, or possibly by direct inhibition of transcription. In our in vitro antitumor study, we found that novel octahydropyrazin[2,1-*a*:5,4-*a*']diisoquinoline derivatives have strong antitumor effect on human cancer cells. Our experiments carried out with flow cytometry assessment of annexin V binding and fluorescent microscopy assay revealed that these compounds inhibited the proliferation of breast cancer cells by increasing the number of apoptotic cells [2]. Our results suggested that apoptosis of cells in the presence of these novel diisoquinoline derivatives follows the mitochondrial pathway, with the decrease in MMP and the activation of caspase 9. However, these compounds also activated caspase 8 suggesting that the extrinsic pathway of apoptosis might be also involved in induced cell death. Activated caspase 8 can cleave and activate effector caspase 3.

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P68

Development of selective GPCR ligands – 5-HT$_{1B/2B}$ case study

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Compounds targeting multiple receptors, have both beneficial and harmful properties. The promiscuity of such compounds results in activating or blocking multiple therapeutic targets, facilitating a more complex response. However, this also means that multiple off-target receptors may be activated, causing undesirable side effects.
Finding new, selective drugs, that target only one type of receptor while not interacting with other receptors, especially with closely related ones, is proving to be quite complicated. In this study, new compounds selective for 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors were designed (with both 1B/2B and 2B/1B selectivity). The study focused on the selectivity of compounds defined by their interactions with the secondary (allosteric) binding pockets of target proteins while retaining standard interactions with the orthosteric binding pockets.
During the study a new type of fingerprint – the Substructural Connectivity Fingerprint (SCFP), a two-dimensional fingerprint containing information on connectivity of substructural features of a compound, was utilized. This novel methodology was used to create multiple machine learning-based classifiers, which were further used in a multi-step compound selection protocol, consisting additionally of docking protocols and multiple scoring methods. In the last step, the compounds were visually inspected and selected for *in vitro* testing. This complex process helped to ensure, that the compounds selected in virtual screening campaign would have the highest probability of being 1B/2B selective. Within the study, a database containing 4.9 million compounds (MCule) was searched for 1B/2B and 2B/1B selective ligands, and 10 structures (5 for each selectivity type) have been highlighted for *in vitro* testing.

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This study was partially funded by the PRELUDIUM grant no. 2015/17/N/ST6/03578, funded by the National Science Center and Hungarian Brain Research Program (KTIA-NAP-13-1-2014-0009).

P69

Synthesis and *in vitro* screening of the new thiazolo[4,5-*d*]pyrimidines

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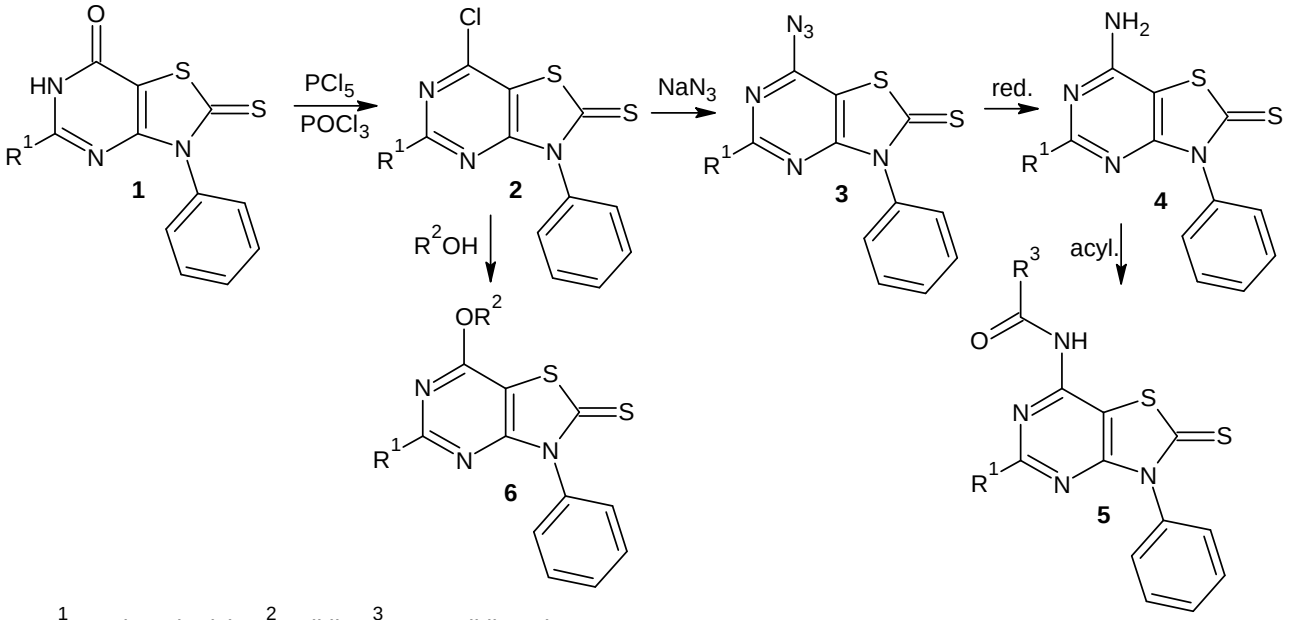
Synthesis of new 7-substituted thiazolo[4,5-*d*]pyrimidines was carried out according to Scheme. Thiazolo[4,5-*d*]pyrimidin-7-ones **1** were prepared from 4-amino-5-carboxamido-2,3-dihydrothiazole-2-thiones by cyclocondensation with aldehydes, following the reaction procedure described previously [1]. Compounds **1** were converted to the corresponding chlorides **2** to introduce a good leaving group and then were treated with an array of alcohols affording 7-alkoxy derivatives **6**. *O*-alkylation required the presence of a basic catalyst such as sodium hydride or sodium alkoxide. The chlorine of intermediate **2** was also nucleophilically substituted by the azide ion. The 7-azide group of **3** was subsequently reduce. The final amides **5** formed by *N*-acylation of amines **4** with different anhydrides and acid chlorides. Anticancer *in vitro* one-dose screening of the selected compounds, towards the full panel of 60 human cancer cell lines was performed by the National Cancer Institute (NCI). Alkoxy derivatives **6** showed no significant activity whereas amines **4** and amides **5** were more potent. Compounds **4** and **5** bearing the electronegative moieties, showed the highest cytostatic potency against cell lines of different origin.

Scheme



R^1= aryl, 3-pirydyl, R^2= alkil, R^3=CF_3, alkil, aryl

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P70

Synthesis and *in vitro* antimicrobial activity of new 1,3-benzothiazin-4-one derivatives containing hydrazone moiety

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Due to the increasing microbial resistance to currently used drugs it is necessary to search for new antimicrobial agents [1]. It is affordable that new chemotherapeutic agents should show different mechanism of action than currently used antibiotics. It seems appropriate to combine two or more systems with different structure, which exhibit complementary mechanism of antimicrobial activity. This would imply an increase in its potency, broadening the spectrum of antimicrobial activity, reduced their toxicity to the human body, and above all, with a reduction in opportunities for the development of drug resistance [1].
Among variety of heterocycles, the benzothiazine derivatives display wide spectrum of biological properties like antibacterial [2,3], antifungal [4,5], antitubercular [6], antimalarial [7], antiviral [8], anti-inflammatory [9], analgesic [10], and antiproliferative activities [11].

In this study we decided to investigate the 1,3-benzothiazin-4-ones as potential antimicrobial agents. New 1,3-benzothiazin-4-one derivatives were obtained by the reaction of appropriate hydrazide-hydrazone derivatives with thiosalicylic acid in the presence of 1,4-dioxane [12]. The structures of synthesized compounds were fully confirmed on the basis of spectral analysis (^{1}H NMR and ^{13}C NMR) [12].
Synthesized 1,3-benzothiazin-4-ones were subjected to *in vitro* antimicrobial assays to determine their activity according to EUCAST [13] and CLSI [14] guidelines. Antimicrobial activity tests revealed that nine of the synthesized compounds showed good activity against Gram-positive and Gram-negative bacteria, especially against *Staphylococcus* spp. (MIC = 15.62–62.5 μg/mL), *Bacillus* spp. (MIC = 7.81–62.5 μg/mL), *Bordetella bronchiseptica* ATCC 4617 (MIC = 62.5–125 μg/mL), and fungistatic activity against *Candida* spp. (MIC = 62.5–125 μg/mL) [12].

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P71

The Cu^{2+} binding properties of branched peptides based on *L*-2,3-diaminopropionic acid. Impact of branching and location of histidine residues on the metal chelation

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Metal ions are essential for the proper functioning of living organisms. The metal ion of great importance for human life is copper. For example Cu^{2+} ions are involved in redox processes and regulate collagen metabolism. Moreover they occur in prosthetic group of several metalloproteins such as superoxide dismutase. On the other hand, disruption of homeostasis of metals result in serious diseases, like Wilson's disease. That is why, ligands that can efficiently chelate metal ions and control their function are in great interest of scientists[1].

Metal binding properties of linear and cyclic peptides have been widely explored[2, 3]. Branching of peptides is new, innovative approach towards biologically active compounds and metal chelation. This new molecules have been applied as models for studying various biological activities[4], including antibacterial, immunological[5], enzyme-like[6-8], associations with receptors[4] and selective targeting[9].

Moreover, branched peptides are very useful in gene delivery[5] and also can act as metal transporters. It was proved already, that selective delivery of Ni^{2+} ions into cell nucleus is possible by elongation of TAT vector with branched peptide based on *L*-2,3-diaminopropionic acid (Dap)[10].

In this study we characterize metal binding properties of new series of peptides: H-Gly-Dap(H-Gly)-Gly-NH2, H-His-Dap(H-His)-Gly-NH2, H-Gly-Dap(H-Gly)-His-NH2 and H-His-Dap(H-His)-His-NH2. The systematic spectroscopic (UV−vis), electron paramagnetic resonance (EPR), circular dichroism (CD), potentiometric and electrospray ionization massspectrometry (ESI-MS) studies shown correlation on the peptide structure to metal binding properties.

Our study provided answers about the influence of histidine residues number and localization on metal binding, as well as, the impact of additional *N*-terminal arm. Investigated branched peptides are much more effective in copper binding than their linear analogues. Moreover, the location of histidine residue is emphatic. When placed at *N*-terminal, it promotes metal bindig in low pH values, whilst the presence of histidine at the *C*-terminal of peptides provides effective Cu^{2+} chelation in basic pH range[11].

New ligands point toward the design of new copper-based models of metallo-enzymes, biosensors and metal-peptide based radiopharmaceuticals.

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P72

Pharmacological studies *in vivo* with a novel group of 1,3,5-triazine derivatives as 5-HT$_6$ agents

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In recent years a large amount of information has been collected on serotonin 5-HT$_6$ receptors and their possible physiological role within central nervous system (CNS). The 5-HT$_6$ receptor mRNA expression and receptor protein are largely confined to CNS, specifically striatum, nucleus accumbens (NAc), olfactory tubercle, cortex, and hippocampus. Such central localization has suggested that ligands of this receptor may be involved in regulating mood changes; thus the 5-HT$_6$ receptor has emerged as an interesting molecular target for the development of drugs for the treatment of mood disorders. Moreover, there is still an unresolved paradox that agonists/partial agonists and antagonists of this receptor evoke similar effects, i.e. pro-cognitive [8], anti-obesity [9], anxiolytic, and antidepressant.

The new series of 1,3,5-triazine derivatives with a high affinity for 5-HT$_6$ (K_i=11 - 30 nM) receptors were synthesized at the Department of Technology and Biotechnology of Drugs Jagiellonian University Medical College. The *in vivo* studies were carried out to determine potential antidepressant activity of these compounds. The forced swim test was conducted on male Wistar rats according to the version modified by Detke et al. (1995). Imipramine (an antidepressant drug) and SB-271046 (a 5-HT$_6$ receptor antagonist) were used as the reference compounds.

The investigated compound MST-4 produced a more distinct antidepressant-like effect after intraperitoneal (i.p.) administration 30 minutes before the experiment rather than after 60 minutes. Thus, all 1,3,5-triazine derivatives were investigated 30 min after i.p. injection. Compound MST-4 produced antidepressant-like activity only in one dose (3 mg/kg), significantly increasing climbing behavior by 65% and slightly decreasing immobility by 10% (not statistically significant effect). The compound TR-12 did not change any parameters in Porsolt's test.

To the best of our knowledge, the triazine motive has not occurred yet among the structures of 5-HT$_6$ agents described and the present results are the first preclinical report indicating that these 5-HT$_6$ receptor ligands may produce antidepressant-like activity detecting in the forced swim test in rats.

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P73

UHPLC study of finasteride in pharmaceutical formulation

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Finasteride is a synthetic 4-azasteroid compound which belongs to the specific inhibitor of steroid 5α-reductase that converts the androgen testosterone into DHT (5α-dihydrotestosterone) [1]. Finasteride tablets are widely used in the treatment of prostatic hyperplasia and reducing prostatic size [2]. Different analytical methods have been described for the determination of finasteride in biological and pharmaceutical samples. Among various chromatographic techniques, liquid chromatography includes high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and thin-layer chromatography (TLC) are the most important in finasteride analysis.

This work is focused on the use of UHPLC (ultra high performance liquid chromatography) combined with classical UV-Vis detector and also equipped with a new universal detector named Charged Aerosol Detection (Corona CAD detector) in the determination of finasteride in its tablet dosage form containing 5 mg of finasteride per tablet. Our results confirm the utility of new developed UHPLC-CAD method in the pharmaceutical analysis of biologically active steroid compound, namely finasteride. On the basis of obtained data, it could be concluded that described UHPLC procedure is suitable for the routine laboratory control of commercially available finasteride tablets.

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This research was financed by the Medical University of Silesia as part of statutory research project KNW-1-024/N/5/0, KNW-1-019/K/6/0 and performed using UHPLC system available at the Educational Center of the School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec.

P74

Monoesters and diesters of benzoyldithiocarbazic acid – a potential tuberculostatics

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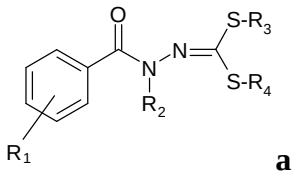
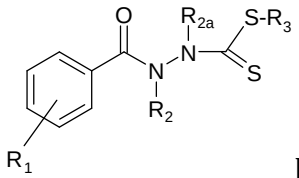
One of the promising chemical classes showing activity against tuberculosis were monoesters and diesters of benzoyldithiocarbazic acid [1]. We report here seven the crystal structures of type **a** and two structures of type **b** (Scheme, Table).



a



b

No	R_1	R_2	R_{2a}	R_3	R_4	Crystal		
						Unit cell parameter [Å,°]		Space group
1a	2-NO_2	H	-	Me	Me	8.1381(5) 18.920(1) 9.270(5)	111.766(4)	$P2_1/c$
2a	2,5-OH	H	-	Me	Me	12.7754(4) 17.5547(5) 7.2881(3)		$P2_12_12_1$
3a	2-OH, 5-Cl	H	-	Me	Me	20.7182(7) 19.3768(7) 12.7013(5)	95.873(1)	Cc
4a	3,4-Cl_2	H	-	Me	Me	9.2225(4) 11.2741(5) 13.0892(5)	95.367(1)	$P2_1$
5a	3,4-Cl_2	Me	-	Me	Me	8.9307(4) 9.0858(7) 10.3873(5)	65.039(6) 69.172(4) 74.892(5)	P-1
6a	4-NO_2	H	-	Me	Me	7.4371(3) 23.1404(10) 8.8737(5)	121.358(3)	$P2_1/c$
7a	4-NO_2	Me	-	Me	Me	6.5716(2) 16.3193(5) 12.8982(4)	91.219(3)	$P2_1/n$
1b	4-NO_2-Ph	H	H	Me	-	27.8016(18) 4.5139(3) 19.4423(12)	109.162(2)	$P2_1/c$
2b	3,4-Cl_2-Ph	H	Me	Me	-	4.5696(3) 24.7384(17) 11.7638(8)	96.940(1)	$P2_1/c$

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P75

Determination of lipophilicity of antibacterial thiosemicarbazide derivatives using the Reversed Phase-High Performance Liquid Chromatography and PCA analysis

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Several studies report the correlation between lipophilicity and antibacterial potency of different classes of compounds. Lipophilicity is usually expressed quantitatively as log *P* where *P* is non-aqueous/aqueous phases partition coefficient and can be characterized by different techniques including solvent/water partitioning, chromatographic methods, immobilized artificial membrane, electrokinetic and calculation methods. It should be kept in mind, however, that while there is plethora of methods of theoretical predicting clogP the results of these methods are quite diverse. Thus, in order to make the clogP as useful descriptor for thiosemicarbazide class of compounds, the lipophilicity of representative tiosemicarbazides with varied antibacterial potency was determined both experimentally and theoretically and correlation of each method with others as well as with antimicrobial potency was investigated. The results of these studies will be presented at poster.

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P76

Synthesis and antibacterial activity of 1-(2,6-difluorophenyl)-4-substituted thiosemicarbazide

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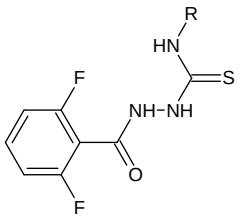
Thiosemicarbazides are an important class of organic compounds because of their biological properties.
Our team is working on synthesis of biologically active thiosemicarbazide derivatives for many years. We focus mainly on the synthesis of compounds with antibacterial activity [1-6].
In recent years we obtained very active derivatives of thiosemicarbazide with 3-chlorophenyl and 2-, 3-, 4-fluorophenyl substituents in the position 1 of thiosemicarbazide scaffold. The results of our investigations suggested that 1-fluorophenyl-4-substituted thiosemicarbazide derivatives are more active than 1-chlorophenyl-4-substituted thiosemicarbazide derivatives.
In continuation of our work, we decided to explore how the presence of additional fluorine atom in phenyl ring affects the antibacterial activity of new compounds.



The *in vitro* antibacterial activity of the MW1-MW9 compounds was primarily screened using agar dilution method (with concentration of 1000 µg/mL). Next, for potentially active compounds MIC (minimal inhibitory concentration) was determined using broth dilution method (with concentration range from 7.81 to 1000 µg/mL). Both Gram-positive (*Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 25923, methycillin resistant *S. aureus* ATCC 43300 (MRSA), *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633), and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 9027, *Bordetella bronchiseptica* ATCC 4617) reference strains were used.

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P77

Synthesis and antiinflammatory properties of new isoxazole derivatives

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A series of new isoxazole derivatives of expected immunosuppressive activities was synthesized. Following *in vitro* screening in the human cell models, the activity of MZO-2 compound (Ethyl N-{4-[(2,4-dimethoxybenzyl)carbamoyl]-3-methylisoxazol-5-yl}acetimidate) in mouse *in vivo* models was evaluated. *In vitro* tests included evaluation of: peripheral blood mononuclear cells (PBMC) viability, phytohemagglutinin (PHA)-induced PBMC proliferation and lipopolysaccharide (LPS)-induced tumor necrosis factor α (TNF α) production in whole blood cell cultures. MZO-2 was studied in mice for its effects on: humoral immune response to sheep erythrocytes (SRBC), delayed type hypersensitivity (DTH) to ovalbumin (OVA), contact sensitivity to oxazolone and carrageenan-induced foot pad edema. In addition, the effect of MZO-2 on expression of caspases in Jurkat cells was determined. The studied compounds exhibited differential, dose-dependent effects to suppress PHA-induced PBMC proliferation and a weak property to suppress LPS-induced production of TNF α. MZO-2 had no effect on the induction phase of the humoral immune response to SRBC *in vitro* and *in vivo*, but moderately suppressed the induction phase of DTH to OVA. Its inhibitory effect on carrageenan-induced paw inflammation was potent. Likewise, MZO-2, applied in ointment, was very effective in reducing ear edema and number of lymphocytes in draining lymph nodes of mice sensitized to oxazolone, comparably to tacrolimus, the reference drug. The expression of caspases 3, 8 and 9 in Jurkat cells was inhibited by the compound. MZO-2, applied systemically or locally, may serve as a potential drug for amelioration of inflammatory processes [1].

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P78

Discovery of amide derivatives of 8-substituted 1,3-dimethylpurine-2,6-dione as a new series of dual PDE4/7 inhibitors with anti-inflammatory properties

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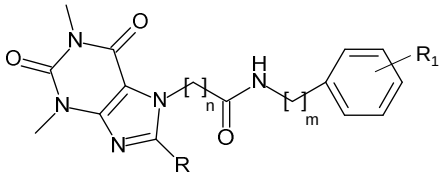
Inflammation is a response of the immune system to physical, chemical or biological injury and is connected with a large number of diseases, such as atherosclerosis, cancer asthma, arthritis, and many others. Most studies on this process have been focused on the role of cytokine overproduction, e.g. tumor necrosis factor alpha (TNF-α) and interleukins. Selective inhibitors of cAMP specific phosphodiesterases - PDE4 or PDE7 which elevate cAMP level and block the T cell component of a disease are regarded as potential anti-inflammatory and immunosuppressant agents. Subsequently, dual PDE7/4 inhibitors which possess synergistic activity and are not significantly limited by emesis, are proposed as a novel class of therapeutics.

In this study we designed and synthesized a new series of amide derivatives of 8-substituted 1,3-dimethylpurine-2,6-dione as dual PDE7/4 inhibitors.



R = alkoxyl, arylalkylamine
R_1 = 2-Cl, 3-Cl, 4-Cl, 4-isopropyl, 4-*tert*-butyl
n = 1, 3; m = 0, 1

For the new compounds, their PDE7A and PDE4B inhibiting activity was determined *in vitro* using PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE7A and PDE4B expressed in Sf9 cells. For the selective compounds with dual PDE7/4 activity their anti-inflammatory properties were tested in *in vitro* (rat whole blood TNF-α release assay) and *in vivo* (formalin, carrageenan-induced edema and allyl isothiocyanate models).

Acknowledgements:
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P79

Synthesis and antiproliferative activity of new pyrazolo[4,3-*e*]tetrazolo[4,5-*b*][1,2,4]triazine derivatives

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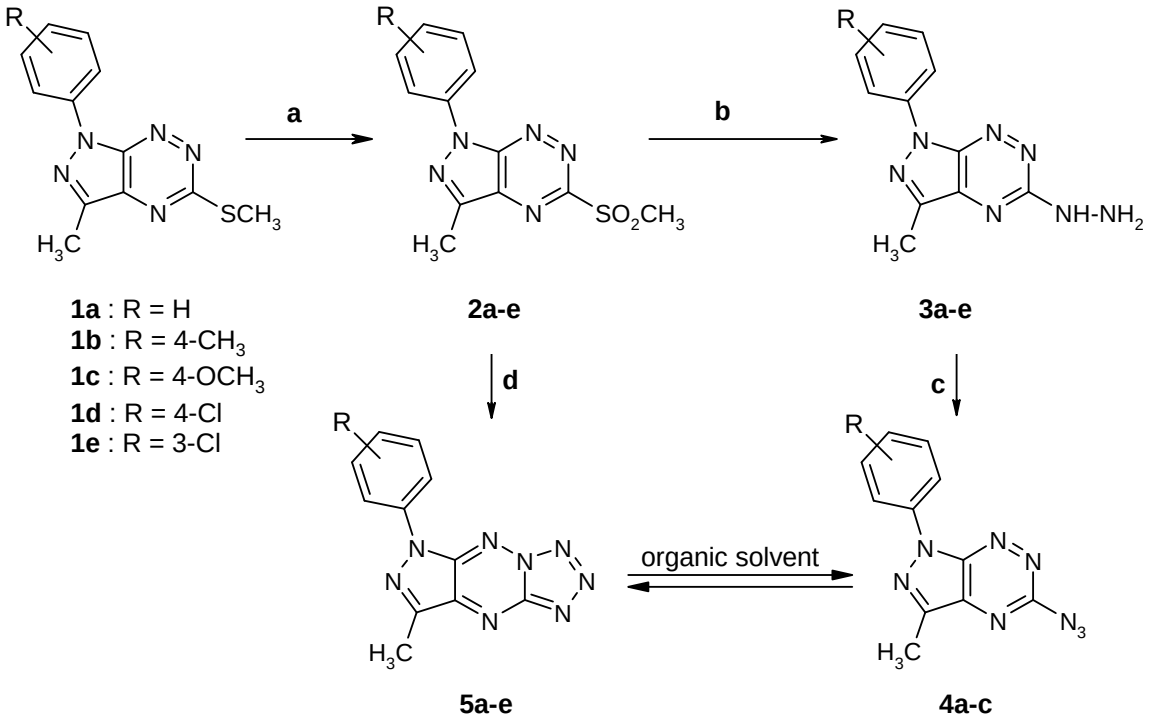
A new series of pyrazolo[4,3-*e*]tetrazolo[4,5-*b*][1,2,4]triazine derivatives have been synthesized, characterized and evaluated for their antiproliferative activity on LS180 colon adenocarcinoma cells. MTT assay revealed that tested compounds inhibited cancer cells divisions in concentration-dependent manner. The synthesis pathway leading to the title compounds is depicted in scheme.



Scheme: Synthesis of new tetrazole derivatives fused with 1*H*-pyrazolo[4,3-*e*][1,2,4]triazine system. Reagents and conditions: (a) $KMnO_4$, Bu_4NBr, CH_3COOH, benzene-H_2O, 20°C, 2h; (b) H_2N-NH_2, THF, 20°C, 12h; (c) $NaNO_2$, CH_3COOH, 0-5°C; (d) NaN_3, EtOH, reflux, 12-24h.

P80

Isomorphism and solid solutions of nitroimidazole derivatives

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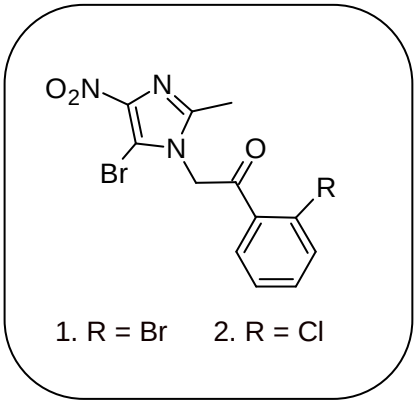
Nitroimidazole derivatives show cytostatic, antitumor, photosensibilizing, antiprotozoal and antibacterial properties. They are common medications used in the treatment of many infections. Various nitroimidazole analogues differ in spectrum activity and bioavailability which is the effect of a variety of functional groups and their position in the heterocyclic ring [1]. Due to their ability to accumulate in hypoxic cells, these class of compounds can also be used in the diagnosis of neoplasm or cardiac and brain ischemia. Therefore, over the last decades, the new nitroimidazole derivatives are synthetized to obtain more selective and less toxic anticancer drugs, antibiotics and markers of hypoxia [2-6].



1. R = Br 2. R = Cl

Formation of complexes, co-crystals or solid solutions of the active pharmaceutical ingredients is another way of designing a new form of drug with improved stability, bioavailability and solubility. The solid solutions play an important role in the modern pharmaceutical industry because they affect physicochemical parameters and pharmacokinetic of an individual components. [7]. Recent studies have shown that nitroimidazole derivatives are able to form solid solutions [8].

The basic motivation for this study was crystallization and X-ray structural analysis of solid solutions of nitroimidazoles obtained from solutions composed of two nitroimidazole derivatives mixed at different stoichiometric ratio. As the crystal structures of derivatives (**1**) and (**2**) were isomorphic they were chosen for further investigations. In this presentation we will discuss the molecular organization in crystals of (**1**) and (**2**) and the ability of these compounds to create solid solution.

We hope that establishing whether the composition of solid solutions of nitroimidazoles is dependent on concentration of individuals in solution will be beneficial to the design of a new generation of drugs based on nitroimidazole derivatives.

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P81

Biopolymers coated magnetic nanoparticles for HSA immobilization

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Magnetic nanoparticles (MNP'S) are promising materials for many of applications: biomedical, catalytic, analytical and industrial. [1,2]. A superparamagnetic core guarantees MNP'S a simple separation from the reaction mixture and good reusability of such material. Pure magnetite (Fe_3O_4) has a poor colloidal stability so it needs stabilization and surface modification. For biomedical applications polymers are most frequently used for MNP'S stabilization. [3]

One of widely applied for biomedical applications especially as a polymer shell covering the magnetic nanoparticles material is chitosan (CS). It is a non-toxic natural polymer easy for chemical modification due to the reactive amino and hydroxyl groups in polymer structure. In this work several types of magnetite superparamagnetic nanoparticles were synthesized. Magnetic core was coated with pure chitosan with three types of cross-linking and aminated chitosan with different content of reactive amino groups on the surface distanced from magnetic core. The structure, size and morphology of nanoparticles were investigated by ATR-FT IR spectroscopy, transmission electron microscopy, X-ray diffraction, dynamic light scattering and low temperature adsorption of nitrogen.

Recently, more attention was paid to the application of magnetic nanoparticles as a support for biomolecules immobilization. [4] All of prepared MNP'S were applied to human serum albumin (HSA) immobilization with wery good results. [5-6]

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P82

X-ray structural analysis of sulfanyl maleonitrile and porphyrazine derivatives

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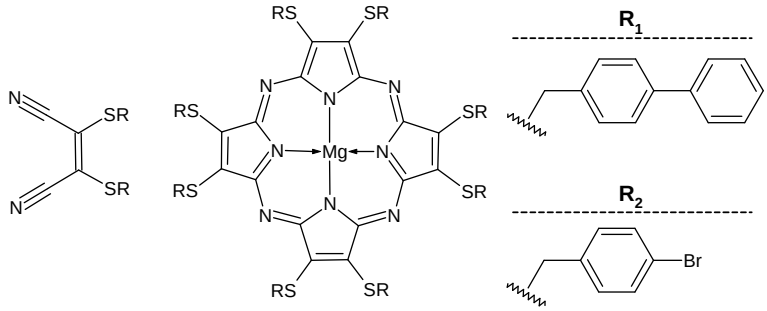
Porphyrazines are tetrapyrrole macrocycles exhibiting unique properties and numerous potential applications e.g. in medicine, in photodynamic therapy of cancer (PDT) and in photodynamic antimicrobial chemotherapy (PACT) which may be considered as an alternative treatment for drug resistant microorganisms [1-3]. Porphyrazines possessing peripheral sulfanyl substituents are considered as potentially useful compounds for medical applications.

Monocrystals of maleonitrile derivatives **1**, **2** and sulfur porphyrazine derivative **3** (Fig. 1) were subjected to X-ray diffraction analysis. Maleonitrile derivatives crystalized in monoclinic crystal system and $P\,2_1/n$ space group, whereas porphyrazine **3** crystalized in triclinic crystal system and $P\bar{1}$ space group. The X-ray crystallography study performed for sulfanyl porphyrazine **3** revealed that the biphenyl residues from two consecutive molecules form the barrel-like cages with macrocyclic core constituting a bottom and a top of the barrel and pyridine molecules enclosed inside.



1 R= R_1
2 R= R_2
3 R= R_1

Fig. 1.

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Acknowledgements
This study was supported by the Polish National Science Centre under grant No. 2012/05/N/NZ7/00624.

P83

Novel hyperbranched sulfanyl porphyrazine/multiwalled carbon nanotube hybrids – improved electrocatalytic response toward hydrogen peroxide reduction

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Dendrimers are relatively new class of polymeric compounds classified as branched polymers. They are spherical macromolecules of a diameter reaching nanometer size, which creates a vast area of applications including nanotechnology and medicine. Particular attention should be paid to dendrimeric porphyrazines and their role as photosensitizers in photodynamic therapy. They exhibit good solubility and considerably high singlet oxygen generation quantum yields. Carbon nanotubes (CNT) have gained considerable attention in research due to their unique electronic properties, chemical stability and affinity to biomolecules. It was found that the CNT can promote effective electron transfer reactions. Moreover, they can be used as a support for immobilization of different electron transfer mediators onto electrode surfaces able to improve their electrochemical properties. CNT can be easily modified by covalent functionalization, electropolymerization or adsorption.

Synthesis of expected dendritic Pzs was performed following a two-step procedure [1].The resulting products were carefully purified via flash column chromatography and characterized using various analytical techniques, especially NMR, MALDI and UV-Vis. Moreover, the morphology of hybrid nanostructures was researched using SEM and AFM microscopy and they were subjected to electrochemical study. The modified electrodes were also utilized for electrocatalytic determination of hydrogen peroxide, an important compound generated in many enzymatic reactions. The strong synergic effect between two nanostructural components, namely multi-walled carbon nanotubes and dendrimeric porphyrazines was evaluated.

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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.

P84

N9-benzyl derivatives of pyrimido[2,1-*f*]purinediones as A_1/A_{2A} adenosine receptor ligands and MAO-B inhibitors

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Adenosine modulates many important physiological processes through extracellular interaction with four subtypes of G-protein-coupled adenosine receptors: A_1, A_{2A}, A_{2B}, A_3. They present different biochemical and pharmacological properties and display distinct distribution in tissues. A_1 and A_{2A} adenosine receptors antagonists could be beneficial for the treatment of neurodegenerative diseases such as Parkinson's Disease (PD) and Alzheimer's Disease (AD). The combination of antagonistic activity toward adenosine A_1 and A_{2A} receptors with simultaneous inhibition of monoamine oxidase B (MAO-B) seems particulary promising for the development of new drugs for cognitive disorders. *m*-Chlorostyrylxanthine (CSC), is one example for an adenosine A_{2A} receptor antagonist with ancillary MAO-B inhibitory potency [1,2].

Previous research in the group of annelated xanthines confirmed their activity toward adenosine receptors and indicated that introduction of a benzyl moiety in position N9 increased affinity for MAO-B. We made an effort to study a group of N9-benzyl-substituted 1,3-dimethylpyrimido[2,1-*f*]purinediones derivatives as adenosine receptors antagonists and MAO-B inhibitors. The designed compounds were synthesized according to previously described procedures [3,4]. Firstly theophylline was oxidatively brominated followed by *N*-alkylation. In the last step the third ring was built on to the xanthine core by condensation with appropriate benzylamines.

We obtained a small library of compounds that differ in their substituents in position N9. The synthesized compounds were evaluated in radioligand binding studies. Affinity for A_1 receptors was tested on rat brain cortical membranes, for A_{2A} receptors on rat brain striatal membranes, for A_{2B} and A_3 receptors on CHO cell membranes recombinantly expressing the respective receptor. The compounds were also tested for their effect on MAO-B. Drug-like properties (logP, logS, toxicity, drug score) of the obtained compounds were evaluated by the OSIRIS program [5].

The results confirmed adenosine receptor antagonistic and MOA-B inhibitory activities of the compounds. Furthermore, our investigation highlighted the significant effect of the benzyl moiety for MAO-B inhibitory activity and adenosine receptor affinity.

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Acknowledgments for support of the Polish National Science Center founding, DEC-2012/04/M/NZ4/00219 and K/ZDS/004689.

P85

Synthesis and biological activity of some new thio/semicarbazide derivatives containing cyano group

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A semicarbazide derivatives are an important structural element which has an impact on biological activity of compounds. Optimization of this structure can contribute to the discovery of a new class of agents with broad spectrum of anticonvulsant activity [1]. In turn, thiosemicarbazide derivatives are known as a antitumor agents against a panel of human cancer cell lines [2]. Furthermore, semicarbazide and thiosemicarbazide derivatives of 4-(adamantan-1-yl)quinoline show good antibacterial, antifungal and anti-tuberculosis activity [3, 4].

In our work we obtained 48 new tio/semicarbazide derivatives containing cyano group. Starting materials for the synthesis of title compounds were carboxylic acid hydrazide and isothiocyanate or isocyanate. All compounds were tested *in vitro* against Gram-positive and Gram-negative bacteria. In addition, cytotoxicity and therapeutic index for the most active compounds was determined.

Obtained data showed that some of the tested substances effectively inhibited growth of the tested strains. MIC value were in the range 1.95µg/ml - 125µg/ml. Furthermore, the one of the various *in vitro* microbiological parameters – MBC – used to determine the bactericidal activity of antimicrobial agents was determined. Most of the tested compounds showed bactericidal activity against aerobic Gram-positive strains. The MIC and MBC values of thio/semicarbazide derivatives correlated well to the screening test results. The cytotoxicity of compounds exhibiting antibacterial activity were assessed and expressed as IC_{50} values. Among tested compounds, the most of them possessed relatively low cytotoxic activity. Two of them exhibited therapeutic safety against all tested Gram-positive bacterial strains with TI values ranged from 12.18 to 24.43 and 16.80 to 68.07, respectively.

In conclusion it was proved that some thiosemicarbazides possessing cyano group may constitute promising agents against Gram-positive bacteria.

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P86

Adsorption of nonsteroidal anti-inflammatory drugs on carbonaceous materials

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In the past decades, carbonaceous materials have attracted considerable industrial and scientific interest. Their outstanding properties offer new ways of solving many technological and environmental problems. Each material reveal unique properties [1].
Examples of such materials are carbon nanotubes (CNT) and graphene. Carbon nanotubes are man-made one-dimensional carbon crystals with different diameters and chiralities. Owing to their superb mechanical and electrical properties, many potential applications have been proposed for them. Graphene (a single layer of graphite) has recently attracted considerable attention owing to its remarkable electronic and structural properties and its possible applications in many emerging areas [2]. In the present study, graphene and nanotubes are applied for adsorption of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, acetylsalicylic acid, ketoprofen, and diclofenac. The experimental part of the study is supported with DFT calculations of drug-material interaction.

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P87

Phenylalanine-based amino acids as AMPA receptor antagonists

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(*S*)-Glu is one of the major neurotransmitters in the mammalian central nervous system and exerts biological effects interacting with two receptor classes: metabotropic receptors and ligand gated ion channels (iGluRs). A family of ionotropic glutamate receptors mediates fast neurotransmission and is suggested to play an important role in neural plasticity. iGluRs are divided into three subclasses: *N*-methyl-D-aspartic acid (NMDA), *α*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) receptors.
AMPA receptors are complex of four subunits (dimers of dimers) and can exist as homo or heterotetramers composed of subunits GluA1-GluA4. These receptors are involved in the processes important for memory, learning, cognition and fast neural transduction. On the other hand, their over-stimulation may cause effects linked to neurodegeneration that occurs in diseases such as Alzheimer's disease, parkinsonism, or amyotrophic lateral sclerosis (ALS).
The current project is a continuation of earlier research on potent and selective competitive antagonists of AMPA receptors among phenylalanine derivatives. A series of unnatural amino acids built on a phenylalanine scaffold was designed and synthesized. The target compounds were pharmacologically characterized by radioligand binding at native AMPA, KA and NMDA receptors. Additionally, the most potent compound was examined at homomeric GluA2 and GluA3 receptors. The results of design, synthesis and pharmacological tests are described.

Acknowledgements:
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P88

Assessment of quantum optimized $mGlu_1R$ in virtual screening

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The mGlu1 and mGlu5 receptors (metabotropic glutamate receptor 1 and 5) are considered promising therapeutic targets to treat diseases including chronic pain, schizophrenia, Alzheimer's disease, anxiety, and autism [1-3]. However, the development of selective small-molecule ligands that might serve as drug candidates for these receptors has been hampered by the conservation of the orthosteric (glutamate) binding site. This can be overcome by using allosteric modulators that act at alternative binding sites; i.e., within the 7TM domain of the receptors [1].

In this study the potential of 23 quantum optimized (ONIOM method) conformations of $mGlu_1R$ in virtual screening was tested. The active site was tuned on structures of thirteen known allosteric modulators (2.4 nM < IC50 > 10000 nM) as well as modeled using 10 different calculation methods (7 different DFT method, 3 different basis sets). Each resulting conformation was evaluated by docking the test set (195 active and 14465 non-active molecules) and several performance metrics was calculated: ROC AUC, BEDROC. Interestingly, the best discriminative model was obtained by optimizing the complex of receptor with CHEMBL565934, for which the experimentally determined affinity was 210 nM.

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Acknowledgements:
Authors acknowledge the computing resources from PL-Grid Infrastructure.

P89

FMO/EDA study of 1-hexyl-4-(2-methoxyphenyl)piperazines as ligands of serotonin receptors

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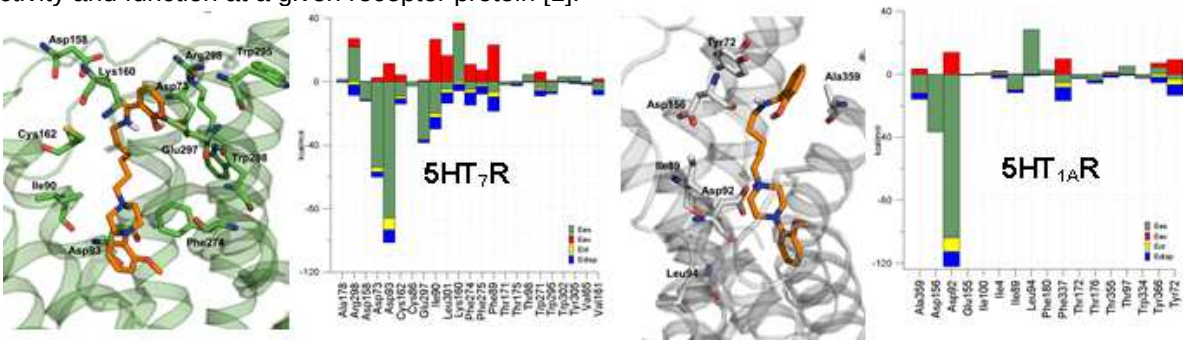
Long-chain arylpiperazines (LCAP) are one of the commonly studied class of bioactive compounds due to their potential therapeutic effects caused by interactions with different subtypes of serotonin receptors. A number of studies have been aimed at examining the impact of LCAP structure modifications on the affinity, selectivity and function at a given receptor protein [1].



In this study the structure of four derivatives of 1-hexyl-4-(2-methoxypneyl)piperazine complexed with two serotonin receptors (5-HT_{1A}R, 5-HT_7R) has been investigated by means of quantum mechanical methods. The FMO/EDA analysis showed that the 2-(piperazin-1-yl)-methoxybenzene moiety of considered ligands forms very strong salt bridge between the protonated nitrogen atom of piperazine and Asp3.32, as well as hydrophobic interactions of the methoxybenzene ring with the aromatic cluster of TMH6 (Phe6.51, Phe6.52), for both studied receptors. The performed calculations can be helpful in the interpretation of the experimental results concerning the affinity to receptors, as well as they provide the reasonable binding energies and binding patterns of ligand-protein interactions.

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Acknowledgements:
The study was financially supported by the National Centre for Research and Development, Project LIDER VI (No. LIDER/015/L-6/14/NCBR/2015). Authors acknowledge the computing resources from PL-Grid Infrastructure.

P90

Synthesis and anticancer activity of new pyrazolo[4,3-*e*]triazolo[4,5-*b*][1,2,4]triazine derivatives

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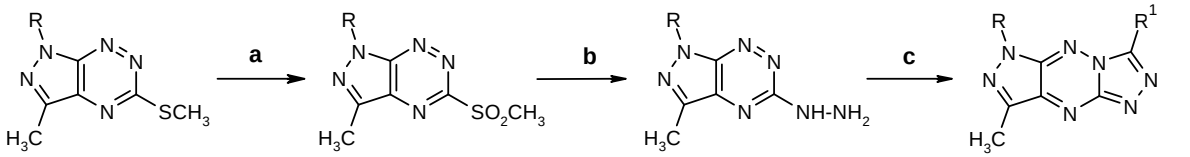
Presented studies are an extension of research topic concerning tricyclic heterocyclic systems. The research refer to earlier work on the synthesis of derivatives of pyrazolo[4,3-*e*][1,2,4]triazine fused with triazole ring.[1] The synthesis pathway leading to the title compounds is depicted in scheme.



1a : R = 4-CH_3Ph
1b : R = 4-OCH_3Ph
1c : R = 4-Cl-Ph
1d : R = 3-Cl-Ph
1e : R = 2-Cl-Ph
1f : R = Ph

2a-f

3a-e

4a : R = 4-CH_3-Ph; R^1 = H
4b : R = 4-CH_3O-Ph; R^1 = H
4c : R = 4-Cl-Ph; R^1 = H
4d : R = 3-Cl-Ph; R^1 = H
4e : R = 4-CH_3-Ph, R^1 = CH_3
4f : R = 4-CH_3O-Ph; R^1 = CH_3
4g : R = 4-Cl-Ph; R^1 = CH_3
4h : R = CH_3; R^1 = H
4i : R = CH_3; R^1 = CH_3
4j : R = CH_3, R^1 = CH_2Cl
4k : R = Ph, R^1 = H
4l : R = Ph, R^1 = CH_3

Scheme: The synthesis of new triazole derivatives condensed with 1*H*-pyrazolo[4,3-*e*][1,2,4]triazine system. Reagents and conditions: (a) $KMnO_4$, Bu_4NBr, CH_3COOH, benzene-H_2O, 20°C, 2h; (b) H_2N-NH_2, THF, 20°C, 12h; (c) R^1-COOH, 100°C.

The preliminary anticancer studies revealed that tested compounds exhibited antiproliferative activity *in vitro.*

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P91

Synthesis of modified chitosanes with surfaces reach of NH_2 groups

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Chitosan is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine and can be derived by partial deacetylation of chitin from crustacean shells [1]. It is a polycationic polymer with a specific structure. Chitosan has very good properties as a biomaterial: it is biodegradable, biocompatible, non-toxic and antithromboganic. These properties made chitosan widely applicable in the pharmaceutical and biomedical fields for controlled release of drugs, for wound management and space filling implants etc.
Three different derivatives of chitosan have been succesfully obtain by chemical modification of reactive amino and hydroxy groups to branch carbonyl group. In the next step, new formed aldehyde moieties of chitosan reacted with ethylenediamine to give chitosan containing long-distanced free amino groups (traditional way and under solvent-free conditions.
The structure of the obtained polymers was proved by the ATR-FTIR and NMR spectroscopy. The morphology and size of the obtained chitosans were characterized by transmission electron microscopy (TEM).

[1] Hlum L. *Pharm. Reaserch* 15 (2008) 1326–1331.

Acknowledgements:
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P92

Nanodiscs - artificial membrane-like environment for *in vitro* studies of membrane proteins

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Membrane proteins constitute a large group of potential drug targets, accounting for almost 30 % of the proteome, and making up approximately half of all known therapeutic targets. Surveys show that the membrane proteins are the second most frequently indicated target class of molecules worked with or plan to work with for binding analysis by respondents. However, studying membrane proteins has proven to be a challenge, since it is highly difficult to form soluble, monodisperse membrane protein preparations that maintain the transmembrane activity of the protein and provide robust biophysical and biochemical assay systems. Several techniques have been developed to overcome these problems, such as detergent-lipid micelles and proteoliposomes. Nevertheless, conventional systems lack stability, result in a random orientation and provide access restricted to only one side of the protein.

Here we present optimization of a protocol for formation of alternative artificial membrane-like environment, so-called nanodiscs. The nanodiscs are highly soluble nanoscale lipid bilayers which can incorporate single molecules of fully functional integral membrane protein targets through simple chemical self-assembly. They have been reported to be more stable than proteoliposomes, while still offering a lipid bilayer environment, which can be composed of different types of lipids. In presented work the lipid part of the nanodiscs was formed using two lipids: POPC and POPG at the ratio reassembling near-native environment of eukaryotic cell membranes. The lipids were circumferentially bound by a MSP1E3D1 variant of membrane scaffold protein (MSP) to form nanodiscs of approximately 12 nm in diameter as it was proved by the dynamic light scattering and transmission electron microscopy. The lipid to MSP ratio and the assembly procedure were carefully optimized in order to obtain homogeneous population of the particles. The nanodisc structures were then directly purified by normal chromatographic procedures. The resulting construct showed to be soluble and stable in aqueous solution.

In future, the presented protocol will be used to form nanodiscs with embedded protein of interest (e.g. GPCR). This will allow for biophysical and biochemical characterization of interactions of antagonists, agonists, and other binding partners (i.e. G proteins) with physiologically intracellular and extracellular faces of the transmembrane protein.

P93

The salt bridge - systematic QM and database search study

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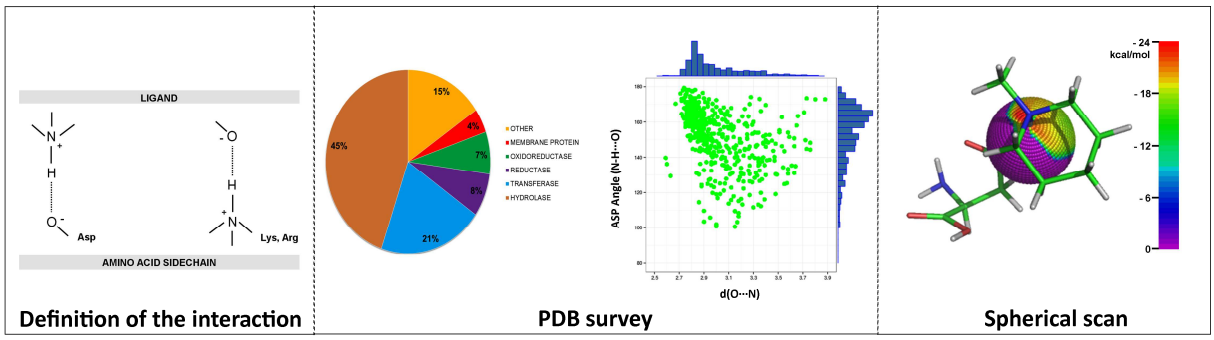
Salt bridges occur frequently in proteins, providing conformational specificity and contributing to molecular recognition [1]. It can be defined as an interaction between two groups of opposite charge in which at least one pair of heavy atoms is within hydrogen bonding distance, moreover, it was also classified as an double charge-assisted hydrogen bond (+/-CAHB) by Gilli et al. [2]. Among all known non-covalent ligand-protein interaction salt bridge is the strongest one [3], however, no comprehensive study of their role and significance in drug design have been performed so far.



Herein we report on a systematic study of the role and nature of salt bridge in biological systems including: comprehensive geometrical and target occurrence analysis of Protein Data Bank survey and quantum mechanical-based study of the geometrical preferences of several model salt bridges. The results indicated that salt bridge is a key interaction in many different drug targets and shows larger hydrogen bond contribution than ionic.

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Acknowledgments:
The study was supported by the National Science Center Grant No DEC-2014/15/D/NZ7/01782. Authors acknowledge the computing resources from PL-Grid Infrastructure.

P94

Combined therapy with disintegrin and cisplatin as a new strategy in inhibition of MDA-MB 231 breast cancer cell line growth

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Breast cancer is one of the most frequently diagnosed cancer in females. The cornerstone treatment for numerous malignancies, including breast cancer is cisplatin. This medicine is an effective cancer chemotherapeutic agent and functions by generating DNA damage, promoting DNA damage-induced cell cycle arrest and apoptosis; however, its efficacy is challenged by the resistance of tumor cells in clinical application. The need for alternatives to cisplatin has consequently inspired further work towards the development of novel platinum-based drugs or combined therapy with other components. It is a promising strategy to generate synergistic anticancer effects, reduce individual drug-related toxicity, suppress multi-drug resistance and maximize the treatment effect.

The aim of the present study was to investigate the effects of echistatin in combination with cisplatin on MDA-MB-231 human breast cancer cells. The echistatin belongs to the family disintegrins which are effective agents in limiting tumor growth and spread. The results of the study suggest that combined treatment of breast cancer cells with echistatin and cisplatin severely inhibits important biological functions of the cells. We showed that such strategy have a potent cytotoxic effect, especially by increase apoptosis in cells. The apoptotic effect, due to treatment cells with combined treatment echistatin and cisplatin, required low doses of those reagents than those components alone. The functional significance of the combined treatment of MDA-MB-231 cells with echistatin and cisplatin was found at the level of DNA biosynthesis. In this case, similar like in concern apoptosis, combined treatment echistatin and cisplatin were more effective and required low doses of those reagents for inhibition DNA biosynthesis than the components alone.

Acknowledgements:
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P95

Synthesis and evaluation of a new indole-based series as non-basic 5-HT6 receptor ligands

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The majority of known 5-HT_6R ligands, like endogenous agonist – serotonin, possess positively charged at physiological pH basic nitrogen atom, which is considered to be necessary for effective interaction with the receptor. However, in last years, progressively grow new generations of 5-HT_6R ligands without a protonable nitrogen atom. The development of such molecules with novel, alternative binding mode, follows from the possibility of improving the pharmacokinetic properties of the known active compounds.[1] The 5-HT_6R ligands with reduced basicity developed so far revealed excellent selectivity over other monoaminergic GPCRs and low hERG affinity. The mechanism of a non-basic ligand-receptor interaction has been studied and some hypotheses were formulated but the phenomenon is still unclear.[2-4]

As a continuation of our investigations on the non-basic 5-HT_6R ligands, the new series of compounds has been synthesized based on structure of the two selected ligands from the previously developed series with 1-(phenylsulfonyl)-1H-indole fragment. The 5-HT_6, 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and D_2 receptor affinities for all the synthesized compounds were assessed in radioligand displacement experiments. The structure-affinity relationships and the results of molecular modelling experiments are discussed.

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P96

The service of *in silico* methods in the development of metabolically stable ligands

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Various *in silico* methods have become an integral part of the modern process of drug design and development. Their service is not only limited to the selection of compounds with potential activity towards particular target, but they also enable the initial evaluation of the physicochemical and pharmacokinetic properties of compounds [1,2].
Within the study, a series of derivatives of the selective agonist of serotonin receptor 5-HT_7, LP-211, were synthesized. The compounds were examined in terms of their activity towards 5-HT_7R, but also their metabolic stability properties were assessed. The promising properties of one of the compounds, determined its selection for further *in vivo* studies.
In order to support the design of new stable 5-HT_7R ligands, a protocol for *in silico* evaluation of metabolic stability was developed. It involved the description of compounds with the use of a hybrid representation of various one-, two- and three-dimensional descriptors generated in the PaDEL-Descriptor [3] and application of the machine learning algorithm – Support Vector Machine adjusted for performing regression tasks [4]. The obtained results were compared with the outcome of the set of online tools allowing for direct or indirect evaluation of compound's metabolic stability properties.

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Acknowledgments:
The study was supported by the project HARMONIA 2015/18/M/NZ7/00377 financed by the National Science Centre, Poland (www.ncn.gov.pl).

P97

Research of fungal polysaccharide immunoactive fraction structure

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Fungal extracts are used all over the world in the treatment of many diseases. In Asian countries, the antitumor drug lentinan is used – an exopolysaccharide demonstrating immunomodulatory activity. Lentinan is isolated from fruiting bodies of *Lentinula edodes* (Shii-take mushroom), which is a species of medicinal fungus belonging to the type Basidiomycota. Lentinan is a highly purified polysaccharide fraction - branched β -D- glucan with the molecular weight of 400 - 800 kDa. It is mainly used in cases of gastrointestinal cancer. Selenium compounds and polysaccharide fractions have a similar pharmacological effect, even though the mechanism by which selenium exerts anticancer and immunomodulatory activity differs. The addition of selenium to the growth medium of *L. edodes* can potentially reinforce letinan's antitumor properties. Currently, research aimed at confirming the synergism of the two factors are under way [1,2].
The aim of the study was to determine the molecular weight and determination of selenium in the polysaccharide fraction of L.edodes mycelium from submerged culture on growth media enriched with selenium from the Japanese Shiitake mycelium.
Isolation of selenopolisaccharide fractions, corresponding to the extraction lentinan from L.edodes mycelial cultures, using the method of Chihara et al. [3]. We obtained efficient growth of the mycelium and high selenium concentration in the biomass (67 mg/ ml). Subsequently, the molecular weight of the polysaccharide fraction was determined with gel permeation chromatography GPC with triple detection, whereby the the components in the mixture were divided with molecular sieves according to particle sizes. Molecular weight determination was performed by the standard curve. For the preparation of the calibration curve 6 patterns of β -glucans with masses of 40 kDa , 123 kDa, 245 kDa , 359 kDa and ~ 500 kDa were used. Based on the determinations, it was found that the molecular weight of the polysaccharide fraction was about 380 kDa.

Investigation on polysaccharides in L.edodes cultures may contribute to the creation of a new type of drug with selective immunosuppressant properties that would be much cheaper and less toxic than those currently available in the pharmaceutical industry .

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[3] Chihara G. et. al. *Cancer Research. (*1970) 30(11):2776-81.

P98

Structure of benzimidazole derivatives – potential tuberculostatics

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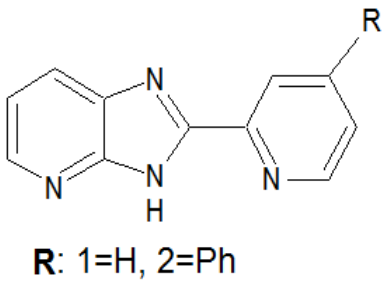
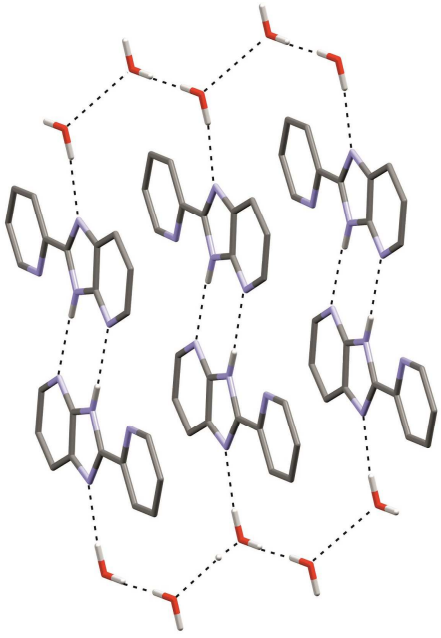
Tuberculosis remains a serious problem, because of increasing resistance of the *Mycobacterium tuberculosis* to known medicines [1]. Benzimidazole derivatives are group of compounds showing tuberculostatic activity. 3D structures of two of them were determined: 2-pyridin-2-yl-*3H*-imidazo[4,5-b]-pyridine (**1)** and 2-(4-phenyl-pyridin-2-yl)-*3H*-imidazo[4,5-b]pyridine (**2**).



R: 1=H, 2=Ph

	1	2
Formula	$C_{11}H_8N_3{*}H_2O$	$C_{17}H_{12}N_3$
Space group	$P2_1/c$	$P2_1/c$
a, b, c [Å]	14.703(3) 4.638(1) 15.356(2)	14.290(7) 14.435(2) 18.995(5)
β [°]	101.388(1)	92.889(3)
Z'	1	3
R	0.0342	0.0444

In structure **1**, molecules of benzimidazole form dimers due to N-H...N hydrogen bonds. Water molecules present in the structure form chains going down [010] direction. On the figure the system of the hydrogen bonds is showed (only hydrogen atoms forming hydrogen bonds are displayed). In the case of structure **2**, molecules form dimers due to N-H...N hydrogen bonds.



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P99

Catalytic properties of novel iron(II) porphyrazines as potential biomimetic systems

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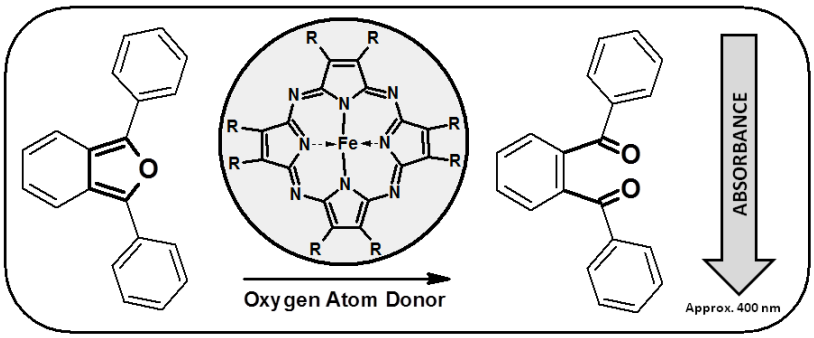
INTRODUCTION

Porphyrazines (Pzs) are macrocyclic compounds related to phthalocyanines. They possess various transition metal cations inside the macrocyclic core (i.e. iron(II/III), zinc(II), cobalt(II), manganese(II/III) and copper(II)) which impacts their electrochemical properties [1,2]. Biomimetic catalytic reactions are basically composed of redox reactions of organic substrates with proper porphyrinoid catalysts and oxygen atom donors e.g. hydrogen peroxide, iodosylbenzene or *tert*-butyl hydroperoxide [3].

METHODOLOGY

Previously synthesized and characterized iron(II) porphyrazines were investigated as potential catalysts in oxidation reaction on diphenylisobenzofurane (DPBF) in organic solvents. Hydrogen peroxide and *tert*-butyl hydroperoxide were utilized as oxygen atom donors. Reactions were performed in DMF in dark to eliminate photodynamic effect of Pzs on DPBF. DMAP was used as a co-catalyst to increase the catalytic effect of pzs. The transformation of DPBF to 1,2-dibenzoylbenzene in the reaction mixture was assessed by the decrease of substrate absorbance at approx. 400 nm by UV-Vis spectroscopy.



RESULTS

All investigated iron(II) porphyrazines demonstrated catalytic activity. There were noticed significant differences in the oxidation efficiency of DPBF as the result of differentiated macrocyclic periphery. The stability of catalysts during oxidation reactions will be presented.

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P100

Design, synthesis and crystal structure of novel chalcone derivatives as potential microtubule targeting agents

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The microtubular system with its dynamic nature characterized by the polymerization and depolymerization of α,β-tubulin heterodimers, is essential in a variety of cellular processes, including maintenance of cell shape, regulation of motility and cell division [1]. Because of the latter function microtubules are one of the significant and more successful molecular target for designing of new active molecules possessing anticancer activity. Among this group of compounds chalcones (1,3-diphenylprop-2-en-1on derivatives) represent a promising class of compounds with a simple structure, taking the possibility of extensive structural modifications that improve their natural anticancer properties.
Their mechanism of action including the inhibition of tubulin assembly by binding to the colchicine binding domain resulting from their structural similarity to other active ligands that have the same molecular target (e.g. combretastatin A-4, CA-4). Our successful investigation on novel potent inhibitors of tubulin polymerization from group of CA-4 thioderivatives prompted us to extend our research on chalcone scaffold.
Herein we present synthesis, docking studies and X-ray structural characteristics of novel chalcone thioderivatives. Their biological activity will be further determined using series of a cancer cell lines, tubulin inhibition, cell cycle and pro-apoptotic analyses.
The multidisciplinary research methodology supported by computer aided drug design methods, standard and high-resolution X-ray structural analysis combined with modelling of the multipole electron density distribution [2] enable to develop of a new, effective chemotherapeutics from the group of chalcone derivatives and for the better understanding of their interaction with tubulin at the molecular level.

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Acknowledgements:
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P101

The synthesis of new biologically active isoxazole derivatives via multicomponent reactions

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Multicomponent reactions (MCRs) are important class of chemical transformations which plays an important role in drug discovery and design. More than two starting materials are used to obtain the final product composed of the atoms of the substrates. A simple one-pot, one-step procedure, instead of multiple sequential steps, makes multicomponent reactions a valuable tool in combinatorial chemistry by giving the opportunity to obtain a large number of compounds in a short time. Among the known MCRs isocyanide-based reactions, such as Passerini three-component reaction (P-3CR), is especially a powerful tool [1,2].

Heterocyclic compounds play an important role in drug design and discovery. Previously we synthesized a series of isoxazole-containing derivatives, presenting the potential immunological activity [3-6]. To allow the rapid synthesis and screening of isoxazole-containing compounds we decided to apply 5-amino-3-methylisoxazole-4-carboxylic acid (AC) in the Passerini three-component reaction.

Here we present our investigations under P-3CR transformation, in the presence of 5-amino-3-methylisoxazole-4-carboxylic acid, isocyanide and carbonyl compound (Figure 1), reaction conditions optimization and the immunological activity analysis of obtained compounds. We found that the applied isoxazole derivative AC, such as β-amino acid, does not undergo Ugi transformation, leading directly to the Passerini product.

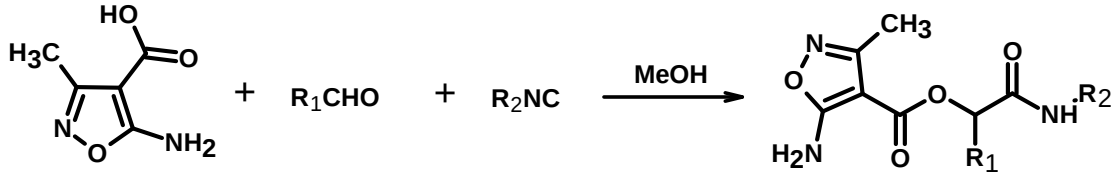

Fig. 1 Schematic presentation of the synthesis of the Passerini products using AC derivative.

The structure of the model P-3CR compound was confirmed by X-ray, NMR and MS analysis. The biological study showed that the obtained products inhibit the phytohemagglutinin A-induced proliferation of human mononuclear blood cells and tumor necrosis factor alpha production.

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P102

Influence of tricyclic xanthine derivatives on activity of monoamine oxidase B

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Monoamine oxidase is an enzyme present within the brain, existing in two isoforms, A and B. Both of them catalyze deamination of molecules like dopamine and serotonin in the presence of oxygen [1, 2]. This reaction yields hydrogen peroxide, and high levels of it can cause oxidative stress and furthermore provoke death of neurons. Because of that, MAO-B has become a drug target in the treatment of Parkinson disease (PD), in which loss of dopaminergic neurons occurs [1, 3]. Another novel target of drugs against PD are A_{2A} and A_1 adenosine receptors [1, 3, 4]. Currently, compounds which targets both MAO-B and A_1 or MAO-B and A_{2A} receptors are being searched for, as an alternative to classical treatment of PD with L-DOPA [1, 3, 4]. Additionally, MAO-A isoform is a drug target in treatment of depression [2].

To determine inhibitory potential of the investigated tricyclic xanthine derivatives towards MAO-B, the Amplex Red® Monoamine Oxidase kit was used. Initially, compounds were tested in 1μM concentration. Their activities were compared with reference MAO-B inhibitor, pargyline. Compounds that had exhibited high values for inhibition of the human recombinant monoamine oxidase B were tested in ranges of different concentrations, which allowed IC_{50} and K_i values to be calculated.

Among investigated compounds, the most potent one is JS16015 with IC_{50} = 82±19 nM. Additionally, most active compounds were tested in similar assays, using human recombinant MAO-A enzyme and the same kit to determine their selectivity. Tested compounds showed selectivity for the MAO-B isoform, which is convenient for dual targeting of enzyme and adenosine receptors described above.

Results of performed experiments provide knowledge about activity of tested tricyclic xanthine derivatives and can help with selection of compounds for further research.

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Acknowledgements:
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P103

Tools of the Systemic Pharmacology in investigation of the biological activity of imidazoline derivatives

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Systemic Pharmacology defined for the first time by Hopkins in 2007, is multidisciplinary scientific domain which comprise pharmacology with biological networks, systemic biology, bioinformatics and other scientific disciplines connected within that domain. At its base we can find results of the research and observations that it is not possible to cure effectively many civilization's diseases just by blocking, even in the most efficacious way, of only one molecular target. It is well known that disease is usually caused not by dysfunction or mutation of a single structure but is a malfunction of a whole network or pathway in an organism.

The goal of the Systemic Pharmacology is therefore an understanding of illness on the system level and recognition of interactions between drug and organism within so called a biological network. Such attitude affected theory and methodology of the drug R&D. On the field of the systemic pharmacology the newest paradigm in drug development was developed – search for selectively unselective, multitarget acting compounds.

Leading *in silico* research on biological activity of imidazoline derivatives, tools allowing pointing out the probable molecular targets were used. Further analyses revealed that the most active compounds acted on the cancer pathways. Due that, as a reference drug for testing the tools used, lapatinig – anticancer drug used in the treatment of metastatic breast cancer interacting with relatively well known, broad spectrum of molecular targets, was applied. The tools were properly identifying main molecular targets for which the drug was projected and 21 additional targets being in very good accordance with results of the experiments. They pointed out the key cancer pathway involved in development of the drug resistance – PI3K-Akt kinases. On the fourth place the ErbB (HER) pathway, main target drug was developed for, was recognized. In the case of imidazoline derivatives we identified derivative for which that pathway is the main one (first place in the rank) without significant interaction with PI3K-Akt pathway. Further search revealed with high probability additional interactions with other pathways like chemokines, broadening the margin of action due to the possible synergistic actions.

P104

Structural characterization of the new anhydrous polymorph of sodium alendronate

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Human bone system related diseases are remarkably deteriorating the quality of life. A very popular remedy in the treatment of these diseases are drugs from bisphosphonates family which can act as selective inhibitors of osteoclastic bone resorption. Currently, they are used in the clinical treatment of a variety bone disorders, for example, osteoporosis, Paget's disease, hypocalcemia [1], and are of some importance in bone cancer therapy [2]. Our work has focused on an anhydrous form of sodium alendronate (Figure 1) - a drug from nitrogen containing group of bisphosphonates – which was crystallized and structurally characterized.

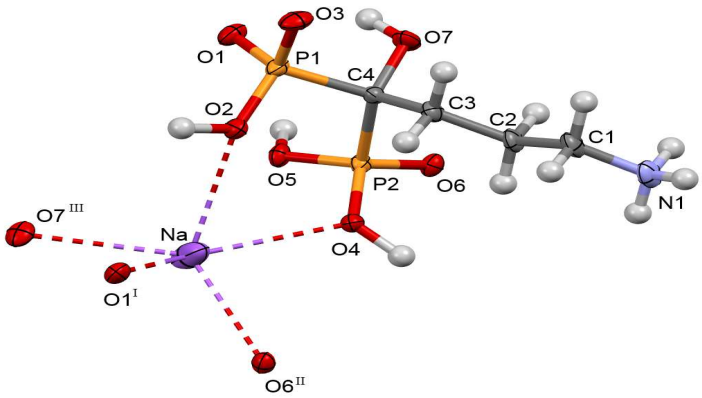

Figure 1. Molecular structure of sodium alendronate

Unlike the presented in the literature the crystal structure of anhydrous sodium alendronate determined from X-ray powder data [3] our single crystal data revealed a big difference between the two structures e.g. distinct coordination of Na^+. Here we would like to present the crystal structure of a new form of anhydrous sodium alendronate. In addition some other properties of studied molecule, including intermolecular interactions using Hirshfeld surfaces (HS) and fingerprint plots (FP) analyses, the spectroscopic studies (FT-IR) and thermal investigation (TG,DTA) will also be presented.

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P105

Synthesis, structure and antimycobacterial activity of new thiosemicarbazide derivatives containing pyridine ring

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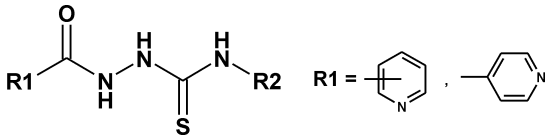
The title compounds were obtained in the reaction of 2-,3-,4-pyridinecarbohydrazide with appropriate isothiocyanate. The reaction was carried out in methanol and the conditions were chosen experimentally.



All newly synthesized substances were tested *in vitro* against *Mycobacterium tuberculosis* H_{37}Ra, *Mycobacterium phlei*, *Mycobacterium smegmatis* and *Mycobacterium timereck*. Obtained data showed that most of the test substances effectively inhibited growth of the tested strains. For the most active compounds MIC value were in the range 7.81 µg/ml – 62.5 µg/ml. The highest activity was observed against Mycobacterium tuberculosis H37Ra (MIC 15.625 µg/ml) and Mycobacterium smegmatis (MIC 7.81 µg/ml).
The molecular modeling studies using DFT and AM1 methods were undertaken to investigate the conformational preferences of searched derivatives. Moreover, the molecular docking procedure was used to study the interaction of thiosemicarbazide ligands with the model protein system using GOLD package.

P106

Chemometric analysis of the thiourea derivatives incorporating 2-aminothiazole scaffold

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Chemical compounds containing the 2-aminothiazole moiety are commonly used in medicinal chemistry in research of chemotherapeutics [1]. Several drugs containing the 2-aminothiazole moiety have been launched (Famotidine, Cefdinir, Meloxcam, etc.) [2]. Research on new compounds containing the 2-aminothiazole moiety is still in progress. The field of chemotherapeutic action of new 2-aminothiazoles is also expanding. Recently, compounds exhibiting the anticancer, antitumor, antidiabetic and anticonvulsant activity have been obtained [2]. Traditional laboratory research of new compounds exhibiting therapeutic properties is expanded by addition of *in silico* research. Such research involves the use of computational systems in computer's virtual space for designing new chemotherapeutics. The QSAR method, involving searching for relationships between compound structure and biological activity, is commonly used for research of this type.

This paper employs chemometric methods to describe relationships between the structure of analyzed compounds and their biological activity or cytotoxicity of thiourea derivatives containing the 2-aminothiazole moiety described in [3]. Commonly used chemometric methods have been utilized for the purpose of this paper, e.g. cluster analysis (Ward's method, k-means method), correlations and regression analysis (Multiple Linear Regression (MLR) [4]). The analyses were conducted using the STATISTICA 12.0 software. The analysis of similarities between the researched compounds in relation to their biological activity against Gram-positive and Gram-negative bacteria and their cytotoxicity against MT-4 cells was performed using the cluster analysis method. The correlation and regression analysis methods were utilized to determine linear dependencies between cytotoxicity versus descriptors describing the electronic, steric and lipophilic properties of the 11 researched compounds exhibiting biological activity against Gram-positive bacteria described in the paper [3]. Moreover, linear equations for the relationship between biological activity for the Staphylococcus epidermidis bacteria and electronic and steric molecular descriptors have been developed. All obtained equations meet the condition of acceptability for model R^2>0.6 and Q^2>0.5 [5], as well as the coincidence condition.

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P107

Exploring the influence of fluorine substitution on tuning the hydrogen bonding properties using theoretical and spectroscopic methods

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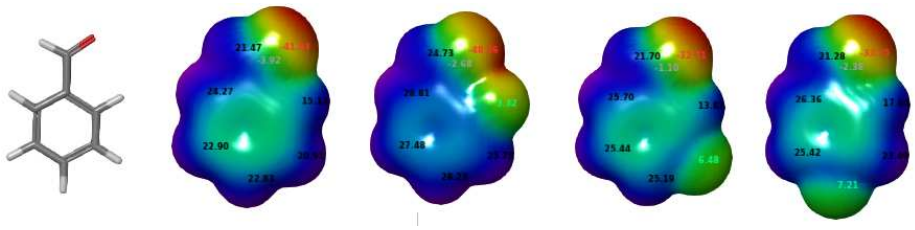
Fluorine substitution affects to a number of different properties of molecules i.e.: electrical charges of neighboring atoms, pK_a perturbation, conformational changes, basicity/acidity and bioavailability, toxicity, modulation of lipophilicity and metabolic stability [1-3].
Since the 1950s, over 150 fluorinated drugs have been released to market and now make up approx. 20% of all pharmaceuticals, with even higher records for agrochemicals (up to 30%) [4].

Herein, we present the effect of fluorine substitution on electrostatic surface potential (ESP) and atomic charge distribution for a series of benzene derivatives with hydrogen bond donors/acceptor (HBD/A) substituents (-OH, $-NH_2$, -CHO, $-OCH_3$, $-SCH_3$) using DFT/B97-1 method and cc-pVTZ-pp++ basis set. Next, the influence of fluorination on the strength of the hydrogen bond was investigated for fluorinated series of aniline – methanol dimers using the same DFT and basis set combination and FTIR spectroscopy.



The results confirmed the impact of fluorine on the acidity of the functional groups, in particular, the effect was the most significant for *para*-substitution. Interestingly, the strength of hydrogen bonds depend on the position of the fluorine substitution in the ring. The obtained results can be used in rational drug design and the lead modification in order to improve its activity as well as pharmacokinetics and pharmacodynamics properties.

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P108

Molecular docking in virtual screening of *trans*-stilbene derivatives as CYP1 family inhibitors

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The cytochrome P450 superfamily comprises the enzymes which contain a heme prosthetic group catalysing oxidation reactions and N- and O-dealkylations of numerous xenobiotics. The cytochrome P450 family 1 (CYP1) is represented by isoforms CYP1A1, CYP1A2 and CYP1B1, which are responsible for the phase I metabolism of endogenous and exogenous substrates including drug compounds and procarcinogens. Isozyme CYP1B1 is involved in pathogenesis of the hormone-induced cancers. The inhibition of CYP1B1 activity by nutraceuticals constitutes one of the chemopreventive strategies, while in cancer therapy the inhibition of CYP1s activities may be helpful in avoiding drug resistance caused by the metabolism of chemotherapeutics catalysed by CYP1s [1]. *Trans*-resveratrol (3,4',5-trihydroxy-*trans*-stilbene) is the best known natural polyphenol showing inhibitory activity toward CYP1s, although its bioavailability in humans was determined as poor due to the fast metabolism. In the last decade, the natural and synthetic *trans*-stilbene derivatives were studied in the context of their interaction with CYP1s. The substitution of hydroxyl groups with methoxy- substituents efficiently improved bioavailability by preventing polyphenol metabolism and concomitantly influenced the affinity of compounds to active sites of cytochromes.

To evaluate docking as virtual screening tool for searching effective inhibitors of CYP1A1 and CYP1B1 two sets of test compounds (only *trans*-stilbene derivatives) taken from bindingDB database and from the literature [2,3] were used. Test sets of compounds were docked into CYP1A1 (PDB ID: 4I8V) and CYP1B1 (PDB ID: 3PM0) binding sites with the use of two algorithms CDOCKER and LigandFit, implemented in Discovery Studio. Binding affinities for protein-ligand complexes obtained in docking were evaluated by scoring functions and binding energy (ΔG) calculations. Next, based on analysis of ROC curves and area under ROC (AUC), the ability to discriminate actives and inactives for different scoring methods was assessed. The present studies fit into the development of molecular docking as a method for the analysis of ligand-enzyme interactions and mechanisms of CYP1-catalyzed reactions. In a future perspective, the developed methods of virtual screening might be helpful in computational structure-based drug design.

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P109

Synthesis and structural characterization of new pyrazolo[4,3-*e*][1,2,4]triazine acyclonucleosides

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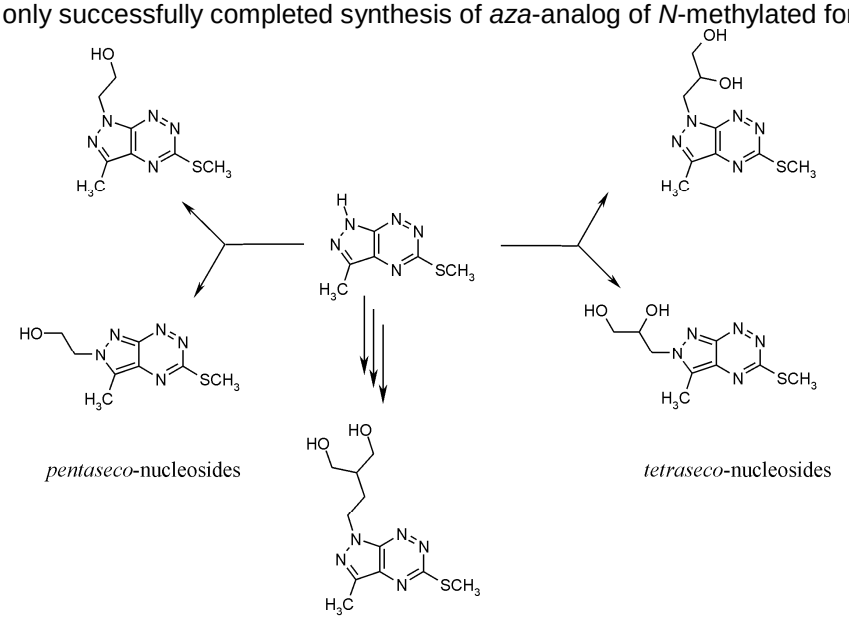
The discovery and use of acyclonucleosides in medicine as useful antiviral therapeutics and the development of viral diseases made the synthesis of new acyclic nucleosides still relevant and very interesting subject of study. Despite the many research papers devoted to the new acyclonucleosides, there is little information in the literature about acyclonucleosides of unappreciated so far pyrazolo[4,3-*e*][1,2,4]triazine ring system.[1,2] Using the possibility of functionalization of the pyrazolo[4,3-*e*][1,2,4]triazine system so far was only successfully completed synthesis of *aza*-analog of *N*-methylated formycine A.[3]



In this communication the synthesis and structure of acyclonucleosides containing pyrazolo[4,3-*e*][1,2,4]triazine core as the nitrogenous base are discussed. The molecular modeling studies using DFT method were undertaken to investigate the energetic, electronic and conformational preferences of searched derivatives.

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P110

Novel tetrahydroacridine derivatives as acetylcholinesterase inhibitors with multifunctional activity

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Alzheimer's disease (AD) is a chronic neurodegenerative disease and the most common neurodegenerative disorder. AD is also one of the main causes of death in the world in people over 65 years old. Due to the aging of the population, by 2050 it is expected that the number of people with dementia will increase over 3 times. In spite of the fact that this disease has been known since 1906, so far there is no effective therapy methods. Unfortunately the drugs currently on the market can only inhibit the development of the disease but not cure it completely. Nowadays the most perspective method of AD treatment is increasing cholinergic neurotransmission by inhibiting the enzyme acetylcholinesterase (AChE). Still it is only one of the many hallmarks of AD pathology. Multifactorial nature of the disease is one of the principles underpinning that the single-target directed drugs have turned out to be palliative. This has led to a new paradigm in medicinal chemistry, the "multitarget-directed ligand" – one compound that is able to modulate multiple targets in the same time and thus have multiple biological properties.

Following this strategy we developed new derivatives that can be presented in the current work. Our main goal was to combine the well-known inhibitor of AChE – tetrahydroacridine with iodobenzoic acid. Afterwards computer simulations of the basic ADME parameters were performed and the best derivative was chosen considering pharmacodynamics properties.

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Aknowledgements:
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P111

Multifunctional activity of the new tetrahydroacridine derivatives as acetylcholinesterase inhibitors. Prediction of the biological properties

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Neurodegenerative diseases belong to dominant disorders for which the risk factor increases with age. Neurodegeneration leads to progressive death of neurons in the brain and loss of function but the mechanism is still not well understood. Alzheimer's disease (AD) is one of the major disorders developing as a result of this process, as well as Parkinson's and Huntington's diseases. AD is the most common cause of dementia and has a large economic, social and health impact. It is estimated that 24 million people worldwide suffer from dementia, most of which are suspected to have AD. The therapy is available, but it can only alleviate the symptoms of the disease. Research indicates that the number of people with AD will increase significantly and that is why it is a research priority nowadays. It is important to understand the pathogenesis deeper, to enable the development of disease-modifying drugs. By now there are several hypotheses that aim to explain the pathogenesis of AD. The first one is cholinergic hypothesis which verifies than the decline in acetylcholine (ACh) levels leads to cognitive deficits and memory loss. Nowadays AD treatment is mainly based on acetylcholinesterase inhibitors (AChEIs) able to increase ACh levels in the cholinergic synapses. Thus far, the number of approved drugs is limited to only three AChEIs: rivastigmine, donepezil and galantamine, and the NMDA antagonist – memantine. The other hypothesis is the amyloid one. It indicates mutations that result in abnormal amyloid precursor protein processing and deposition in the brain. Many published articles pay attention to the relationship between amyloid and AD. And the last hypothesis is the Tau theory. Tau is a protein that stabilizes microtubules and that exist in the neurons. Though, in AD it is hyperphosphorylated and forms tangles. Anti-tau therapies concern inhibition of tau aggregation and phosphorylation but have not been successful yet. Because of the multifactorial nature of AD, the traditional 'one molecule, one target' paradigm is not sufficient. Thus, a strategy named multi-target-directed ligand (MTDL) can be more effective in research on new drugs.

The main goal of the project is to develop new hybrids built of well-known inhibitor of AChE – tetrahydroacridine with fluorobenzoic acid. Afterwards computer simulations of the basic ADME parameters should be performed and the most promising derivative will be chosen to consider pharmacodynamics properties.

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P112

Synthesis and biological activities of palmitic acid derivatives

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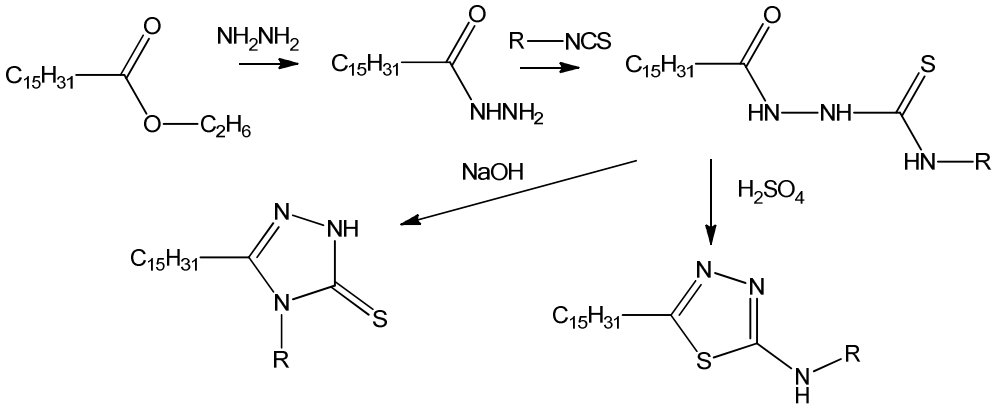
It is known and proved that fatty acids have antibacterial, antimalarial and antifungal activity [1-3]. The development of resistance by microbes, including fungi and yeasts, towards antimicrobial agents which are already in use, necessitates the search for alternative antimicrobials, including fatty acids and their derivatives. A long non-polar chain of fatty acids affects microorganisms' plasma membranes, especially gram-positive bacteria, tuberculosis bacilli and fungi. Biological activity is correlated to the length and to the saturation of the alkyl chain, for example the antifungal efficiency of fatty acids increases with an increase in chain length [2].

Ethyl palmitate was used as a substrate for the synthesis of palmitic acid hydrazide. In the next step the acid hydrazide was reacted with aromatic isothiocyanides to give good yields of thiosemicarbazide derivatives of palmitic acid. The newly syntesized compounds were than transformed using two different pathways - under alkaline conditions into triazloes and under acidic conditions into thiazoles derivatives.



All obtained derivatives were tested for antibacterial activity. Thiosemicarbazides and thiazoles were dissolved in DMSO to be tested, while triazole derivatives were converted into sodium salts. Only some thiosemicarbazide derivatives showed medium-level activity against gram-positive cocci (MIC 8 – 32 μg/ml). All compounds are being tested for antimicrobial activity against the tuberculosis bacilli and pathogenic fungi.

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P113

Evaluation of a new bismuth composite with potential medical application

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The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Since then, antibiotics have transformed modern medicine and saved millions of lives. However, the rapidly emerging resistant bacteria threaten the health benefits that have been achieved with antibiotics. Consequently, there is a pressing need to develop new classes of antibiotics and to design more potent antimicrobial compounds derived from existing antibiotics in clinical use for decades [1,2].

Searching for new antibacterial drugs, we synthesized the composite consisting of ciprofloxacin, derivative of bioactive biguanide and bismuth (Cip-Bi-Big) in order to prepare a new dressing with antimicrobial and anti-inflammatory properties, with potential medical application. The antibacterial activity of the received composite against gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. coli*) was proved using microbiological tests as the inhibition zone and diffusion assays.

The presented studies contributes to the analytical assessment of the developed composite using a simple, accurate, and sensitive thin layer chromatographic method. TLC method was used for the evaluation of synthesis process of Cip-Bi-Big composite in context of its composition and purity (separation of Cip-Bi-Big composite from the starting substrates of synthesis: ciprofloxacin, biguanide and bismuth(III) citrate). TLC method was also applied to assess the stability of Cip-Bi-Big composite which was subjected to the influence of different stress conditions (stress degradation studies).
The various chromatographic systems were tested. The best separation was obtained on HPTLC plates coated with octadecylsilyl-bonded silica gel F_{254} (RP-HPTLC) using mixture of methanol, 10% solution of NH_4Cl, isopropanol (7:2:0.5, *v/v/v*) as the mobile phase. Under chromatographic conditions, a compact, moon-shaped spot of Cip-Bi-Big composite was found at R_F value of 0.6. This system allowed the separation of the product (Cip-Bi-Big composite) from the substrates: bismuth(III) citrate (R_F = 0.2), biguanide (R_F = 0.65), ciprofloxacin (R_F = 0.73). No additional spots were observed in the chromatogram obtained with test solution of composite.
Cip-Bi-Big composite was found to be stable to the exposure of weak organic acids as citric and tartaric acid, but showed degradation under strong acids as acetic and hydrochloric acid, and under relatively strong degradation conditions as oxidation with 30% H_2O_2 and high temperature (100^{o}C). The results showed the importance of appropriate protection from acidic condition during the drug development process and handling.

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P114

Novel derivatives of (+)-usnic acid yielded from *Cunninghamella* sp. biotransformation

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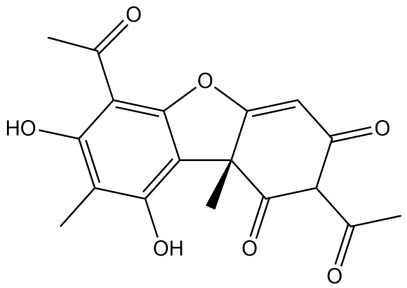
Usnic acid (UA) is a nature-derived compound found only is some lichen species. It occurs especially in *Cladonia, Usnea, Lecanora, Ramalina, Evernia* and *Parmelia* genus. Chemically it is dibenzofurane derivative, optically active, chiral structure, with lipophilic character. More active, right-handed UA, was widely studied in many pharmacological research. Cytotoxic, antiproliferative, antimicrobial, antiviral, antiprotozoal, anti-inflammatory and analgesic activities have been found. Its potential use in treatment of some conditions is patented. However, UA is also known from hepatotoxic properties, which limit its development as a drug [1].

One of possible ways to obtain novel derivatives of a chemical compound is to conduct biotransformation process, using microorganisms. Previously, such approach was successfully used to yield metabolites which, against parent molecule, showed more potent pharmacological activities, favorable pharmacokinetics properties, or better safety profile [2]. Ones of the most widely used microorganisms in drug biotransformation studies are species of *Cunnighamella* genius. *C. echinulata*, *C. elegans* and *C. blakesleeana* have showed high metabolic activity and metabolic profile similar to human one [3].

Aim of this study was to indicate if *Cunninghamella* species are able to conduct biotransformation of (+)-UA in different culture media. For this purpose, 25 mL of culture medium (CSL or GCL) has been inoculated with 200 µL suspension of one of *Cunnighamella* species' spores. After 48 hours incubation, 5 mg of (+)-UA, dissolved in acetone, was added into each biotransformation medium. Then after 7 days of incubation, under appropriate condition of temperature and shaking, biotransformation medium has been extracted with ethyl acetate. Biotransformation process was monitored by LC-MS technique.



(+)-usnic acid

Biotransformation resulted in formation of several metabolites, different according to species and medium. Two metabolites, obtained with the highest yields (M1-24,3% and M2-15,5%) were found in *C. blakesleeana,* cultured in GCL medium. For these, based on mass spectrum, hydroxylation (m/z = [UA] + 16) and oxidation (m/z = [UA] - 1) are expected metabolic pathways. Metabolites' semi-preparative scale biosynthesis, isolation, structure elucidation and activity/toxicity determination are presently implemented steps of research.

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P115

A new method for the synthesis of long-chain arylpiperazines (LCAPs) under microwave irradiation

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Long-chain arylpiperazines (LCAPs) represent the biggest and thoroughly examined class of 5-HT_{1A} receptor ligands [1]. Ligands in this group usually comprise a flexible alkyl chain terminated substituted arylpiperazines and on the opposite side having most frequently imide, an amide or a sulfonamide group. Synthesis of these ligands is most often carried out by a multi-step process, in solvents such as acetonitrile, DMF, THF, acetone, methanol and others [2-4]. A process usually occurs within a few hours, and the purification of the product is associated with the necessity of the use of column chromatography or recrystallization. Within the framework of the research work, we received of phthalimide derivatives belonging to the LCAPs family, using new methodology based on microwave irradiation and catalysts PTC. The method involves use of solvents only in a small proportion weight in relation to substrates, which can significantly reduce the duration of the process, and reducing the use of toxic solvents, which has a beneficial effect on the economy, and ecology of the process. The great advantage of the method used is the high yield (>80%) obtained ligands and purity >90%. Another advantage is the fact that the process takes only a few minutes. The reaction conditions established in the model synthesis of NAN-190, and then the universality of this method was confirmed in synthesis ligands from the group of LCAPs (Long Chain Arylpiperazines). Obtained ligands had a substituents arylpiperazine, a variable length alkyl chain and in the terminal part phthalimide moiety. We considered phthalimide derivatives because they are interesting as potential ligands of 5-HT receptors and they are also important intermediates in the synthesis of bioactive compounds.

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P116

The influence of anti-MUC1 with berenil complex of platinum(II) on concentration of apoptotic markers in human skin fibroblasts

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The mucin MUC1 is a type I transmembrane glycoprotein expressed at the apical border of healthy epithelia that line the respiratory, reproductive and gastrointestinal tracts and is overexpressed in various cancer cells. Tumour-associated MUC1 has shorter and less dense O-glycan chains compared to normal MUC1, which is useful as a promising therapeutic target for the treatment of breast cancer patients. The function of MUC1 in the healthy state is still unclear, but its role in cancer metastasis, anti-apoptosis and immune suppression is well documented. Recently we proved that combined treatment berenil complex of platinum(II)-(Pt12) + anti-MUC1 caused the highest releasement of the proapoptotic markers in MDA-MB-231 breast cancer cells. The concentration of Bax, caspase-3,-8 and -9 statistically increased in cell lysates after 24 hour incubation with drugs used in combination. The effect was stronger than treatment with cisplatin, anti-MUC1, and anti-MUC1 used with cisplatin. The goal of the study was to check the effect of Pt12 together with anti-MUC1 on induction of programmed cell death in human skin fibroblasts. The influence of Pt12 with anti-MUC1 on the level of selected markers of apoptosis such as: p53, Bax, cytochrome c, caspase-8, -9 and caspase-3 was performed using the ELISA technique. The results from combined treatment were compared with those obtained using monotherapy and combined treatment (cisplatin + anti-MUC1). In our study we observed the weakest proapoptotic properties after combined treatment of Pt12 with anti-MUC1 in normal cells. We observed higher concentrations of all tested apoptotic markers such as Bax protein, p53, caspases-3,-8,-9. The pro-apoptotic effect was weaker after combined treatment of cisplatin together with anti-MUC1.

The obtained results proved that only cisplatin in dose 20 μM induced both apoptotic pathways. It activated the death receptor pathway associated with higher concentration of caspase-8 as well as mitochondrial pathway connected with cytochrome c and caspase-9 releasement. The mechanism of action initiated by cisplatin used together with anti-MUC1 induced only the death receptor pathway. We observed higher concentration of caspase-8 in cell lysates as compared with that in control group. The combined treatment with anti-MUC1 is a possible way to improve selectiveness of a chemotherapeutic agent. Monoclonal antibody against MUC1 sensitizes breast cancer cells and improves the effectiveness of such a therapy, but in normal cells used in combination with cytotoxic agents plays a role as a " selective" inducer of apoptotic markers [1].

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P117

Screening for novel $GABA_B$ orthosteric ligands

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Gamma-aminobutyric acid (GABA) is the primary inhibitor neurotransmitter in the central nervous system (CNS) and the GABAergic pathway has been associated with psychiatric disorders such as depression and anxiety1. $GABA_B$ is a heterodimeric receptor ($GABA_{B1}$ and $GABA_{B2}$) that belongs to class C of G-protein coupled receptors (GPCRs). The extracellular part of $GABA_{B1}$ contains the orthosteric binding site, while the transmembrane part of $GABA_{B2}$ contains an allosteric binding site2. About 40% of all marketed drugs bind to GPCRs and most of them to the orthosteric site3. Despite the discovery of the link between GABAB and affective disorders, no current medication targets this receptor. Quite few $GABA_B$ agonists and antagonists have been identified and the chemical diversity among the agonists and the antagonists is low. There are 9 available X-ray crystal structures of the extracellular region of $GABA_{B1}$ in complex with agonists or antagonists. The objective of this study is to combine a ligand-based and a structural based virtual screening approach to identify new orthosteric molecules as potential drug candidates.
Here we will present the ongoing screening. The first step in the multi-step screening approach is filtering of compound libraries by Lipinski's rule of 5 and ADMET. In the ligand-based approach, known ligands are separated into agonists and antagonist, before clustering based on similarity, and generation of pharmacophore models from each cluster. The pharmacophore models are then used for screening of compound libraries. The output from the ligand-based screening will be further filtered by structural based pharmacophores, docking and scoring. The last step will be to predict the binding energy between the identified hits and the target by Linear Interaction approximation (LIA), before selection of compounds for experimental verification.

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Allosteric modulation of the human GABAB receptor

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□-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system (CNS), and dysregulation of the GABAergic system is related to brain disorders. The $GABA_B$ receptor is a heterodimeric class C G-protein coupled receptor (GPCR) consisting of two subunits (gabr1 and gabr2). GPCRs are targets for more than 1/3 of marketed drugs. Most of these drugs are orthosteric drugs. But due to the conservation of the orthosteric binding site among GPCRs family they may lack selectivity.
Allosteric modulators (AMs) have higher specificity than regular orthosteric drugs and hence may trigger fewer side effects. For $GABA_B$ receptor, the allosteric binding pocket is located in the transmembrane domain of gabr2 while gabr1 contains the extracellular orthosteric binding site. No experimental structures of $GABA_B$ receptor are available, hence by using the technique of homology modeling we have generated several hundred models of gabr2 subunit using templates from different GPCR families. A database consisting of 74 known allosteric binders and 2536 decoys was generated and used to evaluate the gabr2 models. The evaluation indicated that the constructed gabr2 models can be used as tools in structure-based virtual ligand screening for new allosteric $GABA_B$ modulators.

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