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- Polskiego Towarzystwa Chemii Medycznej
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# PLAN KONWERSATORIUM

## CZWARTEK, 17.09.2015

**15.00-17.00 – Rejestracja uczestników**

**17.00-17.10 – Ceremonia otwarcia**

**17.10-19.10 – Wykłady Inauguracyjne (WI1, WI2)**

***WI1***

*Thierry Langer, University of Vienna, Austria*
*„Chemical feature-based 3D pharmacophores: current and futures aspects”*

***WI2***

*Janusz Bujnicki, International Institute of Molecular and Cell Biology, Warsaw, Poland; Adam Mickiewicz University, Poznan, Poland*
*„RNA in the spotlight: 3D structure and interactions with small molecule ligands”*

**19.10 – Koncert**

**20.00 – Spotkanie towarzyskie**

## PIĄTEK, 18.09.2015

**9.00-11.00 – Wykład (W1) (45’)**

**Komunikaty (K1–K3) (20’)**

Prowadzący sesję – *prof. Dariusz Matosiuk*,
*prof. Jerzy Tiuryn*

***W1***

*Wiesław I. Gruszecki, Maria Curie-Sklodowska University, Lublin, Poland*
*„Amphotericin B: An old, good antibiotic with very bad reputation”*

***K1***

*Sebastian Kmiecik, University of Warsaw, Poland*
*„Web server tools for modeling of protein structure, its flexibility, aggregation properties and propein-peptide interactions”*

***K2***

*Olga V. Zubkova, Victoria Univerisity of Wellington, New Zealand* |
*„Heparan sulfate fragments and glycomimetic clusters for therapeutic application”*

***K3***

*Małgorzata Kotulska, Wrocław University of Technology, Poland*
*„Bioinformatical studies of amyloid proteins and their contribution to diseases of civilization”*

**11.00-11.30 – Przerwa kawowa**

**11.30-13.10 – Wykład (W2) (45’)**

**Komunikaty (K4) (K5) (20')**

Prowadzący sesję – *prof. Katarzyna Kieć-Kononowicz*,
*prof. Jacek Błażewicz*

***W2***

*Adolfo Rivero-Müller, University of Turku, Finland,*
*Medical University of Lublin, Poland*
*„Molecular analysis of a new mutant lutenising hormone beta subunit that results in hypogonadism*"

***K4***

*Dariusz Plewczyński, The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA*
*Warsaw University, Poland*
*„3D-NOME: an integrated 3 Dimensional NucleOme Modeling Engine for data-driven simulation of spatial genome organization"*

***K5***

*Imin Wushur, UiT-The Arctic University of Norway*
*„Allosteric modulation of GABAB receptor"*

**13.10-14.30 – Przerwa obiadowa**

**14.30-16.30 – Wykład (W3) (45')**

**Komunikaty (K6–K8) (20')**

Prowadzący sesję – *prof. Piotr Formanowicz*,
*prof. Krzysztof Bielawski*

***W3***

*Marek Tchórzewski, Maria Curie-Sklodowska University, Lublin, Poland*
*„Eukaryotic ribosomal T-site as modulator of translational machinery"*

***K6***

*Marek Bajda, Jagiellonian University Medical College, Cracow, Poland*
*„Towards a better understanding of neurodegenerative diseases – beta-amyloid and Alzheimer's disease"*

***K7***

*Szymon Wąsik, Institute of Computing Science, Poznan University of Technology, Poland*
*„Inferring bioinformatics models using crowdsourcing game"*

***K8***

*Thibaud Freyd, UiT The Arctic University of Norway*
*„Allosteric modulation of the human $GABA_B$ receptor"*

**16.30-17.00 – Przerwa kawowa**

**17.00-18.30 – Sesja posterowa**

## SOBOTA, 19.09.2015

### SEKCJA CHEMII MEDYCZNEJ

**9.00-11.00 – Komunikaty (K9–K13) (20')**

*Prowadzący sesję – dr hab. Jadwiga Handzlik,*
*prof. Zbigniew Chilmończyk*

***K9***

*Anna Mrozek-Wilczkiewicz, University of Silesia, Katowice, Poland,*
*Silesian Center for Education and Interdisciplinary Research, University of Silesia, Chorzow, Poland*
*„Combination of PDT therapy with iron chelators"*

***K10***

*Andrzej Zięba, Medical Univeristy of Silesia, Sosnowiec, Poland*
*„New synthesis and in vitro antiproliferative activity of azaphenothiazine derivatives"*

***K11***

*Przemysław Szafrański, Jagiellonian University Medical College, Kraków, Poland*
*„New 'Click chemistry' solutions: water-soluble ligands for the Huisgen cycloaddition"*

***K12***

*Małgorzata Sęczkowska, Maria Curie-Sklodowska University, Lublin, Poland*
*„Study of adsorption kinetic of selected pharmaceuticals on activated carbon"*

***K13***

*Dorota G. Piotrowska, Medical University of Lodz, Poland*
*„Phosphonylated amonafide conjugates"*

**11.00-11.30 – Przerwa kawowa**

**11.30-13.00 – prezentacje posterów (PP1-PP7) (10')**

**sesja posterowa**

Prowadzący sesję – *prof. dr hab. Monika Wujec,*
*dr hab. Paweł Zajdel*

***PP1***

*Agnieszka Jankowska, Jagiellonian University, Medical College, Kraków, Poland*
*„Synthesis of new amide and hydrazide derivatives of 1,3-dimethyl- or 3,7-dimethylpurine-2,6-dione as PDE7 inhibitors"*

***PP2***

*Aneta Pogorzelska, Medical University of Gdansk, Poland*
*„New series of 2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives – synthesis and anticancer activity"*

***PP3***

*Patryk Kasza, Jagiellonian University Medical College, Cracow, Poland*
*Scientific Association of Chemists at the Jagiellonian University, Cracow, Poland*
*„Synthesis and evaluation of fluorescent 1,2,3-triazole derivatives of 3'-azido-3'-deoxythymidine (AZT), using a novel ligand-accelerated [3+2] cycloaddition protocol"*

***PP4***

*Łukasz Popiołek, Medical University of Lublin, Poland*
*„Design, synthesis and in vitro antimicrobial activity of new nalidixic acid – 1,3-thiazolidin-4-one hybrids"*

***PP5***

*Sabina Podlewska, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*
*Jagiellonian University, Kraków, Poland*
*„New non-basic ligands of serotonin receptor 5-$HT_6$ as a result of virtual screening based on machine-learning methods"*

***PP6***

*Waldemar Tejchman, Pedagogical University of Cracow, Poland*
*„Isorhodanines as substrates in the Diels-Alder synthesis of new potential biologically active compounds''*

***PP7***

*Ryszard Bugno, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*
*„Non-basic 5-$HT_6$ receptor ligands"*

**13.00-14.00 – Przerwa obiadowa**

**14.00-15.50 – Komunikaty (K14–K18) (20')**

Prowadzący sesję – *prof. Anna Bielawska*,
*prof. Andrzej Bojarski*

***K14***

*Jarosław Sączewski, Medical University of Gdańsk, Poland*
*„Synthesis and structure-activity relationship analysis of 5-$HT_7$ receptor antagonists: piperazin-1-yl substituted unfused heterobiaryls"*

***K15***

*Tomasz Siódmiak, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University,Toruń, Poland*
*„Kinetic resolution of (R,S)-ibuprofen with the application of lipase from Candida rugosa in free and immobilized form"*

***K16***

*Zdzislaw Chilmonczyk, National Medicines Institute, Warszawa, Poland*
*„Participation of pre- and postsynaptic of 5-$HT_{1A}$ receptors in mood regulation (modulation of depressive like behaviours)"*

***K17***

*Gniewomir Latacz, Jagiellonian University Medical College, Kraków, Poland*
*„In vitro evaluation of metabolic stability of bicyclic imidazole-4-one derivative – the potent and selective antagonist for the orphan G protein-coupled receptor GPR18"*

***K18***

*Tomasz Pańczyk, Polish Academy of Sciences, Kraków, Poland*
*„Carbon nanotubes as drug delivery systems. Insights from molecular simulations"*

**16.00-16.30 – Przerwa kawowa**

**16.30-18.30 –Komunikat (K19) (20')**

**prezentacje posterów (PP8-PP7) (10')**

Prowadzący sesję – *dr hab. Agata Paneth,*
*prof. Stanisław Ryng*

***K19***

*Wojciech Płaziński, Polish Academy of Sciences, Cracow, Poland*
*„Mechanism of the water-assisted ring-opening reaction in hexopyranoses"*

***PP8***

*Anna Boguszewska-Czubara, Medical University of Lublin, Poland*
*„Effects of bergapten on memory processes and oxidative stress"*

***PP9***

*Dorota Chełminiak, Nicolaus Copernicus University, Toruń, Poland*
*„Magnetic nanoparticles coated with modified chitosan rich of long-distanced amino groups – synthesis, characterization, and lipase immobilization"*

***PP10***

*Monika Tomczykowa, Medical University of Białystok, Poland*
*„Dipeptide L-Carnosine analogu es"*

***PP11***

*Anita Płazińska, Medical University of Lublin, Poland*
*„The influence of the β2-adrenergic receptor genetic polymorphism on the interactions with agonists. A molecular modeling study"*

***PP12***

*ABL&E-JASCO Poland*

***PP13***

*Adam Hogendorf, Polish Academy of Sciences, Cracow, Poland*
*Jagiellonian University, Cracow, Poland*
*„The development of mGluR8 PAM agonists"*

***PP14***

*Michał Załuski, Medical College, Jagiellonian University, Kraków, Poland*
*„Evaluation of novel N-9-benzyl-disubstituted derivatives of 1,3-dimethylpyrimido[2,1-f]purinediones as MAO-B inhibitors"*

***PP15***

*Artur Wnorowski, National Institute on Aging, Baltimore, USA*
*Medical University of Lublin, Poland*
*„(R,R')-4-methoxy-1-naphthylfenoterol prevents GPR55 pro-oncogenic signaling in rat C6 glioma cells"*

## SOBOTA, 19.09.2015

**SEKCJA BIOINFORMATYCZNA**

**9.00-11.00 – Komunikaty (K'9) (K'10) (25')**

**Prezentacje posterów (PP'1-PP'5) (15')**

Prowadzący sesję – *prof. Małgorzata Kotulska*

***K'9***

*Marcin Świstak, Faculty of Mathematics Informatics and Mechanics, University of Warsaw, Poland*
*„Comparison of differential expression and coexpression across multiple tissues (skin, fat and LCL) in twins"*

***PP'1***

*Mateusz Łącki, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Poland*
*„IsoStar – the ultimate algorithm for fine isotopic structure calculations"*

***PP'2***

*Marta Kulik, Centre of New Technologies, University of Warsaw, Poland,*
*Department of Chemistry, University of Warsaw, Poland*
*„Energetic and structural investigation of aminoglycoside-RNA complexes"*

***PP'3***

*Tomasz Ratajczak, Institute of Computing Science, Poznan University of Technology, Poland*
*„Robust 3D RNA models comparison with RNAssess"*

***K'10***

*Bogumił M. Konopka, Department of Biomedical Engineering, Wroclaw University of Technology, Poland*
*„Protein Contacts Ontology - a tool for annotation of protein residue-residue contacts"*

***PP'4***

*Natalia Szóstak, Institute of Computing Science, Poznan University of Technology, Poland,*
*European Centre for Bioinformatics and Genomics, Poznan University of Technology, Poland*
*„New in silico approach to assess RNA secondary structures with non-canonical base pairs"*

***PP'5***

*Witold Dyrka, INRIA - Université Bordeaux - CNRS, Team MAGNOME, Talence, France,*
*Institut de Biochimie et Génétique Cellulaires, CNRS, Bordeaux, France, Wrocław University of Technology, Department of Biomedical Engineering, Poland*
*„Deciphering the language of fungal pathogen recognition receptors"*

**11.05-11.30 – Przerwa kawowa**

**11.30-13.00 – Komunikaty (K'11) (K'12)**

**prezentacje posterów (PP'6-PP'7) (15')**

Prowadzący sesję – *dr hab. Dariusz Plewczyński*

***K'11***

*Witold Rudnicki, Interdisciplinary Centre for Mathematical and Computational Modelling, University of Warsaw, Poland, Department of Bioinformatics, University of Bialystok, Poland*
*„Amino acid properties conserved in molecular evolution"*

***PP'6***

*Krzysztof Gogolewski, Institute of Informatics, University of Warsaw, Poland*
*„Activity of NAHR mediating LINE sequences and the distribution of their microhomologies"*

***K'12***

*Michał Burdukiewicz, Department of Genomics, University of Wroclaw, Poland*
*„Biogram: a toolkit for n-gram analysis"*

***PP'7***

*Michał Startek, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Poland*
*„Reconstructing the chronology of transposable element activity from interruption matrix: a Bayesian approach"*

**13.00-14.00 – Przerwa obiadowa**

**14.00-15.40 – Komunikaty (K'13–K'16) (25')**

Prowadzący sesję – *prof. Andrzej Koliński*

***K'13***

*Joanna Ciomborowska, Adam Mickiewicz University, Poland*
*„Functional retrogens in human genome"*

***K'14***

*Julia Herman-Iżycka, Institute of Informatics, University of Warsaw,Poland*
*„Identification of regulatory sequences in mammalian genomes"*

***K'15***

*Jakub Rydzewski, Institute of Physics, N. Copernicus University, Toruń, Poland*
*„Memetic Algorithms for Ligand Expulsion from Buried Receptor Docking Site"*

***K'16***

*Anita Sokołowska, Wroclaw University of Technology, Department of Biomedical Engineering, Poland*
*„Cluster analysis of protein contact sites with regard to protein class and topology"*

**15.40-16.00 – Przerwa kawowa**

**16.00-17.30 – Komunikat (K'17) (25')**

**prezentacje posterów (PP'8-PP'11) (15')**

Prowadzący sesję – *dr hab. Witold Rudnicki*

***K'17***

*Jakub Bartoszewicz*, *Institute of Computing Science, Poznan,University of Technology, Poland,*
*Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poland,*
*Department of Logic and Cognitive Science, Institute of Psychology, Adam Mickiewicz University, Poland*
*„Amplifying bacterial memory controlled by a synthetic RNA thermometer and a nontoxic inducer"*

***PP'8***

*Jarosław Synak, Institute of Computing Science, Poznan University of Technology, Poland*
*„Application of chemical reaction simulation methods in order to verify RNA World hypothesis"*

***PP'9***

*Michał J. Dąbrowski,Institute of Computer Science, Polish Academy of Sciences, Poland*
*„Discovering inerdependencies among features in disease-related genomic data"*

***PP'10***

*Anna Tamulewicz, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Gliwice,Poland*
*Faculty of Biomedical Engineering, Silesian University of Technology, Gliwice, Poland*
*„Hot Spot identification in protein complex using S transform"*

***PP'11***

*Joanna Żyła, Data Mining Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland*
*„Searching for radiosensitivity biomarkers by Monte Carlo feature selection and rough sets approach"*

**19.00 Spotkanie towarzyskie (Hotel Victoria, 11. piętro)**

# Wykłady inauguracyjne (WI1, WI2)

**WI1**

# Chemical feature-based 3D pharmacophores: current and futures aspects

*Thierry Langer*

Department of Pharmaceutical Chemistry, University of Vienna
Althanstrasse 14, 1090 Vienna, Austria

Pharmacophore-based compound modelling, virtual screening, and bio-activity profiling has become one of the most popular in silico techniques for supporting medicinal chemists in their hit finding, hit expansion, hit to lead, and lead optimization programs. [1]

At Inte:Ligand GmbH, we developed the program LigandScout [2] as an integrated software solution containing rapid and efficient tools for automatic interpretation of ligand-protein interactions and subsequent transformation of this information into 3D chemical feature-based pharmacophore models. Additionally, pattern recognition-based algorithms were developed for ligand-based pharmacophore modelling in the absence of a target 3D structure, as well as for establishing novel accurate virtual screening procedures. Recently, also molecular dynamics simulation trajectories have been in the focus of research, in order to develop pharmacophore ensembles representing the dynamic event of binding. As an extension of this approach, parallel pharmacophore-based screening has been introduced as an innovative in silico method to predict the potential biological activities of compounds by screening them with a multitude of pharmacophore models, and made available as a LigandScout extension workflow node within the KNIME platform. [3]

In the presentation, Prof. Langer will give an overview of the pharmacophore technology developed over the last decade and will then present the results of several success stories: Examples range from proof of concept studies employing natural product compounds that were submitted to in silico activity profiling using a subset of the Inte:Ligand Pharmacophore Database [4] to in silico fragment-based discovery of novel enzyme inhibitors.

[1] Langer T. *Mol. Inf*. 29 (2010) 470.

[2] Wolber G., Langer T. *J. Chem. Inf. Model*. 45 (2005) 160.

[3] **K**o**N**stanz **I**nformation **M**in**E**r, available from KNIME.COM AG, Zurich, Switzerland (http://knime.org).

[4] The Inte:Ligand Pharmacophore Database is available from Inte:Ligand GmbH, Vienna, Austria (http://www.inteligand.com).

**WI2**

# RNA in the spotlight: 3D structure and interactions with small molecule ligands

*Janusz M. Bujnicki*

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, ul. Ks. Trojdena 4, PL-02-109 Warsaw, Poland
Faculty of Biology, Adam Mickiewicz University, ul. Umultowska 89, PL-61-614 Poznan, Poland

Computational methods play a pivotal role in the early stages of small molecule drug discovery and are widely applied in virtual screening, structure optimization and compound activity profiling. Over the past half century in medicinal chemistry, almost all the attention has been directed to protein ligand binding and computational tools were created with such targets in mind. However, with growing discoveries of functional RNAs and their possible applications, RNA macromolecules have gained considerable attention as possible drug targets. RNAs are involved in a variety of biological processes, in particular transmission of genetic information, protein synthesis and regulation of gene expression on various levels. Some RNA molecules catalyze chemical reactions, others sense and communicate responses to cellular signals. The function of many RNAs is modulated by small ligand molecules. These can be naturally occurring molecules as well as fully synthetic compounds. This flow of discovery of RNA-ligand interactions was followed by adapting existing protein-ligand computational docking tools for RNA applications as well as active development of new RNA-tailored methods. However, due to the different nature of RNA, especially its tendency to use morphological plasticity (conformational change in ligand binding) the study of RNA-ligand interactions remains a challenging task. The evolution of “protein-based” drug discovery and related computational methods offer some clues on possible future directions and developments in modelling RNA-small molecule ligand interactions.

# Wykłady (W1-W3)

**W1**

# Amphotericin B: An old, good antibiotic with very bad reputation

*Wieslaw I. Gruszecki*

Department of Biophysics, Institute of Physics, Maria Curie-Sklodowska University, Lublin, Poland

Systemic fungal infections are a serious problem of modern medicine owing to growing antibiotic resistance, pandemic of acquired immune deficiency syndrome and organ transplantations. Amphotericin B is one of the most effective antibiotics applied to treat deep seated fungal infections. It is a natural product and was isolated for the first time 60 years ago, from the filamentous bacterium *Streptomyces nodosus*. The drug is in use for several decades despite the fact that treatment of patients with application of amphotericin B is associated with several, severe toxic side effects, including hepatotoxicity and nephrotoxicity and may even lead to a patient death. Activity of numerous research centers around the world is focused on elaborating pharmacological formula of amphotericin B, retaining its antibiotic activity but with minimized toxic side effects. The problem is rather complex, owing to the fact that exact molecular mechanisms responsible for biological activity of the drug are still not fully understood. During our studies we have arrived to the concept that it is highly probable that toxicity of amphotericin B is associated with spontaneous self-organization of the drug, leading to formation of dimeric structures, in the water phase. Such dimeric structures can further self-associate into the tetrameric forms, in the lipid bilayer environment. Molecular modeling shows that such tetrameric structures may act as trans-membranous pores affecting physiological ion transport. These concepts will be presented and discussed during the talk.

**W2**

# Molecular analysis of a new mutant lutenising hormone beta subunit that results in hypogonadism

*Adolfo Rivero-Müller*[1,2]

[1]Institute of Biomedicine, Department of Physiology, University of Turku, Turku, Finland
[2]Department of Biochemistry and Molecular Biology, Medical University of Lublin, Poland

The lutenising hormone (LH) is a heterodimer glycoprotein formed by two subunits: LH beta (LHB) and the common glycoprotein alpha (CGA) subunit of all glycoproteins (hCG, FSH and TSH). Glycoprotein LH plays an essential role in the development and maturation of gonads in both sexes. Therefore, inactivation mutations of LH (LHB) are extremely rare, to-date only 5 of such mutations have ever been described.

Here we report a mutant LHB where the codon for lysine 20 (K20) of the mature peptide is absent (LHB-K20del). The resulting phenotype of the male patient was hypogonadic, characteristic of lack of LHB function and thus very low testosterone production, which could be rescued by hCG injections.

The LHB-K20del mutant is retained intracellularly, and even over-expression results in very small amounts of secreted peptide. Mutagenesis analyses show that K20 can be modified to alanine, arginine or aspargine without functional consequences to the LH heterodimer. Deletion of either glutamate residues flanking K20 also results in intracellular retention which, together with 3D modelling, shows that this region lays in a loop that is required for proper folding and further secretion, but surprisingly it seems not to affect LHB dimerisation with CGA and further activation of its receptor.

**W3**

# Eukaryotic ribosomal T-site as modulator of translational machinery

*Marek Tchórzewski*

Department of Molecular Biology, Maria Curie-Skłodowska University, Lublin, Poland

Protein synthesis is carried out by ribosomal particles, which are regarded as a conveying molecular machine, where tRNA is passing though the structure, delivering amino acids to the growing polypeptide chain. This unidirectional passing is 'catalyzed' by translational factors, called translational GTPases (tGTPases), which promote forward reaction at the expenses of GTP hydrolysis and the T-site represents the landing platform for them. The GTPase associated center (GAC) represents the main element within the T-site, composed of two constituents: conserved fragment of rRNA - called Sarcin-Ricin Loop (SRL) and also preserved protein elements consisting of uL11 and uL10; there are domain/species specific elements with bL12 proteins characteristic only for bacteria and P1 and P2 proteins specific for eukaryotic/archeal cells, forming distinct lateral protuberance, called stalk. The GAC is responsible for stimulation factor dependent GTP hydrolysis, regarded as a driving force of translation. Therefore, it is thought that in general the GAC together with tGTPases provide speed and accuracy for ribosomal action. Thus, the T-site with GAC, being the vital ribosomal element, appears to be attractive target for regulation of eukaryotic translational machinery at various steps, including ribosome biogenesis, translation *per se* and ribosomal quality control system, and exerting an effect on global cellular metabolism.

# Komunikaty (K1-K19) (K'9-K'17)

**K1**

# Web server tools for modeling of protein structure, its flexibility, aggregation properties and protein-peptide interactions

*Sebastian Kmiecik, Michal Jamroz, Maciej Blaszczyk, Mateusz Kurcinski, Agata Szczasiuk, Andrzej Kolinski*

Department of Chemistry, University of Warsaw, Poland

Recently, we developed a series of molecular modeling tools for structure-based studies of protein functions and interactions. The tools are publicly available as web servers that can be easily operated even by non-specialists: CABS-fold server for protein structure prediction [1]; CABS-flex server for modeling of protein structure flexibility [2]; Aggrescan3D server for prediction of protein aggregation propensities and rational design of protein solubility [3]; and CABS-dock server for prediction of peptide binding sites and peptide docking [4]. These web servers provide predicted 3D models together with accompanying analysis and make it all available for convenient online visualization. The web servers are freely available from the laboratory website: http://biocomp.chem.uw.edu.pl/tools

[1] Blaszczyk M., Jamroz M., Kmiecik S. et al. *Nucleic Acids Re*s. 41 (2013) 406.

[2] Jamroz M., Kolinski A., Kmiecik S. *Nucleic Acids Re*s. 41 (2014) 427.

[3] Zambrano R., Jamroz M., Szczasiuk A. et al. *Nucleic Acids Res.* 2015 doi: 10.1093/nar/gkv359.

[4] Kurcinski M., Jamroz M., Blaszczyk M. et al. *Nucleic Acids Res*. 2015 doi: 10.1093/nar/gkv456.

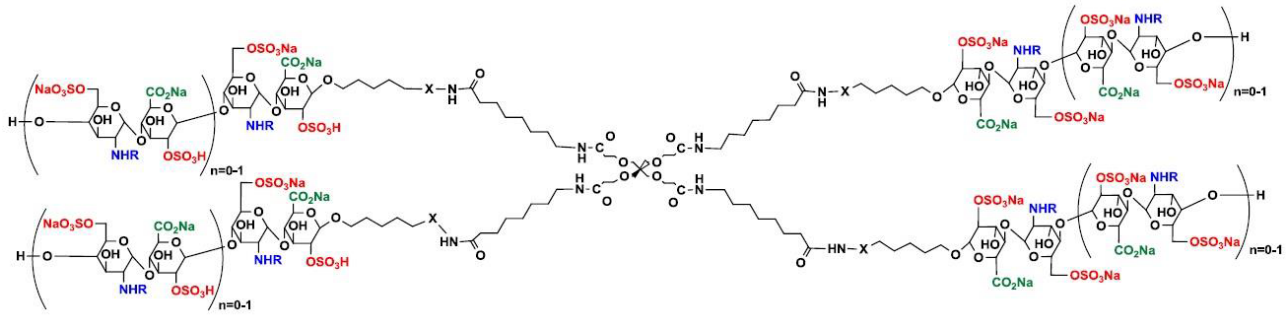
**K2**

# Heparan sulfate fragments and glycomimetic clusters for therapeutic applications

*Olga V. Zubkova, Peter C. Tyler, R. Schwörer, Jeremy E. Turnbull*

Victoria University of Wellington, The Ferrier Research Institute, New Zealand and Center for Glycobiology, Institute of Intergrative Biology, University of Liverpool, United Kingdom

Heparan Sulfate (HS), a highly sulfated glycosaminoglycan, plays a crucial role in a range of essential physiological processes. Functions of HS depend on ionic interactions between negatively charged sulfates and carboxylate groups with a variety of proteins such as cytokines, growth factors, lipases and proteases. HS oligosaccharides can mimic or interfere with HS functions in biological systems but exploitation has been hindered by the complexity of their synthesis. The complex synthesis of HS octa- to dodecasaccharides has been investigated by a number of groups, including ours (Chem. Eur. J., 2013), but despite many useful modifications and improved glycosylation protocols, multi-step syntheses of HS targets remain cumbersome and costly. Polyvalent displays of small specific HS structures on dendritic cores offer more accessible constructs with potential advantages as therapeutics, but the synthesis of single entity HS polyvalent compounds has not previously been achieved. We synthesized a novel targeted library of single entity glycomimetic clusters capped with varied HS saccharides (Angew. Chem Int. Ed., 2015).



They have the ability to mimic longer natural HS in their inhibition of the Alzheimer's disease protease BACE-1. We have identified several single entity HS clusters with low nM IC50 potency. None displayed any measurable ability to accelerate antithrombin-III mediated inactivation of Factor Xa and had no anticoagulant activity. Unlike heparin, such synthetic compounds would thus be expected to have no significant side effects related to anticoagulation. These have also been checked for ex vivo activity in a mouse brain slice assay which replicates many aspects of the in vivo context, crucially including bioavailability. Using an in vivo model, we further demonstrated the passage of C14-labeled clusters of HS through the blood-brain barrier. These novel HS clusters offer a novel framework for the manipulation of HS-protein interactions in general.

**K3**

# Bioinformatical studies of amyloid proteins and their contribution to diseases of civilization

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Many neurodegenerative diseases, regarded as diseases of civilization, result from protein fragments forming clusters, which are labeled amyloids. This occurs when a cell environment fosters the partial unfolding of protein chains or its fragmentation such that the parts prone to joining with other protein fragments are exposed. Initially, the resulting molecules form clusters consisting of a few elements, which are called oligomers. Next, these aggregates grow into protofibrils, whose structures are more regular. Eventually, long fibrils form. These consist of numerous protein fragments and are characterized by a highly regular structure resembling a zipper. The mature fibrils are no longer able to connect with other protein fragments. Such fibrils have been observed in the brains of people suffering from Alzheimer's disease, and they are also associated with Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease, as well as many other diseases, even non-neurodegenerative diseases such as type 2 diabetes and cataracts. Cells in tissues containing these fibrils exhibit extremely high mortality. Initially, researchers believed that the fibrils were responsible for cell death. It turned out, however, that the culprits are immature forms of such aggregates i.e. the oligomers. This is due to the fact that the higher the susceptibility of the cluster to connect with other molecules, the higher its toxicity for cells, while mature fibrils are not toxic for cells. Unfortunately, the reasons for this phenomenon are not fully understood.

Aggregation of proteins and peptides can be influenced by various factors, such as protein high concentration, high temperature, low pH, binding metals. It does not necessarily lead to amyloid formation. Peptide aggregation can be a reversible process, unless it concerns sequences that are amyloidogenic. Amyloid beta-sheet aggregate has a specific and highly ordered structure, resembling a zipper (called steric zipper), which distinguishes them from other protein aggregates of the beta-sheet type. Due to this structure an amyloid is very durable and resistant to activity of proteolytic enzymes and cannot be dissolved.

There are several hypotheses providing feasible mechanisms for development of fully symptomatic neurodegenerative diseases. Currently, it is believed that short peptide sequences of amyloidogenic properties (called hot-spots) can be responsible for aggregation of amyloid proteins. These 4-10 residue long fragments (typically hexapeptides), have a high propensity for strong interactions that lead to aggregation of the protein. Previous studies suggested that amyloidogenic fragments may have a regular characteristic, not only with regard to averaged physicochemical properties of their amino acids, but also the order of amino acids in the sequence. There have been attempts to predict the sequence of such peptides by computational modeling. Recent studies indicate that the neurodegenerative processes may also correspond to incorporation of amyloid oligomers into the cell and organelle membranes, creating weakly cation-selective ion channels that allow uncontrolled influx of calcium into nerve cells. The excessive influx of calcium into the cytoplasm leads to disruption of intracellular pathways, membrane depolarization, ATP depletion, and mitochondrial membrane depolarization with impairment of mitochondrial function.

In the talk we will present the computational methods that recognize peptides which are potentially amyloidogenic, as well as available databases devoted to amyloids. Research into modeling hypothetical structures of the amyloid pores will also be discussed along with other hypotheses regarding mechanisms of the diseases.

**K4**

# 3D-NOME: an integrated 3 Dimensional NucleOme Modeling Engine for data-driven simulation of spatial genome organization

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+Equal contribution

Spatial arrangement of chromosomal folding is an important feature of genome organization inside cell nucleus, and genomic topology is thought to have critical roles to genome functions such as transcription regulation. By rendering linearly distant genomic regions to close spatial proximity through mapping of long range chromatin interactions using 3C-based technologies, some basic principles of spatial genome organization are starting to emerge. Among the available 3D genome mapping technologies, ChIA-PET is unique for its ability to generate multiple datasets simultaneously in one experiment including binding sites and enriched chromatin interactions mediated by specific protein factors of interest as well as non-enriched interaction data that reflect topological neighborhood of higher-order chromatin association. This multiplicity nature of ChIA-PET data represents an important advantage in capturing multiple layers of information from detailed chromatin interactions to higher order topology. Meanwhile, it also imposes a new challenge and opportunity for multi-scale modeling of 3D genome organization. Here we report the development of an integrated software platform, 3D-NOME (***3 D**imensional **N**ucle**O**me **M**odeling **E**ngine*) for processing ChIA-PET data. 3D-NOME includes three components: 1) hierarchical data de-noising, 2) top-down multidimensional scaling and refinement for rapid structure inference, and 3) web-based visualization tools. Using ChIA-PET data derived from a human B-lymphocyte cell line (GM12878), we demonstrate that 3D-NOME can effectively build the 3D models of the human genome at multiple levels, entire nucleome, individual chromosomes, and specific segments in various megabase and kilobase resolutions. Furthermore, the web-based visualization tools allow robust presentation of the simulated models for visual examination at global scale and segmental details. Further refinement of 3D-NOME and application to additional ChIA-PET and other types of 3D genome mapping data will help to advance our understanding of the human genome structure and functions.

**K5**

# Allosteric modulation of GABAB receptor

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Overstimulation of GABAB receptor system could worsen the symptoms of depression [1]. Compound that binds to GABAB receptor and reduce the overstimulation can be a therapeutic strategy of depression. The functional GABAB receptor is a dimer that consists of subunit 1a and 2b. The natural substrate GABA binds to the extracellular part of subunit 1a (orthosteric site), while known allosteric modulators bind into the transmembrane helical bundle of subunit 2b (allosteric site). Based on a homology model of 2b subunit, several potential allosteric hits are identified and will be tested on CHO-K1 cell line that stably overexpressing GABAB receptor. The effects of allosteric binding compounds can be any combination of positive, neutral or negative modulation of natural substrate GABA's affinity and efficacy. In our case, the goal of ongoing experiment is to find negative allosteric modulators as potential antidepressant drugs.

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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).

**K6**

# Towards a better understanding of neurodegenerative diseases - beta-amyloid and Alzheimer's disease

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Alzheimer's disease is a progressive neurodegenerative disorder that leads not only to memory decline but also to speech and abstract thinking impairment as well [1]. Developing slowly, it causes problems with the performance of even the most basic activities and leads to death. In the brain of affected patients the presence of the senile plaques, composed of β-amyloid peptide fragments, can be detected. These senile plaques are responsible, inter alia, for the death of neurons and intercellular signalling disturbances, which consequently lead to the symptoms of the disease. The multifactorial nature of Alzheimer's disease requires administration of comprehensively acting drugs in a potential therapy.

Due to the availability of high-performance computers and molecular dynamics software it became possible to perform simulations that reflect the processes occurring in the brain of patients with Alzheimer's disease as well as to find novel substances that may stop the adverse effects. β-amyloid is produced from the precursor protein APP under the influence of two enzymes, known as β-secretase and γ-secretase, and consequently large units called the senile plaques are being formed [2]. Computer simulations allowed us a better understanding of the transition process of individual β-amyloid fragments into larger units as well as the systematic identification of those structural elements involved in the initiation of that process and stabilization of the formed deposits [3]. Further analyses enabled to understand how the medicines could inhibit that process and to design new, more potent substances. The compounds designed in this way were the subject of further laboratory work. Some of them have been obtained by chemical synthesis, and the first biological tests confirmed their inhibitory activity in the aggregation process.

Moreover, a homology model of presenilin 1, which is a component of γ-secretase enzyme was built and mutations of this protein that lead to a higher incidence of early-onset Alzheimer's disease were studied. This type of analysis allowed to understand how the selected mutations influence the development of Alzheimer's disease. A better understanding of the above-mentioned processes may contribute to the effective therapy of this disease.

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The studies were financially supported by National Science Centre in Poland, postdoctoral research grant no. DEC-2012/04/S/NZ2/00116.

**K7**

# Inferring bioinformatics models using crowdsourcing game

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First formulation of the crowdsourcing problem, understood as outsourcing work to a large network of people in the form of an open call, comes from the 18th century [1]. Since that time the concept of crowdsourcing have been utilized many times, however, the rapid development of this technique started with the development of the Internet in 1990s. The best examples of its capabilities are services like Wikipedia or OpenStreetMap. Another interesting application of crowdsourcing concept is an implementation of computer games which objective is to solve a scientific problem by employing users to play the game, so called Crowdsourced Serious Game [2]. Such approach has already helped to discover several interesting biological phenomena using games such as Foldit [3], EteRNA [4] or EyeWire [5].

The main objective of the presented study was to verify if the crowdsourcing approach can be successfully applied for finding mathematical equations that explains data gathered from the biological experiments. Moreover, we wanted to compare it with the approach based on artificial intelligence that uses symbolic regression to find such formulas automatically [6]. To achieve this we designed and implemented the game in which players tries to design a spaceship representing an equation that models the observed system. The game was designed keeping in mind that it should be easy to use for people without strong mathematical background and it was integrated with Facebook to easily reach plenty of players. Moreover, we tried to make use of the collective intelligence observed in crowdsourced systems [7] by making it possible to collaborate on a single solution by many players.

The game was tested by playing almost 10000 games by several hundred players and by conducting users opinion survey. The objective of the test instance was to find the equations modelling HCV infection observed during one of the clinical studies described by Dahari [8]. Results prove that the proposed solution has very high potential. The function generated during week long tests was almost as precise as the analytic solution of the system of differential equations and it explained data better than the solution generated automatically by the Eureqa – the leading software implementing symbolic regression [9]. Moreover, we observed benefits from the use of the crowdsourcing technique – the chain of consecutive solutions that leaded to the best solution was obtained by continues collaboration of several players.

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**K8**

# Allosteric modulation of the human $GABA_B$ receptor

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γ-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.

**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).
Thibaud Freyd is a fellow of the National graduate school in structural biology (BioStruct).

**K9**

# Combination of PDT therapy with iron chelators

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We present here the results concerning the combination of two different anticancer therapies. Namely the exploration of influence of the thiosemicarbazones on photodynamic reaction triggered by photosensitizer. Iron chelators are a new class of compounds suggested for anticancer therapy. Their mechanism of action is multitargeted and consist of inhibition of activity of rybonucleotide reductase, generation of reactive oxygen species and deprivation of iron form cytosol[1]. Iron is a common factor participates in a variety of important processes such DNA synthesis, electron transport, oxygen delivery and erythropoiesis. A consequence of binding describing microelement is inhibition of the cell cycle and arrest cells at the G1/S interface[2]. In addition, depletion of iron affects the regulation of important genes such as BNIP3 and NDRG1, which are crucial for triggering apoptosis of cancer cells. This multi-targeted mechanism of action makes this type of compounds an especially attractive material for the study of possible application in the anticancer therapy.
In the last few years we combined some active chelators with ALA-PDT therapy and we observed strong synergistic effect[3]. In this communication combination with chlorins – porphyrine like compounds is presented. Reaction of the photosensitizer with oxygen and the light of specific wavelength led to the production of singlet oxygen and free radicals. These trigger chain reactions in the cell, causing various types of damage and finally the destruction of the tumour. It is worth noting that specific structure of the irradiated compound and also construction of the tumour tissue together contribute to the selectivity of the photodynamic therapy. This method of treatment is one of the least invasive and the safest therapies. In our approach the drugs incteractions were calculated as combination indexes according to Chou-Talay method. To elucidated the plausible mechanism of action we measured the level of the reactive oxygen species and lipids peroxidation after joined therapy.

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**Acknowledgements**
The financial support of the NCN grant 2014/13/D/NZ7/00322 is greatly appreciated.

**K10**

# New synthesis and in vitro antiproliferative activity of azaphenothiazine derivatives

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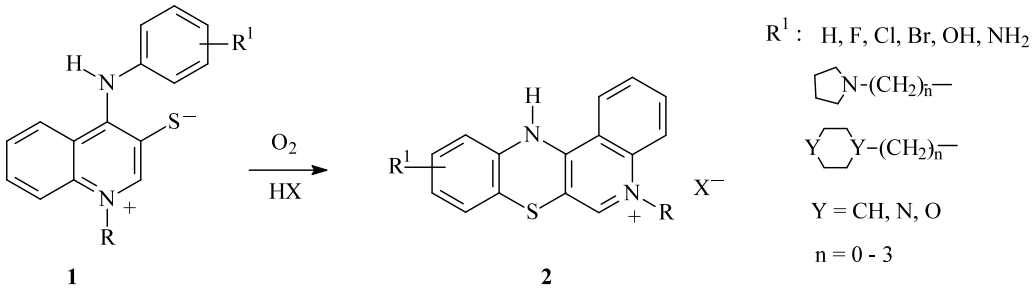
A new method of 1,4-thiazine system synthesis was developed consisting of hydrogen atom substitution in the phenyl ring by thiolate sulphur atom. The mechanism of cyclization reaction was studied. Using the new method several novel tetracyclic phenothiazine derivatives were synthesized that contain a quinoline moiety [1-3]. The structure of particular compounds was modified by introducing several different substituents or functional groups into the quinobenzothiazine system. The new compounds structure was analyzed by $^{1}H$ and $^{13}C$ spectroscopy as well as $^{15}N$ NMR and X-ray.




**1** **2**

Antiproliferative activity mechanism of the compounds (2) was assessed *in vitro* using four cancer cell lines (Hct116, SNB-119, MDA-MA-231 and LLC) and doxorubicin as a reference. Most of the studied phenothiazine derivatives showed activity against all cell lines investigated (0.5 - 19.6 mg/mL concentration range). A structure-activity relationship was established. The mechanism of antiproliferative activity has been analyzed.

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**Acknowledgements**
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**K11**

# *New "Click chemistry"* solutions: water-soluble ligands for the Huisgen cycloaddition

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The copper-catalyzed azide-alkyne cycloaddition (CuAAC) is the World's most known *Click chemistry* reaction[1], which has found a myriad of applications in modern pharmaceutical sciences and chemical biology[2,3]. However, for a number of cases, this reaction is inefficient in its original form[4,5]. In 2004, Sharpless presented a solution to this problem: the application of tris-triazolyl amine ligands such as TBTA, which improve the solubility of copper(I) species and stabilize the +I oxidation state required for CuAAC[6]. The application of TBTA allowed to significantly reduce the amounts of copper needed for efficient cycloaddition and thus it quickly found its place as a basic *Click chemistry* reagent. However, high lipophilicity of TBTA made it difficult to separate from reaction the reaction product, using non-chromatographic methods.

The problem of ligand separation and a growing number of applications requiring aqueous environment (such as bioconjugation), gave rise to the need for water-soluble CuAAC ligands. The most notable ligand so far is THPTA, another member of the tris-triazolyl amine family developed by the Sharpless group[6,7]. At present it is considered a "gold standard" for ligand-assisted CuAAC in aqueous solutions and for bioconjugation purposes.

Within this work we present a novel water-soluble ligand, AMTC, which is highly efficient for the copper-catalyzed cycloaddition of aliphatic azides and alkynes. For a broader view, a comparison with TBTA and THPTA will be presented, together with general guidelines on catalytic ligand choice.

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**K12**

# Study of adsorption kinetic of selected pharmaceuticals on activated carbon

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Current data say about the growth of consumption and production of medicines and pharmaceuticals. Increasing demand for medicines available without prescription, and prescribed by a doctor. The most commonly used pharmaceuticals are analgesics and anti-inflammatory heart medicines, medicines for colds and flu, vitamins and mineral supplements. In the era of intensive development of the pharmaceutical industry we observed significant quantities of pharmaceutical preparations in water and industrial wastewater and municipal. This in turn created the need to develop technologies to effectively remove or reduce the concentration of drugs in water and wastewater. A highly effective method of purification of pharmaceutical fluids is adsorption process using activated carbon as an adsorbent. The course of the adsorption process is limited by several factors relating to the characteristics of the adsorbent and adsorbate, and the environment of operation. This process takes place in several stages. Each step of the various substances takes place in different times. The kinetics of this multistage process will be determined by the slowest phase [1-2].

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**K13**

# Phosphonylated amonafide conjugates

*Dorota G. Piotrowska*[1], *Andrzej E. Wróblewski*[1], *Iwona E. Głowacka*[1]

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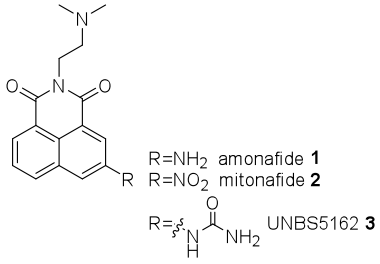
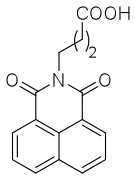
Since the discovery of intercalating properties of amonafide **1** [1,2] naphthalimide derivatives are of special interest. Amonafide **1** as well as some of its derivatives, including mitonafide **2** [3] and UNBS5162 **3** [4], reached clinical trials but showed poor therapeutic indices linked to their metabolisms. On the other hand, substituted naphthalimides **4** and **5** have been designed and tested as inhibitors of RNA viruses [5].



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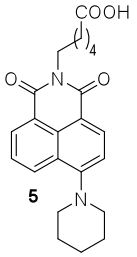
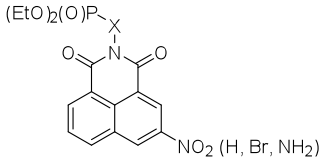
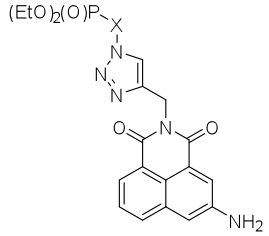


**5**

As a continuation of our ongoing project concerning the synthesis of various naphthalimide-conjugates we designed new series of compounds of general formulae **6** and **7** and their antiviral and anticancer properties were assayed.




**6**




**7**

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**K14**

# Synthesis and structure-activity relationship analysis of 5-HT$_7$ receptor antagonists: piperazin-1-yl substituted unfused heterobiaryls

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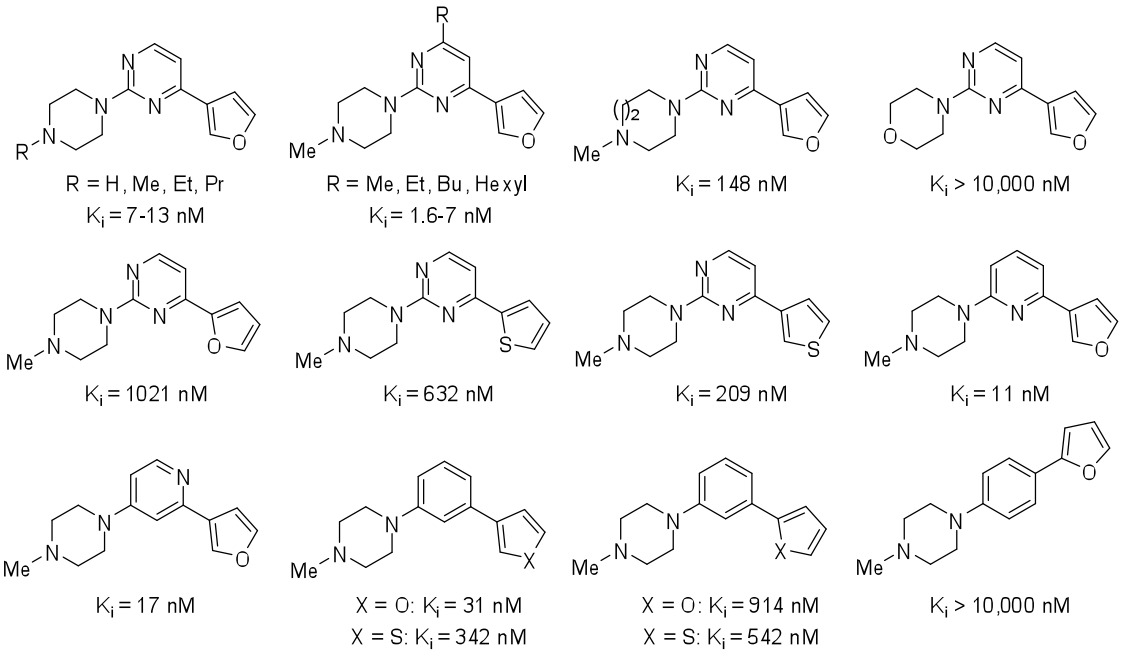
Serotonin (5-hydroxytryptamine or 5-HT) is involved in cognitive and behavioral functions. Activation of the 5-HT$_7$ receptor plays a role in smooth muscle relaxation, thermoregulation, circadian rhythm, learning, memory, and sleep. On the other hand, the 5-HT$_7$ antagonism has been linked to diverse antidepressant-like behavioral effects [1, 2].

Many amino-substituted heterobiaryls are CNS antagonists [2, 3]. More than 1000 such compounds were synthesized and assayed for binding to different 5-HT receptors in our laboratories. The binding results of a variety of heterobiaryl antagonists with the 5-HT$_7$ receptor, expressed by inhibition constants ($K_i$), are discussed in this presentation. The representative molecules are shown below for illustration. These agents were selected to show how a small alteration of the structure has a profound effect on the binding to the 5-HT$_7$ receptor. For example, 4-(furan-3-yl)pyrimidines are much more active than their furan-2-yl analogs.

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**K15**

# Kinetic resolution of (*R,S*)-ibuprofen with the application of lipase from *Candida rugosa* in free and immobilized form

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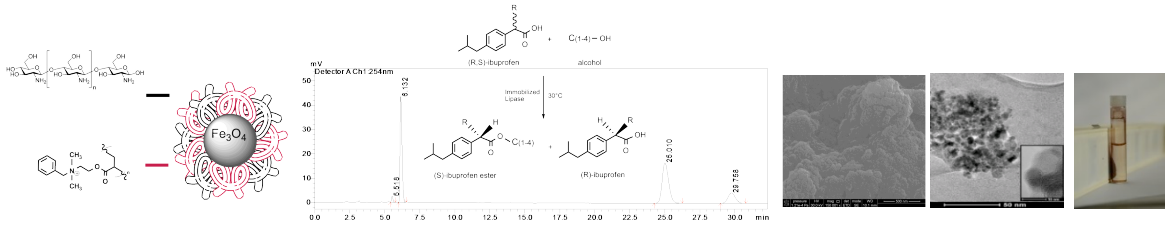
The development of new strategies for synthesis of enantiomerically pure compounds is still an open challenge in the chemical and pharmaceutical industry. The biotechnology is an alternative approach offering more environmentally and economically attractive way to obtain bioactive and valuable compounds [1]. Our study is focused on the kinetic resolution of (*R*,*S*)-ibuprofen with the use of free and immobilized form of lipases from *Candida rugosa* and the evaluation of their catalytic activity.




a) b) c) d) e)

Fig. Characterization and application of superparamagnetic nanoparticles: a) nanoparticles with chitosan and amphiphilic polymer; b) kinetic resolution of (*R*,*S*)-ibuprofen and HPLC chromatogram; c) SEM image of nanoparticles; d) TEM image of nanoparticles; e) nanoparticles with immobilized lipase attracted by magnet;

Based on the preliminary data of the activity of *Candida rugosa* lipase, the enzymes with the best parameters were selected and immobilized onto magnetic particles. After optimizing the immobilization procedures of biocatalysts onto magnetic material, they were used in the enantioselective esterification of (*R*,*S*)-ibuprofen and allowed to obtain high enantioselectivity of kinetic resolution (E=50.6, ee = 93.5%). Immobilization of the lipases was carried out on commercial carriers - magnetic beads and carriers synthesized with the use of chitosan and amphiphilic polymer. Study of catalytic activity of the formed enzymatic systems (immobilized lipase onto magnetic particles) confirmed the possibility of easy separation from the reaction media and the ability to reuse them and to apply in subsequent reaction cycles. Received data demonstrated high lipolytic activity, as well as operational stability of the immobilized lipase from *Candida rugosa* and also activity recovery (higher than 80%) [1-3].

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**K16**

# Participation of pre- and postsynaptic of 5-$HT_{1A}$ receptors in mood regulation (modulation of depressive like behaviours)

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Serotonin (5-HT) is a monoamine neurotransmitter that plays an important role in physiological functions as sleep, feeding, sexual bahaviour, temperature regulation, pain, and cognition as well as in pathological states including mood and anxiety disorders, psychosis and pain. The seven 5-HT receptor classes consist of 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_5$, 5-$HT_6$, 5-$HT_7$, which are further subdivided into 14 receptor subfamilies. All of these receptors - except for 5-$HT_3$ receptor which belongs to the family of ionic channels - belong to superfamily of seven-transmembrane-domain, G protein-coupled receptors (GPCRs). For serotonin GPCRs three main types of primary coupling to G proteins have been described. The 5-$HT_{1A}$ receptors activates $G_i/G_o$ proteins, the 5-$HT_{2A}$ receptors activate $G_q/G_{11}$, and the 5-$HT_4$, 5-$HT_6$ and 5-$HT_7$ activate $G_s$. The 5-$HT_{1A}$ receptor is found in presynaptic as well as in postsynaptic part of the serotonergic tract. Presynaptically, the receptor is the major somatodendritic autoreceptor on the soma and dendrites of serotonergic neurons where it acts as a "brake" to inhibit the activity of the entire 5-HT system and is thought to delay antidepressant response. The 5-$HT_{1A}$ heteroreceptors are located on non-serotonergic neurons, primarily in the limbic areas, such as on the dendrites and soma of glutamatergic pyramidal neurons, and axon terminals of GABAergic and cholinergic neurons. It is supposed that autoreceptors impact the establishment of anxiety-like behavior and heteroreceptors affect behavior in the forced swim test, a depression-related test. Selective inactivation of presynaptic receptors results in antidepressant-like effects in rodents. Increased transcription of 5-$HT_{1A}$ autoreceptor associates with depression and resistance to chronic SSRI treatment. Increases postsynaptic signaling (SSRI, TCA, Li, valproate, electroconvultion). Chronic treatment with SSRI desentizises presynaptic receptors. However, according to clinical data full 5-$HT_{1A}$ blockade neither enhances nor cancels the antidepressant effect of fluoxetine in MDD patients. Suggesting the involvement of other 5-HT receptors (e.g., 5-$HT_4$ receptor).

In the present paper the influence of the 5-$HT_{1A}$ receptor ligands on the biochemical pathways and the implication on depressive like behaviour wil be discussed.

**Acknowledgements**
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**K17**

# *In vitro* evaluation of metabolic stability of bicyclic imidazole-4-one derivative – the potent and selective antagonist for the orphan G protein-coupled receptor GPR18

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The orphan G protein-coupled receptors GPR18 and GPR55 have been recently reported as potential candidates for another cannabinoid CB receptors. GPR18 is highly expressed in the spleen, the thymus, leukocytes, testes, and the endometrium [1]. In addition, the receptor was shown to be highly expressed in several cancer cell lines, such as: glioblastoma, astrocytoma, breast cancer, prostate, and ovarian carcinomas. Therefore GPR18 ligands may be useful novel therapeutics for the treatment of several types of cancer [2-3].

In our recent study we presented compound CB-5, (Z)-2-(3-(4-chlorobenzyloxy)benzylidene)-6,7-dihydro-2H-imidazo[2,1b][1,3]-thiazin-3(5H)-one, identified as the potent and selective GPR18 antagonist with calculated by β-arrestin recruitment assay $IC_{50}$ value = 0.279mM, >36-fold selective vs.$CB_1$ and GPR55,14-fold selective vs.$CB_2$ [4]. In the present study we examined CB-5 metabolic stability using *in vitro* and *in silico* methods. For prediction the routes of the metabolic biotransformation as well as the most probably structures of metabolites we used The MetaSite 4 computational method. The metabolic stability was examined *in vitro* by human liver microsomes (HLMs) and rat liver microsomes (RLMs). The *in silico* results, LC-MS spectra and the precise ion fragments analysis produced by obtained *in vitro* metabolites allowed to determine their most probably structures. For prediction the potential further drug-drug interactions we used the luminescent CYP3A4 P450-Glo™Assay which allowed to evaluate the influence of CB-5 on cytochrome CYP3A4 activity.

The LC-MS analysis of the reaction mixture after 2h CB-5 incubation with HLMs at 37ºC showed the presence of the metabolites with masses 401,01m/z and 417,03 m/z, whereas after incubation with RLMs under the same conditions similar metabolites: 401,01m/z, 417,03 m/z and additionally metabolite 403,07 m/z. Additionally, in compare to the strong CYP3A4 inhibitor ketoconazole ($IC_{50}$ = 0,14 μM), CB-5 may be considered as a moderate inhibitor with calculate $IC_{50}$ = 3.57 μM.

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**K18**

# Carbon nanotubes as drug delivery systems. Insights from molecular simulations

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Carbon nanotubes (CNT) are widely studied as drug delivery systems due to their unique physical and chemical properties. Fabrication of biologically active CNT-drug conjugates utilizes two general strategies: covalent attachment of functional moieties or non-covalent physical adsorption. Chemical attachment of drugs takes normally place at the CNTs sidewalls and often such composite systems reveal better pharmacological activity than drugs alone. Physically adsorbed drug molecules can be located either on the sidewalls or in the inner cavity of the nanotubes. The latter approach allows for perfect isolation of drugs from the environment during the transportation stage or storage phase. Whatever architecture of a drug delivery system it must always realize the drug release at the target site. Many physical, chemical and biochemical triggering factors are considered for that purpose. Among them a particularly interesting seems to be the pH change from neutral to acidic one occurring naturally in tumour tissue or inflammation site or application of safe physical factors like exposition to an external magnetic field.

Selected systems involving the above mentioned triggering factors will be discussed according to results available from molecular dynamics simulations. That methodology provides insights into molecular mechanism of structural transformations occurring during the drug release. Therefore, results obtained from computer simulations might be very useful in designing of smart drug delivery systems without involving time consuming and probably expensive experimental methods.

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**K19**

# Mechanism of the water-assisted ring-opening reaction in hexopyranoses

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The ring-opening reaction occurring in hexopyranoses is an important focus for carbohydrate chemistry and biochemistry for many years. However, some basis aspects of its mechanism remain enigmatic. We focus on the ring-opening reactions of a- and b-glucose molecules (treated as model systems) catalyzed by water. We apply the computational methods based on the combination of the transition path sampling (TPS) approach with the molecular dynamics (MD) protocols employing the DFT potentials. Such combination allows to study the unbiased MD trajectories representing the favourable reaction paths of the process of interest.

The TPS simulations of the ring-opening, water-catalyzed reaction of a and b glucose anomers allowed to elucidate the molecular details of the process. This includes: (i) description of the catalytic role of water; (ii) identifying the particularly crucial steps of the process; (iii) analysis of the conformational preferences of the aldehyde residue in the aldehyde form of glucose and its influence on the product of the ring-closing reaction. The results indicate that the most probable mechanism of the ring-opening reaction involves the contribution of only one water molecule. In the initial steps of the reaction the water molecule deprotonates the O1 atom acting as a Brønsted base. Then, after series of proton transfers, the O5 ring oxygen atom becomes protonated. This protonation is identified as the 'bottleneck' of the whole process which triggers the cleavage of the C5-O1 bond. According to the additional series of simulations, the orientational preferences of the aldehyde moiety determine the product of the ring-closing reaction.

The presented computational methodology is of a general nature and can be applied to other molecular system of biological importance in which the ring-opening reaction plays a significant role.

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**K'9**

# Comparison of differential expression and coexpression across multiple tissues (skin, fat and LCL) in twins.

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While most studies on gene expression focus on single tissues and analyse data gathered from different non-related subjects, complexity of tissue-specificity still remains elusive. Here we have obtained microarray expression data from healthy female twins (TwinsUK cohort). The subjects originate from the MuTHER resource which contains information on well-phenotyped individuals. The samples were gathered from three tissues: lymphoblastoid cell lines (LCL), skin, and fat (571, 478 and 569 samples respectively). It is the first skin dataset not only of this size but the first ever available.

In these three datasets we have analysed differential expression finding sets of differentially expressed genes across tissues. Furthermore, we have performed differential coexpression analysis on each of the datasets. This analysis enabled us to distinguish clusters of co-regulated genes. Each such module has been correlated with extensive phenotypic traits showing significant associations. Within the modules functional gene clusters and groups of genes from the same families (eg. keratins) have been found.

**K'10**

# Protein Contacts Ontology – a tool for annotation of protein residua- residue contacts

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The accuracy of protein structure prediction methods ca be enhanced by inclusion of the information regarding residue-residue contacts, that is, pairs of amino acid residues that are in close proximity in three dimensional space [1-2]. Unfortunately, accuracy and reliability of state-of-the-art residue-residue contact predictors are low [3], the major problem being the high rate of false positive predictions [4]. This low performance may be caused by the fact that most methods treat all contacts as equal, based on a simple geometrical definition, despite that only some of the contacts are real interactions which truly impact the protein structure. Moreover, the physicochemical phenomena that keep residues together may also be different. Taking into account these facts may be crucial in order to improve prediction accuracy.

Residue-residue contact prediction methods benefit greatly from available structural data – there are over 100 000 experimentally solved protein structures deposited in the Protein Data Bank [5], each containing information on approximately 300-500 residue-residue contacts. Currently, there are no tools that would allow for detailed, high-throughput annotation of available structural data. In this work we present the Protein Contacts Ontology (PCO). The ontology provides a standardized vocabulary that allows to formally describe protein residue-residue contacts and their environments.

At the most general level the ontology has three distinct classes: i)'contact_attribute', ii)'residue_attribute', iii)'entity'. The part of the ontology related to 'contact_attribute' defines attributes/properties that can be used to describe protein contact sites e.g. the type of observed physico-chemical interaction. The part related to 'residue_attribute' includes terms that allow to describe amino acid residues. Finally, the terms grouped under 'entity' are used to model objects such as protein structural regions, amino acid residues or contact sites. Following the guidelines provided by OBO consortium (Open Biomedical Ontologies Consortium) [6], fragments of other ontologies were reused, where possible.

The presented PCO ontology allows precise annotation of available structural data with the focus on inter-residue contacts. Based on that, a detailed classification of inter-residue contacts can be performed. This will allow to decompose the residue-residue contact prediction problem into a set of simpler problems of predicting certain types of contacts. We hypothesize that this will improve the accuracy of predictions performed by machine learning methods.

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**K'11**

# Amino acid properties conserved in molecular evolution

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**Motivation:**

That amino acid properties are responsible for the way protein molecules evolve is natural and is also reasonably well supported both by the structure of the genetic code and, to a large extent, by the experimental measures of the amino acid similarity. Nevertheless, there remains a significant gap between observed similarity matrices and their reconstructions from amino acid properties.

**Methods:**

Therefore, we introduce a simple theoretical model of amino acid similarity matrices, which allows splitting the matrix into two parts – one that depends only on mutabilities of amino acids and another that depends on pairwise similarities between them. Then the new synthetic amino acid properties are derived from the pairwise similarities and used to reconstruct similarity matrices covering a wide range of information entropies.

**Results:**

Our model allows us to explain up to 94% of the variability in the BLOSUM family of the amino acids similarity matrices in terms of amino acid properties. The new properties derived from amino acid similarity matrices correlate highly with properties known to be important for molecular evolution such as hydrophobicity, size, shape and charge of amino acids.

This result closes the gap in our understanding of the influence of amino acids on evolution at the molecular level. The methods were applied to the single family of similarity matrices used often in general sequence homology searches, but it is general and can be used also for more specific matrices. The new synthetic properties can be used in analyzes of protein sequences in various biological applications.

**K'12**

# Biogram: a toolkit for n-gram analysis

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N-grams (k-mers) are vectors of *n* characters derived from input sequences. Originally developed for natural language processing, they are also widely used in genomics [1], transcriptomics [2] and proteomics [3].

Despite the continuous interest in the sequence analysis, there are only a few tools tailored for comparative n-gram studies. Moreover, they often do not contain efficient feature-filtering methods, which severely hampers the application of these methods.

To facilitate comprehensive analysis of n-grams, we created **biogram** software. Aside from essential functionalities, like efficient data storage, we also implemented a feature selection method. QuiPT (**Qui**ck **P**ermutation **T**est) uses several filtering criteria such as information gained to choose significant features. To speed up the computation and allow precise estimation of small p-values, QuiPT performs an exact test instead of a large number of permutations.

N-grams may be used only as a data encoding method. In this case, they can be freely combined with any machine learning technique. As a proof of concept, we prepared a simple predictor of amyloids, short proteins associated with the number of clinical disorders, for example Alzheimer's or Creutzfeldt-Jakob's diseases. Due to the presence of characteristic short sequences of amino acids, called hot-spots, amyloids can create harmful zipper-like β-structures. In this case, we expect that n-gram analysis, as an addition to commonly acclaimed methods as FISH Amyloid [4], will shed more light on the putative motifs of hot spots. The n-gram model of the amyloidogenicity, trained on the data from AmyLoad database, is validated through simple yet accurate amyloid prediction framework using random forests. The mean AUC in 5-fold cross-validation was 0.84.

Moreover, n-grams may be also used as an extension of a complex stochastic model. Here we implemented n-grams as hidden states in a hidden semi-Markov model of signal peptides called signalHsmm. These N-terminal targeting signals are responsible for targeting of proteins to endomembrane system and their export outside the cell. After reaching the destination, signal peptides are cut off by the signal peptides. In our model, the cleavage site is modelled by a set of n-grams reflecting variability of this region. This approach not only yields the largest AUC value (AUC = 0.98) in comparison to other software, but also allow more precise prediction of the exact cleavage site. Another advantage of the combined HSMM – n-gram approach is its flexibility. Our model recognises malaria parasites *Plasmodium* and their relatives with AUC = 0.92, i.e. more accurately than popular programs (AUC = 0.84).

**biogram** is a R package available on CRAN (http://cran.r-project.org/web/packages/biogram/).

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**K'13**

# Functional retrogenes in the human genome

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Retrogenes are copies of genes originated from reverse transcription of mRNA and incorporation of cDNA into a genomic sequence. This process is called retroposition and it results in a formation of a single-exon copy from a multi-exon parental gene. The main goal of my PhD project was the analysis of retroposition in the human genome with a special focus on accompanying evolutionary phenomena such as replacement of parental genes by retrogenes and intron gain. All performed research led not only to the discovery of abovementioned processes but also to our better understanding of the human genome, which despite the fact of finished sequencing and great number of analyses still hides many secrets.

Our bioinformatics and experimental analyses of retrogenes allowed identification of novel, species-specific introns and showed that intron formation process is active also in mammals. As a result of studies of genes *RNF113* and *DCAF12* retroposition, we identified in the human genome two single-exon retrogenes (*RNF113A, DCAF12L1*) as well as retrocopies with introns (*RNF113B* and *DCAF12L2*). Structural verification of obtained candidates was performed through the analysis of EST sequences. As an outcome we identified one case of "intronization" in primate-specific retrogene *RNF113B* and two independent "intronization" events in the retrogene *DCAF12L2* – one took place in the common ancestor of primates and rodents and another one in the rodent lineage. Additionally, as a first group in the world, we found and experimentally confirmed retrogenes with splicing variants.

Retrosequences for a long time have been considered as "genomic junk" mainly because they were regarded as inactive and disappearing over the time genes (pseudogenes) Only recently it has been showed that some retrosequences may remain in the genome and play a crucial role by giving birth to new genes or regulatory RNAs. Aspects of pseudogenization together with neo- and sub-functionalization were dominating in all previous analyses and so far nobody took into consideration a situation in which not the retrogene but the parental gene is pseudogenized. Theoretically we cannot exclude such scenario and therefore we decided to take on this challenging task, focusing on identifying in the human genome cases where retrogene overtook the function of its parent. Utilizing innovative approach and a number of bioinformatics tools we performed comparative analyses of human, chicken and nematode genomes, which led us to identification of 25 "orphan" retrogenes in the human genome. These results clearly showed, for the first time, that retrogenes not only can be pseudogenized, neo- or subfuncionalized, but also are able to replace their progenitors.

**K'14**

# Identification of regulatory sequences in mammalian genomes

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Identifying tissue specific enhancers has been an important field of study in biology for a number of years now. It is necessary for understanding the differentiation of tissues in higher organisms. Many experimental techniques have been used to characterize hundreds of functional enhancers, however given the number of different cell types it is difficult to identify all specific enhancers experimentally. The other option is to find computational methods for identification of regulatory elements. They usually base on our knowledge of characteristic features of such DNA fragments, including evolutionary conservation, specific sequence motifs or epigenetic markers. Despite these important developments, the quality of predictions has been so far limited and difficult to assess due to the limited knowledge of truly functional sequences in different contexts.

We defined recognition of regulatory sequences as a classification problem of partitioning genomic regions into two groups: active enhancers and other regions. We used supervised machine learning approach to distinguish between enhancers active in particular tissue (heart, brain and limb) from VISTA database[1] and other regions, chosen randomly from the genome but maintaining similar length distribution. We use random forest algorithm as a classifier and train it on histone modification as well as sequence features. As histone modifications features we use signal from ChIP-seq expreriments data published in ENCODE Project[2] which come from several cell types from Tier 1 (including H1 human embryonic stem cells – H1hesc) and Tier 2. As sequence features we use frequencies of 4-mers occurrence in the sequence.

Various variants of feature sets where tested under 10-fold cross-validation, showing that even usage of sequence features alone enables classifications with AUC between 0.79 to 0.88. Using only histone modifications from embryonic stem cells (H1hESC) results in AUC from 0.74 for limb up to 0.85 for heart enhancers. Inclusion of histone modifications data from other cell types slightly improved prediction (maximally by 0.03 points) with cost of 10-fold increase of feature space. Moreover, combining sequence features with histone modifications gives improvement in every case but one and results in classifiers with AUC over 0.9 for heart and brain.

Importance of single features was computed with Boruta package [4] in R, which utilizes random forest classifiers to compute feature importance relative to a randomized control. Both histone modifications and 4-mers where among highly important features. Not surprisingly, H3K4me1 (from H1hESC), known to be a marker of regulatory regions, was the most important feature for almost all classifiers, but also some of the 4-mers were found to be very important. Comparison of important 4-mers showed that some of those are tissue-specific (were found important only for classifiers for one of the tissues), but did not depend on included histone modifications.

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**K'15**

# Memetic algorithims for ligand expulsion from buried receptor docking site

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Ligand travel through a protein interior is a fundamental process governing biological signaling and enzymatic catalysis. At a single molecule level, this process is hard to study experimentally. Moreover, even in standard molecular dynamics simulations, a complex topology of channels in proteins leads often to difficulties in modeling ligand escape pathways. We have developed two novel memetic methods for searching the exit paths and cavity space exploration: Memory Enhanced Random Acceleration (MERA) Molecular Dynamics and Immune Algorithm (IA). In MERA, a pheromone concept is introduced to optimize an expulsion force. In IA, hybrid learning protocols are exploited to predict ligand exit paths [1].

The new algorithms are tested on three model protein systems with increasingly buried binding sites: M2 muscarinic GPCR receptor, industrial enzyme nitrile hydratase and heme-protein P450cam. The memetic methods outperform Simulated Annealing and Random Acceleration Molecular Dynamics [2]. Moreover, collision statistics and the RMSD values generated during ligand dissociation trajectories for each system tested, are much lower in IA and MERA, comparing to RAMD. It is worth noting that despite the high percentage of successful dissociations, IA and MERA do not introduce excessive artifacts in the sampling of the protein conformations. The proposed algorithms are general and appropriate in all problems where an accelerated transport of an object through a network of channels is studied.

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**K'16**

# Cluster analysis of protein contact sites with regard to protein class and topology

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Knowledge of the spatial structure of protein is critical for understanding its function and biological activity. Protein structure modeling is the main task of the dynamically developing structural bioinformatics. One of the latest protein modeling approaches is the use of a contact map [1, 2], which provides information about distances between the amino acids in the protein sequence [3]. The protein spatial structure is determined by the sequence of amino acids, which have specific physicochemical properties, resulting from the structure of their molecules. Some studies show a possibility of increasing the accuracy of protein structure prediction by proper selection of the analyzed amino acids properties [4, 5]. The goal of the reported study was to investigate, whether in selected protein classes and topologies there is specific physicochemical characteristics of the amino acids forming contact sites or a particular protein contact site content. For this purpose, 11 amino acids physicochemical properties, mainly derived from AAindex database, were selected. The research material was a set of 4180 protein domains, identically described by SCOP and CATH databases [6, 7]. The algorithms we implemented included unsupervised learning algorithms (K-means clustering, hierarchical clustering and self-organizing maps) as well as classification algorithms (neural networks for pattern recognition). The research results showed no evidence of specific physicochemical characteristics of the protein contact sites. In the second part of the study it has been reported that, on the basis of the frequency of a contact site for a pair of amino acids, it is possible to distinguish a protein class (between alpha and beta classes) and certain topologies within one protein class (alpha or beta). Assessing the study results and assumptions, a few ways of modification and extending of the study methodology were proposed.

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**K'17**

# Amplifying bacterial memory controlled by a synthetic RNA thermometer and a nontoxic indu cer

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Information processing subsystems are crucial for designing reliable and efficient synthetic biological devices. While growing demand for detection of low-level signals (e.g. traces of heavy metals or other contaminants) makes systems capable of signal amplification highly desirable, they may also be used to increase protein expression after induction of a relatively weak promoter dependent on a commercially available nontoxic inducer, e.g. rhamnose. We have developed a synthetic bacterial memory based on previously described transctiptors [1, 2, 3], biological analogs of transistors using serine recombinases for specific DNA edition. Detection of an inducer results in discretization of the input signal and its storage across hundreds of *E. coli* generations. As glucose has been shown to inhibit expression in systems dependent on promoters containing a Crp binding site [4], it was possible to use a promoter that would be induced by a sugar inducer and strongly repressed by glucose, ensuring tight control over the input signal. In order to make the system more reliable and less noise-sensitive, the promoter was coupled with a synthetic RNA thermometer based on a previously described U10 thermometer [5, 6], modified to become compatible with the BioBrick standard. Secondary structures and free energies of designed candidate thermometers have been predicted using mfold web server [7, 8]. Resulting promoter forms a biological AND logic gate, demanding a certain inducer to enable transcription, and a certain temperature to enable translation of a target recombinase. If those two conditions are satisfied, high expression of an output protein is enabled. The functioning of the system has been assessed using his-tagged superfolder GFP as a reporter.

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# Prezentacje posterów (PP1-PP15) (PP'1-PP'11)

**PP1**

# Synthesis of new amide and hydrazide derivatives of 1,3-dimethyl- or 3,7-dimethylpurine-2,6-dione as PDE7 inhibitors

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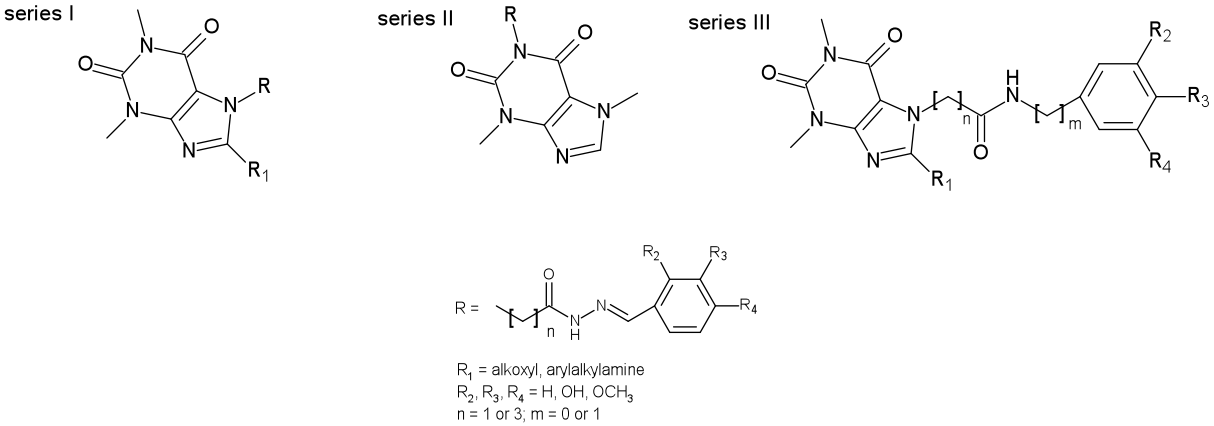
Among 11 known families of phosphodiesterases (PDEs) special interest has been focused on PDE7 that is expressed in immune cells and specifically controls intracellular levels of cAMP. The PDE7 inhibitors which maintain high levels of intracellular cAMP and exert immunosuppressant and anti-inflammatory effects are regarded as potential agents for the treatment of many neurological, immune and inflammatory disorders [1-3].

Taking into account the chemical structures of known PDE7 inhibitors which cover a wide range heterocyclic systems [4], we designed and synthesized new series of compounds, chemically based around purine scaffold, containing hydrazide or amide moieties in a 7 (series I and III) or 1 (series II) of purine-2,6-dione system.




For the new compounds their ability to inhibit PDE7A was tested *in vitro* using PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE7A expressed in Sf9 cells. The results of this study indicated that some of the tested compounds inhibited PDE7A at concentration close to that of isobutylmethylxanthine (IBMX).

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**Acknowledgements**
The study represents preliminary evaluation performed for the Polish National Science Centre grant No UMO-2014/15/B/NZ7/00885.

**PP2**

# New series of 2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives - synthesis and anticancer activity

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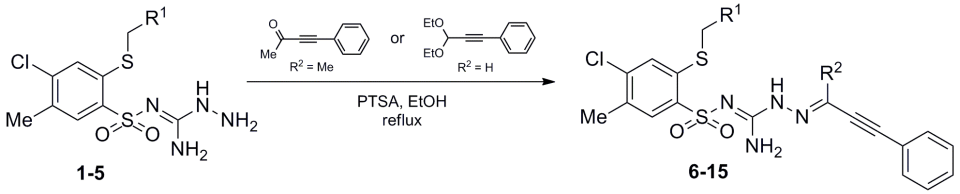
According to the search for new anticancer benzenesulfonamides [1-3], new series of 2-(2-alkylthiobenzenesulfonyl)-3-(3-phenylprop-2-ynylideneamino)guanidine derivatives **6**-**15** have been synthesized. The final compounds were obtained by reaction of the appropriate 1-amino-2-benzenesulfonylguanidine **1**-**5** with 4-phenyl-3-butyn-2-one or phenylpropiolaldehyde diethyl acetal.




**7** - $R^1$ = Ph, $R^2$ = Me

The *in vitro* cytotoxic activity of the synthesized derivatives was evaluated against three human cancer cell lines: colon cancer HCT-116, breast cancer MCF-7 and cervical cancer HeLa. All tested compounds have been shown to exert cytostatic activity. The results indicated that the HCT-116 was the most sensitive cell line - of the 10 tested derivatives, 9 compounds displayed $IC_{50}$ value between 8 - 80 μM. The compound **7** was submitted for extended *in vitro* studies to National Cancer Institute (USA) for an evaluation of activity against 60 human cancer cell lines. This research have shown the high ability of derivative **7** to inhibit the growth of 18 cell lines from eight different cancer types ($GI_{50}$ in the range of 1.84 to 4.99 μM).

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**Acknowledgements**
Project was financed by National Science Centre based on the decision No. DEC-2013/09/B/NZ7/00048.

**PP3**

# Synthesis and evaluation of fluorescent 1,2,3-triazole derivatives of 3'-azido-3'-deoxythymidine (AZT), using a novel ligand-accelerated [3+2] cycloaddition protocol

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3'-azido-3'-deoxythymidine (AZT, Fig. 1), also known as *Zidovudine* is an FDA-approved nucleoside antiviral drug which could also be used for the treatment of acquired immunodeficiency syndrome (AIDS).[1] Conjugates or functional hybrids consisting of nucleoside derivatives linked with fluorescence marker by a 1,2,3-tiazole unit have already been reported.[2,3]

Copper-catalyzed azide-alkyne cycloaddition, widely known as the flagship reaction of *Click chemistry*, is a powerful tool in organic synthesis due to the ease of application, high yields, modularity and wide scope.[4] Unfortunately there are limitations of this cycloaddition, such as redox stability of the copper(I).[5] These can be overcome by the use of copper(I)-stabilizing ligands, which are effective for improving the reaction outcome.[6]

The aim of our study was to synthesize fluorescent 1,2,3-triazole derivatives of 3'-azido-3'-deoxythymidine using a novel ligand accelerated copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) protocol based on a water-soluble ligand (AMTC) and perform fluorescence emission studies of the obtained compounds.

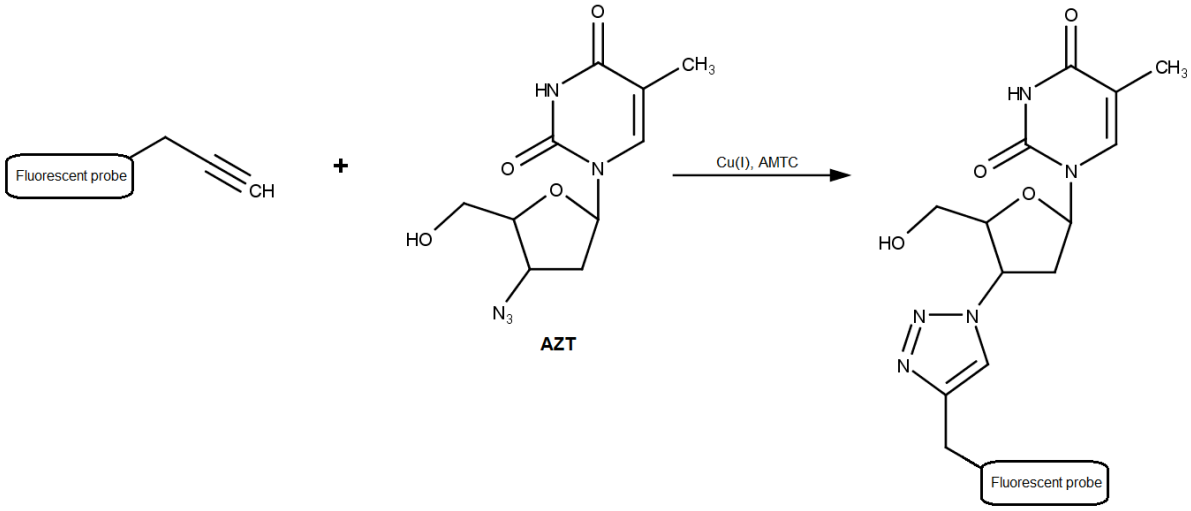


**Fig. 1** The main step of obtaining 1,2,3-triazole derivatives of 3'-azido-3'-deoxythymidine.

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**PP4**

# Design, synthesis and *in vitro* antimicrobial activity of new nalidixic acid – 1,3-thiazolidin-4-one hybrids

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Quinolones are the first-line therapy that has made a major impact in the field of antimicrobial chemotherapy [1]. Nalidixic acid (1,8-naphthyridine derivative) was the first synthetic quinolone antibiotic introduced in 1960s by Leisher. It inhibits the DNA gyrase enzyme that is responsible for the initiation and propagation of DNA synthesis. It showed activity against Gram-negative organisms including most of the enterobacteria and was mainly used in the treatment of urinary infections [2]. The structure-activity relationship study of nalidixic acid derivatives suggested that the carboxylic group at C-3 position is essential for biological activity [3].

4-Thiazolidinones contain β-lactam ring with sulfur atom in the ring. Its derivatives interact with MurB enzyme and inhibit the biosynthesis of the peptidoglycan, polymer essential for cell wall of bacteria. MurB enzyme is a unique target for antibacterial activity of thiazolidinones [4]. They also have significant activity, like: anti-inflammatory, antibacterial, antifungal, antitubercular, antiviral, antitumor, anticancer, anticonvulsant, and antihistaminic activity [5,6].

In the present work, antimicrobial activity associated with both 1,3-thiazolidinones and nalidixic acid moieties prompted us to synthesize new nalidixic acid derivatives carrying the 1,3-thiazolidin-4-one heterocyclic system at position 3 with an objective to obtain biheterocycles with enhanced biological activity. New derivatives were prepared by cyclization reaction of nalidixic acid-base hydrazones with thioglycolic acid. Obtained compounds were *in vitro* screened for antimicrobial activity against a panel of strains including Gram-positive bacteria, Gram-negative bacteria and fungi belonging to *Candida* spp. [7,8].

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**PP5**

# New non-basic ligands of serotonin receptor 5-HT$_6$ as a result of virtual screening based on machine-learning methods

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Virtual screening is one of the most popular techniques in computer-aided drug design with machine learning methods as representatives of the group of extensively explored methodologies in this field.[1] In the study, a variation of Support Vector Machines – a Sequential Minimal Optimization algorithm[2] was applied in the search of new 5-HT$_6$R ligands in the ChemBridge and ChemDiv databases. Three different fingerprints were used for molecules representation and consensus prediction was taken as the final answer. Selected compounds indicated as active were purchased and their affinity towards four serotonin receptors (5-HT$_6$, 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$) was examined in *in vitro* experiments. Two structurally new compounds were found to be characterized by a significant 5-HT$_6$R activity (119 and 670 nM).

The compounds do not possess positive ionisable group in their structure, therefore they belong to the group of atypical non-basic 5-HT$_6$R ligands. Although several reports have proven that the presence of a basic nitrogen atom enabling formation of the interaction of its protonated form and D3.32 is not indispensable for 5-HT$_6$R anchoring,[3] the fraction of non-basic compounds within known 5-HT$_6$R ligands is low (about 7%) and the majority of 5-HT$_6$R ligands keep fitting the standard pharmacophore model, which requires the possession of the positive ionisable group.[4]

One of the hits was selective over the remaining serotonin receptors, whereas for the other, the affinity for the 5-HT$_{2A}$R subtype was also marked. Docking and molecular dynamic simulation experiments proved that the obtained hits fit well in the 5-HT$_6$R binding cavity interacting with the amino acid residues reported as important for 5-HT$_6$R activity. Moreover, *in silico* evaluation of ADMET properties indicated their drug-like character.[5]

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The study was supported by the National Science Center Grant No DEC-014/15/D/NZ7/01782 in the frame of SONATA 8 Program.

**PP6**

# Isorhodanines as substrates in the Diels-Alder synthesis of new potential biologically active compounds

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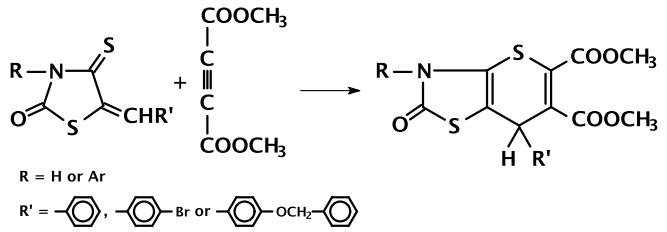
Cycloaddition reactions were described for the first time by Otto Diels and Kurt Alder in 1928.[1] These reactions are particularly useful for the synthesis of complex cyclic and bicyclic compounds and therefore are widely applied in organic chemistry. In cycloaddition reactions not only systems composed exclusively of carbon and hydrogen atoms are used, but also systems including heteroatoms, such as oxygen and sulfur are engaged.

Among others, particularly interesting groups of diens are 2-thiothiazolidyno-4-on derivatives (rhodanines) and 4-thiothiazolidyno-2-on derivatives (isorhodanines), having arylidene substituent at C-5 position. The Diels-Alder reactions involving 5-arylidenorhodanines and 5-arylidenoisorhodanines are a convenient method of the synthesis of condensed thiazoles having potential biological activity.[2]




Literature sources indicate that 5- arylidenorhodanines are much less reactive than 5-arylidenoisorhodanines.[3] Therefore we put our attention on reactions of the second group with dienophiles such as maleicanhydride, acrolein, dimethylmaleate, and dimethylacetylenedicarboxylate. The reactions were carried out using microwaves, under solvent-free conditions. The obtained products were purified by crystallization and their structures were initially confirmed by ES-MS and IR.

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**PP7**

# Non-basic 5-HT$_6$ Receptor Ligands

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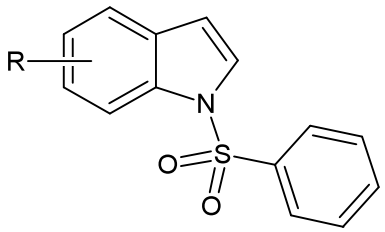
Recently a progress has been made in finding new non-basic ligands of serotonin receptors – mainly 5-HT$_6$ subtype. Until recently it was believed that only compounds with a basic nitrogen atom can act as aminergic receptor ligands. The discovery of the non-basic ligands has changed the longstanding views in medicinal chemistry. This phenomenon has been recently studied and some hypotheses were formulated, [1,2] but the mechanism of non-basic ligands-receptor interaction is still unclear.

As a part of our study on 5-HT$_6$R the consistent series of indole derivatives has been designed in an attempt to describe the interactions of non-basic ligands in the binding pocket. Following the examples of literature ligands with 1-(phenylsulfonyl)-1H-indole fragment and basic nitrogen atom, their counterparts with reduced and/or removed basicity were synthesized.




R = 1-methylpiperazinyl, 1-acetylpiperazinyl, 1-(2,2,2-trifluoroethyl)piperazinyl, 1-piperidinyl

The 5-HT$_6$, 5-HT$_{2A}$, 5-HT$_7$ and D$_2$ receptor affinities for all the synthesized compounds were assessed in radioligand binding experiments. The structure-affinity relationships and results of molecular modelling experiments are discussed.

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**Acknowledgements**

The study was partly supported by the grant OPUS 2014/13/B/NZ7/02210 financed by the Polish National Science Centre.

**PP8**

# Effects of bergapten on memory processes and oxidative stress

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Bergapten (5-methoxypsoralen, 5-MOP), a furanocoumarin found in many medicinal plants, has been used in combination with UV radiation in skin photochemotherapy for decades, and also possesses slight antioxidative activity as evidenced from *in vitro* studies. Literature data have demonstrated that bergapten, exerts both anti-proliferative effects and induces pro-apoptotic responses in human breast cancer cells. Some studies also found that it has anticancer, antidepressant, anticonvulsion, and anti-inflammatory effects. Bergapten also inhibits the butyrylcholinesterase and acetylcholinesterase activity. These enzymes degrade ACh, which prevents the formation of senile plaques in Alzheimer's disease

The aim of the present study was to examine the effects of acute administration of bergapten on memory processes in the passive avoidance (PA) paradigm and anxiety-like behaviors in the elevated plus maze test (EPM) in Swiss mice. We also assessed the relation of this drug in the level of oxidative stress in brain. Bergapten was purified by high-performance counter-current chromatography from dichloromethane extract of the fruits of Heracleum leskovii Grossh. *W*e revealed that acute injections of bergapten at the dose 25, 50 and 100 mg/kg, i.p. improved processes of memory acquisition whereas the doses of 25 and 100 mg/kg improved processes of memory consolidation in the PA task. Oxidative stress was assessed by determination of antioxidant enzymes (glutathione peroxidases (GPx), superoxide dismutase (SOD),) activities as well as of malondialdehyde (MDA) concentration in the whole brain. The results of our research suggest bergapten to be an interesting therapeutical option in disorders with memory deficits.

**Acknowledgements**
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**PP9**

# Magnetic nanoparticles coated with modified chitosan rich of long-distanced amino groups – synthesis, characterization, and lipase immobilization

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

In this study the synthesis of new three types of chitosan coated nanoparticles rich of long-distanced amino groups on the surface is presented. All of prepared materials have been used for lipase from *Candida rugosa* covalent immobilization via standard procedure [4-6]. Prepared nanoparticles were characterized by analytical methods and the activity, reusability and the amount of immobilized lipase were determined. The effect of amount of free amino groups on the surface, surface morphology, particle and pore size, and the surface area on lipase loading capacity and activity have been investigated.

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**PP10**

# Dipeptide L-Carnosine analogues

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L-carnosine (β-alanyl-L-histidine) is an endogenous dipeptide present in some mammalian tissues. It is a natural scavenger of reactive aldehydes generated in the degradative oxidative pathway of endogenous molecules (sugars, polyunsaturated fatty acids, proteins). It is also a selective scavenger of α,β-unsaturated aldehydes - the by-products of peroxidation of membrane lipids. Carnosine prevents glycation and oxidation processes and related to that anti-crosslinking activity. It has been demonstrated that carnosine may be used as a treatment in neurodegenerative disorders such as Alzheimer's or Parkinson's diseases, in an inflammatory diseases, as well as in cardiovascular ischemic damage. It may play a role as a physiological buffer and a ion-chelating agent. That biologically active peptide can be very easily destroyed by its specific peptidase – carnosinase, present in human plasma. This fact greatly limits the possibility of therapeutic use of carnosine. The synthesis of modified carnosine is intended to obtain more stable and bioavailable derivatives which may have the same therapeutic applications.

The derivatives of carnosine were synthesized by manual SPPS method using Fmoc-based strategy. Two series of peptide synthesis were carried out. In the first one in P1 L-histidine was present. In the second series P1 has been substituted by D-histidine. The received modified peptide derivatives of L-carnosine instead of β–alanine contained in P2: L- and D-alanine, 6-aminohexanoic acid, 5-aminohexanoic acid and 4-aminohexanoic acid and were obtained as acids and amides $I^o$. The lipoic, acetyl, palmityl and lauryl residues were attached to N-terminal end of each of the compounds. The products were characterized using mass spectroscopy. The resulting compounds are not cytotoxic as checked on selected cell lines. In the next step will investigate some aspects of synthetized compounds on oxidative stress. We expect that our dipeptide carnosine derivatives will prove more resistant to carnosinase. This will cause their higher concentration and prolong the duration of action in a human body. Preserved fairly strong resemblance in molecular structure to carnosine, raises a presumption that obtained analogues will demonstrate similar biological activity as carnosine. They can be a potential source of factors that prevent oxidative stress and reduce its effects which would allow them to be used as therapeutic agent and an active cosmetic ingredient.

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**PP11**

# The influence of the β2-adrenergic receptor genetic polymorphism on the interactions with agonists. A molecular modeling study

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β2 adrenergic receptors (β2-AR) participate in the physiologic responses of the lung, including bronchodilation and bronchoprotection and may also play an important role in the pathophysiology of asthma. The gene encoding β2-AR (ADRB2) is extremely polymorphic, and the studies fucused on it improves our understanding of asthma and may possibly lead to new methods of prevention, diagnose and treatment. Experimental studies revealed that the polymorphisms at codon 16 (β2-AR-16) and codon 27 (β2-AR-27) of the β2-AR might affect the response to treatment of long and short-acting beta mimetics.

We theoretically examined the role of the residue at position 16 in the native protein (β2-AR$^{Gly16}$) and its polymorph (β2-AR$^{Gly16Arg}$) played in interactions of β2-AR with β2-agonists. The molecular dynamics enhanced-sampling simulations based on the umbrella sampling (US) protocol allowed to assign the energetic characteristics to the association/dissociation paths of the agonistic ligand ((*R*,*R*)-fenoterol) entering/leaving the binding cavity of both β2-AR$^{Gly16}$ and β2-AR$^{Gly16Arg}$.

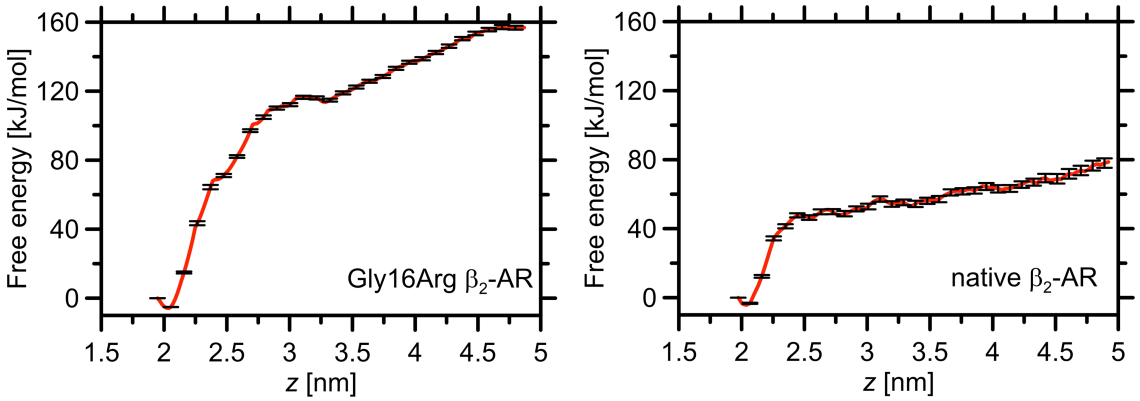


**Fig. 1.** The free energy profiles calculated by using the US simulations for the distance between ligand-receptor (*z*).

The results indicate that the main differences between β2-AR$^{Gly16}$ and β2-AR$^{Gly16Arg}$ are connected with the altered pattern of interactions between ligand and the N-terminus region. Strong, repulsive interactions between arginine and ligand (both positively charged) affect the overall free energy profile associated with ligand binding/unbinding, increasing the height of the barrier for the unbinding process. The same repulsive interactions prevent the formation of the Arg16-ligand hydrogen bond, present in the case of Gly16-ligand pair. Other aspects of the ligand-β2-AR interactions (e.g. intermediate, metastable states present on the binding/unbinding path or the topology of this path) are common for both β2-AR$^{Gly16}$ and β2-AR$^{Gly16Arg}$.

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**PP12**

**Komunikat firmy ABL&E-JASCO Poland**

**PP13**

# The development of mGluR8 PAM agonists

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Glutamate is the main excitatory neurotransmitter in the central nervous system, which is essential for cognitive functions such as memory formation and learning.[1] Group III metabotropic glutamate receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are considered promising drug targets for treatment of neurological disorders e.g. Parkinson's disease, schizophrenia, major depressive disorder and pain.[2]

We have synthesized and evaluated chemical scaffold (compounds **AH-48**, **MAH-14** and **MAH-15**) exhibiting mGluR8 Positive Allosteric Modulator activity along with a strong agonistic component. **AH-48** has the following characteristics:

- activates mGluR8 as an agonist ($EC_{50}$ = 2.6 uM),
- acts as a Positive Allosteric Modulator ($EC_{50}$ = 4.3 uM in the presence of 1 uM L-Glu),
- activity of AH-48 with or without presence of L-Glu is completely abolished by 10uM of **LY341495**,
- **AH-48** acts as mGluR8 full PAM-agonist in contrast to benchmark compound **AZ12216052** which activates the receptor only partially,
- **AH-48** activates mGluR4 and mGluR7,
- **MAH-14** acts as a mGluR8 PAM ($EC_{50}$ = 4.4uM),
- **MAH-15** acts as a mGluR8 PAM ($EC_{50}$ = 5.7uM).

We plan further tests (metabolic stability, genotoxicity, anti-target assays) which will help us establish lead structure in the study.

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Databases in this study were created using ChemAxon JChem software.

**PP14**

# Evaluation of novel N-9-benzyl-disubstituted derivatives of 1,3-dimethylpyrimido[2,1-f]purinediones as MAO-B inhibitors

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Parkinson's disease is a neurodegenerative disorder that manifests itself by movement-related symptoms such as: shaking, rigidity, slowness of movement and difficulty with walking and gait. The PD is currently second in terms of prevalence of neurodegenerative disease in the world. Available drugs despite the relatively high efficiency are not without adverse effects and in later stages of the disease often become insufficient due to the progressive degeneration of dopaminergic neurons and the severity of side effects. One of the more interesting strategy for finding new drugs for Parkinson's disease is the combination of the antagonistic activity toward adenosine A1 and A2A receptors, with inhibition of MAO-B. As an example would be a chlorostirylxanthine, that is adenosine A2A receptors antagonist and MAO-B inhibitor [1,2].

Previous research in the group of annelated xanthines confirmed their activity toward adenosine receptors and indicated that introduction of benzyl moiety in position N-9 increased affinity for MAO-B. We made an effort to study a group of N-9 benzyl-substituted 1,3-dimethylpyrimido[2,1-f]purinediones derivatives as adenosine receptors antagonists and MAO-B inhibitors. The designed compounds were synthesized according previous described procedure [3,4]. Firstly theophiline was oxidative brominated then N-alkylated. In the last step third ring was build on to the xanthine core by the condensation with proper benzylamine.

We received the small library of compounds that differ in the substituent in position N-9. The synthesized compounds were tested for their effect on MAO-B enzyme. Structure activity relationship analysis has shown higher MAO-B inhibitory activity in the group with halogens subsituents in position ortho and meta of benzene ring. The most potent compound presents $IC_{50}$ value 217 nM. Halogens subsitutents in position ortho and meta seem to be also preferable. 3-bromo-4-fluorobezyl derivatives has $IC_{50}$ value 346 nM. The compound with 4-chloro-3-trifluoromethylbenzyl substituent present $IC_{50}$ value 310 nM.

Drug-like properties (logP, logS, toxicity, drug score) of the obtained compounds were evaluated by the OSIRIS program[5].

The results confirm the MOA-B inhibitory activity of obtained compounds. Furthermore, our investigation has shown the significant effect of benzyl moiety on the MAO-B inhibitory activity.

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**PP15**

# (*R,R'*)-4-methoxy-1-naphthylfenoterol prevents GPR55 pro-oncogenic signaling in rat C6 glioma cells

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The bitopic compound (*R,R'*)-4-methoxy-1-naphthylfenoterol (MNF) elicits growth inhibitory and pro-apoptotic activities in a number of cell lines from diverse tumor types and in a xenograft model of glioblastoma. Here, we investigated the contribution of β2-adrenergic receptor (β2-AR) and the recently deorphanized GPR55 in the anti-tumorigenic effects of MNF in rat C6 glioblastoma cells *in vitro*. Analysis of cell cycle and apoptosis detection by flow cytometry, wound-healing scratch test, and immunoblotting techniques were carried out in response to MNF and various ligands. MNF elicited growth arrest in G1 phase of the cell cycle and promoted apoptosis, with a maximum effect at 20nM. Serum-inducible cell migration and constitutive activation of AKT and ERK were dose-dependently inhibited by MNF, with $IC_{50}$ of 4 nM, 1.22 nM and 1.45 nM, respectively. Consequently, MNF induced phosphorylation of inhibitory Ser-33 on β-catenin, transcriptional regulator of protumorigenic genes expression, leading to downregulation of Cyclin D1 and MMP-9. C6 cells remained sensitive to MNF signaling even after pretreatment with the selective β2-AR antagonist ICI-118,551. We then investigated MNF's ability to block GPR55 pro-oncogenic responses and found that the cellular uptake of a fluorescently-labeled GPR55 agonist and GPR55 ligand-mediated increases in ERK phosphorylation and cell migration were significantly reduced by MNF. Moreover, GPR55-dependent activation of β-catenin was abrogated by MNF. These results demonstrate the usefulness of MNF as a novel approach to chemoprevention of glioblastoma cancer and may offer a potential mechanism for its anticancer action through modulation of GPR55 function.

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**PP'1**

# IsoStar – the ultimate algorithm for fine isotopic structure calculations

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Mass spectrometry has paved way for high-throughput proteomics. Among numerous of its applications one finds protein identification, quantization, analysis of cross-linking, and other. It is acknowledged, that one of the most important qualities of these instruments is their power to precisely resolve masses of the introduced samples [1]. There exists a well established mathematical model theoretically describing the distribution of mass of a given chemical substance, potentially observable on the instrument. The model takes into account the presence of isotopes appearing in nature at random. However, direct enumeration of all possible configurations (pairs [mass, probability]) was problematic for bigger molecules due to memory requirements [2].

Traditional approaches to solve this problem consisted in averaging pairs within well established clusters in the mass domain, e.g. [3,4]. Recently, direct calculations of the *fine structure* configurations was performed via multidimensional Fourier transform [5] and by pruned transition trees [6]. Reduction in the state space were possible because the distribution under study is highly concentrated around the mean mass.

With the present approach we use to the full the concentration properties. Similar to [6], we also divide the problem into two stages. First, we enumerate a representative subset of the marginal configurations, i.e. masses and probabilities of individual elements forming the molecule. Then, we combine the results via the *prunned exterior product.* By doing so, we manage to retrieve almost exactly the minimal set of configurations with a given probability threshold, i.e. the critical set of configurations for this distribution. Not only do we save space, but also we provide the end user with configurations that have the highest chance of appearance in the mass spectrometer itself. We manage to prove the optimality of our method up to a set of configurations of negligible probability. Our approach is up to 60 times faster than [6].

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**PP'2**

# Energetic and structural investigation of aminoglycoside-RNA complexes

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Aminoglycoside antibiotics are frequently used against serious infections caused by Gram-negative bacteria. Unfortunately, bacterial resistance to aminoglycosides is spreading fast. Understanding the interactions which determine their binding to ribosomal RNA is crucial to design new modifications of these antibiotics.

The main role in the recognition of ligands by nucleic acids is played by electrostatic interactions [1]. Therefore, we performed a detailed energetic analysis of twelve aminoglycosides in complexes with bacterial decoding site (A-site) [2]. We reconstructed the electron densities of the structures using a pseudoatom database (University at Buffalo Databank) [3] to calculate the energies of electrostatic interactions with the Exact Potential Multipole Method [4]. The energies between various aminoglycosides and their binding sites revealed a significant correlation with experimentally obtained binding free energies. Additionally, a number of water molecules mediating interactions between antibiotic and RNA allowed us to propose modifications which would enhance the binding specificity.

We have also investigated the influence of different aminoglycosides on the structure of RNA A-site region [5]. For this purpose, we used MINT software [6], which enables analysis of three-dimensional full-atom structures of RNA. We have investigated the hydrogen bond patterns, solvent accessibility, and stacking energies of the nucleobases. We pointed out some antibiotic-induced RNA structural differences that depend on the type of bound aminoglycoside.

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**PP'3**

# Robust 3D RNA models comparison with RNAssess

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Quality evaluation of multiple 3D RNA models against reference structure is a difficult and time consuming task. To capture subtle differences between alternative structures and reliably rank them, many different comparison measures need to be calculated and usually manual inspection is also required.

Here we present RNAssess [1], a web server that allows users to assess quality of multiple 3D RNA models with many integrated measures that can be applied from global and local perspective. Local comparison of 3D RNA structures gives the ability to identify well and poorly predicted regions of molecule. Together with interactive user interface and embedded structure visualization, RNAssess makes 3D RNA structure comparison easier and faster. RNAssess is available as a free service open to all users at http://rnassess.cs.put.poznan.pl/

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**PP'4**

# New *in silico* approach to assess RNA secondary structures with non-canonical base pairs

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RNA function depends on its structure, therefore an appropriate recognition of the latter is of great importance. One particular concern is the assessment of base-base interactions, described as the secondary structure. It greatly facilitates an interpretation of RNA function and allows for structure analysis on the tertiary level. The RNA secondary structure can be predicted from sequence using in silico methods often adjusted with experimental data, or assessed from 3D structure atom coordinates. Computational approaches consider mostly Watson-Crick and wobble base pairs. Handling of non-canonical interactions, important for a full description of RNA structure, is still a challenge.

Here we present novel two-step in silico approach to asses RNA secondary structures with non-canonical base pairs. Its idea is based on predicting the RNA 3D structure from sequence or secondary structure that describes canonical base pairs only, and next, back-calculating the extended secondary structure from atom coordinates. We have integrate in a computational pipeline the functionality of two fully automated, high fidelity methods: RNAComposer for the 3D RNA structure prediction and RNApdbee for base pair annotation. We have benchmarked our pipeline on 2559 RNAs sequences with the size up to 500 nucleotides obtaining better accuracy in non-canonical base pair assessment than the compared methods that directly predict RNA secondary structure.

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**PP'5**

# Deciphering the language of fungal pathogen recognition receptors

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The immune system searches for pathogen invasion markers, which include pathogen proteins and host proteins modified in the course of the invasion. While some pathogen-associated molecular patterns are relatively invariant, numerous pathogen-specific markers change quickly. Therefore, to win the arms race with pathogens, host recognition receptors must adapt quickly to varied and modulating markers. To achieve this goal, the recognition domain of the receptor requires the capacity to recognize diverse possible pathogen molecule epitopes, and ability to quickly learn new ones. In plants and fungi, which lack an adaptive immune system, this key role in the immunity is played by the NLR family of receptors, which adapt to ever-changing pathogen-specific invasion markers thanks to their repeat-based architecture, which can produce diversity of recognition paratopes through unequal crossing-over and mutation [1]. The unequal crossing-over is a repeat shuffling process 10,000 to 100,000 times quicker than the standard point mutation, however it requires (and promotes) high sequence similarity between consecutive repeat units. At the same time certain positions in the repeats are highly variable under positive diversifying selection; they are believed to form the actual recognition paratope [2, 3]. Characterizing computationally the language of these pathogen recognition receptors can provide insight into the molecular mechanisms of immune response and describe the limits of the pathogen targets that can be recognized. In this work, we modeled generation and selection of the recognition paratopes as a stochastic string rewriting system with constraints, tuned by analysis of observed evolutionary processes and validated with regard to a large data set of fungal NLR [3]. First, we cross-checked the unequal crossing-over and mutation model with the existing experimental data. Second, we analyzed mathematical properties of the model and showed that its convergence to stationary distribution. Then, we compared real and simulated data. Among others, analyzing the feasible set of solutions revealed that the model explained the i=i+2 (even-odd) periodicity observed in the repeat number distribution of a family of receptors. Next, we explored discrepancies between real and simulated data in order to discover constraints acting on the paratopes. For example, by comparing amino acid content in the model and original populations, we confirmed that highly variable sites identified on the basis of entropy, were subject to constraints towards composition typical for binding sites, which was coherent with the suggested role of recognition paratopes. Finally, we proposed an interactive approach to exploring the solution space of amino acid repeats by means of 2-d projections and significance tests in sectors of the space. In a preliminary analysis, we found an overrepresented pattern R-[SYQFW](1,3)-R at one of the highly variable positions in a family of receptors, which potentially has functional importance. The methodology developed in this work is general and therefore can be applied to any class of amino acid repeats generated by unequal crossing-over for which an equivalent high quality data set is available. An appealing future application of our model would be testing of hypotheses regarding repeat origin or function encoded as constraints on the string rewriting system.

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**PP'6**

# Activity of NAHR mediating LINE sequences and the distribution of their microhomologies

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Over 3 billions nucleotides constituting human genome, encode every information that organism needs to live. However, such complex structure is susceptible to various genetic changes including: somatic point mutations [1] and copy-number variations (CNV) [2]. Most recently we showed that non-allelic homologous recombination (NAHR) can be mediated by transposable elements that are placed relatively close to one another [3].

In general, the nature of such genetic disorders can have various phenotypical consequences. On one hand, there are mutational changes that potentially do not affect an organism's phenotype (e.g. silent point mutations or duplication of dosage insensitive genes), on the other hand, they can induce cancerogenous genomic rearrangements (e.g. sequence duplications [4] or multiple mutations of suppressor genes [5].

In our work, we analyse sequences from patients with genomic disorders. Specifically we are interested in NAHR mediated by transposable elements from the LINE family. We concentrate on discovering the common motif (microhomology), which characterises the known cases of NAHR events stored in the Baylor Collage of Medicine databases. Our preliminary results indicate that there is an significant enrichment in microhomologies in the first 1500 bp (for the sequences of length 60 kbp approx.).

Furthermore, we hypothesise that there is a correlation between the time of activity of particular LINE families and their susceptibility to induce NAHR events. Interestingly, over 90% of all sequences reported in the databases belong to one of the *L1PA3* or *L1PA4* subfamilies. Currently, there is an ongoing research to verify the hypothesis by formulation of the adequate model of evolution of transposable element's sequences.

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**PP'7**

# Reconstructing the chronology of transposable element activity from interruption matrix: a Bayesian approach

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Transposable elements are short genomic sequences capable of copying and/or relocating themselves within the genome in a process called transposition. Their ability to cause genetic disease by inserting themselves into a gene (causing its disruption) or in its vicinity (causing its misregulation) has caused them to become the focus of study of both population geneticists [1] and of the medical community [2]. The abundance of DNA sequence data for multiple organisms, as well as the availability of efficient tools for mass detection of TEs make it possible to attempt to reconstruct the history of evolution of TEs within species.

The unique nature of TEs makes it possible to supplement the standard phylogeny-based approach with another one, based on the TEs ability to insert into one another. From these insertions a so-called *interruption matrix* may be reconstructed, and its analysis makes it possible to reconstruct the chronology of TE activity.

In contrast to the previous approach [4] (which worked by using a heuristic approach to find a good solution to the Feedback Arc Problem) we propose a Bayesian model of the interruption matrix with added noise, and use it to recover the chronology of activity of TEs. Unlike the previous approach, our model makes it possible to take into account the concurrency of activity of certain TE families, creating cycles in the interruption graph, as well as to estimate the length of each family's activity.

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**PP'8**

# Application of chemical reaction simulation methods in order to verify RNA World hypothesis

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The RNA World hypothesis [1] is the most popular and well substantiated theory which tries to explain origins of life on Earth. It is a collaborative effect of work of many scientists who try to guess possible reaction paths and mechanisms which resulted in life in the form that we know today. Taking into account an enormous space of possible reactions and an uncertainty of primordial conditions, these attempts require effective methods of verification and estimation of various possibilities. Such methods should be fast, based on existing knowledge and, at the same time, as accurate as possible.

This presentation demonstrates a variety of algorithms which can be used to simulate interactions between molecules on prebiotic Earth [2], based on our three years long experience on simulating such systems. We underline the accurateness and efficiency of each method. Moreover we present a new approach that treats RNA chains as regular chemical molecules. RNAs diffuse, react with other RNAs, replicate and are the subject of mutations. Some variants of the model built on this assumption have been implemented as computer simulations. Based on these simulations we were able to infer first, biologically significant conclusions regarding evolutionary dynamics of first living systems and verify already formulated hypothesis. [3]

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**PP'9**

# Discovering interdependencies among features in disease-related genomic data

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Contemporary computational methods discover single variables associated with phenotypes under the degenerate assumption that biological systems can be characterized by single parameters. This diverges from the principle that a variety of variables (features) are to define the fate of the living organisms, through non-linear complexity.

Here we aim to apply a new approach established on classification-based feature selection and rule-based modeling. Interactions among features are modeled as a network of interdependencies, extracted from the classification trees, constructed by the MCFS-ID algorithm. The network is given in the form of directed graph. On a final level, classification rule sets are provided by the ROSETTA system to assign feature values to specific decision classes.

This approach was successfully used in a number of applications. Here we showed how this combined approach helped identify interactions and analyzed them in detail. Importantly, our approach is suitable to analyze heterogeneous data and is likely to support well various studies related to different genomic data, such as, for instance, immune system or pan-cancer analysis. Our methodology is illustrated here with examples from the study of immune responses in CD4+ T cells and examples of breast (BRCA) and lung (LUAD) cancers using data from The Cancer Genome Atlas.

We discovered interdependencies in the human immune system responses to various stimuli of CD4+ T-cells depending on the racial background. Biologically, generic-function protein-coding genes (i.e. UTS2) point to function-specific ones (i.e. LYZ). The direction of the significant feature connections followed the time increment of treatment (i.e. genes measured in the 4th hour point to the ones measured in the 48th one). We learnt that gene-responses related to bacteria characterized Afro-Americans; to viruses, Caucasians; and to both characterized Asians. Finally, the refinement to the level of rules showed that the distribution of the attribute values across the classes suggested that African-Americans and Asians were much more homogeneous than Caucasians for the selected genes.

In the case of pan-cancer analysis, we detected 603 genes that contribute to classifying/discerning BRCA-LUAD. Next, the ID-graph showed several genes with plausible roles in BRCA and LUAD. Finally, the rule nets for decision classes showed detailed interactions between several genes with a possible function in BRCA and LUAD. This pilot study needs a significant follow up to investigate in detail the hypothesized interactions, and to validate them.

**PP'10**

# Hot spot identification in protein complex using S transform

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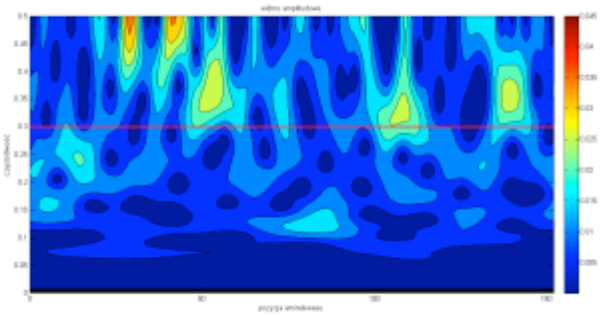
Some of amino acid residues – called hot spots – contribute most of the energy into interaction between proteins. Detection of such residues is important due to the fact that most of the biological processes are controlled by protein complexes. Experimental method of hot spot identification requires a lot of effort, which induces need for developing computational methods. One of the computational approach is applying digital signal processing methods for hot spot prediction. The basis of such algorithms is so-called Resonant Recognition Model introduced by Veljković et al. [1]. According to the model, there is a correlation between protein function and its spectrum. One of the digital signal processing tool that can be applied for hot spot residues prediction is S transform [2], which combines properties of Short Time Fourier Transform (STFT) and wavelet transform (WT). It can be derived as STFT with window width depending on frequency or as WT with phase correction and provides direct correlation with Fourier spectrum as well as frequency dependent resolution [3-5]. Properties of S transform makes it useful for biomedical data analysis. Sequence analysis using S transform gives satisfying results on finding hot spots residues in protein complexes.
Figure shows an example of protein spectrum (human Interleukin-4) obtain with the aid of S transform.



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**PP'11**

# Searching for radiosensitivity biomarkers by Monte Carlo feature selection and rough sets approach

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**Aim:** We propose a data analysis method based on Monte Carlo feature selection for data pre-filtering and rough sets classifier for radiosensitivity phenomena biomarkers investigation.

**Materials and methods:** The population under investigation is composed of 60 patients with a breast cancer, where from each the lymphocytes were collected before radiotherapy. Patients were divided into two stratified groups: radioresistant (RR) and radiosensitive (RS). Gene expressions were measured in two conditions - before and after the low dose irradiation (Microarrays HU Gene 1.0 ST Affymetrix). First step of the analysis relays on data quality assessment where NUSE and RLE measures [1] were used accompanied by MicroImage software. Re-annotation of probes was performed and batch effect was removed. Feature selection analysis was done with usage of Monte Carlo based approach (MCFS) [2]. The intersection of significant genes between 0Gy and 0.2Gy were used in rough set classification through ROSETTA system [3] with discretization based on entropy, Johnson rule reduction method and object tracking voter classification. Independently *in silico* functional analysis were performed using GSEA-Pre-Ranked method.

**Results:** Five microarrays with poor quality were removed from analysis which gave 27 investigated controls and 28 cases. Four batches were recognized and corrected by Combat algorithm. Monte Carlo Feature Selection was run with 500 permutations on two datasets (0Gy and low dose) which gives 13 significant genes common for both conditions. Using 5-fold cross-validation accuracy equal to 0.79 was obtained and AUC from was equal to 0.83. Three rules from eleven were statistically significant after applying hypergeometric test. One of the gene was discover as significant and validated in literature as a possible biomarker of radiosensitivity. The independent in silico validation on GSEA, where Relative Importance was used as rank metrics showed 5 gene sets relevant to investigated phenomena. The most prominent discovery are gene set related to Reactive Oxygen Species (ROS) and PRKDC gene regulators (p-value=0.004 and p-value=0.022 respectively). Significant gene found using rough sets was not observed in significant gene sets found by GSEA.

**Conclusions:** The procedure based on MCFS and rough sets was used to create a model of breast cancer patients radiosensitivity. Potential biomarker, relevant to describe radiosensitivity phenomena, was obtained, but the biological experiment validation is needed. Independently, group of relevant genes in gene set enrichment analysis was obtained which show high relation to radiation response.

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# SEKCJA BIOINFORMATYCZNA

## Postery (P1-P22)

**P1**

# A filtration of cerebrospinal fluid signal based on Gaussian mixture model decomposition of magnetic resonance diffusion weighted imaging data

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**Aim:** Aim of this work is to propose a cerebrospinal fluid (CSF) filtering method based on Gaussian mixture model (GMM) decomposition of diffusion weighted imaging only.

**Introduction:** Magnetic resonance diffusion weighted imaging (DWI) is a popular sequence that is used for detection of different tissues in examined specimen, basing on information about water molecule displacement over time. One of possible application is detection of brain tumour tissue. The crucial step of every analysis is data pre-processing that is mainly devoted to the filtration of CSF signal that has similar features to the tumour tissue. The standard method is to use T2 FLAIR sequence on which CSF as well as all fluids are attenuated and visible as pixels of low signal intensity, very easy to detect. However, this special sequence is not always available what makes CSF filtration a problematic part of the whole analysis.

**Data:** The data set consists of 370 “non –tumour” images of brain collected on a group of patients from Cancer Centre and Institute of Oncology in Gliwice. For all 370 cases both sequences: DWI represented as apparent diffusion coefficient (ADC) together with T2 FLAIR were available.

**Material and methods:** At first step all images were decomposed into GMM and in the next step k-means clustering were performed in a set of all obtained Gaussian components. Such an approach allows to determine two groups for which separate mixture models (using information about components assigned to the group) were calculated. In a last step, with the use of maximal probability rule, a cut off threshold dividing brain tissue from CSF was estimated.

In parallel the same 370 images were processed using a standard method for which CSF voxels are labelled basing on T2FLAIR sequence.

**Results:** As a result of decomposition into GMM, a set of 793 Gaussian components has been identified. The k-means clustering results in a two groups containing: 388 and 405 components respectively. The estimated ADC cut off threshold for CSF was equal to: $1.08 * 10^{-3} mm^2/s$. The comparison of CSF detection performed with the use of proposed methodology together with standard method based on T2FLAIR sequence, given in Dice similarity coefficient was equal to: 90.7% +/- 0.4 %.

**Conclusion:** It was proven that CSF filtration performed with diffusion imaging only gives similar result (almost 91%) as standard method based on T2FLAIR, while it does not require availability of this additional sequence as well as image registration.

**Acknowledgments**
The work was financed by BK/265/RAU1/2014/10. Calculations were carried out using infrastructure of GeCONiI (POIG.02.03.01-24-099/13).

**P2**

# RECKONER – a new tool for DNA read error correction

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DNA sequencing is dominated by next-generation sequencing (NGS) technologies nowadays. Their ability to produce huge amount of data at moderated cost have enabled various applications in biomedical sciences. Unfortunately, reads produced by NGS techniques are affected by significant amount of errors. This drawback causes many problems in reads utilization. Bioinformatical algorithms processing defected reads produce results with lower quality and often require more computational resources, especially memory amount.

We present a new algorithm for correction of reads produced by Illumina's sequencers, called RECKONER. The algorithm is based on correction algorithm BLESS [1] and exploits tool KMC [2] for k-mer counting and storing a k-mers database. The idea of RECKONER is utilization of both quality indicators of reads obtained from input FASTQ files and numbers of particular k-mers in the input data. We have introduced a new method of rating of potential single read corrections to choose the best one. RECKONER is capable to correct set of reads from eucaryotic organism with genome of length 300 Mbp in less than 1.5 hour on 16-core CPU with consumption 4 GB of memory.

We have performed evaluation of RECKONER's ability to error detection and correction on both simulated and real data. Quality of obtained results is similar or better than other correction algorithms. Tests have also proved positive affect of error correction to performing typical genomic data processes, like de novo assembly and reassembly.

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**P3**

# High-quality DNA de-novo assembly of long heavily repetitive genome

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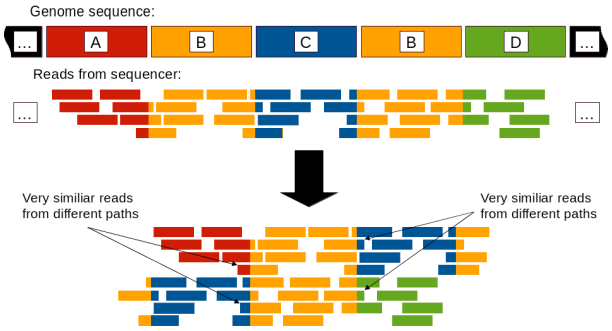
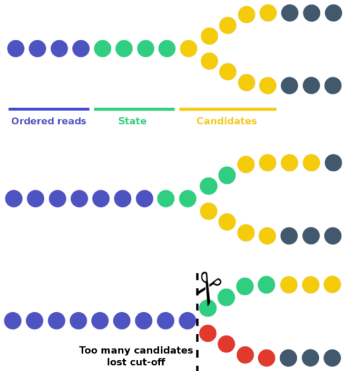
The era of NGS had dramatic impact in decreasing of sequencing cost. The NGS experiments brought the great material to expand our knowledge of the genomes of the number of organisms. However the boom in sequencing has just began and the main growth in the area is yet to come with the sequencing as a standard medical procedure in each health care facility.

In this context the most challenging remains processing continuously growing sequencing data using assembly methods. The main goal of the assembly methods is still to achieve high quality results. This however obviously stands in opposition to gaining the results in a fast manner. The limitation in the speed of processors enforces assembly programs to gain the efficiency by parallelization which makes the methods even more complex.

Another aspect of the assembly problem is the case of repetitive fragments of the genome. The more genome is repetitive the more advanced technique must be used to detect certain fragments of genome.




Repetitive genome fragments and their influence on sequenced reads.




Detecting a fork in genome graph.

Detecting the certain fragments of genome boils down to finding the genome graph forks. It can be perceived as a process of finding ambiguity points of the genome.

The presentation will concern our approach that tries to overcome mentioned assembly problems.

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**P4**

# BioShell software in structure biology applications

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BioShell project has been started in 2005 as a set of stand-alone programs aimed on simplification of typical bioinformatics tasks. Since its beginnings it has been focused on focused on biomolecular modelling and structural bioinformatics. The software provides a wide range of methods to handle, analyse and model structures of biomolecules, most notably proteins. The most recent version of BioShell includes a few web applications (i.e. programs operated from a web-browser) devised to visualise various biomolecular features and utilities for protein structure predictions.

**P5**

# Molgears - cheminformatic tools for medicinal chemists

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Computational tools are urgently needed to make sense of the wealth of data volumes from modern medical chemistry. Despite the number of commercial tools it is very difficult to find a free open source software enabling the efficient analysis of the large chemical, biological and pre-clinical datasets currently being produced by both academic institutions and pharmaceutical companies during drug discovery process.

Molgears is free, opensource web-based application designed to support drug discovery process. The aim of the project is to build an open and comprehensive system that helps users to interrogate the wealth of scientific data stored within the database. It encompasses elements of electronic lab notebooks, sample management, data acquisition, data processing, reporting and scientific data management. Molgears has, among others, such functional features as importing/exporting from multiple molecular file formats, structure/substructure search capabilities, rapports generation, automated/self-programed calculations and plotting tools for interpretation and analysis of data.

The application is licensed under the BSD License and freely available from GitHub: https://github.com/admed/molgears.

**P6**

# Operations on k-mer‘ sets

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One of the first steps in many bioinformatics applications is k-mer counting. k-mer is a substring of length k. Histogram of k-mers' counters is used e.g. in reads correctors, genome assemblers, fast multiple sequence alignment, repeat detection etc. Most often k-mers' database is used to check if k-mer exists (and getting value of its counter) or listing all k-mers with counters. In some applications such operations are not sufficient.

We present a tool that provide more operations on k-mers' databases produced by efficient k-mer counting algorithm KMC [1]. These operations are divided into the following groups. The first group contains operations that process two input k-mers' databases. In this group there are such operations: intersection (output database contains only k-mers present in both input databases, counter is equal to smaller one of both inputs), k-mers subtraction (output contains only k-mers present in first input, but absent in the second one), counters subtraction (output contains k-mers present in first input, counter is equal to difference between counter from first input and counter from second input), union (output contains k-mers from both inputs, for equal k-mers counter is equal to sum of counters). There is also operation that can process more than two input databases (which can improve performance over processing databases in pairs separately). The next group of operations are the ones with single input database. In this group there are: converting database to text format, sorting k-mers in database, producing a histogram of k-mers counts, removing counters of k-mers, filtering out too rare or too frequent k-mers. The last supported operation takes one input k-mers' database and set of sequencing reads, as a result it produces set of reads reduced by the ones which contains not enough k-mers.

The most important operation is the one which process list of k-mers' databases. It can be applied to algorithms that use sequencing data from couple of individuals (for example from family members) or tissues (for example diseased and normal). Example of application which use family trio sequencing data is DIAMUND [2]. The purpose of this algorithm is to discover mutations responsible for some kinds of diseases. Originally authors perform operation on k-mers' databases in a text form. It could be performed more efficient with the proposed tool.

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**Acknowledgements**

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**P7**

# Molecular dynamics simulation study of lutein intercalation into a model lipid membrane

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Carotenoids constitute a large group of plant and bacterial dyes with diverse structures and functions. In plants, carotenoids take part in protein complex assembly, light absorption in photosynthesis and photoprotection. They are also components of bacterial photosynthetic complexes, where they act as light harvesting particles. In the animal cell, carotenoids play a vital role in energy dissipation, protection against oxidative stress as well as in membrane stabilization. In our study, we focus on one group of carotenoids – xanthophylls, particularly on lutein and zeaxanthin. They are the only two carotenoids in the human eye, responsible for light screening [1] and protection against oxidative stress [2].

However, most animals are not able to produce carotenoids on their own and must obtain them through their diet. Intestinal absorption of lutein and zeaxanthin from food occurs by passive diffusion [3]. After initial absorption, carotenoid molecules are being transported and stored in various tissues. In our previous study [4], orientation of the lutein molecule in the palmitoyl-oleoyl-phosphatidylcholine (POPC) bilayer was analyzed. In this study, we analyze the process of carotenoid molecules entering the POPC bilayer using molecular dynamics simulation. We record subsequent steps of the β and ε ionone rings of lutein intercalation into the bilayer/water interface and associated with this process hydrogen bond formation and lipid rearrangement as well as rearrangement of water molecules around the polyene hydrocarbon chain when it inserts into the bilayer.

Our results indicate a crucial role of water in lutein intercalation into the bilayer. Initially, water inhibits entering of the lutein. But, water bridges that form between polar groups of POPC and lutein anchor the ring in the interface and prevent escaping of the molecule into the water phase. Further intercalation of lutein is correlated with dehydration of its polyene chain. Once the lutein molecule is fully intercalated, water stabilizes its position in the bilayer by forming multiple hydrogen bonds with the terminal hydroxyl groups of the ionone rings.

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**P8**

# Efficient algorithm for Gaussian mixture modeling of 1D biological spectra

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High-throughput technologies opened a new chapter in biomedical research allowing characterization of low-molecular-weight fractions of the human transcriptome, genome, proteome or metabolome in a relatively short time. 1D spectra are high-throughput data that in fact are complex histograms of the measured values. In biological experiments we can find many measurement technologies which produces similar signal shape: chromatograms, mass spectra or Nuclear Magnetic Resonance spectra. All of them are classified as 1D spectra and differ mostly in resolution and the degree of complexity. Most important fragments of the spectrum are signal peaks, which correspond to the compounds contained in the analyzed sample.

In our analysis spectrum is decomposed into a sum of Gaussian bell-shaped curves where a single model component (or sum of few components) represent a signal peak. Mixture modeling of spectra is an interesting approach with many potential applications, but existing algorithms do not allow for automatic analyses of whole spectra containing from hundreds to thousands of peaks. Proposed algorithm for Gaussian mixture modeling is based on signal partitioning into smaller fragments [1]. The partition is obtained by using existing peak detection algorithms and introduced idea of 'splitters'. The obtained fragments are separately decomposed into Gaussian mixture models by the iterative expectation maximization (EM) algorithm. Next, the parameters of the segment models and 'splitters' are aggregated to form a whole spectrum Gaussian mixture decomposition. Last step is post-processing of model components.

We verify efficiency of the developed algorithm using different type of datasets. In the first step we use our algorithm as a tool for improving peak detection in simulated low resolution mass spectra. We present comparisons of our algorithm to the two peak detection algorithms of high efficiency published in the literature, MassSpecWavelet (based on continuous wavelet transform) [2] and Cromwell (based on spectra differentiation) [3]. On a large number of artificially generated datasets we demonstrate the improvements in peak detection sensitivity achieved by using Gaussian mixture modeling. In the second step of verification of the methodology we show Gaussian mixture decompositions of three real proteomic datasets. For the high-resolution Aurum dataset we demonstrate the improvement of the accuracy of estimation of positions of peaks by using MS-GMM. For other low-resolution datasets we highlight abilities of modeling method to detect hidden spectral components which represent skewed shapes of spectral signals.

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**P9**

# Iterative clustering procedure for automated identification of peptide signature of molecularly heterogeneous tissue regions in tumor samples

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**Introduction:** MALDI-IMS is a recently developed technique of molecular imaging, which combines Matrix Assisted Laser Desorption and Ionization (MALDI) spectroscopy with targeting laser shots on a raster of points on the surface of the analyzed tissue sample. Application of MALDI-IMS in experiments supporting research in cancer biology leads to generation of datasets of significant complication and massive volumes and retrieval of useful information is highly dependent of development of appropriate computational tools for processing and analyzing of this data. **Aim:** The aim of this study was to develop k-means clustering based MSI data analysis pipeline allowing for distinguishing different types of tissues or cancer heterogeneous regions basing on their peptide profile. **Material and methods:** The set of 9493 spectra with 109,568 mass channels from training head and neck cancer sample was analyzed. Second dataset of 12962 spectra of the same resolution collected on independent cancer sample was used for molecular tumor signature validation. Signal preprocessing included: baseline detection and removal, alignment, normalization and Gaussian Mixture (GM) model based peak identification. Peaks with relatively low Signal-to-Noise Ratio (SNR) and low variance across all spectra were filtered out. K-means clustering algorithm was used for detection of molecularly homogeneous regions. The novel data-driven initialization of clustering algorithm was conducted in the following manner: a linear model for all spectra was built. Then, the most distant outlying spectrum (with the highest error) was chosen as first cluster center. The next *K-1* cluster centers were chosen to maximize their smallest distance to every existing cluster. As a distance metric the Pearson correlation was used. The percentage change of Dunn's index less than a priori assumed threshold was applied as stopping criterion. Due to high similarity between tumor and epithelium peptide signatures, the clustering was performed in two steps. During the first step, a rough main region estimates were obtained, while the second step, with clustering performed within each of these regions separately allowed for identification of heterogeneous subregions. The final stage of developed signal analysis pipeline required performing ANOVA test supported by Games-Howell pairwise comparisons on every feature to find tumor specific peptide signature. P less than 2.5e-06 was assumed statistically significant. Cohen's d metric of relative effect size was used as additional feature filtration and it was required to be more than 0.8. The obtained tumor molecular signature was validated on the independent sample. **Results:** Spectra preprocessing and modeling resulted in dimensionality reduction from 7.91 GB for raw data to 431 MB (6216 peaks) before non-informative feature removal. Filtering of low SNR features allowed for additional reduction to 3884 peaks (213 MB), while limitation to high variance peaks only left 2113 of them. Measured by Dice index similarity between tumor region defined by pathologist and core tumor identified by iterative k-means clustering was equal to 67% only, demonstrating extremely high heterogeneity of tumor tissue. The detailed inspection of subregions obtained within epithelium region revealed identification of tumor molecular margin (46.4% of spectra) in between healthy epithelium and core tumor. Core tumor specific signature included 512 upregulated and 74 downregulated peptides, among which 127 and 43 with large or extremely large effect size. The signature restrictively validated on independent sample B at 93.7% level.

**Acknowledgements:** The work was partially supported by SUT grant BK/Rau-1/2015 and NCBiR GeCONiI project (POIG 02.03.01-24-099).

**P10**

# A priori identifiability analysis as an approach to experimental design for systems biology models

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Dynamical models in quantitive biology are characterised by much more complex structures and substantially larger sets of parameters than models used in physics and engineering. Moreover available experiments usually provide limited set of data, usually corresponding only to fragments of studied systems. In consequence the reliability of used models is limited and our knowledge of studied processes misrepresented.

Potential remedy is provided by experimental design techniques. Such tools aim at selection of optimal experimental setup to maximise missing information about model parameters or model structure. As models in quantitative biology differ qualitatively from conventional models, experimental design tools need to be adapted to their specificity.

Here we present a method specifically tailored for multi-parameter models of quantitative biology. Our framework enables to determine which parameters of a given model can be identified in a given experiment and predict which experiment should be performed next to maximise the number of identifiable parameters. Our tool is different from methods developed so far as it is focused in verifying identifiability of individual parameters in large dynamical models, which contain even hundreds of parameters. We present applicability of our tool analysing JAK-STAT signalling model. Our method helps to guide experimental design in order to render such parameters identifiable.

**P11**

# Duplication clustering problems for unrooted gene trees

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One of the fundamental issues in evolutionary molecular biology is to discover the locations of gene duplications and multiple gene duplication episodes. We investigate into the *gene duplication episode clustering* problem introduced by Guigo [1]. The problem is to find a mapping from a collection of rooted, binary gene family trees onto theirs corresponding rooted binary species tree in such a way that the total number of multiple gene duplication episodes is minimized. In the literature there are several approaches presenting models that specify how gene duplications from evolutionary history of gene families can be interpreted as one duplication episode. In [2] Bansal and Eulenstein studied existing models and they provided their own neat model. However, none of the hitherto approaches consider unrooted gene trees. This restriction limits the applicability, since unrooted gene family trees are frequently inferred by phylogenetic methods.

For the first time we would like to propose the episode clustering problems where the input gene family tree(s) are unrooted. We present theoretical properties of that problem for two variants of an input. In particular, by using the properties of unrooted reconciliation [3,4], we show an efficient linear-time algorithm that reduces our problem into the episode clustering problems defined for rooted trees. The latter problem can be solved in linear-time [5]. In consequence, the unrooted episode clustering problem can be solved in linear time. Furthermore, we discuss properties and propose algorithm for episode clustering problem for multiple unrooted gene trees.

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**P12**

# Chromosomal conformation capturing from 2D ChIA-PET matrices using graph distance and Multi-Dimensional Scaling

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More than a decade ago, an ISOMAP [1] was introduced into machine learning framework, which is aimed at transforming the feature space into a low-dimensional projection space for the data clustering purposes, but not only. We coined and designed similar methodology while having the dimensionality of the problem fixed at 3D, as we deal with real structures of biomolecules in our research. Thus initially, a novel bioinformatics framework was proposed in the protein 2D-to-3D retrieval [2]. Recently then, we designed a similar, but much simpler approach within genomic structure retrieval from ChIA-PET [3] experimental 2D interactions counts, in which literally no 3D biophysical modelling is used [4]. We use the three main statistical components introduced previously in ISOMAP, which are (1) neighbourhood definition, (2) the proximity graph distance calculation and (3) the multi-dimensional scaling (MDS) technique [5], which embeds the points representing residues or genomic segments into their location in Euclidean space. By using this novel approach enriched with both 2D noise removal and fuzzy T-norms as arithmetic operators' substitutes, we can deliver real-scale, fine-grained 3D ensembles of single chromosomes and also, crude 3D model of the whole nucleus. We thus believe that our approach can be successfully applied to other bio-polymers, like ncRNAs as this turned out to be both novel and generic 2D-to-3D retrieval protocol.

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**P13**

# Functional analysis of P2X7 receptors

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Activation of the cell P2X7 receptor by extracellular adenosine 5-triphosphate (ATP) causes rapid flux of $Na^+$, $K^+$ and $Ca^{2+}$ ions [1]. Continued activation of P2X7 leads to the formation of large, non-specific pores, which allow for transport of small organic compounds (e.g. analogs of nucleosides) through the cell membrane [1, 2].

Looking for biomolecular differences between normal and cancer cells we decided to measure the expression and functionality of P2X7 receptor in K562, A549, HeLa, THP-1 and HEK 293T cell lines. First, we measured the level of P2X7 mRNA (by *real-time* RT PCR) and found rather small differences (up to 30%), with the highest and the lowest level in the A549 line and in the THP-1 line, respectively. Then, the level of the P2X7 protein was determined by a western blot and the differences did correlate with the levels of mRNA. We assumed that the channels getting reversibly open/closed by extracellular ATP or Bz-ATP, might be used to introduce some therapeutic analogs of nucleosides into the cytoplasm. Thus, that functionality of P2X7R was evaluated by flow cytometry measurements of the uptake of ethidium bromide or propidium iodide after ATP- and BzATP stimulation of the cells [2]. The process was investigated in different buffers (NaCl, KCl or Sucrose buffer).

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**P14**

# Molecular dynamics of mammalian blood coagulation factor XI

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Blood coagulation factor XI (FXI) is a protein regarded as an important risk factor of thrombosis and a medication target in humans. [1] It consists of four N-terminal apple domains (A1, A2, A3 and A4) and one C-terminal serine protease domain (SP). Two polypeptide chains form homodimer through A4 domains. The structure of the human dimer was solved by X-ray crystallography and NMR about ten years ago [2,3].

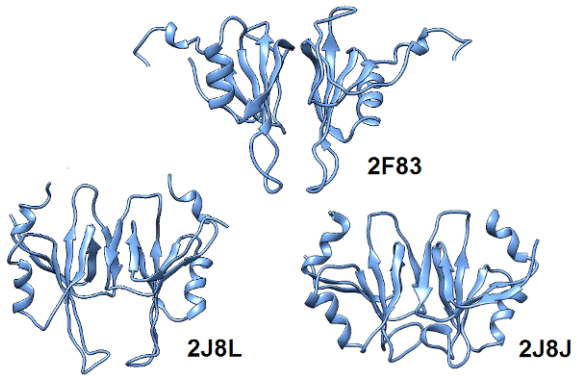


Figure 1. The human A4 dimer variability based on 2F83, 2J8L and 2J8J structures visualised using UCSF Chimera software (http://www.cgl.ucsf.edu/chimera/).

Comparisons of crystal and NMR structures indicate differences and outline some changes that occur upon activation of the zymogen to the active enzyme. According to the NMR results human A4 dimer is a dynamic structure and differs from the crystal structure e.g. in localization of loops connected by an interchain disulphide bridge between two Cys 321. The A4 dimer structure is rebuilt after FXI activation, contributing to bringing together the both catalytic SP domains with their active sites, which is crucial for the activation of factor IX (FIX). The mentioned findings make the dynamics of FXI very interesting scientific problem, worth to be solved by molecular dynamics (MD) simulations, taking into account evolutionary differences between mammals. The objective of the current presentation is to elucidate a mechanism of dynamics of dimeric structures formed by A4 domains through a comparative analysis of modelled structures of mammalian orthologs. Preliminary 1 ns MD simulations of structural changes resulting from evolutionary differences were computed by GROMACS 5.0.4 (http://www.gromacs.org/) [4]. Even one nanosecond simulations already show some evolutionary differences in the dynamics between the models obtained for different mammals. Longer simulations are obviously planned using appropriate computer clusters.

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**P15**

# ModeLang – controlled natural language for viral infections modelling

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There is a growing need for more advanced solutions for mathematical modelling. Main way of modelling – systems of differential equations are requiring a lot of time for adopting models for specific purpose and, which is the case, require to go into deep mathematical definitions. One of the answers is multi-agent systems modelling, where simulation is based on the interactions between agents. Definition of such models can be done by User Interface software or specific language, that is prepared for simulation program. There is a third way which is Controlled Natural Language analysis. Reading the subset of natural language for preparation of multi agent system allow to speed up preparation of the system and allow self-documentation. Avoids the need of using User Interface software, but allows still to convert the model stored that way into more machine-friendly code.

**P16**

# Feature selection on GPU

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**Motivation**

Datasets in molecular biology have number of features reaching several thousands (gene expression, proteomics) and even millions (SNPs). Identification of the features that truly contribute to the studied phenomena is a key step, necessary for their understanding. The number of features is so large that only univariate significance tests for each variable are routinely performed, hence any effects that depend on interactions of several features may be overlooked.

**Solution**

We have developed very fast CUDA based engine that allows for exhaustive multidimensional searches of all combinations of features that may lead to statistically significant relationships between k-tuple of descriptive variables and a decision variable, with k = {2,3,4,5}. We present the service based on this engine, that aims at performing feature selection that takes into account joint influence of subsets features in the studied phenomena for data sets described with very large number of features. The first module is devoted to search epistatic interactions due to structural variations in genomes, nevertheless, the algorithm is easily extensible to more general problems and this work is under development.

**Results**

In the case of 2-dimensional problem with three values per variable, corresponding to the analysis of epistasis in SNP data computations for 1024 objects and 3 276 800 variables take 4018 s on NVIDIA GeForce Titan Black GPU. The GPU engine performance is $1.35 * 10^9$ model evaluations per sec. With such performance it is feasible to compute 2-dimensional GWAS analysis for 10 mln SNPs on a single GPU card.

**Perspectives**

The same same computational engine used here for GWAS study can be applied for the general feature selection for datasets with real-valued and categorical data. The dataset will be discretised randomly and treated as categorical data with few values. Then the GWAS like statistics will be computed for the discretised data and compared with the randomised contrast. The procedure will be repeated multiple times to obtain reliable results. The engine for general feature selection is currently under development in my team.

**P17**

# Non-canonical imperfect RNA base pair predictor

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Identification of intramolecular interactions is one of the most important aspects in computational three-dimensionial RNA model building. Particularly non-canonical interactions play a key role in native structure stabilization, so the ability to predict them, even for imperfect tentative model can help to speed up the whole modeling process and to increase the quality of final models. In this work I propose a machine learning method capable to predict both canonical and non-canonical interactions even for incomplete or imperfect structures. This can be a valuable tool for prediction or validation RNA 3D models.

I constructed a predictor that can identify both canonical and non-canonical base pair interactions within a given structure. The main advantages of this predictor are:

1) the ability to work with incomplete input structures,

2) the ability to correctly predict base pair type even for imperfect (fuzzy) input atoms coordinates.

The predictor is based on the set of SVM multi-class classifiers and is able to decide to which of 18 recognized pair types a given base pair belongs to. The predictor was trained on the experimental high quality data set and tested on different (not used for training), imperfect (not ideal atom coordinates) and incomplete (coarse-grained) structures. The average quality of predictor for tested fuzzy nucleotide pairs is at the level about 96% of correct recognitions.

**P18**

# The bottleneck of metastasis formation: insights from a stochastic model

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Metastasis formation is a complex process in which cancer cells spread from their primary tumor of origin to distant organs where they initiate new tumors. However, only a tiny fraction of disseminating tumor cells succeed in establishing a stable colony. There is evidence that while the early steps of this process, including release to the vascular system and infiltration of the secondary organ are efficient, the bottleneck is the initial expansion of the founder cell in the new environment. Here, we study this rate-limiting step of metastasis initiation in a quantitative fashion using a size-dependent branching process model. Our model adds to a systematic understanding of metastasis formation and may help defining medical intervention strategies.

Major Findings. We compute the probability of metastatic colony survival and derive critical colony sizes under plausible initial growth assumptions, where tumor cells benefit from each others company when exposed to detrimental forces of the alien environment. Using established models of primary tumor growth together with our metastasis initiation model, we further obtain the probability of metastatic invasion and median patient survival given the tumor size. These models fit well to epidemiological data collected for eleven cancers, were validated with independent datasets, and used to predict the impact of treatment delay on metastasis incidence and survival.

**P19**

# DANIR as a near-infrared fluorescence probe for *in vivo* detection of β-amyloid deposit

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The deposition of β-amyloid (Aβ) plaques in the brain (in its paranchymal and cortical parts) is accepted as the main pathological hallmark of Alzheimer's disease (AD). Up to date, the molecular mechanism is not so known and clinical treatments are not available to stop or reserve the progression of disease just a few drugs that could alleviate the symptoms. Thus, early detection of amyloid plaques is of great importance. Currently, the clinical diagnosis based on the patient history, collateral history from relatives or neurological and neuropsychological observation. In most cases, these approaches are not sufficient. In spite of this, with the assistance of molecular imaging agents specifically targeting Aβ plaques in the brain may lead to the early diagnosis of AD.

DANIR is one of the promising *in vivo* fluorescent markers developed due to its high emission at λ>650 nm and its ability to cross the blood-brain barrier, and target Aβ deposits. Moreover, DANIR conjugated with Aβ fibrils exhibit higher fluorescence enhancement than separately.

Computational simulations were performed to investigate the properties of DANIR molecule as a marker for Aβ detection. The ground and excited state properties were calculated by using Density Functional Theory, DFT and Time-Dependent Density Functional Theory, TD-DFT with the Gaussian09 package. Several functionals and basis sets were tested, since charge transfer transitions are known to be challenging for TD-DFT. Results show that the CAM-B3LYP functional in combination with the 6-31+G** basis set provides values in good accordance with experimental data. The binding site search and refinement of DANIR interacting with fibril's models was carried out by the Protein Energy Landscape Exploration program. PELE is a combination of Monte Carlo stochastic approach and protein structure prediction algorithm.

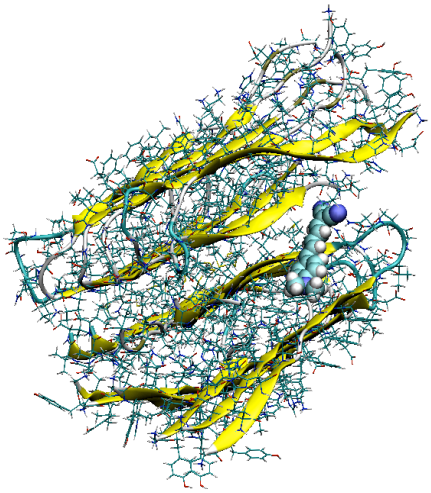

**Figure.** Structure of interacting DANIR's molecule with β-amyloid fibriles.

**P20**

# Genetic code optimality as a multiobjective optimization problem

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We tried to verify a hypothesis assuming that the canonical genetic code evolved to minimize the effects of deleterious mutations and translational errors [1, 2]. Therefore, we compared the canonical genetic code with the best and the worst possible alternatives. However, under the assumption that all hypothetical codes consist of 64 nucleotide triplets encoding 20 amino acids and stop translation signals, the total number of possible codes is greater than $1.51 \times 10^{84}$. Hence, it seems reasonable to adopt some additional restrictions on the set of hypothetical genetic codes to reduce the potential search space. In addition, these limitations allow us to consider various aspects of possible optimality of the canonical genetic code. In this work, we examined the two models of genetic codes:

1. Canonical block model (CB), which preserves the characteristic codon block structure of the canonical genetic code. To generate potential genetic codes we just permuted the assignments of amino acids to the codon blocks.
2. Unrestricted structure model (US). To generate potential codes, we randomly divided 64 codons into 21 non-overlapping sets ensuring, that each of these sets is not empty.

For each of these models, we assumed an objective function, which was the difference in polarity measure of amino acids substituted by a single point mutation. We calculated such substitution costs separately for three codon positions. The obtained three functions were used to find the best and the worst possible alternative codes. It was done by applying a version of SPEA2 algorithm [3], which is widely used as a procedure to explore search spaces of potential solutions under multiobjective constraints. The results showed that the canonical genetic code has a tendency to minimize the effects of harmful mutations. The code was closer to the best solutions than to the worst ones. In the CB model, the canonical code was closer to the best codes than in the US model because of different restrictions imposed on the models. Nevertheless, we confirmed the hypothesis about minimization properties of the canonical genetic code.

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**P21**

# AmyLoad – web service dedicated to amyloidogenic proteins

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A significant growth in the number of patients with neurodegenerative diseases, such as Alzheimer's or Parkinson's disease, has been observed recently. Studies show that these diseases are related to the occurrence of specific, amyloidogenic protein sequence fragments, which are prone to aggregate. The analysis of these fragments can provide new knowledge about mechanisms of neurodegenerative diseases development. In the Internet, different websites can be found, which contain sets of amyloidogenic sequences. However, these sets are typically represented by the plain text, hence difficult to browse or use for more advanced analysis or modelling.

We present the AmyLoad web portal which gathers amyloidogenic sequence fragments from different sources, such as WALTZ-DB [1], AmylHex [2], AmylFrag [2], and a great number of publications reporting such sequences. AmyLoad provides easy way to filter the data, e.g. according to fragment length, subsequence occurrence, or the protein name. Selected fragments, along with more extended information and references, can be downloaded in one of several supported file formats. Also, AmyLoad allows users to add their own sequences, which can be later available in the database. Finally, our portal provides the tools for analysis of FASTA sequences with regard to the occurrence of amyloidogenic fragments. For this purpose, different methods, such as FoldAmyloid [3], AGGRESCAN [4], and FISH [5] are implemented. The AmyLoad website provides more advanced and comfortable ways of studying amyloidogenic sequences.

The website is available at http://comprec-lin.iiar.pwr.edu.pl/amyload/.

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**P22**

# Constructing a library of 3D RNA conformations

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Understanding of RNA 3D structure is becoming more and more important, especially nowadays, when the number of experimentally derived structures is constantly increasing and new methods for structure *in silico* modeling are developed. It seems even more crucial if one takes into account the unprecedented lengths and complexities of newly discovered RNA structures. This induces the necessity of constant improvement of the methods for structure analysis. To support this issue, we have constructed a library of 3D conformations which contains representative samples of nucleotides derived from experimentally confirmed RNA structures.

In our routine of library construction, nucleotides are first filtered, preprocessed and transformed into real-value feature vectors. The data are next clustered to obtain prototypes of the inherent classes including common substructures. A selection of clustering algorithm and good data representation is crucial for the scheme. In our approach, each RNA nucleotide is transformed into a vector of torsion angle values. This trigonometric representation is useful and in some applications more advantageous than a standard Cartesian coordinate system [1]. Furthermore, a vector of torsion angle values can be easily separated into subvectors for backbone, ribose and base residue components. We employed such data partitioning and performed clustering with Median Neural Gas in a hierarchical manner separately for subvectors. The final cluster centers were selected as representatives of a larger group of similar shapes. When extracted and properly annotated, these cluster centers became part of a library of 3D RNA conformations.

Hereby, we present the aforementioned routine that resulted in constructing a library of representative 3D RNA conformations [2]. It was applied for a set of high resolution, experimentally determined RNA structures. The resulting library was evaluated in domain independent ways as well as from a structural point of view. The proposed pipeline can be reused for a different set of RNA structures or with another clustering algorithm to obtain a variety of libraries. Then, another application may apply the knowledge gathered in such library to assess the quality of a given structure, predict a new three-dimensional model or refine an existing one.

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# SEKCJA CHEMII MEDYCZNEJ

## Postery (P23-P109)

**P23**

# Positive allosteric modulator of μ opioid receptor disrupts sodium ion-related receptor inactivation

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Allostery is a source of great complexity of function of biological macromolecules. Allosteric modulators have great potential for being used as potent and safe drugs. The benefits would include preservation of physiological spatial and temporal patterns of protein action, limited risk of overdosage, improved receptor subtype selectivity and, possibly, functional selectivity. The number of known positive and negative allosteric modulators (PAM and NAM, respectively) of GPCRs is increasing, as well as number of GPCRs targeted by modulators. One of such new compounds is BMS986122, selective PAM of human μ opioid receptor (MOR) [1]. The modulator exerts complicated action apparently related to extracellular sodium ions concentration, with pronounced probe dependence [2]. In order to investigate the interplay between full agonist, modulator, sodium ion and MOR itself we employed molecular docking and molecular dynamics simulations. A full agonist R-methadone (RME) and BMS986122 were docked to active-state model of MOR in complex with a G protein [3]. The model has proven to be of high quality – comparison to the recently solved active-state murine MOR in complex with a camelid antibody [4] shows Cα RMSD value of 2.6 Å for complete structures and 1.91 Å for transmembrane bundle. The most promising docking positions were simulated for 20 ns in raft-like membrane. Stable positions were simulated for additional 30 ns. The best pose was selected. A set of 400 ns-long simulations of the receptor with RME, RME+sodium ion at Asp 2.50, as well as RME, sodium ion and BMS986122 were performed. Simulations were run in triplicate, and additional simulation of the unliganded receptor was performed for reference. Analysis of the results reveals that $Na^+$ disturb intra-receptor transmembrane water chain formation. Presence of BMS986122 reverse this effect. The allosteric signal is probably transmitted through the $7^{th}$ transmembrane helix of MOR.

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**Acknowledgements**
The research was partially performed during the postdoctoral fellowship of Agnieszka A. Kaczor at University of Eastern Finland, Kuopio, Finland under Marie Curie fellowship.The work was supported by the Foundation for Polish Science (TEAM 2009-4/5 Program). Calculations were partially performed under a computational grant by Interdisciplinary Center for Mathematical and Computational Modeling (ICM), Warsaw, Poland, grant number G30-18, under resources and licences from CSC, Finland, and under computational grant by Partnership for Advanced Computing in Europe (PRACE), Tier-1 project ALLOTRANS.

**P24**

# Alkane, alkene and alkyne derivatives of betulin

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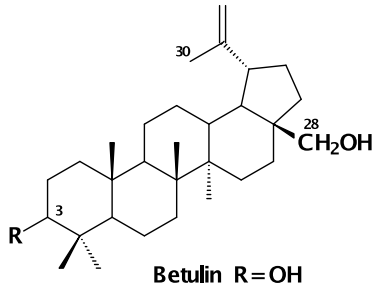
Betulin (betulinic alcohol) is a naturally occurring pentacyclic triterpene, which was first isolated in 1788 by Lowitz through sublimation of white birch bark. Betulin can by modified at C28, C3 and C30 positions into derivatives, which reveal a wide spectrum of biological activity such as anticancer, antiviral, antibacterial, antimalarial and anti-inflammatory [1-3].




**Betulin R=OH**
**Betulone R=O**

The aim of this study was the synthesis and evaluation of cytotoxic activity of derivatives of betulin and betulone, which contained alkane, alkene or alkyne groups at the position C28. The starting material, betulin was isolated from the birch bark with dichloromethane extraction. Treatment of betulin with carboxylic acids and chloroformates produced esters, which oxidation with pyridinium chlorochromate (PCC) gave derivatives of betulone. The chemical structure of new derivatives were determined on the basis of their $^{1}H$, $^{13}C$-NMR, IR and MS spectra. The obtained compounds were tested for anticancer activity *in vivo* against human and murine cell lines. The described method is experimentally simple and good in the yield and practically, seems to be available for the obtainment compounds. Resulting derivatives exhibit interesting anticancer activities.

[1] Alakurtti S., Mäkelä T., Koskimies S. et al. *Eur. J. Pharm. Sci. 29* (2006) 1.

[2] Drąg-Zalesińska M., Wysocka T., Borska S. et al. *Biomed. Pharmacother*. 72 (2015) 91.

[3] Boryczka S., Bębenek E., Wietrzyk J. et al. *Molecules.* 18 (2013) 4526.

**Acknowledgements**
This work was supported by the Medical University of Silesia in Katowice, Poland. Grand No KNW-1-002/N/5/0.

**P25**

# The combined treatment with novel platinum(II) complex and anti-MUC1 increases apoptotic response in MCF-7 breast cancer cells

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MUC1 was ranked by the National Cancer Institute as the most promising candidate tumor antigen with a high clinical potential. Mucin 1 is also involved in the phenomenon of drug resistance and in the inhibition of induction of apoptosis. Increased expression of MUC1 induces thyroid cancer cell resistance to cisplatin, docetaxel and doxorubicin and it induces the resistance to trastuzumab in breast cancer cells. Understanding the pathways by which new platinum(II) complex with anti-MUC1 induce cell death can provide information necessary to target specific cell death pathways in the treatment of breast cancer. In our study the induction of apoptosis by new platinum complex (Pt12) with anti-MUC1 in human MCF-7 breast cancer cells was confirmed by several biochemical markers, such as: phosphatidylserine externalization, loss of mitochondrial membrane potential ΔΨm, and DNA degradation, Bax and caspase -8, -9 levels.

Our experiments carried out with flow cytometry assessment of annexin V binding revealed that Pt12 with anti-MUC1 inhibited the proliferation of MCF-7 cells by increasing the number of apoptotic and necrotic cells. Most apoptotic pathways converge on the mitochondria, inducing the disruption of the mitochondrial membrane potential. It is an early and already irreversible stage of apoptosis. Exposure to Pt12 with anti-MUC1 resulted in a decrease of mitochondrial membrane potential. DNA fragmentation is also associated with apoptosis. The TUNEL assay was performed after treating MCF-7 cells with compounds for 24 hours. A highest increase in the percent of TUNEL positive cells was observed after incubation of Pt12 with anti-MUC1 in comparison to monotherapy (cisplatin) or combined treatment (cisplatin + anti-MUC1). The highest concentration of pro-apoptotic markers (Bax, caspase-8 and caspase-9) was detected after 24 hours of incubation with Pt12 and anti-MUC1.

The compounds used in combination induced apoptosis of breast cancer cells via mechanisms dependent on caspases activation and associated with mitochondrial membrane potential disruption.

**Acknowledgments**

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**P26**

# Synthesis and antitumor activity of novel lutidine-platinum complexes

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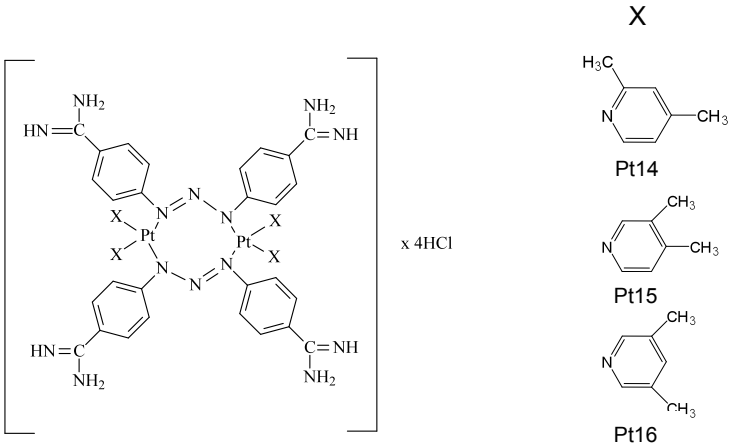
Polynuclear platinum complexes constitute a novel class of prospective anticancer agents that have shown some peculiar activities as compared with mononuclear platinum compounds [1]. The adducts formed by multi-nuclear platinum complexes are vastly different from the adducts formed by cisplatin [1, 2]. It has been suggested that the distortions induced by these complexes are only weakly recognised by DNA repair proteins. Structurally novel platinum complexes that bind to DNA differently than cisplatin may have distinct cytotoxicity and side effect profiles. The present study was undertaken to extend our recent findings related to the antineoplastic activity of novel dinuclear platinum(II) complexes with berenil and amine ligands [1, 2].




In this study we examined the impact of the compounds of formula $[Pt_2L_4(berenil)_2]Cl_4$ where L is 2,4-lutidine (Pt14), 3,4-lutidine (Pt15) or 3,5-lutidine (Pt16) (Fig. 1) on viability of breast cancer cells using the MTT assay and inhibition of [$^3$H]thymidine into DNA in both MDA-MB-231 and MCF-7 breast cancer cells. The cellular responses of human breast cancer cells to dinuclear platinum(II) complexes has been studied using cisplatin as a reference. To determine the nature of cell death induced by Pt14 – Pt16 in human MDA-MB-231 and MCF-7 breast cancer cells, we measured cell death by flow cytometric analysis after annexin V-FITC and propidium iodide staining. Evaluation of the cytotoxicity of Pt14–Pt16 employing a MTT assay and inhibition of [$^3$H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that these compounds were more potent antiproliferative agents than cisplatin.

[1] Bielawski K., Czarnomysy R., Muszyńska A. et al. *Environ. Toxicol. Pharmacol. 35*(2) (2013) 254.
[2] Bielawska A., Popławska B., Surażyński A. et al. *Eur. J. Pharmacol.* 643 (2010) 34.

**P27**

# *N*-alkylated arylsulfonamides of (aryloxy)ethyl piperidines: 5-HT$_7$ receptor selectivity *vs* multireceptor profile

*Vittorio Canale[1], Rafał Kurczab[2], Grzegorz Satała[2], Anna Partyka[3], Magdalena Jastrzębska-Więsek[3], Karolina Słoczyńska[4], Tomasz Kos[5], Elżbieta Pękala[4], Piotr Popik[5], Andrzej J Bojarski[2], Anna Wesołowska[3], Paweł Zajdel[1]*

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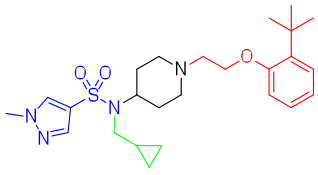
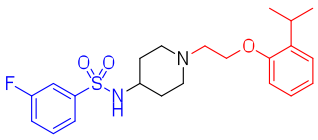
According to recent preclinical data, antagonism at 5-HT$_7$ receptors (5-HT$_7$Rs) may represent a clinically relevant target for the treatment of depression, negative symptoms of psychosis as well as for the treatment of memory dysfunction in cognitive disorders [1,2]. Continuing our efforts in development of potent 5-HT$_7$R antagonists (i.e. PZ-1404) [3,4], we designed a series of *N*-alkylated arylsulfonamide derivatives of (aryloxy)ethyl piperidines. Structural modifications, which comprised the introduction of an *N*-methyl and *N*-cyclopropylmethyl moiety at the sulfonamide as well as the diversification of an *ortho* substituent at the (aryloxy)ethyl fragment, were aimed to establish the influence of these modifications on 5-HT$_7$ receptor affinity and selectivity over related monoaminergic receptors (i.e., 5-HT$_{1A}$, 5-HT$_{2A}$, D$_2$).



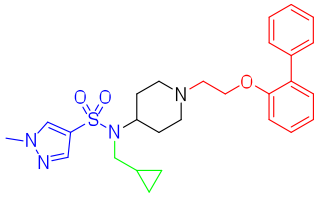
**31**

**$K_i$ (5-HT$_7$) = 49 nM $K_i$ (5-HT$_{1A}$) = 17770nM**
**$K_i$ (5-HT$_{2A}$) = 1479 nM $K_i$ (D$_2$) = 230 nM**
**FST (MED) = 1.25 mg/kg**
**NOR (MED) = 1 mg/kg**



**PZ-1404**

**$K_i$ (5-HT$_7$) = 9 nM $K_i$ (5-HT$_{1A}$) = 356 nM**
**$K_i$ (5-HT$_{2A}$) = 178 nM $K_i$ (D$_2$) = 102 nM**
**FST (MED) = 2.5 mg/kg**
**NOR (MED) = 1 mg/kg**



**33**

**$K_i$ (5-HT$_7$) = 10 nM $K_i$ (5-HT$_{1A}$) = 159 nM**
**$K_i$ (5-HT$_{2A}$) = 30 nM $K_i$ (D$_2$) = 16 nM**
**FST (MED) = 1.25 mg/kg**
**NOR (MED) = 1 mg/kg**

Synthetized compounds were identified as potent and selective 5-HT$_7$ receptor antagonists (i.e. **17** and **31**) or multimodal 5-HT/dopamine ligands with significant 5-HT$_7$/5-HT$_{2A}$/D$_2$ receptor antagonist properties (i.e. **20** and **33**). The most metabolically stable compounds **31** and **33** were further *in vivo* evaluated in forced swim test (FST) in mice and novel object recognition (NOR) task in rats, demonstrating distinct antidepressant-like and pro-cognitive properties (MED = 1.25 mg/kg and 1 mg/kg, *i.p*, respectively). Further studies in the area of selective 5-HT$_7$ receptor antagonist or mixed 5-HT/dopamine ligands might be beneficial to confirm their potential application in the treatment of CNS disorders.

[1] Matthys A. et al. *Mol. Neurobiol. 230* (2011) 555.

[2] Nikiforuk A. *CNS Drugs. 29* (2015) 265.

[3] Zajdel P. et al. *Eur. J. Med. Chem. 56* (2012) 348.

[4] Zajdel P. et al. *Med. Chem. Comm. 6* (2015) 1272.

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Supported by the National Science Center Grant No DEC-2012/05/B/NZ7/03076.

**P28**

# Synthesis and pharmacological evaluation of the phenylacetamide and arylsulfonamide derivatives of tricyclic theophylline

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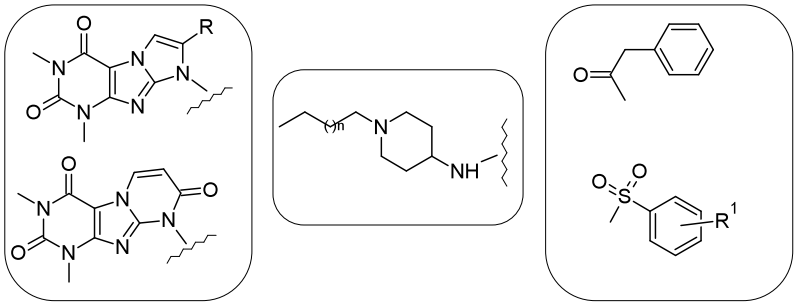
For years, our attention has been focused on the development of long-chain arylpiperazines (LCAPs) with the complex terminal part based on a purine moiety. Annulated derivatives of theophylline with an LCAP moiety demonstrated high affinity towards serotonin 5-$HT_{1A}$ and moderate affinity to 5-$HT_{2A}$ and 5-$HT_7$, and the dopaminergic $D_2$ receptor site. The fusion of an additional heterocyclic ring with the 7,8-double bond of theophylline was found to produce the most potent compounds for the 5-$HT_{1A}$ receptor. The study allowed us to identify some potent 5-$HT_{1A}$, 5-$HT_7$ and mixed 5-$HT_{1A}$/5-$HT_7$ receptor ligands with additional affinity for dopamine $D_2$ receptors [1,2].



For further exploration of the multimodal strategy for obtaining novel antipsychotic agents, in the current studies the library of new tricyclic theophylline derivatives with 4-amino-piperidine moiety instead of 1,4-piperazine was synthesized. Moreover, the phenylacetamide or arylsulfonamide derivatives of them were prepared, in analogy to PZ-766 [3] and Lu AE51090 [4]. This structural modification was made to provide compounds with increased affinity for 5-$HT_{2A}$, 5-$HT_7$ and $D_2$ receptors. Moreover, compounds were designed as potential ligands, which may effect on muscarinic ($M_1$) receptors and inhibit of PDE4/PDE10. Muscarinic receptor expression is decreased in cortical regions of schizophrenia patients, hence, it has been suggested that $M_1$ receptor agonism has a role in the treatment of Alzheimer's disease and cognitive impairment associated with schizophrenia. Since PDE4/PDE10 have been shown to play distinct roles in processes of emotion and related learning and memory processes, selective PDE inhibitors, by preventing the breakdown of cAMP and/or cGMP, modulate mood and related cognitive activity.

[1] Zagórska A., Pawłowski M., Siwek A. et al. *Arch. Pharm. Chem. Life Sci.* 346 (2013) 832.

[2] Zagórska A., Kołaczkowski M., Bucki A. et al. *Eur. J. Med. Chem*. 97 (2015) 142.

[3] Zajdel P., Kurczab R., Grychowska K. et al. *Eur. J. Med. Chem.* 56 (2012) 348.

[4] Sams A. G., Hentzer M., Mikkelsen G. K. et al. *J. Med. Chem.* 53 (2010) 6386.

**Acknowlegements**
The studies were supported by Polish National Science Centre grant DEC-2012/07/B/NZ7/01173 and Jagiellonian University Medical College grant K/ZDS/004654.

**P29**

# Cytotoxic activity of new 30-substituted derivatives of betulin

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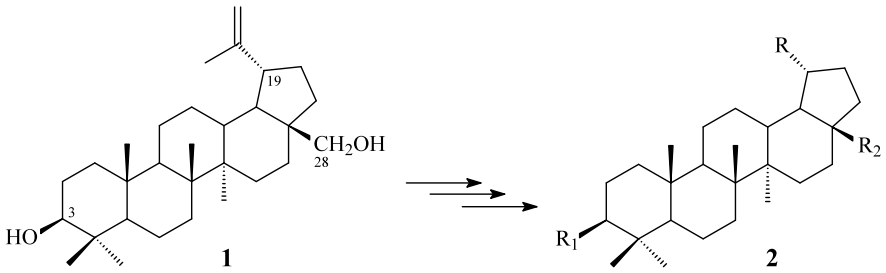
Natural plant products are an important source of the exploration and development of new anticancer drugs. Betulin, known for over 200 years, pentacyclic triterpene is isolated from bark of many species of birch. Betulin **1**, having two hydroxyl groups at C-3 and C-28 and an isopropenyl group at C-19, is a very good starting material for the preparation of new derivatives with a broad spectrum of biological activities, such as anticancer, antiviral, antimalarial, antibacterial, antiinflammatory, and hepatoprotective [1].

Introduction substituents containing a triple bond into the positions C-3 ($R_1$) and C-28 ($R_2$) of betulin, results in derivatives with increased cytotoxicity, and provides a starting point for further modification of the compound [2,3].




In this work we have synthesized new betulin derivatives with moiety containing phosphorus atom at the C-19 position. The obtained compounds **2** were tested for their antiproliferative *in vitro* activity.

[1] Alakurtti S., Mäkelä T., Koskimies S., et al. *Eur. J. Pharm. Sci.* 29 (2006) 1.

[2] Boryczka S., Bębenek E., Wietrzyk J. et al. *Molecules* 18 (2013) 4526.

[3] Majeed R., Sangwan P.L., Chinthakindi P.K. et al. *Eur. J. Med. Chem*. 63 (2013) 782.

**Acknowledgements**
This work was supported by the Medical University of Silesia in Katowice, Poland. Grant No KNW-1-002/N/5/0.

**P30**

# Synthesis and physicochemical properties of potential new alkylating drugs for anticancer therapy

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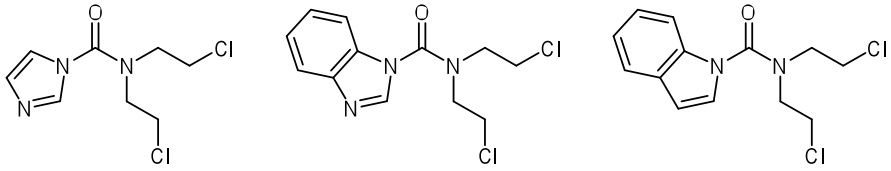
Tumors are a growing health problem worldwide. They are one of the major causes of death. World Cancer Report is reporting that global cancer rates could increase to 27 million new cases in the year 2030. In the world during the year from cancer die more than 8 million people, where in Poland the number of deaths is more than 90 thousands per year.[1] The report also reveals that cancer has emerged as a major public health problem in developing countries, matching its effect in industrialized nations. It is therefore still very much attention is paid to the search for the new chemotherapeutic agents and the new cancer therapies. The poor selectivity and very low efficiency of cytostatic drugs currently used in conventional cancer chemotherapy causes that studies are conducted to find new drugs more effective, more selective to cancer cells, and with lower side effects. Among the drugs used against cancer are alkylating agents. This is a group of cytostatics commonly used and best known. Their mechanism of action involves the formation of chemical bonds with the functional groups of molecules such as DNA or protein. Among many of the agents isophosphoramide mustard (iPAM) was selected, which is active, cytotoxic metabolite of ifosfamide (IF), a widely used anticancer alkilating drug.[2] Our reaserch concentrated on the synthesis, the selected chemical properties, stability, antitumor activity and toxicity of new compounds, potential drugs for anticancer therapy. The aim of research was to find a potential drug, which is minimally metabolized by liver enzymes, and will be find more effective, selective to cancer cells. We designed and synthesized such drugs, which contain a imidazole, benzimidazole or indole coupled with isophosphoramide mustard, a DNA alkylating agent, by means of urea group:




Drugs should have a good stability under phisiological conditions. Solubility and hydrolytical stability of our potential drugs was examined. Further studies of the antitumor activity of the obtained compounds are in progress.

[1] Stewart B.W., Wild C.P. *World cancer report 2014*, World Health Organization, International Agency for Research on Cancer. (2014) 16.

[2] Cytarska J., Skowerski K., Jaworski S. et al. *Letters in Drug Design & Discovery*. 12 (2015) 172.

**Acknowledgements**
This study was supported by Nicolaus Copernicus University (Project No. 786/2015).

**P31**

# Spectroscopic studies of *econazole* and *sulconazole* – experimental analysis and theoretical calculations

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[2]Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, 6 Grunwaldzka St, 60-780 Poznań, Poland
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Imidazole derivatives are used mainly as antifungal, antibacterial and antiprotozoal agents. As antifungal drugs, imidazole derivatives inhibit the synthesis of normal membrane sterols in fungi [1]. Two of the most important compounds among antifungal imidazoles are *econazole* **1** and *sulconazole* **2** (**Fig. 1**). Continuing our investigations on the interactions of biologically important azahetarenes with the environment [2,3], we focused our attention on the IR, UV and NMR spectral analyses of **1** and **2**. The calculated spectra were correlated with experimental data using the DFT formalism and B3LYP/6-31G(d,p), CAM-B3LYP/6-31G(d,p), B3LYP/6-311+G(d,p) and PBE1PBE/6-31G(d,p) approaches both in gaseous phase, as well as the CPCM solvation model and water, methanol and acetonitrile as solvents (for UV calculations we used TD DFT and LR PCM approach). The PBE0 functional revealed a stronger correlation of the IR spectrum with the corresponding experimental one (**1**, **Fig. 2**), i.e. lower values of the relative percentage errors and mean absolute deviation parameters. The similarity of the estimated UV spectrum of **1** and **2** with the experimental data depends on the solvent used. However, the calculations in gaseous phase (**2**, **Fig. 3**) gave more correct results and showed the PBE0 to be a suitable functional. The NMR calculations also revealed convergence with the experimental data.

**Fig. 1**

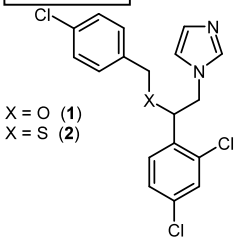



**Fig. 2**

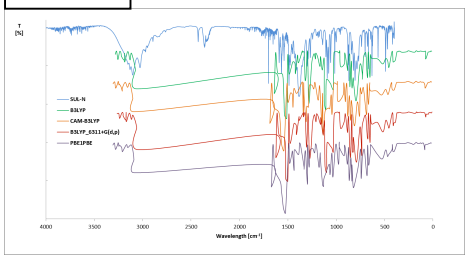
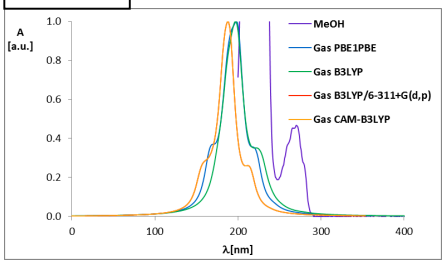



**Fig. 3**




[1] Mital A. *Sci. Pharm*. 77 (2009) 497.

[2] Kujawski J., Bernard M.K., Jodłowska E. et al. *J. Mol. Model.* 21 (2015) 105.

[3] Kujawski J., Czaja K., Doskocz M. et al. *Comput. Theor. Chem.* 1059 (2015) 45.

**Acknowledgements**
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**P32**

# The cytotoxic activity of *Nigella sativa* oil in human breast cancer cells

*Anna Czajkowska*[1], *Natalia Pawłowska*[2], *Agnieszka Gornowicz*[1], *Bożena Popławska*[1], *Krzysztof Bielawski*[2], *Anna Bielawska*[1]

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*Nigella sativa* Linn. is an annual herb from the *Ranunculaceae* family, that is commonly known as black seed or black cumin [1,2]. It exhibits numerous medicinal properties and has been used as a natural remedy for the prevention and treatment of different diseases and ailments for thousands of years [1]. The source of active ingredients are the seeds of this plant. They contain fixed oils, proteins, alkaloids, saponin and essential oil. The seeds and produced from them oil have similar therapeutic values. Both forms possess anti-inflammatory, antioxidant, antidiabetic and antimicrobial properties [1,2]. Their pharmacological activity also include protection against nephrotoxicity and hepatotoxicity induced by chemicals. Moreover, they are known to be effective against several cancer cell lines and have a very low degree of toxicity in normal cells [1,2].

Our study was conducted to evaluate *Nigella sativa* seed oil for its cytotoxic activity in human breast cancer cells. We examined the impact of the tested oil on viability and DNA biosynthesis in MCF-7 and MDA-MB-231 breast cancer cells. The viability of breast cancer cells was performed using MTT assay. The DNA biosynthesis was checked by incorporation of [$^3$H]-thymidine into DNA. The viability and [$^3$H]-thymidine incorporation were analyzed in MCF-7 and MDA-MB-231 breast cancer cells after 24 hours of incubation with different concentrations of the tested oil.

Performed experiments showed that the *Nigella sativa* seed oil had cytotoxic effect in MCF-7 and MDA-MB-231 breast cancer cell lines. The $IC_{50}$ values in MCF-7 and MDA-MB-231 breast cancer cells were 5.9 mg/ml and 1.2 mg/ml, respectively. Moreover, the oil of *Nigella sativa* seed inhibited the DNA biosynthesis in both cell lines. The tested oil led to higher antiproliferative effect in MDA-MB-231 breast cancer cells in comparison to MCF-7 cells. The $IC_{50}$ value in MCF-7 breast cancer cells was 8.2 mg/ml and in MDA-MB-231 cells it was 0.7 mg/ml.

Experimental validation showed that the *Nigella sativa* seed oil was effective as cytotoxic agent in MCF-7 and MDA-MB-231 breast cancer cells. Our results provide promising directions for cancer chemoprevention and treatment.

[1] Aftab A., Asif H., Mohd M. et al. *Asian Pac. J. Trop. Biomed*. 3(5) (2013) 337.
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**P33**

# Characterization of new green fluorophores based on quinoline scaffold

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Fluorescence phenomenon is widely used in chemical and life sciences. As a tool of investigation, it allows us to gain insight into a world of biological structures and cellular mechanisms. Moreover, fluorescent dyes are commonly used to imaging of cancer and neurological diseases, as indicators for aminoacids and sugars or as micro- and macroelements sesnsors [1]. Molecule has to meet a number of conditions to become a fluorescent dye, whose are not always easy to gain. That is why searching for new, better fluorophores is still of interest.This work presents synthesis and characterization of over a dozen of novel Schiff bases based on a quinoline scaffold that exhibit fluorescence properties.

Starting from 2-methylquinoline, we obtained 2-(4-amino-trans-styryl)quinoline, which constituted a starting material for further Shiff bases synthesis [2]. In result we synthesized thirteen hitherto undescribed compounds. They exhibit fluorescence properties with emission in green light region and quite large Stokes shifts (around 100 nm). Moreover, they show positive and negative solvatochromism dependent on change of dipole moment of molecule in ground and excited state.

TD DFT calculations were carried out with use of two exchange-correlation functionals – B3LYP and CAM-B3LYP, the latter seems to be a better choice. Examination of biological activity did not implicated any significant results – in general, all of them turned out to be inactive.

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**P34**

# The mechanism of induction of apoptosis by the novel lutidine-platinum complexes in breast cancer cells

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The cisplatin is the most commonly used chemotherapeutic drugs. Despite its success, the clinical usefulness of cisplatin is limited by its severe side effects such as dose-dependent nephrotoxicity, nausea and vomiting, ototoxicity, neurotoxicity, and myelosuppression. The need for alternatives to cisplatin has consequently inspired further work towards the development of novel platinum-based drugs with improved and or complementary properties. The search for more effective derivatives and selective in action led to synthesize new lutidine-platinum(II) complexes of formula: $[Pt_2L_4(berenil)_2]Cl_4$, where L is 2,4-lutidine (Pt14), 3,4-2,4-lutidine (Pt15), 2,4-lutidine (Pt16).

The aim of this study was to examine the influence of three to lutidine-platinum(II) complexes (Pt 14 – Pt 16) on the mechanism of apoptosis of breast cancer cells MCF-7 and MDA-MB-231. Results showed high probability that obtained compounds possess antineoplastic activity. Our results confirm that compounds Pt14 – Pt16 are more potent antiproliferative agents than cisplatin on the breast cancer cells. The evaluation of the effects of the compounds on the induction of apoptosis have been made using Annexin V-FITC/propodium iodide stainig procedure. Moreover we investigated the effect of novel compounds on the mitochondrial potential changes, activation of caspase 3, 8 and 9, and DNA fragmentation. Our results suggest that apoptosis of cells in the presence of Pt14 – Pt16 follows the mitochondrial pathway, with the decrease in mitochondrial membrane potential and activation of caspase 9, as well as by the external pathway with the significant increase expression caspase 8. The activation of caspase 3 concomitantly with increase DNA fragmentation confirmed also that apoptosis was the main response of breast cancer cells to Pt14 – Pt16 treatment. The obtained results in the present study demonstrated the cytotoxic activity of new lutidine-platinium(II) complexes can be connected with their ability to induction of apoptosis.

**Acknowledgements**
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**P35**

# Lipophilic side chain – influence on the activity of bicyclic imidazole-4-one derivatives on GPR18 and GPR55

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GPR18 and GPR55 are the orphan G protein-coupled receptors (GPCRs) that interact with certain cannabinoid (CB) receptor ligands. GPR18 was reported to be activated by $\Delta^9$-THC and endogenous CB receptor agonist annandamide [1]. GPR55 has been found to be activated by certain CB receptor ligands, including plant cannabinoids: $\Delta^9$-THC and cannabidiol as well as $CB_1R$ inverse agonist - rimonabant. Although so far very little is known about (patho-)physiological roles of GPR18; as it is highly expressed in testes, spleen, endometrium and metastatic melanoma cells, it is thought to may be useful in novel therapy of endometriosis and cancer. GPR55 antagonists are considered as potential candidates for treatment of cancer, neuropathic pain and osteoporosis [2]. The development of potent and selective ligands for GPR18 and GPR55 is required to further study the receptors role in health and disease and to explore their potential as drug targets. In our studies bicyclic imidazole-4-one derivatives were discovered as the first structures showing antagonistic activity for GPR18 (and GPR55) [3].

In the present investigation, influence of lipophilic side chain of 3-substituted arylidene imidazo-[2,1-b][1,3]thiazin-3-ones on the activity of this bicyclic scaffold towards GPR18 and GPR55 was examined. The length of linker connecting (un-)substituted aryl with arylidene part of moieties was changed. Additionally, oxygen atom was introduced to the linker. It was stated that changes in the character of lipophilic part of new compounds influenced their activity and selectivity. Compounds showing selectivity towards GPR18 and GPR55 were found among the new series of structures.

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**Acknowledgements**
Partial support by Polish National Science Center (DEC. 2013/11/8/N27/04865) and GLISTEN: COST Action CM1207 financed by EU-FP7 is greatly acknowledged.

**P36**

# Studies on influence of linker length on pharmacological and „drugability" properties in group of 3-benzyl-5,5-dimetylhydantoin GPCR-agents

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Arylpiperazine moiety is a very popular chemical class present in many biologically active compounds. The arylpiperazine agents are particularly widespread for serotonin receptor 5-$HT_{1A}$, 5-$HT_7$ and all $\alpha_1$-adrenoceptors subtypes. The latest lines of evidence indicated their antiarrhythmic and antihypertensive action [1]. In this context, the search for selective $\alpha_1$-adrenoceptor antagonists has been, and still is, an important topic in medicinal chemistry [2]. Our previous studies for hydantoin derivatives have shown that, a role of substituent at 3-position of hydantoin was important for selective interactions with the GPCRs. The current study is concentrated on newly developed phenylpiperazine derivatives of aromatic methylhydantoin differing in mutual positions of methyl and phenyl moieties [1]. We moved aromatic moiety at 5-position of hydantoin [1] to 3-position [3]. It allowed to define the lead structure **Cpd 22a** with the highest activity for $5HT_{1A}$, $5HT_7$ and $\alpha_1$-receptors. It contributed to searching for new $\alpha_1$-adrenoceptor antagonists among phenylpiperazine derivatives of methylhydantoin. In the present work, further modifications of the lead have been performed. The aim was to synthesize 14 compounds and study on their $\alpha_1$-adrenoceptor affinity, drug-likeness, and structure–activity relationship (SAR). All the compounds were obtained as hydrochlorides by the three-step synthesis, using 5,5-dimethylhydantoin as a starting product.

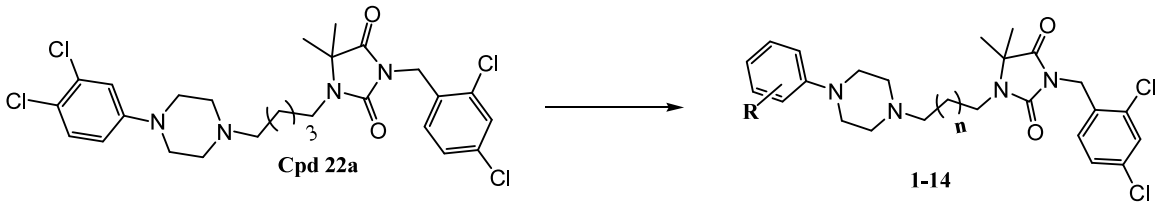


**Fig. 1**

The affinity for $\alpha_1$-adrenoceptors were evaluated in radioligand binding assays using [$^3$H]-prazosin as a selective radioligand. Compounds were evaluated on their "drugability" and toxic effects using OSIRIS program. The obtained results showed the best pharmacological properties for two compounds possessing 2-methoxyphenylpiperazine moiety with butyl and pentyl linker, respectively.

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**P37**

# Acyclic analogues of guanosine with carbamoyl linker

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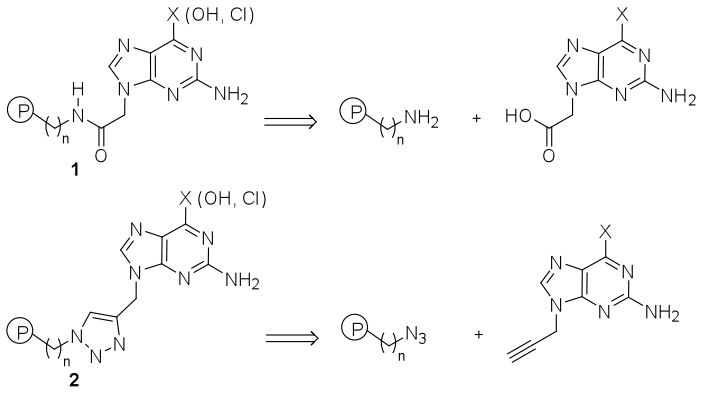
Acyclic phosphonate analogues of nucleosides belong to the important group of medications used in treatment of viral infections. Adefovir and tenofovir are active against DNA viruses and retroviruses. Ganciclovir and its prodrug valganciclovir are commonly used for treatment of cytomegalovirus infections. The specificity of antiviral activity of these compounds strongly depends on structural features of aliphatic chain installed as a sugar replacer, whereas a choice of nucleobases is mostly limited to adenine, guanine and 2,6-diaminopurine. Various guanine-containing analogues of nucleosides have been reported as potent antiviral agents [1-4].

As a continuation of our ongoing project we proposed new acyclic phosphonate nucleoside analogues containing 2-amino-6-chloropurine and guanine as nucleobases of general formulae **1** and **2** with intension to study their antiviral and cytostatic properties.

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[3] Vertuani S., Baldisserotto A., Varani K. et al. *Eur. J. Med. Chem.* 54 (2012) 202.

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

**P38**

# Search for butyrylcholinesterase inhibitors among polycyclic derivatives of azaphenothiazines

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Alzheimer's disease (AD), considered to be the most common form of dementia, is an incurable, progressive neurodegenerative disorder. It affects 4-8% of the elderly population worldwide [1]. The pathophysiology of AD is complex, multifactorial and not fully understood. Histopathologically it is characterized by the presence of extracellular senile plaques and intracellular neurofibrillary tangles. The senile plaques are formed by the accumulation of the amyloid β protein while neurofibrillary tangles are composed of hyperphosphorylated tau protein. AD is also characterized by massive cell loss, especially of cholinergic neurons in the basal nuclei, leading to decrease of cholinergic neurotransmission and imbalance between acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity. As the cholinergic system is mainly affected, the cholinergic pathway is still one of targets in novel anti-AD agents discovery. Both enzymes remain valuable targets in anti-AD drug discovery studies [1,2]. Our recent studies led to the identification of potent BuChE inhibitors among tetra- or pentacyclic derivatives of azaphenothiazines [3]. The aim of the presented study was to discover novel BuChE inhibitors among novel tricyclic derivatives of dipyridothiazines and tetracyclic derivatives of quinobenzothiazines using in silico and in vitro screening methods. From an in-house library of compounds, 120 heterocyclic molecules derived from the azaphenothiazine scaffold were chosen for virtual screening. Based on results of the docking procedure, 25 derivatives of azaphenothiazine were selected for biochemical tests using spectrophotometric Ellman's assay. They represented tricyclic series of dipyridothiazines, tetracyclic derivatives of 9-fluoroquinobenzothiazine and quinobenzothiazine. Most of the tested compounds displayed moderate BuChE and weak AChE inhibitory potency at screening concentrations of 10 mM. The $IC_{50}$ values for active BuChE inhibitors were in the low micromolar range.

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**Acknowledgements**
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**P39**

# The apoptotic activity of novel platinum(II) complex together with anti-MUC1 in MDA-MB-231 breast cancer cells

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An important goal of targeted therapy appears to be a transmembrane glycoprotein type I - mucin 1 (MUC1), which is overexpressed especially in breast cancer. The goal of the study was to check the effect of novel platinum(II) complex (Pt12) used with anti-MUC1 on the concentration of selected markers of apoptosis such as: Bax, caspase-8, -9 and caspase-3 by ELISA technique. The results were compared to treatment with cisplatin and cisplatin used in combination with anti-MUC1. The highest increase in pro-apoptotic Bax protein concentration was observed after combined treatment of Pt12 (20 μM) together with anti-MUC1 (10 μg/mL). The concentration of Bax was 145 ng/mL compared to reference compound cisplatin used with anti-MUC1 in the same doses, where the level of Bax was: 140 ng/mL. The increase was almost 3 times stronger in comparison to control, where cells were untreated. The strongest effect on caspase-9 releasement was determined after combined treatment with Pt12 (20 μM) and anti-MUC1 (10 μg/mL). The concentration of caspase-9 was 39 ng/mL. After cisplatin (20 μM) and anti-MUC1 (10 μg/mL) the level of caspase-9 was 29 ng/mL. All compounds increased the concentration of caspase-8 compared to control value, but the highest statistically significant increase was observed after Pt12 (20 μM) and anti-MUC1 (10 μg/mL), in comparison with cisplatin (20 μM) and anti-MUC1 (10 μg/mL), where the concentrations were: 1.02 and 0.86 ng/mL, respectively. Finally, we checked the concentration of caspase-3 in cell lysates after 24 hours of incubation with drugs used in monotherapy and combination therapy. The strongest statistically significant effect was observed after Pt12 (20μM) with anti-MUC1 (4.4 ng/mL). Our results proved that combination therapy is more effective strategy in increasing the levels of pro-apoptotic markers compared to monotherapy.

Our study proved that Pt12 together with anti-MUC1 strongly induced apoptosis in estrogen negative breast cancer cell line (MDA-MB-231). The apoptosis may go through extrinsic pathway associated with caspase-8 as well as intrinsic pathway connected with caspase-9 [1].

[1] Gornowicz A., Bielawska A., Czarnomysy R., et al. *Moll Cell Biochem* 2015 doi: 10.1007/s11010-015-2486-z.

**Acknowledgments**
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**P40**

# Application of the ring-closing metathesis for the synthesis of building blocks for biologically active compounds

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[2]Department of Green Chemistry and Innovative Technologies, Institute of Biomolecules Max Mousseron, University Montpellier 2, cc 1703, Place Eugène Bataillon, 34-095, Montpellier, France
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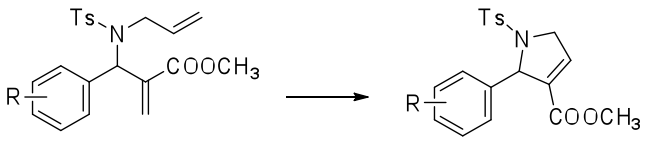
Ring-closing metathesis (RCM) has become an efficient tool widely used for the formation of carbon-carbon double bonds. In particular RCM has a large impact on pharmaceutical industry because the reaction allows for the formation of medium-to-large size carbocycles and heterocycles from acyclic dienes [1].

As a part of our ongoing project, focused on the identification of compounds with CNS activity based on a new scaffold consisting in three fused heterocylic rings, we decided to develop a method for obtaining substituted pyrroline **2**. Such compound might be regarded as a key building block in the synthetic pathway of various 5-HT receptor ligands.




**1** **2**

Herein we report a comparative study of the efficiency of selected RCM catalysts as well as the influence of solvent and reaction kinetics on the above transformation. Further investigation regarded the impact of various electrondonating and electronwithdrawing substituents in the phenyl ring.

The elaborated protocol allowed for the preparation of pyrroline **2** and its analogs in the more environmentally friendly way at the lower energy and money costs.

[1] Dassonneville, B.; Delaude, L.; Demonceau, A. et al. *Current Organic Chemistry* 17 (2013) 2609.

**Acknowledgements**
The study was partially financed by the grant-in-aid for young researchers and PhD students, Grant No K/DSC/001428.

**P41**

# Role of the aromatic substituent at position 5 for $D_2$/ $\alpha_1$-adrenoceptor action of novel ester-hydantoin derivatives of arylpiperazines

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The dopaminergic receptors $D_2$ as well as $\alpha_1$--adrenoceptors are important GPCRs biological targets involving in various diseases of central- or peripheral nervous systems. The dopamine $D_2$ receptors play an important role in neurodegenerative diseases, e.g. schizophrenia and Parkinson's disease as well as they influence on mood, mindfulness and sleep [1]. The $\alpha_1$-adrenergic receptors play a role in cardiac hypertrophy, effects on heart contractile function, cardiac rhythm and protection from ischemic injury [2]. Thus, their antagonists can have therapeutic usage as antiarrhythmic drugs. Our previous studies focused on hydantoin phenylpiperazine dervatives allowed to find the 3-ester compound **JH-38** that dispalyed significant and comparable affinity to both of the GPCRs. The compound has been selected as lead structure for further chemical modifications. This work is concentrated on the lead modifications to obtain a series of new compounds with conserved ester moiety at position 3, various substituent at phenylpiperzine phenyl ring and 5-methyl-5-aryl substitution at position 5 of hydantoin (Fig.1).

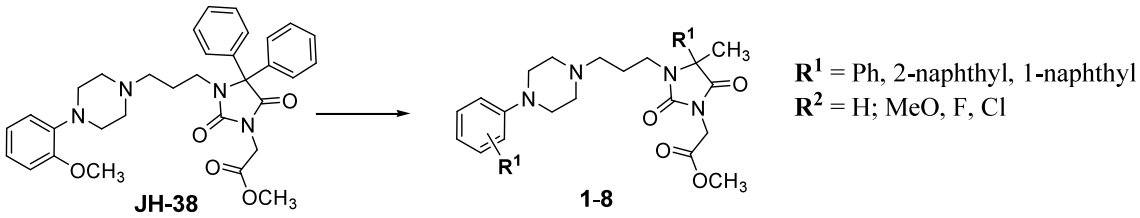


Fig. 1

The 4-step synthesis was carried to give the 8 final products (1-8, Fig. 1). The compounds were investigated on their affinity for the dopamine $D_2R$ and $\alpha_1$-AR in the radioligand binding assays. All of the tested compounds displayed higher activity for $\alpha_1$-AR than that for $D_2R$. The 5-naphthyl substituents were more profitable than the 5-phenyl ones.

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[2] Handzlik J., Bajda M., Zygmunt M. et al. *Bioorg. Med. Chem.* 20 (2012) 2290.

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**P42**

# The synthesis of fused heterocyclic building blocks and their application as core structures of GPCR ligands

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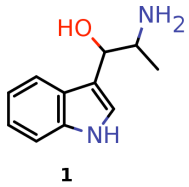
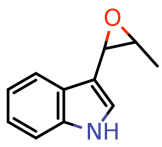
Indole is an exceptionally electron rich fused ring heterocycle. It is among the most widely distributed scaffolds in nature as well as one of the most important building blocks in medicinal chemistry. The unusually high abundance of indole nucleus in ligands of different biological activity led to its classification as 'priviledged structure'.[1]

Our search for selective aminergic GPCR ligands led us to the syntheses of heterocyclic molecules which can serve as substrates for derivatization, e.g. attachment of basic aminergic moiety, ring closure, reactions with various nucleophiles. Our efforts to obtain 2-amino-1-(1H-indol-3-yl)ethan-1-ol (**1**), yielded a straightforward method of synthesis of 3-(3-methyloxiran-2-yl)-1H-indole (**2**) - a very attractive building block for drug discovery which has not been mentioned in literature till date. This epoxide can be readily opened by numerous nucleophiles to yield potential GPCR ligands.

The synthesis of appropriate halocarbonyl compounds enables a very large chemical space to be covered. Herein we describe our way to concise method of preparation of 1-bromo-1-(1H-indol-3-yl)propan-2-one (**3**) and 1-bromo-3-(1H-indol-3-yl)propan-2-one (**4**). Despite the great versatility of **3** and **4** as starting materials for the total syntheses of alkaloids and potentially bioactive compounds, the synthesis of **3** has not been yet described, while the synthesis of **4** involves the use of extremely toxic reagent - diazomethane.[2]




**1**

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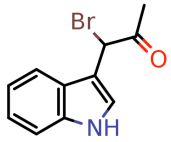
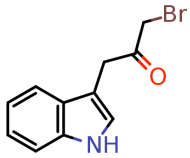



**3**




**4**

[1] Evans B. E., Rittle K. E., Bock M. G. et al. *J. Med. Chem.* 31 (1988) 2235.

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

**P43**

# Synthesis and $\alpha_1$-adrenoceptor affinity of novel hydroxypropyl derivatives of dimethylhydantoin

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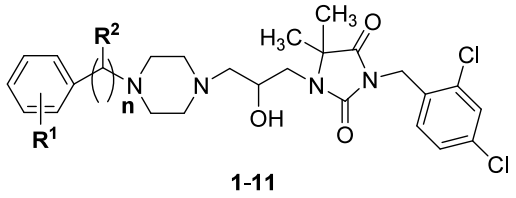
The selective $\alpha_1$-adrenergic receptor antagonists have important therapeutic perspectives as they are able to improve the urodynamic parameters and reduces the symptoms of benign prostatic hypertrophy. In this context, the search for selective $\alpha_1$-adrenoceptor antagonists has been, and still is, an important topic in medicinal chemistry. Analysis of a number of chemical structures of selective $\alpha_1$-adrenoceptor antagonists indicates a crucial role for arylpiperazine moiety, which fits in the pharmacophore model. Furthermore, another hydrophobic/aromatic feature is needed within compounds structure to interact with the $\alpha_1$—adrenoceptor. In the previous studies we investigated a number of phenylpiperazine derivatives of 5,5-diphenyllhydantoin with hydroxypropyl linker [1], which possessed two additional aromatic fragments at position 5. The compounds were selective in respect to dopaminergic and serotonin receptor 5-HT7 but their affinity for $\alpha_1$-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. In this context, a series of arylpiperazine derivatives of 3-benzyl-5,5-dimethylhydantoin were synthesized (Fig.1).




**1-11**

**$R^1$** = H; 2-MeO; 2-CN; 2-F; 4-F; 2,4-diF; 3,4-diMe; 3-MeO; 3,4-diCl; 2,3-diCl
**$R^2$** = Ph
**n**= 0, 1

**Fig. 1**

The compounds **1**-**11** were obtained within three-step synthesis, including microwave-aided synthesis in the last step, and tested on their affinity for $\alpha_1$--adrenoceptor in radioligand binding assays using [$^3$H]-prazosin as selective radioligand. Structures-activity analysis indicated that the chemical modifications significantly improved the affinity for $\alpha_1$-AR of the compounds, in comparison to their 5,5-diphenylhydantoin analogues. The best activity was found for the 2-methoxyphenylpiperazine derivative. Partly supported by K/ZDS/005593.

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**Acknowledgements**
Partly supported by K/ZDS/005593.

**P44**

# New propargyl quino[3,2-b]benzothiazines – synthesis and biological activities

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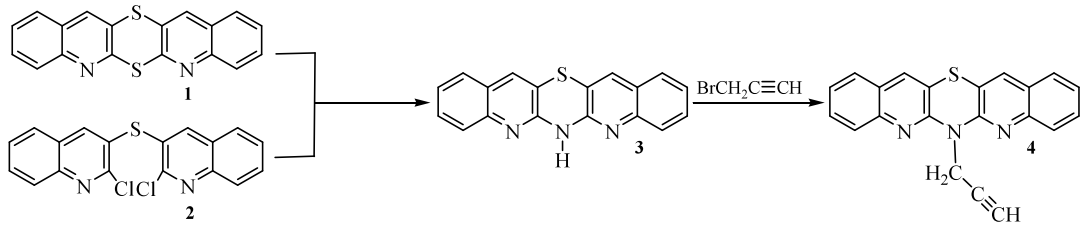
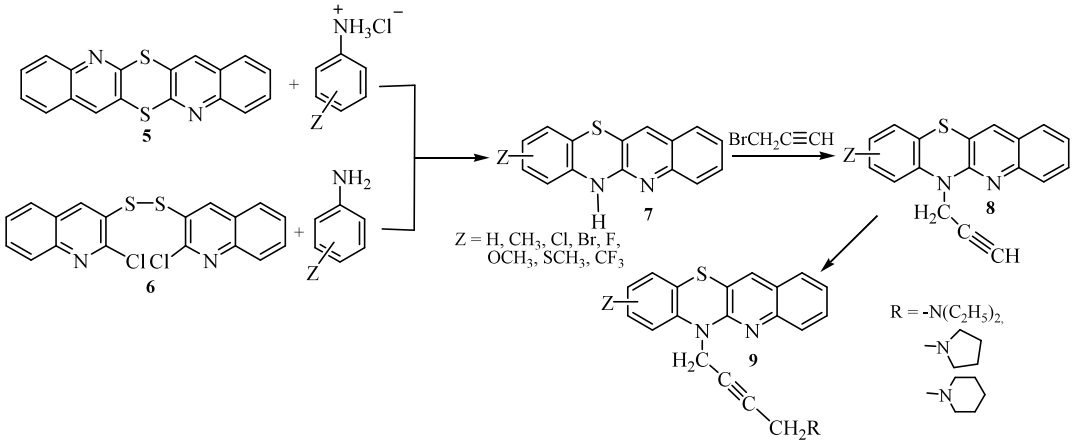
Phenothiazines, the oldest synthetic antipsychotic drug, are used in the treatment of schizophrenia and anxiety. Recent reports describe very promising anticancer and antibacterial activities, also reversal of multidrug resistance and possibility of treatment of Creutzfeld-Jakob's, Alzheimer's and AIDS diseases for classical and newly synthetised phenothiazines. The most perspective modifications of the phenothiazine structure can be achieved by substitution of the benzene ring with an azine ring to form azaphenothiazines [1,2]. We modified the phenothiazine structure with the quinoline moiety in the reactions of pentacyclic diquinodithiin **1** or 2,2'-dichloro-3,3'-diquinolinyl sulfide **2** with amines and ammonia to pentacyclic diquinothiazines **3** of significant anticancer and immunosuppressive activities [3].




Another reactions of diquinodithiin **5** or 2,2'-dichloro-3,3'-diquinolinyl sulfide **6** with various o-, m- and p-substituted anilines led to tetracyclic quinobenzothiazines **7**. Substituted 6*H*-quinobenzothiazines **7** was alkylated with propargyl bromide in the presence of potassium tert-butoxide in DMF to derivatives **8**. 9-Methylthio-6-propargylquinobenzothiazine was transformed into aminobutynyl derivatives **9** via the Mannich reaction, using propargylquinobenzothiazine **8**, the selected amines and formaldehyde.




Compounds **4, 8** and **9** were examined for their antiproliferative and anticancer activities. Anticancer activities were examined against cell lines of leukemia (L-1210) and colon cancer (SV-948). The most active compounds exhibited anticancer activity comparable to that of cisplatin.

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**P45**

# Novel dopamine $D_2$ receptor antagonists identified in structure-based virtual screening

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Schizophrenia is a mental illness the causes of which are not adequately understood and the pathomechanism of which is not satisfactorily treated by the currently available antipsychotics. A growing body of evidence links schizophrenia with excessive stimulation of dopamine $D_2$ receptors in the associative striatum, with a lack of stimulation of dopamine $D_1$ receptors in the prefrontal cortex, and with modifications in prefrontal neuronal connectivity involving glutamate transmission at NMDA receptors.

Virtual screening is nowadays a standard tool in drug discovery used to identify new compounds targeting a protein of interest. We constructed a homology model of dopamine $D_2$ receptor based on dopamine $D_3$ receptor template (PDB ID: 3PBL, in complex with an antagonist eticlopride). We used induced-fit docking to obtain complexes of $D_2$ receptor with chlorprothixene and olanzapine and used both for structure-based virtual screening with automated workflow of Schrödinger suite of software. First, the approach was validated by docking a set of hits and decoys. Next, the Enamine database was screened for new active compounds. As a result we identified 20 compounds which were subjected to experimental validation.

The affinity of the compounds for the cloned human dopamine $D_2$ receptor was evaluated by in vitro ([$^3$H]-Spiperone) binding assays. The selected compounds were also subjected to evaluation of their affinities for the cloned human dopamine $D_1$ and $D_3$, and serotonin 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors in vitro radioligand binding assays. The compounds selected in base of their affinity for $D_2$ receptors were evaluated in in vitro functional assays (inhibition of forskolin-stimulated cAMP production) in order to determine their behavior as agonists or antagonists of $D_2$ receptors. From 20 compounds tested we found 10 $D_2$ receptor antagonist possessing additional affinity to other receptors tested, in particular to 5-$HT_{2A}$ receptors (50% success rate). The affinity of the compounds ranged from 58 nM to about 1μM. Importantly, we identified the first $D_2$ receptor antagonist without a protonable nitrogen atom which is a key element of the classical pharmacophore model.

**P46**

# Structure of benzimidazole derivatives – potential tuberculostatics

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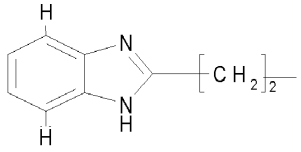
Tuberculosis remains a serious problem, because of increasing resistance of *Mycobacterium tuberculosis* to known medicines [1]. Some benzimidazole derivatives show tuberculostatic activity, which aroused our interest in their structures and SAR analysis. Crystal structures of the four of them will be presented: 2-(2-cyclohexylethyl)-5,6-dimethyl- (**I**), 5,6-dimethyl-2-phenethyl- (**II**), 2-(3-cyclohexylpropyl)-5,6-dimethyl- (**III**) and 5,6-dimethyl-2-(3-phenylpropyl)-1H-benzoimidazole (**IV**).

| | **I** | **II** | **III** | **IV** |
|---|---|---|---|---|
| Space group | Cc | P-1 | $P2_1/c$ | Cc |
| a, b, c [Å] | a=15.2276(3)<br>b=12.7173(3)<br>c=9.7501(2) | a=8.16859(1)<br>b=11.4832(2)<br>c=30.7638(4) | a=14.2078(4)<br>b=9.9210(3)<br>c=11.5761(4) | a=28.8210(9)<br>b=15.4123(5)<br>c=10.1759(3) |
| α, β, γ [°] | β= 126.196(1) | α=99.9705(5)<br>β=94.1047(5)<br>γ =90.9084(6) | β=104.799(1) | β=98.247(1) |
| Z' | 1 | 4 | 1 | 3 |
| R | 0.0270 | 0.0390 | 0.0349 | 0.0365 |

In the structures **I, III** and **IV** C(4) chains are formed based on N-H···N hydrogen bonds. In case of structure **II** different system of hydrogen bonds is formed due to presence of water molecules in the crystal.

Fifty one similar compounds (comprising a fragment drawn below) were found in the Cambridge Structural Database [2] in order to find conformational preferences of benzimidazole derivatives.

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**P47**

# Activity of the 1,3,5-triazine derivatives – potent histamine $H_4$ receptor ligands

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Expression of the most recently discovered histamine $H_4$ receptor (2000/2001) occurs mainly in monocytes, mast cells, eosinophils and basophils [1], what suggests that $H_4R$ is involved in inflammatory processes and immune responses [2]. Recent studies results indicate also that $H_4R$ antagonists have been shown to be effective in several models of pain [3]. As physiological role of $H_4R$ is not clear - new, potent and selective ligands are required to investigate its action. Among $H_4R$ ligands already described in the literature a large group of triazine derivatives can be found [4,5,6]. This kind of derivatives has been synthesized in our Department for the past several years.

The aim of this study was to evaluate *in vivo* activity of five 4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives (KB-4, KB-30, JN-38, TR-11 and TR-40). Compounds were tested in croton oil-induced ear edema model and ear pruritus model *in vivo* in mice. Compounds examined in the presented studies were selected from the library of compounds synthesized in our Department after preliminary screening conducted *in vivo* in mice.

The obtained results showed that pre-treatment with KB-4, KB-30 or TR-11 has strong to moderate influence on ear edema and pruritus. Among the tested compounds, KB-4 and TR-11 seem to have the most favorable profile, combining a good affinity at the human histamine $H_4$ receptor with a high efficacy in the intact animal. The results in detail will be presented and discussed.

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

**P48**

# Design, synthesis and biological activity of new hybrid anticonvulsants derived from 3-methyl- and 3,3-dimethyl-1-[1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl]pyrrolidine-2,5-diones

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The purpose of this study was to synthetize the focused library of 34 new piperazinamides of 3-methyl- and 3,3-dimethyl-(2,5-dioxopyrrolidin-1-yl)propanoic or butanoic acids as potential new hybrid anticonvulsants. These hybrid molecules join the chemical fragments of well-known antiepileptic drugs (AEDs) such as ethosuximide, levetiracetam, and lacosamide. Compounds **5**–**38** were prepared in a coupling reaction of the 2-(2,5-dioxopyrrolidin-1-yl)propanoic (**1**, **2**) or butanoic acids (**3**, **4**) with the appropriately substituted secondary amines in the presence of the *N,N*-carbonyldiimidazole reagent. The initial anticonvulsant screening was performed in mice (*i.p.*) using the 'classical' maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests as well as in the six-hertz (6 Hz) model of pharmacoresistant limbic seizures. The acute neurological toxicity was determined applying the chimney test. The broad spectra of activity across the preclinical seizure models in mice *i.p.* displayed compounds **7**, **15**, and **36**. The most favorable anticonvulsant properties demonstrated **15** ($ED_{50}$ MES = 74.8 mg/kg, $ED_{50}$ *sc*PTZ = 51.6 mg/kg, $ED_{50}$ 6 Hz = 16.8 mg/kg) which showed $TD_{50}$ = 213.3 mg/kg in the chimney test that yielded satisfying protective indexes (PI MES = 2.85, PI PTZ = 4.13, PI 6 Hz = 12.70) at time point of 0.5 h. As a result, **15** displayed comparable or better safety profile than clinically relevant AEDs: ethosuximide, lacosamide or valproic acid. In the *in vitro* assays compound **15** was observed as relatively effective binder to the neuronal voltage-sensitive sodium and L-type calcium channels. Beyond the anticonvulsant properties 6 compounds diminished the pain responses in the formalin model of tonic pain in mice.

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**P49**

# Spectral characteristics of selected rhodanine and hydantoin derivatives

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Rhodanine derivatives, five-membered heterocyclic molecules containing thiazole nucleus with carbonyl group on fourth carbon, have broad spectrum of pharmacological activities. Their antidiabetic, antifungal, antimicrobial, pesticidal and antiapoptotic activity is widely reported [1-6]. Similarly, imidazolidyne-2,4-dione derivatives (hydantoins), possessing related structure, are well known mainly as anticonvulsants [7]. Recent articles show also the role of thiohydantoins in detection of some pathological changes Alzheimer's brains [8].

Although some of these compounds are already used in the therapy, there is still a need for new xenobiotics which are characterized by a broad spectrum of pharmacological activity and low toxicity.

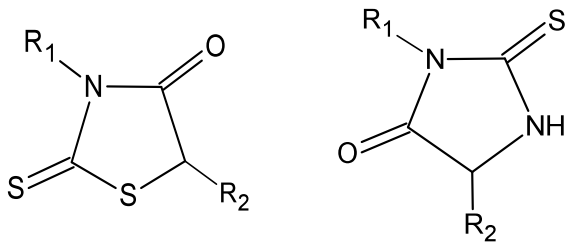


Fig. 1 The basic chemical formulas of rhodanine and tiohydantoin.

In order to characterize and compare both the already known as well as new obtained rhodanine and 2-thiohydantoin derivatives (Fig. 1), we decided to investigate their stability and the interactions of these compounds with human serum albumin (HSA), the most abundant protein in the blood plasma. The following compounds were tested: [5-(pyridine-2'-ylethylidene)-rhodaninel]-3-propionic acid, [5-(pyridine-2'-ylmethylidene)-rhodaninel]-3-butyric acid, [5-(4'-N,N-dimethylaminobenzylidene)-rhodanine]-3-propionic acid, [5-(4'-N,N-dimethylaminobenzylidene)-2-thiohydantoin]-3-acetic acid and [5-(4'-N,N–diethylamino-benzylidene)-rhodanine]-3-acetic acid. Here, we present the results of our investigation carried out using UV-Vis and emission spectroscopy. Additionally, we present the crystallographic structure and mass spectrometry spectra of selected compounds. The obtained results show a good stability of the tested compounds and indicate for a strong interactions with HSA.

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**P50**

# Modeling of piperidin-4-ol and 3-aminepropan-1-ol derivatives interaction with histamine $H_3$ receptor

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Previously, a series of 5-[(1-substituedpiperidin-4-yl)oxy]-*N*-methyl-*N*-propylpentan-1-amines has been described and characterized as a histamine $H_3$ receptor antagonists [1]. Based on the structure-activity relationships of piperidin-4-ol derivatives the highest activity was shown for compounds carrying (1-benzofuran-2-yl)methyl ($pA_2$=8.47) and benzyl moiety ($pA_2$=7.79) (for reference thioperamide $pA_2$ = 8.67). For compounds showing the highest activity two derivatives, where the piperidin-4-ol ring was replaced by flexible aminepropan-1-ol chain, were obtained. Surprisingly results of activity in both series were obtained.. In the case of derivatives carrying (1-benzofuran-2-yl)methyl substituent, replacement of piperidin-4-ol ring by aminepropan-1-ol chain resulted in a drastic reduction of activity ($pA_2$=6.23). For compounds bearing benzyl substituent an inverse relationship was observed - 5-{3-[benzyl(methyl)amino]propoxy}-*N*-methyl-*N*-propylpentan-1-amine ($pA_2$=8.06) has higher activity than its piperidin-4-ol analogue ($pA_2$=7.79).

In this work we tried to explain the reasons of these interesting experimental data by molecular dynamic simulation of aforementioned ligands in the receptor binding site. We also performed computational docking studies to examine the binding mode of the these four ligands. The model of the receptor was created by homology modelling method, placed in the system of lipid membrane and solvent, and optimized by molecular dynamic simulation. All calculations were performed in Discovery Studio [2].

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**Acknowledgements**

Calculations have been carried out using resources provided by Wroclaw Centre for Networking and Supercomputing (http://wcss.pl), grant No. 313.

This study was supported by a departmental sources of the Medical University of Lodz (grant number 503/3/016-01/503-01).

**P51**

# Influence of the substituent(s) at aromatic ring of arylidene hydantoin and of the length of carbon chain on affinity for $5HT_6$ serotonin receptor in the group of 3,4-dichlorophenylpiperazine derivatives

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Recently serotonin receptors have been the subject of intense research because of their potential role in many neurological disorders. They are family of G-coupled (excluded $5HT_3$ type), seven transmembrane receptors which have many subtypes.

One of them are $5HT_6$ receptors which play role in functions like cognitive impairment, emotionality. Modulation of this type of receptor could be also useful for dementia patients and in AD disease [1, 2]

Studies of these type of receptors have shown that the numerous compounds with affinity to serotonin receptors contain arylpiperazine moiety.

In our recent studies 3,4-dichlorophenylpiperazine derivatives of arylidene hydantoins were obtained. Chemical modifications were focused on introduction of one or two- methoxy substituents at arylidene ring of the lead and different carbon chain (from 3 to 8 atoms of carbons, excluded 4 and 7- carbon chain) between hydantoin and phenylpiperazine.

The new compounds were obtained within four-step synthesis [3]: (1) Knoevenagel condensation, (2) Mitsunobu reaction, (3) N-alkylation under microwave irradiation and (4) conversion of the obtained basic derivatives into the corresponding hydrochloric form.

The new hydantoin derivatives were evaluated on their affinity for $5HT_6$ radioligand binding assay. Ki values in the group of investigated compounds were in the very wide range: 37- 1693nM.

SAR-studies indicated a profitable influence of only one methoxy substituent at arylidene ring. We also noticed that the compounds with longer carbon chain have shown better affinity for $5HT_6$ serotonin receptors.

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Partly support of K/ZDS/004689 is kindly acknowledged.

**P52**

# Aromatase inhibitors in the treatment of human diseases

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Aromatase, an enzyme belonging to CYP450 family, is responsible for converting androgens to estrogens. The relationship between concentration of these steroids, pivotal in hormonal homeostasis, can be modified by aromatase inhibitors [1]. Aromatase inhibitors have various applications and the goal of the communication is to show the current use and future potentials of these drugs.

Aromatase inhibitors are used in the treatment of estrogen-dependent breast cancer, mainly in postmenopausal women [1]. Due to the presence of estrogenic receptors in the cancer cells, estrogens play crucial role in cells proliferation and cancer development. Another disease, strongly dependent on estrogens synthesis, is endometriosis because of the steroids influence on endometrium growth. Aromatase inhibitors interfere in the pathological proliferation of endometrium cells and also lessen the pain connected with the disease [2]. These drugs are indicated for the treatment of polycystic ovary syndrome because they stimulate ovulation and reduce PGE-2 level [3].

Aromatase inhibitors can be applied in male diseases as well because of their influence on sperm quality [4]. Moreover, the modification of androgens levels by aromatase inhibitors hold therapeutic promise in delayed pubescence [5] and benign prostate hyperplasia [6]. By decreasing estrogens synthesis, aromatase inhibitors increase the testosterone level that leads to muscle and body mass development. Thus they can be used in illegal doping [7].

The recent studies have revealed that aromatase is active in brain where it influences sex orientation [8], inflammation and other pathological processes [9]. This shows a future potential of aromatase inhibitors in the treatment of CNS diseases [10].

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**P53**

# Synthesis of potential aromatase inhibitors

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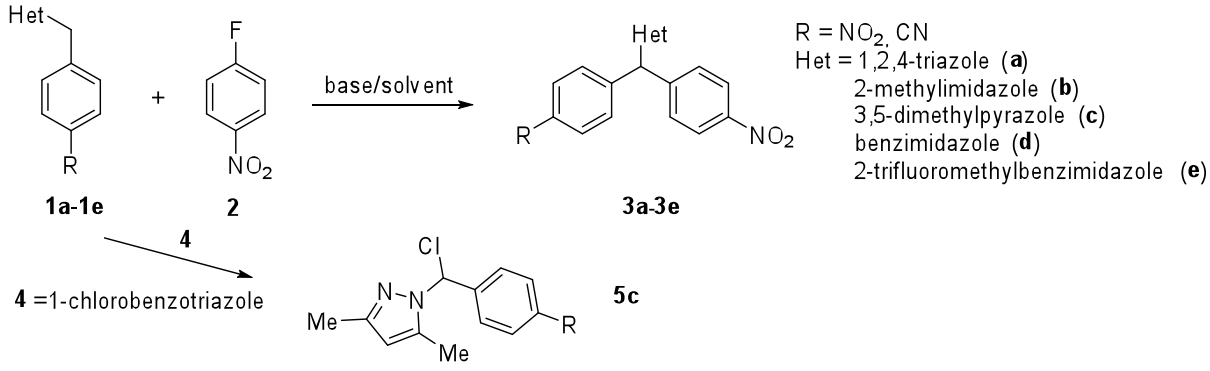
The aim of the project has been to design and synthesize compounds analogous to letrozole, a well-known and currently used in medicine aromatase inhibitor. Selected azoles were N-substituted with 4-cyano or 4-nitrobenzyl residue in which the nitro group was an equivalent of the letrozole cyano functionality (compounds **1a**-**1e**).

Attempts undertaken to attach a second 4-nitrophenyl substituent to the above azole derivatives **1a**-**1e** posed difficulties. A variety of reaction conditions were used, including different reaction temperatures, solvents and bases but the applied methods provided the desired products **3a**-**3e** usually in unsatisfactory yields. However, when 1-chlorobenzotriazole was used as a reagent, one of the isolated in a pure form compounds was 1-[chloro(4-nitrophenyl)methyl]-3,5-dimethylpyrazole **5c**. This compound can be useful in further syntheses of potential aromatase inhibitors because the chlorine atom can be substituted by a variety of nucleophiles, including azoles. Considering the fact that the reaction is probably radical in nature, similar chloroderivatives of benzylazoles **1a**-**1e** can be obtained using a radical initiator.




**Acknowledgements**

This work has been supported by Poznan University of Medical Sciences, grants no. 502-14-03308417-10167 (for young scientists) and 88/2015 (SBN UMP).

**P54**

# Computional study of a nanocontainer composed of carbon nanotube and gold nanoparticles covered by pH-clevable hydrazone linkers

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This work deals with the hydrazone bond (HZB) hydrolysis, occurring under the acidic conditions, [1] as a method of controlling the capped/uncapped state of carbon nanotubes (CNT). We consider two systems – before and after the hydrolysis of HZB. In the first case the gold nanoparticles (GNP) are connected with CNT tips by some linkers containing the HZB. The second case is the system corresponding to acidic pH, that is with the HZB cleaved and free GNPs. Structural transformations leading to detachment of GNPs from the CNT tips or their shift from tips to the sidewalls are thus the key problem analyzed in this study. Potential energy changes accompanied such transitions are very useful in prediction of the likelihood of considered states. However, the potential energy minima are often separated by energy barriers which, in fact, determine the dynamic properties of the system; particularly the likelihood of transitions between energy minima are affected by those barriers. Because sampling of an energy barrier is normally ineffective in molecular dynamics simulations, many methods involving biased simulations have been proposed in the literature. In our particular case two methods seemed to be particularly appropriate, namely umbrella sampling with the weighted histogram analysis [2,3] or steered molecular dynamics [4,5]. However, their standard application turned out to be ineffective as well, mainly due to big size and complex structure of the GNPs and large distances needed for sampling. Therefore, we had to make some adaptations of these algorithms in order to obtain reliable results with acceptable computational costs.

The most important results obtained in this study concern the free energy profiles associated with the uncapping process of CNT. We show that spontaneous detachment of GNPs from CNT tips is normally a very slow process. However, under certain conditions the uncapping might be fast enough when coupled with simultaneous release of cisplatin from the CNT interior.

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**Acknowledgements**
This work was supported by Polish National Science Center (NCN) grant UMO-2012/07/E/ST4/00763.

**P55**

# Influence of cholinesterase inhibitors, donepezil and rivastigmine, on the development of tolerance to antinociceptive effects of morphine in mice

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Published data indicate that cholinergic system is involved in antinociceptive effects of opioids. The aim of our study was to determine whether donepezil and rivastigmine influence the development of tolerance to antinociceptive effects of morphine. Donepezil is a selective inhibitor of acetylcholinesterase but rivastigmine is also an inhibitor of butyrylcholinesterase, the enzymes taking part in neuronal acetylcholine degradation. The experiments were performed in the hot-plate test in mice. Donepezil (0.5, 1 or 3 mg/kg, i.p.) or rivastigmine (0.03, 0.5 or 1 mg/kg, i.p.) were given 20 min before morphine (5 mg/kg, i.p.) administration, once daily for 7 days. Thirty min after morphine administration the latency response (in seconds) for the mouse to lift either of the hind paws or a jump with all four feet off of the hot-plate induced by the thermal stimulus was measured. On the 8 day of experiment all mice were challenged with morphine (5 mg/kg, i.p.) and 30 min later their behavior on the hot-plate test was measured (expression). Our results indicated that donepezil and rivastigmine dose-dependently potentiated the antinociceptive effects of morphine during 7 days administrations. The effects were more pronounced after rivastigmine administration, presumably due to a broader inhibitory spectrum of this drug. On the 8 day of the experiments the challenge dose of morphine did not change the behavior in morphine-treated mice as compare to acute morphine injection, suggesting the development of tolerance to antinociceptive effects of morphine. Our experiments also demonstrated that co-administration the donepezil and rivastigmine with morphine inhibited the development of tolerance to antinociceptive effects of morphine measured on the 8 day of experiments after morphine challenge. Thus, the present study found that stimulation of cholinergic neurotransmission by cholinesterase inhibitors potentiated antinociceptive effects of morphine and inhibited the development of tolerance to morphine antinociceptive effects.

**P56**

# Crystallographic and theoretical studies on potential interactions between aromatase and its inhibitors

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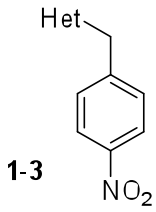
Aromatase is similar in its structure to other enzymes of the CYP450 cytochrome group. The main problem in the design of new selective antiaromatase agents is to recognize typical interactions between the enzyme and its potential inhibitors. The atoms involved in binding to the cytochrome iron and the enzyme aminoacids (usually serine) are pyridinic nitrogen and another nitrogen capable of forming a weak hydrogen bond, respectively.

Having in hand 4-nitrobenzyl derivatives of some azoles (compounds **1**-**3**, Fig 1), we were able to analyse possible interactions between the pyridinic nitrogen atom present in the heterocyclic ring as well as the nitro group and the structural elements of aromatase. The crystallographic structures of compounds **1**-**3** revealed that the nitro group can indeed be involved in weak hydrogen bonding (an example is shown in Fig. 2). Moreover, interesting p and T-stacking interactions can be observed especially in the elemental cell of benzimidazole derivative **3**. The experimental studies were supported by theoretical studies using the *Gaussian G09* platform.

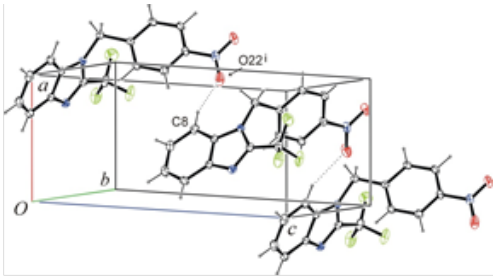
Fig. 1




**1**-**3**

Het = **1**: 1,2,4-triazole
**2**: 2,5-dimethylpyrazole
**3**: 2-trifluoromethylbenzimidazole

Fig. 2

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**P57**

# Influence of presence of aromatic ring(s) at position 5 of hydantoin on activity of potential 5-HT$_7$R ligands

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Search for new antipsychotic medications is required scientific approach, since many patients show incomplete response to currently available treatment. The most important is finding new drugs with reduced side effects such as metabolic abnormalities, QTc prolongation, cognitive and motor dysfunctions.

Recently published studies indicate that blockade of 5-HT$_7$Rs displays e.g. an antidepressant-like activity, anxiolytic-like effect and improvement of reference memory. Hence, design, synthesis and evaluation of new molecules with high affinity towards 5-HT$_7$ receptor is promising strategy for future effective CNS diseases treatment.

Our previous studies, allowed to obtain series of 14 novel hydantoin derivatives with attractive activity to above-mentioned receptor (3 nM < *Ki* < 79 nM). Compounds with the most interesting properties were chosen for further modifications. This work is continuation of mentioned studies and is focused on evaluation how presence/absence of aromatic ring(s) in position 5 of hydantoin influences on activity. Within the research, 9 compounds (5-methyl-5-phenylhydantoin, 5,5-diphenylhydantoin and 5-methyl-5-α-naphtylhydantoin derivatives were synthesized and evaluated, while synthesis of 6 compounds more is ongoing (5,5-dimethylhydantoin and 5-methyl-5-β-naphtyl hydantoin derivatives). However, for the time being, the most favourable, regarding 5-HTRs affinity, seems to be 5-phenyl-5-methylhydantoin moiety.

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**Acknowledgements**
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**P58**

# The potential role of halogen bonding in interactions of ligands with class A GPCRs – the β2 adrenergic receptor case study

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Halogen atoms are one of common features in biologically active compounds and drugs. 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]. Although, 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 formation of specific, direct interactions called halogen bonds.

To date, systematic and comprehensive studies on the role and significance of halogen bonds in family A GPCRs have not been published. There are also no studies showing the use of the concept of halogen bonds in the rational design of potential ligands of these receptors.

Herein we report on a systematic molecular modeling approach, i.e. generation of X-SAR sets fetched from ChEMBL database, molecular docking and hybrid QM/MM calculations, used to study the different role of halogen atoms in ligand-receptor interaction (i.e. steric hindrances, interaction of positive σ-hole with negatively charged atoms of the protein and interaction of the negative electrostatic potential of fluorine with positively charged atoms of the protein). The results obtained by application of developed computational workflow are discussed on the example of β2 adrenergic receptor.

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**Acknowledgements**

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**P59**

# Characterization of some ADME-TOX parameters of the series selected serotonin 5-HT7 receptor ligands

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The selective blockade of 5-$HT_7$ serotonin receptor (5-$HT_7$R) may be used for potential treatment of central nervous system disorders [1]. For instance, studies performed on mice with 5-$HT_7$R knock-out and known 5-$HT_7$R antagonist – SB266970, showed its significant influence on depression mechanism [2]. In our previous studies, the lead compound MF-8 was found as a potent and selective 5-$HT_7$R ligand (Ki = 3 nM), in respect to other GPCRs, including: $\alpha_1$-adrenergic (Ki = 181 nM), 5-$HT_{1A}$ (Ki = 121 nM), 5-$HT_6$ (Ki = 10790 nM) and $D_2$ (Ki = 715 nM) [3]. Moreover, according to our recent data obtained using *in vitro* methods, MF-8 seems to be a safe compound. The cytotoxic effect of MF-8 was seen only at the very high concentrations (above 100 μM). Additionally, MF-8 may not be involved in further potential drug-drug interactions, due to the observed reduction of CYP 3A4 activity only at the concentrations ≥10 μM [4].

The aim of this study was to examine *in vitro* the safety and metabolic stability of the series analogues of compound MF-8, potent and selective ligands of 5-$HT_7$R. The MetaSite 4 computational method was used for prediction the routes of the metabolic biotransformation of 5-$HT_7$R ligands *in silico*. The metabolic stability was examined *in vitro* by human liver microsomes (HLMs). The LC-MS spectra and the precise ion fragments analysis produced by obtained metabolites allowed to determine their most probably structures. The luminescent CYP3A4 P450-Glo™ Assay was used for prediction of potential further drug-drug interactions by testing the effects of 5-$HT_7$R ligands on cytochrome CYP3A4 activity. The preliminary toxicity studies were also performed in human embryonic kidney HEK-293 cells by using colorimetric EZ4U assay.

The only one compound (KKB41) showed similar to MF-8, lack of significant cytotoxicity, whereas other 5-$HT_7$R ligands showed moderate, dose dependent antiproliferative effect at the concentrations ≥50 μM. Almost all compounds were metabolized into two metabolites and inhibited CYP3A4 activity in similar, moderate level. However, in case of one compound (KKB-16) only the trace amount of one metabolite was found and no activity against CYP3A4 was determined, indicating its very high metabolic stability.

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**Acknowledgements**
Partly supported by K/ZDS/005593 and K/ZDS/004689.

**P60**

# Synergistic effects of novel derivatives thiosemicarbazones in combination with photodynamic therapy in human colon cancer

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Photodynamic therapy (PDT) is a promising and developing approach in the treatment of cancer. The basis of photodynamic therapy is combined action of a photosensitizer, light and molecular oxygen within malignant tissue. Under these conditions, administration of the photosensitizer (PS) to the tumor and local exposure to light of a specific wavelength can lead to a series of photochemical reactions and consequently the generation of singlet oxygen and reactive oxygen species (ROS) [1]. Accumulation of ROS may cause induction of protein damage, DNA disruption, lipids peroxidation and consequently - triggering of apoptosis. Currently, the combination therapy is growing approach to increase the overall therapeutic efficacy of PDT. The basis of the combination therapy is the combination of two or more drugs, that can exert preferably additive or synergistic effects [2]. In our group, the promising results were obtained when novel thiosemicarbazones derivatives (TSC) were used in combination with 5-aminolevulinic acid (ALA) which is precursor in ALA-PDT treatment [3].

Recently we focused deeper on the interactions of novel highly active thiosemicarbazones with known PS - chlorine and temoporfin (Foscan) in combined PDT. We performed an *in vitro* assay of cell viability on human colon cancer cell lines (HCT116 +/+) to examine the dark- and photo-toxicity effects of the drugs (TSC and PS) alone and in combination, respectively. Accumulation sites for those drugs were evaluated in co-localization experiments on confocal scanning microscopy. In addition, we measured the production of singlet oxygen as well as lipids peroxidation by TSC, PS and combination thereof as major factors leading to the apoptosis induction.

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**Acknowledgements**
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**P61**

# Structural characterization of ticagrelor and its DMSO solvate

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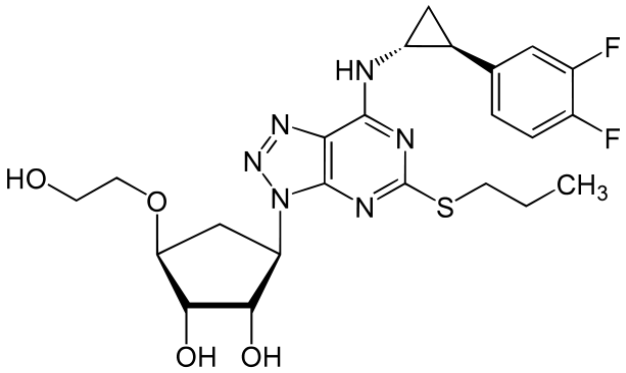
Ticagrelor (scheme), is a new direct-acting, reversible $P2Y_{12}$ – adenosine diphosphate receptor blocker, used in the treatment of cardiovascular diseases, especially in acute coronary syndromes (ACS) [1,2]. The recommended treatment for ACS to reduce the rate of recurrent ischaemic events and stent thrombosis, is dual antiplatelet treatment with aspirin and thienopyridines, such as: clopidogrel or prasugrel, which are oral prodrugs that need to be converted to active metabolites to irreversibly bind to the $P2Y_{12}$ receptor. Unfortunately, about 85% of clopidogrel is hydrolysed to an inactive metabolite. Recently, it has been proved that ticagrerol has higher biological efficacy than popular clopidogrel [3].




Scheme. A molecular diagram of ticagrerol.

At the conference we would like to present the crystal structures of ticagrelor and its DMSO solvate. In addition some other properties of studied molecules, including Hirshfeld surface (HS) analysis with fingerprint plots (FP), spectroscopic studies FT-IR, NMR and thermal investigation will also be presented.

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**P62**

# Synthesis and evaluation of the antiproliferative action of a new group of propargylthio- and propargylselenoquinolines

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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].

Inspired by the biological importance of acetylenic compounds as anticancer agents, we herein reported the synthesis of novel propargylselenosulfamoyl-quinolines and propargylthiosulfamoyl-quinolines. The title acetylenic sulfamoylquinolines were synthesized using halogeno-quinolinesulfonyl chlorides as the starting compounds. Yields in the range 89%–96% were obtained. The ability of all of the synthesized compounds to inhibit the proliferation of the selected cell lines was determined with the WST-1 assay.

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**Acknowledgements**
This research was supported by the Medical University of Silesia, Grant No. KNW-1-002/N/5/0.

**P63**

# Biotransformations developed by a *Cunninghamella* model on new 8-methoxy-purine-2,6-dione derivatives with analgesic and anti-inflammatory activity

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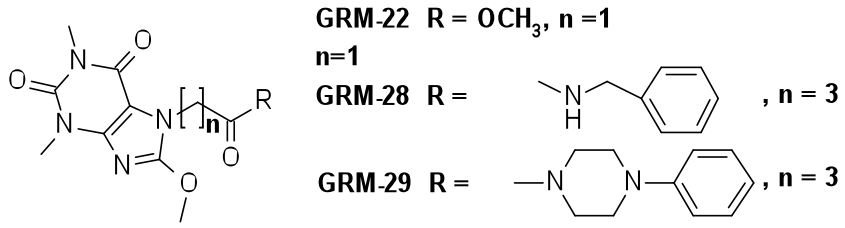
Among the newly synthesized derivatives of 8-methoxy-purine-2,6-diones preliminary pharmacology studies demonstrate the promising potent anti-inflammatory and analgesic properties superior to the reference drug [1,2]. Additional studies have confirmed that the substances are safe, devoid of mutagenic potential, showing a moderate chemopreventive activity.




The aim of the study was to investigate the metabolic stability and to establish a pathway for the biotransformation of three selected, novel compounds from the group of derivatives of 8-methoxy-purine-2,6-diones with defined analgesic and anti-inflammatory activity.

In the studies was used an alternative model of microbial biotransformation [3] utilizing 3 strains of *Cunninghamella* filamentous fungi, because this microorganism contains a rich set of enzymes, with activity similar to the cytochrome P450 enzymes. To monitor the progress of the reaction of biotransformation and analysis of potential metabolites has been used LC / MS / MS methodology [4].

Among the tested compounds GRM-22 was the most stable in the *Cunninghamella* model, did not undergo biotransformation. Two other compounds, GRM-28 and GRM-29, possess less metabolic stablility, depending on the strain, 1-9%, and 53-87% of parent compounds underwent biotransformation reactions, respectively. The metabolites, which were formed during the biotransformation of GRM-28 and GRM-29 are the products of hydroxylation.

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**P64**

# Synthesis and biological activity of 6-aminocaproic acid derivatives as potential plasmin inhibitors

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The blood fibrinolytic system comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin. Plasmin degrades fibrin into soluble fibrin degradation products, by two physiological plasminogen activators (PA), the tissue type PA (t-PA) and the urokinase type PA (u-PA). t-PA mediated plasminogen activation is mainly involved in the dissolution of fibrin in the circulation. Inhibition of the fibrinolytic system may occur either at the level of the PA, by specific plasminogen activator inhibitors (PAI), or at the level of plasmin, mainly by alpha 2-antiplasmin.

Aminocaproic acid (also known as Amicar, ε-aminocaproic acid, ε-Ahx, or 6-aminohexanoic acid) is a α-deaminated derivative of lysine, which is an effective inhibitor of plasmin that bind that to the binding-lysines sites of plasmin.

Aminocaproic acid is used to treat excessive postoperative bleeding, especially after procedures in which a great amount of bleeding is expected, such as cardiac surgery.

A new 15 derivatives of EACA as plasmin inhibitors was described. Compounds were designed by modifications of known linker tumor of recognition sites -Ala-Phe-Lys-. The target compounds were synthesized with good yields using a solid phase methodology. The inhibitory amidolytic enzymatic tests confirmed activity of compounds.

**P65**

# Tuberculosis may be just around the corner! Pyridine derivatives – a potential tuberculostatics

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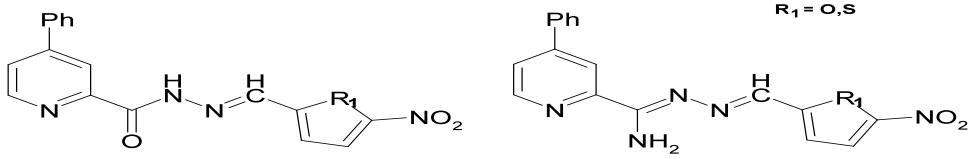
In 2013, an estimated 9.0 million people developed tuberculosis and 1.5 million died because of it [1].

The main problem in the treatment of this disease proves to be an acquisition of drug resistance by the Mycobacterium tuberculosis. It became then necessary to search for new, effective tuberculostatics.

We will present the molecular and crystal structures of four new compounds (Scheme) with potential anti-tuberculostatic activity. Though the chemical structure of the four compounds is very similar, they exibit significant differences in their biological activity against Mycobacterium strains. This prompted us to determine the geometry of the molecules (Fig.) with X-ray crystallography method. The determined structures will be compared with analogous structures contained in the Cambridge Structural Database [2].




**Scheme**

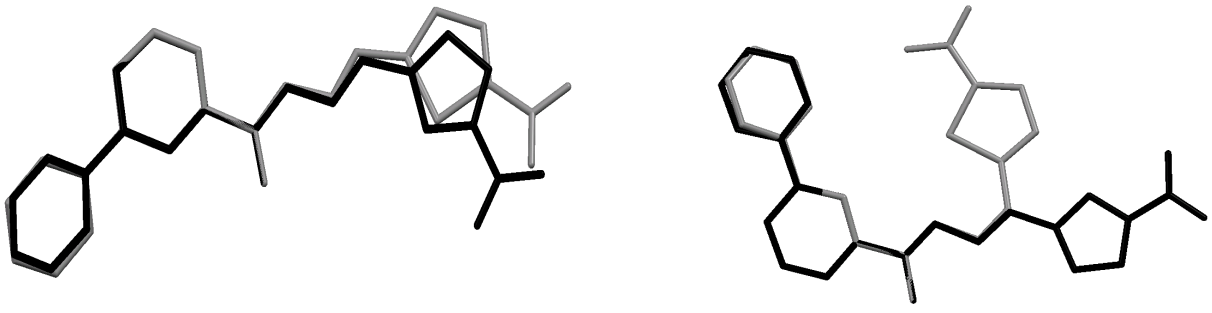


**Figure.** Overlay of the respective derivatives (gray color for $R_1$=S and black for $R_1$=O)

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

**P66**

# Mechanisms of energetic transitions in the molecule of isoxazole derivative - theoretical studies

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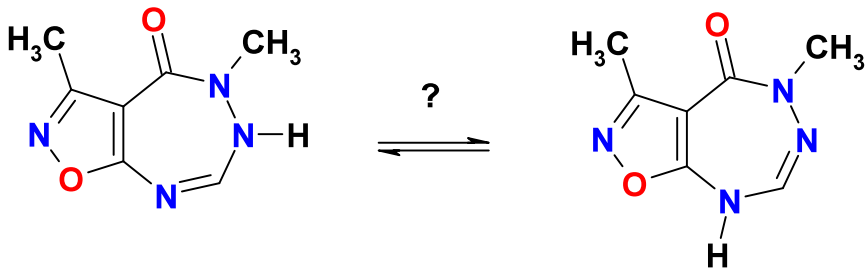
In 2014, we presented preliminary calculation about theoretical studies on mechanisms of energetic transitions in the molecule of isoxazole derivative. Quantum chemical calculations on the level B3LYP were performed to answer the question which of two isomers of 3,5-dimethylizoxazolo[5,4-*e*][1,2,4]-triazepin-4-one is more stable and what is the transition state between these two forms in different solvents. Activation energy of transition was established. Gas phase calculations give 70 kcal/mol of activation energy. Calculations in water (PCM – model, Gaussian 0.9) give decrease of activation energy of the transitions and Δ energy activation is on the level of 30kcal/mol. Now we calculated energy of the transitions in isoxazole complex with cationic and anionic form.

Results achieved in water phase showed that only one of molecules obtained in the experiment is in solution and biological activity is conned with the one form. Intermediate state in water was established which is a local minimum being about 30 kcal/mol above the stationary state. Additional calculations with one, two, three molecules of water showed that same result.

In our research we have checked the influence of cationic and anionic complexes to level of activation energy of the transitions and we want to definitely described the possibility of coexistence of both forms of 3,5-dimethylizoxazolo[5,4-*e*][1,2,4]-triazepin-4-one in presented models.



**P67**

# p-21 mediated synergic cytostatic effects of combined treatment with 5-fluorouracil and sulforaphane

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5-fluorouracil is used to treat many types of cancers, such as: breast, ovarian, colon and gastric ones. It is a pyrimidine analogue. It is a proapoptotic agent and it blocks the cell cycle in the S phase.

Sulforaphane (sfn) is a natural isothiocyanate. In vitro studies have proved that sfn potentiates cytotoxicity of anticancer drugs, for example Oxaliplatin. Anticancer activity of sfn is connected with induction of apoptosis and the cell cycle arrest.Cell cycle arrests depend on the sfn dose and on the cell line. Sfn mainly blocks the G2/M phase. One of mechanisms of G2/M cycle arrests is an increase in the p21 protein level. Both 5-fluorouracil and sfn can influence the p21 level.

The aim of the study was to investigate interactions between 5-fluorouracil and sfn and mechanism of the cytostatic effect of their combination in the breast cancer cell line MDA-MB-231.The antiproliferative effects were evaluated by the MTT assay. The types of interaction were determined as described by Chou and Talalay. The cycle progression was tested with the flow cytometry. The level of cyclin B was estimated by the western blot analysis. The level of p21 and its were assessed with a confocal microscopy.

Antiproliferative effects of combined treatment were stronger than those of separate treatments (a synergic effect). 5-fluorouracil and sfn combination increased cell population accumulation in the G2/M phase in comparison to treatment with sfn only. After 72 hours of incubation with combination of compounds, the level of cyclin B was lower than after incubation with single compounds. After combined treatment, the level of p21 was increased in comparison to single treatments. p21 is an inhibitor of cyclin dependent kinase and cyclin dependent kinase-1. Cyclin B1-cyclin–dependent kinase-1 complex was inactivated and the cell cycle did not enter the mitotic phase.

The results show that combination of 5-fluorouracil with sfn synergically blocks the G2/M of the cell cycle phase,which is mediated by p21.

**Acknowledgements**
The project was partially supported by: the National Science Centre, N/NZ5/02634 and a grant Pol-Nor/198887/73/2013.

**P68**

# An active conformation of mGlu2 receptor induced by molecular dynamics simulation with C-terminal $G_i$ peptide

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Recent progress in crystallization of the G Protein-Coupled Receptors opens new avenues for structure-based drug design approaches. Emergence of new crystal templates allows development of more precise models, and although already published research point out the importance of crystal structure resolution, quality and selection of proper template (or even using multiple ones)[1], homology modelling remains bread and butter of structure-based studies.

Homology modelling procedures are inherently connected with the problem of the presence of the template co-crystalized with the desired type of a ligand. Agonists, as they force active state of the receptor are significantly less represented in the available crystal structures, and the process of transforming homology model or even crystal structure into its activated conformation is a challenging task, involving use of either long term Molecular Dynamics simulations or homology modelling based upon more distant templates.

Here we present a novel method of inducing active conformations of GPCRs with the help of the C-terminal peptide of G protein as a cofactor restraining simulation system into desired active form. The case study of non-trivial target, Metabotropic Glutamate Receptor 2, being a class C GPCR, where the conformations received from simulation runs with and without G peptide probe, is evaluated in retrospective virtual screening procedure. The results show undoubtful advantage of the former approach, leading to relatively simple MD procedure and shorter simulation times and providing significantly better results in screening-like experiments.

[1] Rataj K., Witek J., Mordalski S. et al. *J. Chem. Inf. Model.* 54 (2014) 1661.

**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).

**P69**

# Tyrosine kinase inhibitors based on quinazoline framework Synthesis and evaluation of bioactivity

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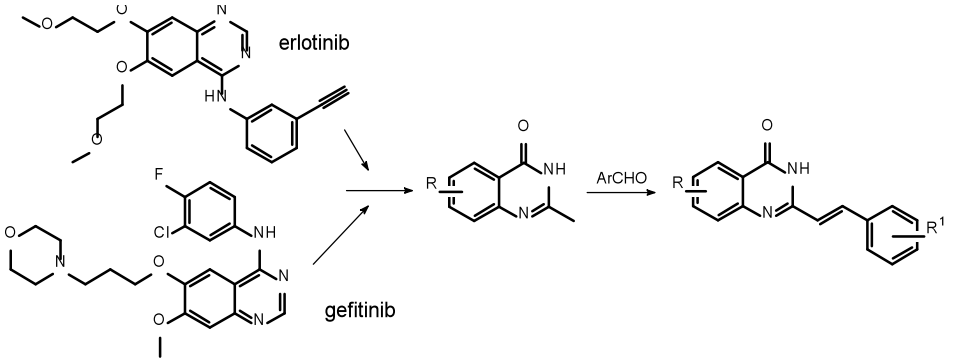
Protein kinases have become the pharmaceutical industry's most important class of anticancer drug target. It has been estimated that approximately 30% of current R&D spend in pharmaceutical companies is focused on the development of kinase inhibitors. In 2001 imatinib was the first FDA approved treatment, and by April 2015, a total of 28 small molecule kinase inhibitors have been approved [1].

Certain structural features trigger biological effects more often than others [2]. In fact it has been found that some structures are overrepresented among drug molecules. Quinazoline moiety is an exceptional example of such features. Its heterocyclic structure has been found as a privileged pattern within many commercially available drugs. Thus, molecules based on its skeleton are interesting synthetic target.

Within this project we wish to exploit the privileged structure idea and polypharmacology as combination for alternative drug design and to show how new combinations of those concepts may provide good results in relatively well explored field. Moreover we utilize a fragment based molecular design approach. In this way, we're able to engineer and compose diverse collection of target molecules represented by a general structures as shown on a scheme below. Our preliminary studies show good to excellent anticancer activity of such substituted quinazolines [3].




Synthesized compounds will be tested *in vitro* for their anticancer activity. This will be fulfilled within two steps. First, we plan to screen our compounds against human colon carcinoma cells. Cytotoxicity assays will be performed on two cell lines: HCT116-wild type (+/+) and HCT116 with knock-out TP53 gene. In addition, the therapeutic index will be specified by means of cytotoxicity measures conducted on normal cell line - fibroblast (NHDF). The next step of studies will consist of *in vitro* tyrosine kinase assays to evaluate the impact of those synthesized compounds on tyrosine kinases signalling pathway.

[1] Wu P., Nielsen T. E., Clausen M. H. *Trends Pharmacol. Sci.* 36 (2015) 422.

[2] Costantino L., Barlocco D. *Curr. Med. Chem.* 13 (2006) 65.

[3] Mrozek-Wilczkiewicz A., Musiol R., Jampilek J. et al. *Bioorg. Med. Chem.* 18 (2010) 2664.

**P70**

# Synthesis, physicochemical properties of new piperazinamides of arylalkyl acids with potential anticonvulsant activity

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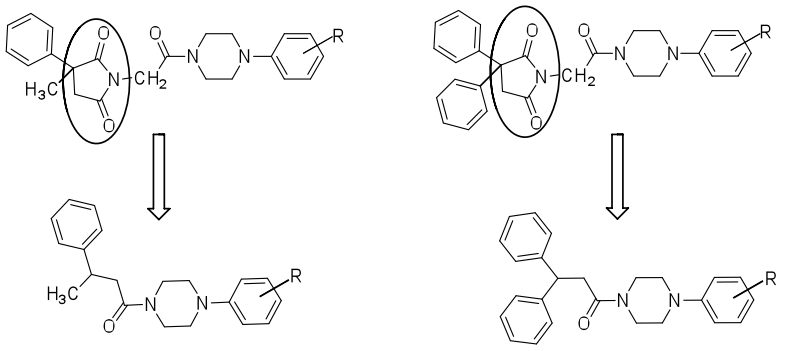
Recently, we have shown that a great number of amides derived from 3,3-disubstituted pyrrolidine-2,5-dione-acetic acid containing as basic fragment 4-arylpiperazin-1-yl moiety exhibited anticonvulsant activity in the MES and/or scPTZ. In this series of compounds the most active were 3-methyl-1-[(2-oxo-2-(4-phenylpiperazin-1-yl)-ethyl]-3-phenyl-pyrrolidine-2,5-dione with an $ED_{50}$ = 40.87 mg/kg in the scPTZ test and 1-(2-oxo-2-(3-trifluoromethylphenyl-piperazin-1-yl)-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione with an $ED_{50}$ = 20.78 mg/kg in the MES test [1,2]. Therefore, we decided to design and synthesize several analogues of compounds mentioned above, which are devoid of pyrrolidine-2,5-dione system as a core fragment.



R= H, 2-Cl, 3-Cl, 4-Cl, 2-F, 4-F, 2-$CH_3$, 3-$CH_3$

The target compounds were prepared in a coupling reaction of 3-phenyl-butyric or 3,3-diphenyl-propionic acids 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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[2] Obniska J. et al. *non published data.*
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**Acknowledgements**
This study was supported by grant of the Polish National Scientific Centre, No. DEC-2013/11/B/NZ7/02081.

**P71**

# Searching for anticancer properties - preliminary evaluation of xanthine derivatives' anticancer activity

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Xanthines are the large group of natural and synthetic compounds, which present various types of activity. Depending on the type of substituents, xanthine derivatives are known as bronchodilators, psychostimulants and cardiac stimulants. Many of the xanthines present antagonist potency towards adenosine receptor subtypes, therefore their role as neuroprotective, cardioprotective and antiasthmatic agents is widely suggested and can be developed in the future. It is also reported, that xanthines show antiviral, antitumor and antimicrobial activity. [1]

Cancer is still the second most common cause of death in Europe in last few years. Since year 2002 incidence and mortality on account of all types of cancer in the World increased rapidly. [2,3]

Among the large group of anticancer drugs only few are used because of their interaction with GPCRs' signaling pathway. However, an increasing body of evidence that can connect GPCRs ligands and cancer development and progression has recently emerged. [4]

The National Cancer Institute (NCI) is the part of the United States National Institute of Health. The Institute was established in 1937 and from that time is addressed for research and training needs for cause, diagnosis, and treatment of cancer. Developmental Therapeutics Program (DTP) is the drug discovery and development arm of the NCI. One of the leading DTPs is anti-cancer compound screening program for identifying novel chemical leads and biological mechanisms of drugs actions. [5]

As the result of our cooperation with NCI, a series of new xanthine derivatives, were accepted for a primary pharmacological screening in DTP program. Compounds were tested in one concentration (10 μM) at 60 different human cancer cell lines: prostate, breast, ovarian, colon, renal, central nervous system, non-small cell lung cancer, melanoma and leukaemia. Evaluated structures exhibit low, moderate or high effect on cancer cells growth. The most active compounds were selected for screening in cancer cell lines in dose-dependent manner.

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**Acknowledgements**
Support of K/ZDS/004689 is kindly acknowledged.

**P72**

# Synthesis of piperidine and pentanediamine derivatives as a potential histamine $H_3$ receptor antagonists

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Previously we reported the synthesis and biological evaluation of a series of 1-substituted-4-[5-(*N*-methyl-*N*-propylamine)pentyloxy]piperidines [1,2] as non-imidazole histamine $H_3$-receptor antagonists. It was shown that the most potent compounds under *in vitro* screening conditions were among others the: 1-(benzyl)- and 1-[2-benzofuranylmethyl]-4-[5-(*N*-methyl-*N*-propylamine)pentyloxy]piperidine ($pA_2$=7,79 and $pA_2$=8,47, respectively). For both of the aforementioned compounds replacement of 4-hydroxypiperidine scaffold by a highly flexible 3-(methylamine)propyloxy chain led to 1-[(*N*-benzyl-*N*-methyl)-3-propyloxy]-5-(*N*-methyl-*N*-propyl)pentanediamine with higher ($pA_2$=8.02) and 1-[(*N*-(2-benzofuranylmethyl)-*N*-methyl)-3-propyloxy]-5-(*N*-methyl-*N*-propyl)pentanediamine with lower ($pA_2$=6.23) affinity at $H_3$ receptor than their piperidine analogues.

These unexpected results prompted us to synthesis and pharmacological evaluated series of 4-hydroxypiperidine and pentanediamine derivatives in which the aliphatic chain between the piperidine nitrogen, 3-(methylamine)propyloxy nitrogen and lipophilic residue was elongated from 1 to 3 methylene groups.

Additionally, we also wanted to explain whether earlier observed tendency is kept or it was an exceptional case only for short methyl chain.

In the present work, we report the synthesis and preliminary pharmacological investigation (functionally on *in vitro* test system using guinea pig jejunum preparations) of new series of 1-(ω-phenylalkyl)-, 1-[ω-(2 benzofuranyl)alkyl]-4-[5-(*N*-methyl-*N*-propylamine)pentyloxy]piperidines and 1-[(*N*-(ω-phenylalkyl)-*N*-methyl), 1-[(*N*-(ω-(2-benzofuranyl)alkyl)-*N*-methyl)-3-propyloxy]-5-(*N*-methyl-*N*-propyl)pentanediamines as $H_3$ histamine receptor antagonists.

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**Acknowledgements**

This work was supported by the Medical University of Lodz (statutory activity number 503/3/016-01/503-01).

**P73**

# Synthesis and the *in vitro* evaluation of cytotoxicity of some thiosemicarbazides and dicarboximides

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Derivatives of thiosemicarbazide are a class of small molecules which displayed extensive spectrum of biological activity. This compounds possess antibacterial [1], antifungal [2] and antitumor [3] properties. Additionally, some of them have low cytotoxicity against normal cell lines [2] and high cytotoxicity against cancer cell lines [3].

In this study, we presented synthesis and *in vitro* evaluation of cytotoxicity of some thiosemicarbazides (**1,3,5,6**) and dicarboximides (**2,4,7,8**). These compounds were obtained by the reaction of selected dicarboxylic acid anhydrides with 4-subsituted-3-thiosemicarbazides in boiling dry chloroform. Some of the derivatives (**1,2,6,7,8**) was obtained and examined for their antimicrobial and antituberculosis activities before [4]. The structure of all compounds was confirmed by different spectroscopic methods, including $^{1}H$ and $^{13}C$ NMR and also by elemental analysis.

In order to assess the cytotoxicity of compounds **1-8** the normal animal cell line (Vero - African green monkey kidney) and human epithelial cell line, FaDu, from a squamous cell carcinoma of the hypopharynx, incubated for 24 hours, have been used. The compounds were dissolved in DMSO to obtain stock solutions (50 mg/ml). Subsequently, for the use in *in vitro* tests, those stock solutions were further diluted in growth media containing 2% of fetal bovine serum. Assessment of cytotoxicity was carried out towards both cell lines in increasing concentrations of tested substances, ranging from 1.9 to 1000 µg/ml.

Data obtained after performing the MTT test were used to calculate $IC_{50}$ values which were the measure of cytotoxicity of tested compounds. All experiments were performed in triplicate.

The $IC_{50}$ of tested substances ranged from 23.66 – 509.57 µg/ml in Vero and 8.85 – 334.23 µg/ml in FaDu cell lines. The lowest cytotoxicity towards Vero was observed for compound **1** (509.57 µg/ml) and towards FaDu for compound **7** (334.23 µg/ml). The highest cytotoxicity was noted for compound **5** in both Vero and FaDu cell lines ($IC_{50}$ = 23.66 µg/ml and 8.85 µg/ml, respectively).

Further studies will involve the assessment of antiviral activity of compounds **1-8** against Human herpesvirus 1(HSV-1) and Coxsackievirus B3 (CVB3).

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**P74**

# The positive allosteric modulator of the α7 nicotinic receptor, 3-furan-2-yl-N-p-tolyl-acrylamide, has anxiolytic activity without toxicity

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Nicotinic acetylcholine receptors (AChRs) are promising molecular targets for the treatment of neurological disorders including drug addiction, cognitive impairments, depression and anxiety. The objectives of this study were to determine the anxiolytic activity of 3-furan-2-yl-N-p-tolyl-acrylamide (PAM-2), a positive allosteric modulator of α7 AChRs, in mice, by the elevated plus maze method, and whether this compound affects neuronal and organ toxicity.

The *in vivo* study shows that PAM-2 mediates anxiolytic behavior in male, but not female, mice, after acute treatment, and this activity lasted for one more day after injection. The chronic treatment (three weeks) produces higher anxiolytic activity compared to the acute treatment in male, whereas there is no residual activity after one week of treatment cessation. To determine the mechanism for the observed anxiolytic activity, PAM-2-induced upregulation of hα7 AChRs was measured in SH-SY5Y cells overexpressing human(h) α7 AChRs by [$^3$H]-epibatidine binding at different temperatures (24 and 37 °C) and periods (1, 3 and 7 days). PAM-2 did not affect the hα7 AChR level under these experimental.

The toxic activity of PAM-2 was compared to that for PAM-4 (3-furan-2-yl-N-phenylacrylamide) by measuring the cellular viability of SH-SY5Y-hα7 cells after 1, 3, and 5 days of incubation. For both compounds, no cellular toxicity was observed up to the concentration of 2.5 μM. The preservative effect of PAM-2 on okadaic acid(OA)-induced neurotoxicity (model of neuronal death) in SH-SY5Y-hα7 cells was also studied. The results show that PAM-2 protects the deleterious effects elicited by OA up to concentrations of 2.5 μM.

To determine the organ toxicity of PAM-2, the levels of several enzymes related to liver, kidneys and heart function were measured. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), creatinine kinase (CK), and bilirubin were measured in blood serum from mice administrated with different doses of PAM-2 (0, 0.5, 1.0 and 2.0 mg/kg). No increase in the examined enzymes and bilirubin was observed in response to any dose of PAM-2, indicating that PAM-2 treatment does not induce toxic effects in important organs.

Our findings suggest that PAM-2 might be a novel therapeutical option for the treatment of anxiety-related diseases without toxic effects.

**Acknowledgements**
This work was supported by the Polish National Science Centre (SONATA funding, UMO-2013/09/D/NZ7/04549).

**P75**

# Mutagenicity assessment of some new *N*-[phenoxyalkyl]- and *N*-2-[2-(phenoxyethoxy)ethyl]aminoalkanols

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The aim of the study was preliminary evaluation of mutagenic potential of new compounds (I-VII) exhibiting anticonvulsant activity *in vivo* in maximum electroshock seizure (MES) test. All tested compounds were aminoalkanol derivatives containing phenoxyalkyl or phenoxyethoxyethyl scaffold substituted in the aromatic ring.

The evaluations were performed with the use of bacterial *in vitro* mutagenicity test (Ames test [1]). It involves genetically modified species of *Salmonella typhimurium,* which lack the ability to produce histidine due to a point mutation. In the presence of mutagen the reverse mutation occurs, resulting in restoration of the ability to produce histidine and grow on a medium lacking histidine. In the current study two species of *Salmonella typhimurium* were used (TA100 and TA98), varying in a type of detected reversed mutations – base-pair substitution in case of TA100 and frameshifts in case of TA98 [2].

Additionally, *in silico* methods were used to predict mutagenic potencial of compounds I-VII. The evaluations were carried out using several programs varying in algorithm of mutagenic properties estimations (*e.g.* Toxtree [3], LAZAR [4]).

All tested compounds I-VII showed no mutagenic potential in the Ames test involving both TA100 and TA98 strains. The outcomes of *in silico* studies were consistent with *in vitro* evaluations, which suggests the usefulness of *in silico* methods in prediction of mutagenicity in the group of phenoxyalkyl and phenoxyethoxyethyl derivatives of aminoalkanols.

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**P76**

# The effect of *Spirulina platensis* extract on cultured human skin fibroblast cells

*Natalia Pawłowska[1], Anna Czajkowska[2], Agnieszka Gornowicz[2], Bożena Popławska[2], Marta Mieleszko[2], Anna Bielawska[2], Krzysztof Bielawski[1]*

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*Spirulina platensis*, also known as blue-green algae, is filamentous, undifferentiated, non-toxigenic cyanobacteria largely distributed in nature. This bacteria belongs to *Arthrospira* genus and it has been taken as supplement in human and animal food. Early interest in *Spirulina* was focused in particular on its potential as a rich source of variety nutrients such as protein, essential fatty acids like gamma-linolenic acid (GLA) and vitamins, especially vitamin B12 and beta-carotene. Over the last few years, Spirulina has been found to have many pharmacological properties mainly through its active constituent C-phycocyanin, which exhibit anti-inflammatory, neuroprotective and immunomodulatory effects. Moreover, extracts from cyanobacteria have antimutagenic and anticancer activity and can prevent the growth of tumor. *Spirulina* is also widely used in cosmetology as a source of compounds with high antioxidant activity.

The aim of the present study was to investigate the effect of the *Spirulina* extract on cultured human skin fibroblasts. The proliferation of skin fibroblasts was evaluated by analyzing the viability and DNA biosynthesis in these cells. The impact of the *Spirulina* extract on fibroblasts viability was performed using MTT assay. The DNA biosynthesis was checked by incorporation of [$^3$H]-thymidine into DNA. The viability of cells and [$^3$H]-thymidine incorporation were analyzed in human skin fibroblasts after 24 hours of incubation with different concentrations of the tested extract (50, 100, 200 and 400 µg/ml).

The *Spirulina platensis* extract was found to stimulate proliferation of human skin fibroblast cells at given concentrations. The addition of the *Spirulina* extract to the cells culture resulted in significant increase of fibroblasts viability. At the lowest concentration of tested extract (50 µg/ml), fibroblast cells have shown 121% of cell viability. Further, increase in extract concentration led to gradual enhance in cell viability. Moreover, *Spirulina platensis* extract enhanced the DNA biosynthesis in fibroblast cells. The higher proliferation corresponded to an increase in concentration of tested extract.

Our study proved that the extract from *Spirulina platensis* has a beneficial effect on proliferation of human skin fibroblast cells. The obtained results suggest that *Spirulina* extract might improve the condition of skin and use in cosmetology as an ingredient of natural cosmetics.

**P77**

# Cytotoxic and mutagenic effect of *Centella asiatica* aqueous extract and its biotechnological modification

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*Centella asiatica* also known as Gotu Kola, belongs to the *Apiaceae* family, which mainly grows in Asia, equatorial Africa, central America and in the tropical region of Oceania. Major constituent of the plant, with reported pharmacological activities, are pentacyclic triterpenes which include: asiaticoside, madecassoside, asiatic and madecassic acid. *Centella asiatica* is a medicinal plant which has been used in Chinese medicine for three thousand years and in folk medicine for hundreds of years. The plant is primarily used for dermatological conditions, to improve small wounds, burns, scratches, insect bites and even eczema healing. It is also recommended in the treatment of vein insufficiency, epilepsy, and as an anti-cancer agent [1,2,3].

The aim of this study was to investigate the mutagenicity and cytotoxicity of *Centella asiatica* aqueous extracts against human skin fibroblasts (HSF). Aqueous extract of *Centella asiatica* and aqueous extract of its biotechnological modification was studied and compared. Human skin fibroblasts were cultivated in the suitable conditions: medium (DMEM low glucose medium with antibiotic, 10% concentration of fetal bowine serum and stable glutamine), at the temperature of 37°C and with an access to 5% $CO_2$. Cells were cultivated with the addition of both aqueous extracts at five different concentrations: 0µg/ml, 10µg/ml, 15µg/ml, 25µg/ml, 50µg/ml, 100µg/ml. A cytotoxicity test was performed by using trypan blue staining. Our results showed that extracts were non cytotoxic against human skin fibroblasts for all tested concentrations.

The mutagenic potential of *Centella asiatica* extracts were determined using the Ames test. The method involved pre-incubation on *Salmonella typhimurium* TA 100 bacterial strains. The results showed that aqueous extract of *Centella asiatica* plant and its biotechnological modification were not mutagenic towards TA 100 strain for all concentrations studied.

In conclusion, both *Centella asiatica* aqueous extracts were non cytotoxic against human skin fibroblasts and non mutagenic on *Salmonella typhimurium* TA 100 strain, however further tests must be carried out, to say that the extracts are safe and can be used as ingredients of herbal pharmaceutics and cosmetics.

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**P78**

# Study on piperlongumine as a multifunctional agent modulating doxorubicin activity

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*Introduction:* Doxorubicin (DOX) is one of the most commonly used drugs in cancers therapy. Despite great efficacy and activity against broad spectrum of neoplasms, its application is limited by several adverse drug reactions – especially cardiotoxicity, which affect even 11% of patients, treated with approved doses[1]. Mechanism of cardiotoxicity is not clear, however it is associated with reduction of DOX to cardiotoxic metabolite – doxorubicinol[2], catalysed by carbonyl reductase 1 (CBR1). Another clinical problem is cancer cells resistance to doxorubicin, which decreases response to treatment. 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 is inhibitor of CBR1, GSTπ and ABC transporters. PL has also its own cytotoxic activity, which does not affect normal cells[4]. Above informations were encouragement to undertake studies on DOX and PL combination.

*Aim:* Aim of the study was to investigate a.) influence of DOX and PL combination on cancer cells viability and motility. b.) changes in kinetics of DOX reduction to doxorubicinol in presence of PL.

*Materials and methods***:** 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 in incubation mixture was 5 μM.

*Results:* 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.

*Conclusions:* 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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**P79**

# Molecular properties and antimicrobial activity of novel thiourea derivatives

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Untreated pathologies in the oral cavity can cause diabetes, hypertension, heart disease, and cardiovascular diseases, sepsis, cancer, allergies, nephritis, gastrointestinal disease. The most dangerous for overall health are destroying the structures of the tooth bacteria and microorganisms that live in diseased periodontal tissues. Among the bacteria responsible for the development of dental caries oral streptococci such as *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus mitis* dominate. Additionally, the cancer patients undergoing chemotherapy often suffer from oral complications including e.g. dental caries oral mucositis.

In our study we synthesized a group of thiosemicarbazide derivatives as substances with potential antibacterial activity. Taking into account the literature data we introduced the thiourea group which was thought to be determining for biological activity. Among tested derivatives seven compounds exhibited good antibacterial activity against microaerobic Gram-positive bacterial strains (MIC 7.81–31.25 μg /ml). The most active 4-(2,4-dichlorophenyl)-1-(pyridin-2-yl)carbonylthiosemicarbazide exhibited growth inhibition of *Streptococcus sanguinis* with the concentration of 7.81μg/ml. This compound showed activity equal to that of chlorhexidine (CLX) and its cytotoxicity was lower than for two reference antibacterial agents (CLX and ethacridine lactate) displaying $CC_{50}$ values of 8.46 ± 3.6 μg/ml and 6.88 ± 2.7 μg/ml, respectively.

The results are very promising so we decided to investigate the molecular properties determining antibacterial activity for this compound. Furthermore, we postulated that the mechanism of antibacterial activity of the investigating compounds might be connected with the inhibition of bacteria biofilm formation. The bacteria use the enzyme glucansucrase to build long, sticky chains of sugars called glucans, using the sugar from human diet. These glucans glue the bacteria to the surfaces of teeth, forming a biofilm that is difficult to remove. In order to demonstrate that the investigated compounds may inhibit the enzyme glucansucrase and thus, biofilm formation, we performed molecular docking of the studied compounds to glucansucrase crystal structure (PDB ID: 3AIC).

**P80**

# Preliminary safety assessment of selected arylsulfonamide derivatives of (aryloxy)alkylamines using alternative methods

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Genotoxicity and mutagenicity are among the toxicological effects that cause the highest concern for humans; thus, they are the object of intense research activity, as well as of recognized regulatory testing methods [1]. Both *in vivo* and *in vitro* methods have been extensively used for genotoxicity and mutagenicity testing, moreover, *in silico* methods have been used in combination with *in vitro* analysis. In the present study, mutagenic and genotoxic effects of selected arylsulfonamide derivatives of (aryloxy)alkylamines, classified as 5HT7 receptor antagonists were evaluated *in vitro* using the *Vibrio harveyi* [2] assay and the SOS/*umu*-test, respectively. Mutagenicity compounds was also analyzed *in silico*. Firstly, we performed *in silico* mutagenicity screening. It was found that evaluated compounds had no structural alerts and were predicted to be non-mutagenic. In a subsequent step, the results obtained *in silico* study were verified *in vitro* by conducting an assay with *V. harveyi* strains. Similarly to the preliminary *in silico* data, none of the investigated compounds showed mutagenic activity on *V. harveyi* BB7, BB7M, BB7X and BB7XM strains, as the number of revertants never exceeded the critical value of a 2-fold increase in comparison to the negative control. Additionally, none of the tested samples revealed genotoxic activity in the SOS/*umu*-test. The IR value never reached the threshold value of 1.5. The obtained results encourage further studies in eukaryotic models to confirm the lack of genotoxicity of tested compounds, as some of tested molecules possess mutagenic and toxic constituents.

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**P81**

# The rearrangement in synthesis of new arylideneimidazolone derivatives as a way to obtain biologically active compounds

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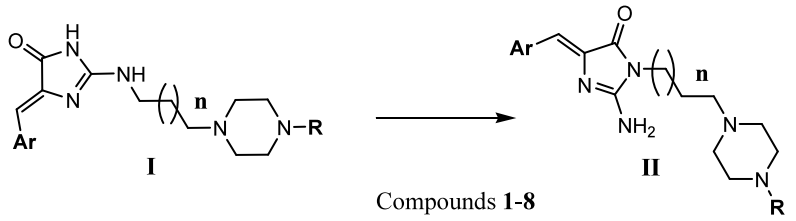
Piperazine derivatives of arylidenoimidazolones are an interesting group of chemical compounds that was found as modulators of bacterial and/or cancer multidrug resistance (MDR) mechanisms [1, 2]. During the studies on anti-MDR properties of piperazine imidazolones, we tried to synthesize the 1-aminopropyl-methylpiperazine derivative **1** (Fig.1 I, Ar: *p*-Cl-phenyl), by condensation of the S-methylimidazolone with the suitable amine. Although spectral and elemental analyses suggested that we obtained the desirable product I (Fig.1), the X-ray analysis confirmed that the compound 1 fitted in the structure II (Fig.1), which indicated a rearrangement within imidazolone ring during the synthesis way performed. For wider studies on this interesting mechanism, we decided to investigate a series of reactions performed in similar conditions, using S-methyl arylideneimidazolones with different substituents at position 5 and various primary amines as reactants.




n = 1,2
Ar = (un)substituted phenyl, naphthalene, phenanthrene, anthracene
R = Me, 2-MeOPh

**Fig. 1**

The synthesis of seven new compounds **2-7** (Fig.1) has been carried out, including the Knoevenagel condensation, S-methylation and reaction with suitable aminealkylpiperazine derivatives. Crystallographic studies have been performed, as well. Both structures (I and II) seem to be interesting target in the search for anti-MDR or GPCR-agents, respectively. Compounds **2**-**5** will be investigated on their bacterial efflux pump inhibitory actions, whereas the phenylpiperazine compounds **6**-**8** on their affinity for adrenergic and serotonin receptors, as well.

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**P82**

# Arylsulfonamide derivatives of aryloxyalkilamines as new uroselective $\alpha_1$-adrenolytics in BPH

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$\alpha_1$-adrenoceptor blockers are currently the first-line treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia (BPH). $\alpha_1$-adrenoceptors (αAR) antagonists have high therapeutic efficacy as they relax prostate smooth muscle and decrease urethral resistance. In contrast, the use of αARs blockers may promote blood pressure–related side-effects, particularly hypotension. It was shown that blockade of subtypes A and D of αAR is important in relieving obstructive and storage symptoms, whereas blockade of subtype B is responsible for side effects[1,2]. Taking that into consideration we are looking for new, highly selective compounds, targeting both prostate $\alpha_{1A}$ ARs and bladder $\alpha_{1D}$ ARs.

A series of arylsulfonamide derivatives of aryloxyalkilamines was synthesized and evaluated in preliminary pharmacological tests. We have assessed the affinity to both $\alpha_1$ and $\alpha_2$ ARs, the selectivity to the subtypes A and B of αARs, antihypertensive properties and the impact on the pressor response elicited by catecholamines.

Radioligand binding studies showed that most of the newly synthesized derivatives have significant affinity for $\alpha_1$ ARs (Ki = 19 - 130 nM), therefore they displayed approximately 10-13 fold selectivity to $\alpha_1$ ARs versus $\alpha_2$ ARs (Ki ca. 300-800 nM). Subsequently it was demonstrated that all selected compounds have antagonistic effect on the pressor response elicited by methoxamine in the range of 90-95%, what confirmed their adrenolytic activity. Furthermore the influence on blood pressure for selected compounds after one-time *i.v.* and long-term *i.p.* administration were assessed. Several derivatives did not significantly decrease systolic and diastolic blood pressure in normotensive anesthetized rats within a dose range of 2-5 mg/kg *i.v.* In comparison, tamsulozin decreased both blood pressure parameters already in a dose of 2 mg/kg *i.v*. Selected compounds did not significantly decrease blood pressure in rats in a dose of 10 mg/kg *i.p.* after long-term administration as well. In the end the calculated EC 50 values for $\alpha_{1A}/\alpha_{1B}$ showed that a number of compounds have preferential activity to the subtype A of $\alpha_1$ ARs (in the range of 1.5 - 6 fold).

The obtained results indicate that tested structures are uroselective which may be a result of their preferential activity within a specific receptor subtypes of $\alpha_1$-adrenoceptor ($\alpha_{1A}/\alpha_{1D}$). In order to confirm this hypothesis there is a need to perform further pharmacological studies. The evaluation of urodynamic parameters in rats with testosterone- induced prostate hyperplasia is the last crucial step of research for the confirmation of the therapeutic efficacy of new $\alpha_1$-adrenolytics.

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**P83**

# Structural connectivity fingerprints – a new method of compound representation

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The current generation of substructural fingerprints, bit-string methods of representation of chemical compounds' structure, is based on sets of well-designed, pre-defined substructural keys. They are a commonly used method during virtual screening campaigns, since they are able to reject compounds that do not possess the vital chemical moieties. However, the methodology is somewhat flawed, since the fingerprints do not describe the positions of the groups, only their existence. This implies, that two compounds with dissimilar structures (but similar composition) can be described by almost identical fingerprints.

In this research we addressed this issue by designing new substructural fingerprint – the Substructural Connectivity Fingerprint. This new method of compound representation contains additional information about the interconnectivity of chemical groups within compound. To properly analyze this kind of data, currently existing methods of machine learning data analysis were employed, and a brand new method, Extreme Entropy Machines was implemented.

The initial tests conducted on compounds for 9 GPCRs and 5 protein kinases yielded very promising results, outperforming all currently available fingerprints. The fingerprint and the new method of data analysis will be expanded and validated in further research.

**P84**

# Molecular basis of stereoselective sulfonation of selected substrates

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Sulfate conjugation is an important pathway in the biotransformation of many endogenous and exogenous compounds. The mammalian cytosolic enzymes that catalyze sulfonation constitute a superfamily of sulfotransferases (SULT) that use a broad array of hormones, neurotransmitters, drugs, and carcinogens as substrates. For the vast number of drugs their physiological effects are terminated by this metabolic pathway. In the scope of our interest are the drugs that follow a sulfate conjugation reaction at the hydroxyl group and the structural factors that govern their biotransformation.

One of the structural characteristics that influence the catalytic efficiency of human sulfotransferases is stereochemistry of the molecule that serves as the sulfuryl acceptor. In the present work, the role of stereoselectivity of the human sulfotransferases is examined. Inferring from the existing literature, we gathered a set of compounds with experimentally determined catalytic activities and reported stereoselectivities. The compounds were subjected to docking to appropriate sulfotransferase isoform(s) to detect substrate-enzyme interactions that determine the SULT preference.

The basis of substrate stereoselectivities of sulfotransferases may be critically important in determining their overall roles in metabolism of drugs, carcinogens, and other xenobiotics.

**P85**

# Design, synthesis of new amides derived from 3-phenyl-2,5-dioxo-pyrrolidine-1-yl-acetic acid as potential anticonvulsant agents

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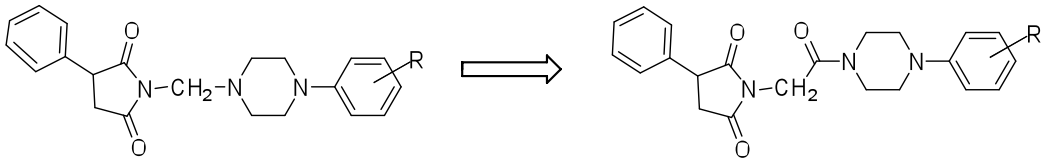
The previous research from our laboratory have demonstrated diversified anticonvulsant activities among the differently substituted pyrrolidine-2,5-diones. The most promising were N-Mannich bases with two aromatic substituents at the position-3 and the phenylpiperazine moiety at the position-1 of imide ring, whereas derivatives, which contain one aromatic ring at the position-3 of pyrrolidine-2,5-dione were devoid of anticonvulsant activity [1,2].

Bearing these considerations in mind, as a continuation of studies among variously substituted succinimides, we have synthesized new series of piperazinamide derivatives of phenyl-2,5-dioxo-pyrrolidine-1-yl-acetic acid. These compounds have been designed as analogues of previously obtained N-Mannich bases, consequently, the proposed modifications enable to assess the role of supplementary amide function on an anticonvulsant properties in this group of derivatives.



R=H, 2-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 3,4-Cl, 3-$CF_3$

The obtained compounds were evaluated for their anticonvulsant activity in the maximum electroshock (MES) and subcutaneous pentylenetetrazole seizure tests (*sc*PTZ). Moreover, the acute neurological toxicity was determined in the minimal motor impairment rotorod screen [3]. The results revealed that the majority of this type of derivatives exhibited anticonvulsant activity, both in the MES and *sc*PTZ tests.

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**P86**

# Novel 1-aryl-6-arylimidazo[1,2-a][1,3,5]triazines - synthesis and antiviral activity

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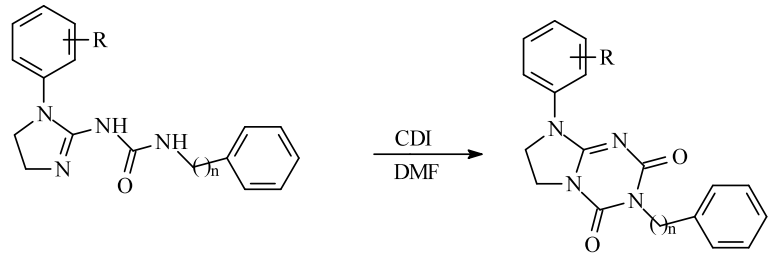
Viral infections are a permanent health problem of mankind. More and more often used therapeutic methods like organ or bone marrow transplantations, when patients are subject to strong immunosuppression, result in numerous viral infections caused by viruses from the Herpesviridae family. *Herpes simplex* virus type I infection can cause several clinical conditions such as keratitis, cutaneous herpes and encephalitis.[1-3]

In order to elaborate novel treatment against Herpes simplex virus and Coxsackie virus compounds 2a-2p which are 1-aryl-6-arylalkyl-5,6(1*H*)-dioxo-2,3-dihydroimidazo[1,2-a][1,3,5]triazines were obtained.

New compounds were obtained from appropriate 1-(1-aryl-2-amine-4,5-dihydro-1*H*-imidazoline)-3-arylalkylurea and 1,1'-carbonyldiimidazole (CDI) in DMF (Scheme 1). Ten compounds were biologically tested. $CC_{50}$ values of the investigated compounds ranged from 54.1to 641.4 μg/mL.

**Scheme 1.** The synthesis scheme of the investigated compounds.




1a-p 2a-p

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**P87**

# Pharmacological characterization of zinc interaction with 5-$HT_{1A}$

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Zinc, as an essential trace element in living organisms, plays an important role in the number of biological processes, especially within the central nervous system [1]. There is an evidence for the involvement of Zn ions in depression and so constitutes potential angle of therapy, as emphasized by numerous preclinical and clinical trials. However, its exact molecular mechanism of action is still not fully understood [2]. Our interests are focused on its effects mediated by serotonin receptors, which are key players in the etiology of anxiety and mood disorders [3].

Here we present radioligand binding assays used to characterization of the pharmacological profile of $Zn^{2+}$ at serotonin 5-$HT_{1A}$ receptor (5-$HT_{1A}$R). The direct influence of $Zn^{2+}$ on agonist binding to human 5-$HT_{1A}$R, stably expressed in HEK293 cells, was investigated by a set of *in vitro* radioligand binding methods (saturation, competition and both association and dissociation kinetic studies) using [$^3$H]8-OH-DPAT, as a selective agonist tool compound. It has been demonstrated that in addition to previously reported negative allosteric modulation [4], for both antagonist and agonist binding, $Zn^{2+}$ at low concentration may also potentiate binding of endogenous serotonin to 5-$HT_{1A}$R.

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**P88**

# Synthesis of Safirinium P, Q and its analogues – novel tunable fluorescent dyes

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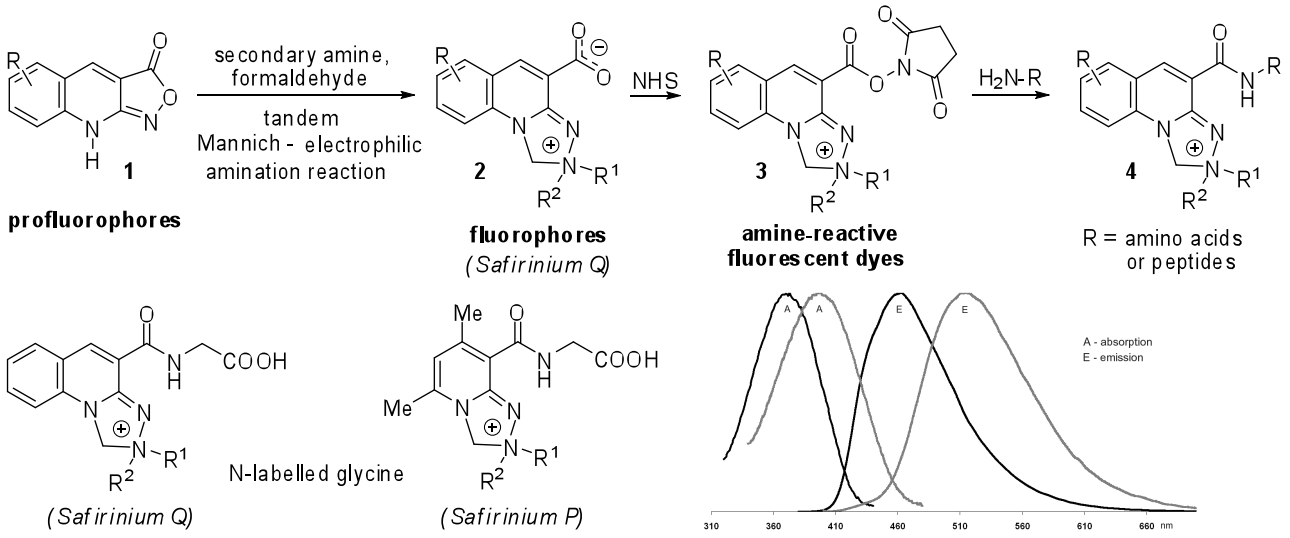
Non-fluorescent profluorophores **1** treated with secondary aliphatic amines and formaldehyde give rise to the formation of fluorescent 1,2,4-triazolo[4,3-*a*]quinolin-2-ium-4-carboxylates (*Safirinium Q*) which are stable in aqueous solution, redox-stable, and exhibit: high quantum yields, large Stokes shift, lack of solvatochromism, remarkable water solubility and adjustable polarity achieved by introduction of alkyl chains of various size.

The above *click* tandem Mannich – electrophilic amination reaction that proceeds quantitatively at room temperature yielding a fluorescent product can be used as a versatile platform for fast and highly sensitive detection and environmental monitoring of formaldehyde and secondary aliphatic amines [1,2].

Consecutively, the *Safirinium* dyes upon esterification with *N*-hydroxysuccinimide (NHS) provide amine-reactive probes useful for fluorescent labeling of amino-acids, lysine-containing peptides and proteins, as exemplified by labeling of bacterial spores [2].




Excitation/emission spectra of the dyes can be tuned in the ranges 350-400 nm and 465-515 nm (blue-green), respectively, by introduction of electron-donating substituents into positions 5, 6, 7 and 8 of the quinolinium moiety.

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**Acknowledgements**
This work was supported by Polish Ministry of Science and Higher Education and National Science Centre, research grant IP2012 055472.

**P89**

# Tricyclic xanthine derivatives as MAO-B inhibitors

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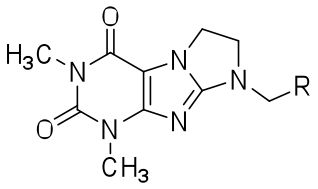
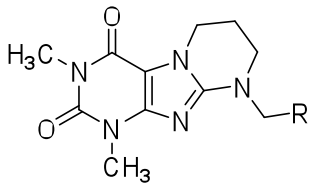
Parkinson's disease is a neurodegenerative disorder related primary to the progressive degeneration of dopaminergic neurons, especially in substantia nigra of the brain. It causes a disturbance in the execution of arbitrary and involuntary movement sequences. One of treatment methods is to use the inhibitors of dopamine degrading enzyme – monoamine oxidase B (MAO-B) [1-3]. Based on recent studies, two series of tricyclic xanthenes derivatives with five or six membered annelated ring were designed [2,3].

Table 1. Structures of tricyclic xanthine derivatives **I** and **II**.



**I**



**II**

Inhibitory activity of the synthesized tricyclic xanthine derivatives towards MAO-B enzyme were determined with the useof commercially available human recombinant enzyme MAO-B expressed in baculovirus infected insect cells, and Amplex Red monoamino oxidase assay kit (Life Technologies).

Drug-like properties (logP, logS, toxicity, drug score) of the obtained compounds were evaluated by using the OSIRIS program [4].

The obtained compounds possess submicromolar inhibitory activity. Derivatives with six-membered annelated ring showed higher activity toward MAO-B enzyme than tricyclic structures with five-membered ring.

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**Acknowledgements**

Partially supported by Polish National Science Center funds, NCN-DEC- 2012/04/M/N24/00219.

**P90**

# Synthesis and anticancer activity of *S*-substituted *N*-(quinazolin-2-yl)-2-mercaptobenzenesulfonamide derivatives

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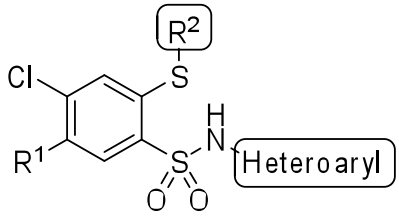
In continuation of our long lasting scientific program which aimed at searching of novel anticancer compounds from among novel series of *N*-aryl-2-mercaptobenzenesulfonamide derivatives we pay special attention to pharmacophoric quinazoline moiety that appearing in many chemotherapeutic agents which acts as tyrosine kinase inhibitors EGFR [1-3].

A series of novel *S*-substituted *N*-(quinazolin-2-yl)-2-mercaptobenzenesulfonamides was synthesized by the reaction of *N*-(benzenesulfonyl)cyanamide potassium salt with the appropriate 2-amino-(aceto/benzo)phenones in glacial acetic acid at reflux.

Anticancer activity of dozen compounds obtained were tested *in vitro* at National Cancer Institute (Bethesda, the USA) on the panel of 60 human cancers cell lines. The highest sensitivity towards the tested compounds was found for several cell lines of leukemia, non-small cell lung cancer (NSCLC), melanoma and CNS cancer cell lines.

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**Acknowledgements**
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**P91**

# Study the spectrum of biological activity of styrylquinoline derivatives

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Compounds bearing a quinoline moiety are well known due to their broad biological activities. A number of them have been widely investigated and clinically used as antifungal or antibacterial agents [1,2]. Styrylquinoline derivatives have gained strong attention recently due to their activity as perspective HIV integrase inhibitors [3]. There are many reports on the synthesis and applications of styryl dyes build on quinoline [4-6]. Recently, some quinoline-based compounds have been synthesized and reported as potent antitumor agents [7]. With these in mind we focused our attention on biological activity quinoline analogues and fluorescent compounds capable of staining cancer cells. Novel styrylquinoline was designed by combining molecular fragments from known anti-proliferative agents on the diazanaphtalene moiety. Series of quinoline derivatives were obtained with the use of microwave-assisted synthesis. The lipophilicity of the compounds was measured by RP-HPLC. All studied compounds were tested for their in vitro antitumor activity. Cellular proliferation was determined using the MTS assay against the human colon carcinoma (HCT 116) cell lines with wild type p53 (p53+/+) and with a p53 deletion (p53-/-). The most active compounds were also tested for their cytotoxicity againts normal cells – human fibroblasts (GM 07492). Compounds localisation in living cell cultures was studied using fluorescence microscopy. Caspase activation (-3 and -7) was measured using the luminescent Caspase-Glo 3/7 assay in HCT116 cells. Styrylquinoline derivatives inhibit the proliferation of tumor cells. The subcellular localisation of the compounds, immunodetection of the protein p53 and activating the caspase cascade suggest the mechanism of action through mitochondrial pathway to apoptosis in p53 independent manner.

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**Acknowledgements**
Ewelina Spaczyńska appreciates the support of the DoktoRIS studentship.

**P92**

# Derivatives of N-[2-(dimethylamine)ethyl]-N-(2-phenylethyl)-aniline as potential polypharmacological ligands of SERT/5-HT6/5-HT7

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Depression is a serious mental disorder that cripples the lives of hundreds of millions of people. It is estimated that annual cost associated with depression reaches 800 billion euro (only in Europe).[1]

Most commonly used antidepressant drugs (e.g. Fluoxetine, Sertraline) acts as a selective serotonin reuptake inhibitors (SSRI) by inhibiting the serotonin transporter (SERT). Unfortunately, these substances sometime exhibit low efficacy together with many side effects.[2]

It was revealed, that compounds acting on serotonin receptors (e.g. 5-HT1A, 5-HT6 and 5-HT7) may possess antidepressant properties.[3] This led to the development of an augmentation therapy, where SSRI treatment is supplemented by, for example, buspirone which is a partial agonist of 5-HT1A receptor.[4]. Unfortunately, this method brings with it a possibility of dangerous drug interactions and cumulative side effects which can be possibly avoided by a fusion of SERT and agonistic/antagonistic activity in one compound.

Derivatives of N-[2-(dimethylamine)ethyl]-N-(2-phenylethyl)aniline revealed to possess high affinity towards 5-HT6 and 5-HT7 receptors, what more, molecular modeling studies showed their possible high affinity towards SERT. This led to a conclusion that these compounds may act as substances with dual SERT/5-HT6 and/or 5-HT7 activity.

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**Acknowledgements**

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**P93**

# Synthesis and *in vitro* preliminary pharmacological investigation of novel guanidine derivatives as non-imidazole histamine $H_3$ receptor antagonists

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Guanidine derivatives are well-known as biological active compounds, both a natural – L-arginine – and synthetic, guanidino-containing drugs – Metformin, Famotidine, Streptomycin. Guanidine due to $pK_a$=13.6 is often associated with low bioavailability, but substituted guanidines as orally available Guanfacine can reach CNS [1], where histamine $H_3$ receptor is mostly located. Histamine $H_3$ receptor antagonists are considered to be potential CNS diseases treatment as Alzheimer's disease, ADHD or memory and learning deficits.

In the recent years, 1-substituted and 1,1-disubstituted guanidine derivatives were synthesized in Department of Synthesis and Technology of Drugs and *in vitro* tested as histamine $H_3$ receptor antagonists [2]. The 1,1-disubstituted guanidines showed higher affinity to the investigated receptor reached $pA_{2gpi}$ value at the level 8.21-8.49. These results prompted us to synthesize and investigate several novel guanidine derivatives based on the lead compounds (Figure 1) .

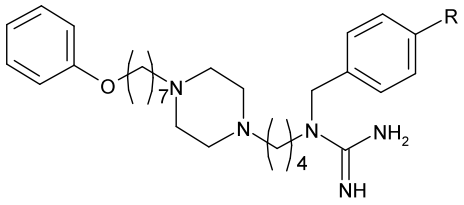


Figure 1. General structure of the lead compounds.

The aim of this study was to modify structure of the lead compound (Figure 1) in search for further increasing affinity to histamine $H_3$ receptor. Firstly, we have planned to replace the central piperazine ring by piperidine. Than we focused on some modifications within guanidino group, therefore 1,3-disubstitutedguanidine, 1,1,2-trisubstitutedguanidines and *bis*-guanidines were synthesized . All new compounds were tested as histamine $H_3$ receptor antagonists *in vitro* in electrically stimulated to the contraction guinea pig ileum [3] and all of them showed lower affinity compared with the lead compounds.

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**P94**

# Design and synthesis of novel chalcone derivatives and evaluation of their effect on microtubule dynamics

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The high incidence of cancer and the high cost of its treatment are important factors driving the search for new and effective chemotherapeutic substances with multi-target activity and no toxicity to normal cells.

Tubulin is regarded as one of the main molecular targets in cancer chemotherapies. Tubulin binding agents interfere with dynamic process of tubulin polymerization leading to abnormal mitotic spindle formation, cell cycle arrest and apoptotic death of cells [1].

In our previous study on novel inhibitors of tubulin polymerization we found very potent combretastatin A-4 (3'-hydroxy-3,4,4',5-tetramethoxy-*cis*-stilbene, CA-4) thioderivatives. That prompted us to investigate the structurally similar compounds like chalcones (1,3-diphenylprop-2-en-1-on derivatives) modified by methylthio substituents. Chalcones as well as CA-4 derivatives are being widely studied due to their multidirectional mechanism of anticancer action, which involves the antimitotic, antiangiogenic, anti-estrogenic and pro-apoptotic activities [2].

In the present study, we designed a series of chalcone thioderivatives using virtual screening protocol of reaction based combinatorial library. In virtual screening, molecular docking to the colchicine binding site of tubulin (PDB id: 1SA0) was used. Consensus score and calculations of binding free energy were used to identify potentially active derivatives. The compounds that have a high scores were synthesized using Claisen-Schmidt condensation of appropriate benzaldehydes and acetophenones and their antitubulin activity was studied *in vitro* with the use of tubulin polymerization assay kit (Cytoskeleton, USA).

The structural similarity to other ligands with the same molecular target as well as relatively simple molecular structure make chalcones very promising object of modifications which – together with the CADD methods – create a chance for the development of new, effective chemotherapeutics and for the better understanding of their interaction with tubulin at the molecular level.

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**Acknowledgements**
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**P95**

# 1-(1-Aryl-4-aryl-4,5-dihydro-1h-imidazo)-3-substituted urea deriva-tives – synthesis, cns activity and molecular modeling

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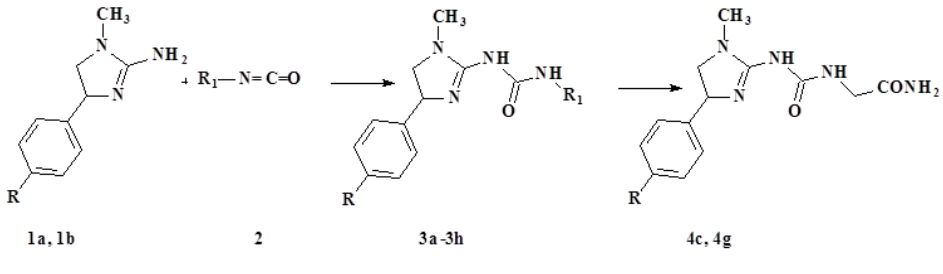
It is well known that many diseases are accompanied by inflammation and pain. Therefore, the search for new antinociceptive compounds is an important focus of attention for chemists as well as for pharmacologists [1,2].

A series of 10 novel urea derivatives has been synthesized and evaluated for their central nervous system activity. Compounds **3a**–**3h** were prepared in the reaction between the respective 1-alkyl-4-aryl-4,5-dihydro-1*H*-imidazol-2-amines **1a** and **1b** and appropriate benzyl-, phenethyl-isocyanate or ethyl 4-isocyanatobenzoate and ethyl isocyanatoacetate **2** in dichloromethane. Derivatives **4c** and **4g** resulted from the conversion of **3c** and **3g** into the respective amides due to action of an aqueous ammonia solution. The results obtained in this study, based on literature data suggest a possible involvement of serotonin system and/or the opioid system in the effects of tested compounds, and especially in the effect of compound **3h**. The best activity of compound **3h** may be primarily attributed to its favourable ADMET properties, *i.e.*, higher lipophilicity (related to lower polar surface area and greater molecular surface, volume and mass than for other compounds) and good blood-brain permeation. This compound has also the greatest polarizability and ovality. The HOMO and LUMO energies do not seem to be directly related to activity.

**Scheme 1.** The synthesis scheme of the investigated compounds.




| | R | $R_1$ |
|---|---|---|
| 3a | H | $-CH_2C_6H_5$ |
| 3b | H | $-CH_2CH_2C_6H_5$ |
| 3c | H | $-CH_2COOC_2H_5$ |
| 3d | H | $C_6H_5$ $COOC_2H_5$ |
| 3e | $CH_3$ | $-CH_2C_6H_5$ |
| 3f | $CH_3$ | $-CH_2CH_2C_6H_5$ |
| 3g | $CH_3$ | $-CH_2COOC_2H_5$ |
| 3h | $CH_3$ | $C_6H_5$ $COOC_2H_5$ |
| 4c | H | - |
| 4g | $CH_3$ | - |

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**P96**

# Synthesis and antitumor activity of novel 2-benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives

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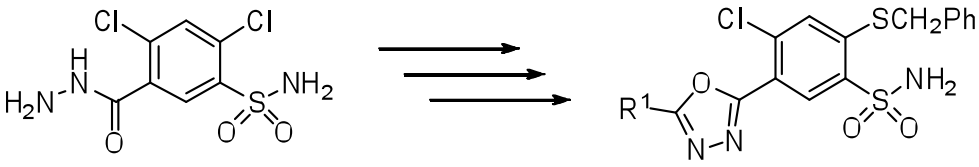
[1]Department of Organic Chemistry, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland
[2]Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG & MUG, Kładki 24, 80-822 Gdańsk, Poland

Our previous investigations focused on the anticancer activity of 2-mercaptobenzenesulfonamide (MBSA) derivatives has shown that compounds containing five-membered heteroaromatic ring attached in position 5 of MBSA scaffold, exhibit remarkable *in vitro* anticancer activity [1,2]. Thus, we have undertaken synthesis of 2-(benzylthio)-4-chloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives substituted in position 5 of oxadiazole ring. Target compounds were obtained in a multistep reactions from 2,4-dichloro-5-(carbazoyl)benzenesulfonamide and were characterized with spectroscopic methods: $^{1}$H NMR, $^{13}$C NMR, IR, and elemental analysis.




Several compounds were tested in the National Cancer Institute (Bethesda MD, USA) at single concentration of 10 μM against panel of 60 human cancer cell lines and at the Department of Biotechnology Intercollegiate Faculty of Biotechnology UG & MUG in 5-dose test on 3 cell lines: MCF-7 (breast), HCT116 (colon), HeLa (cervical cancer). Some compounds exhibited moderate ability to inhibit growth of a certain cancer cell lines, with a selectivity towards leukemia, central nervous system and ovarian cancer.

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**Acknowledgements**
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**P97**

# Synthesis and docking of series of novel diazine derivatives to histamine $H_3$ receptor homology model

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Histamine H3 receptors (H3R) are constitutively active G-protein coupled receptors mostly expressed in CNS, described as presynaptically located autoreceptors as well as heteroreceptors. Interaction with these receptors results in modulation of histamine levels as well as that of other neurotransmitters such as ACh, NA, 5-HT etc. Therefore blockade of these receptors could be useful in the treatment of different CNS disorders [1,2].

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

In order to determinate protein-ligand interactions and it's possible influence on in vitro activity, a set of obtained ligands was docked to histamine $H_3$ homology receptor using Hybrid (OEDocking) [4,5,6].

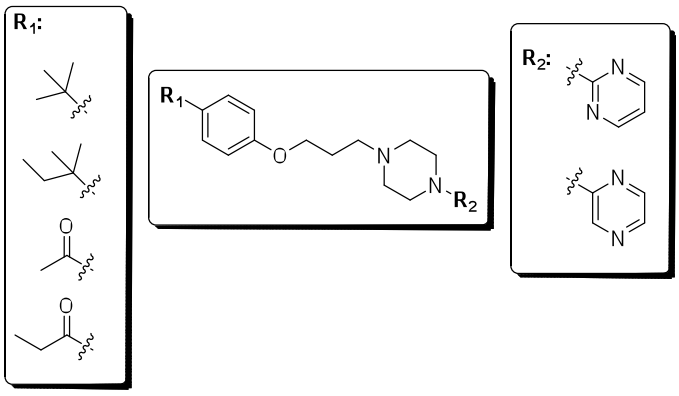


**Fig. 1** General scheme of obtained compounds

[1] Stark H. et al. *Mini Rev. Med. Chem.* 4 (2004) 965.

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**Acknowledgements**

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**P98**

# Long-Chain Aryl-Piperazines - structure and activity

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Long-Chain Aryl-Piperazines (LCAPs) are well known serotonin receptor ligands used as active agents of several antidepressant drugs [1]. LCAPs consist of three structural units (Scheme).


Both arylpiperazine and terminal group have limited conformational freedom. The two units are linked through a flexible spacer. Our X-ray studies on LCAPs of known affinities to 5-HT(1A) receptor and LCAPs found in the Cambridge Structural Database [2] enable us to indicate common conformations and their relationships to their chemical structure.

Protonated LCAPs predominate in the physiological environment and are considered biologically active forms. Protonation of piperazine ring in LCAPs restricts conformational freedom of the spacer and favours its extended geometry. Replacement of the methylene units of the spacer by other groups or atoms affects the affinities of the analogs to serotonin receptors due to changes in their geometries and hydrogen bond functionalities of the new groups.

We will show that a common conformation of LCAPs does exist and that relative orientation of the terminal groups critically depends on the number of methylene units in the spacers.

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

**P99**

# Search on phenylalanine-based AMPA receptor ligands

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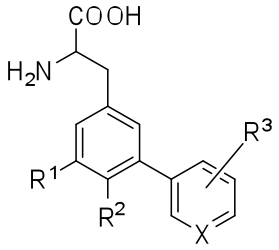
[2]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

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

The present project is a continuation of earlier studies on potent and selective competitive AMPA and/or KA receptors ligands among phenylalanine derivatives [1]. In the process of molecular modelling and docking to known X-ray structures of the glutamate ionotropic receptors binding sites, a new group of compounds were designed on the basis of previously described results [1].

Candidates contained in their structure small polar or lipophyllic substituents in 3rd and/or 4th position of the phenyl ring ($R^1$, $R^2$). Some modification into the distal phenyl ring were also introduced.

A series of the most promising compounds with the structure shown on the Figure below was synthesized and pharmacologically characterized on native NMDA, AMPA, KA receptors. The results of design, synthesis and pharmacological tests are described. Within the performed research the structure-activity relationship was studied. The influence of the substitution pattern ($R^1$, $R^2$ ) as well as structural modifications at the distal phenyl ring (X, $R^3$) were investigated.




$R^1$ = H, Cl, $NO_2$
$R^2$ = H, Cl, $NO_2$, $NH_2$, OH, OMe
$R^3$ = 3-OH, 4-OH, 2,5-diOH, 3,4-diOH, 3,5-diOH, 3-F-5-OH
X = CH, N

[1] Szymańska E., Pickering D.S., Nielsen B. et al. *Bioorg. Med. Chem.* 17 (2009) 6390.

**Acknowledgements**
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**P100**

# The mechanism of synergism between Selol and sulforaphane in the colon cancer cell line HT-29

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Selol is the original Polish patented discovery. It is an organic selenium compound in which selenium has an oxidation state of +4. Currently Selol is a subject of intensive research that suggests the possibility of its use in the therapy of cancer [1,2,3]. Sulforaphane is known for its chemopreventive activity [4]. Numerous studies also showed that sulforaphane potentiates the cytotoxic effect of doxorubicin and oxaliplatin used in antitumor therapy [5,6]. The aim of this study was to investigate the type of interactions between Selol and sulfraphane and mechanism of interaction in HT-29 colon cancer cell line.

The cytotoxic effect of Selol and sulforaphane was assessed after simultaneous and separate administrations of compunds. Cell number after treatment was analyzed after 72 h incubation by CVDE assay. The type of interaction was established by the Chou-Talaly method [7]. The mechanism of interaction was investigated for the strongest antiproliferative effect. The type of cell death was assessed by a confocal microscopy with AnexinV-FITC/propidium iodide staining. The cycle progression was tested with flow cytometry-propidium iodide staining.

The study allowed to emerge new beneficial combinations of compounds. Synergistic effect was observed on HT-29 cell line. Combined administration of Selol and sulforaphane resulted in a stronger cytotoxic effect than monotherapy. There was observed a significant block in S phase of the cell cycle after Selol and combined treatments. Simultaneously large amount of apoptotic cells was detected.

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**P101**

# Synthesis and potent antibacterial activity of new 4,6-dimethylpyridine derivatives

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Research of new compounds with potential antibacterial activity are made mainly in group of heterocyclic compounds. A number of papers about pyridine derivatives has been published. 1,2,4-Triazoles, 1,3,4-thiadiazoles and their condensed derivatives constitute an important class of organic compounds with antimicrobial activities.

Prompted by these findings and in continuation of our efforts in synthesizing various bioactive molecules, we have combined 4,6-dimethyl-2-thiopyridine with 1,2,4-triazoles and 1,3,4-thiadiazoles as potent antibacterial compounds.

The synthesis of title compounds started from acetic acid hydrazide containing 2,4-dimethyl-2-thiopyridine. Thiosemicarbazide derivatives were afforded by the reaction of hydrazide with substituted isothiocyanates. The cyclization of corresponding thiosemicarbazides in the presence of 2% NaOH resulted in the formation of compounds containing 1,2,4-triazole ring. On the other hand, the treatment of thiosemicarbazides with concentr. $H_2SO_4$ caused the conversion of side chain into 1,3,4-thiadiazole ring. The structures of the new compounds were elucidated by spectral and elemental analysis. An antibacterial activity of new molecules is now evaluated.

**P102**

# An analysis of molecular interactions between the 5-HT$_6$ receptor and non-basic ligands

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The newest version of the ChEMBL database, the largest collection of information about the biological activity of chemical compounds, contains 1626 compounds acting on the 5-HT$_6$ receptor ($K_i$ or equivalent equal or less than 100 nM) [1]. Despite the fact that typical 5-HT$_6$R ligands possess positively polarizable nitrogen which forms a crucial, charge-assisted interaction with the D3.32 residue, nearly 15% of actives have low basicity (basic p$K_a$ less than 6) [2,3]. To examine binding modes of non-basic 5-HT$_6$R ligands, class-specific homology models based on seven different templates were generated utilizing previously applied methodology [4]. Models characterized by the highest values of the AUROC parameter in virtual screening experiments were selected for binding mode evaluation. This analysis indicated that the non-basic ligands bind to the receptor through hydrogen bonding with the D3.32 although the charge assisted contribution is missing; hydrogen bonds with T5.46 and T7.39 are also widespread. The most common way of non-basic ligands binding are hydrophobic interactions, among which stackings with aromatic cluster (W6.48, F6,51 and F6.52) are crucial.

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**Acknowledgements**
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**P103**

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

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Epilepsy concerns 1% of world population, and in spite of large progress in development of new antiepileptic drugs, still 30% of seizures are considered pharmacoresistant. Therefore, there are still premises for search of new potential anticonvulsants.

Within the group of aminoalkanol derivatives achieved in our team the most promising so far was *R*-(-)-2*N*-[(2,6-dimethylphenoxy)ethyl]aminopropan-1-ol with $ED_{50}$=5.34 (3.50 – 7.40) mg/kg b.w. (MES, mice, *i.p.*) and this compound was reference for our further research [1] We also found anticonvulsant activity among *N*-[(phenoxyethoxy)ethyl]aminoalkanols which are also subject to intellectual protection. [2]

The present research constitutes nine new variously substituted phenoxyalkyl or phenoxyethoxyethylaminoalkanols, their physicochemical properties and evaluation of their anticonvulsant activity. All achieved compounds were evaluated pharmacologically in maximum electroshock seizure test (MES), maximum electroshock seizure threshold test (MEST), and pentylenetetrazol test (PTZ), as well for neurotoxicity in rotarod test and in chimney test. Additionally, analgesic activity was also evaluated at $ED_{50}$ in MES (respectively) in the formalin test.

Among the achieved compounds, the most active in MES was 1*N*-[3-(2,4,6-trimethylphenoxy)propyl]piperidin-3-ol with $ED_{50}$=20.87 (12.56-34.69) mg/kg b.w. (MES, mice, *i.p.*), however, it proved neurotoxic in the rotarod test ($TD_{50}$=81.95 (70.45-95.32) mg/kg b.w. (mice, *i.p.*). Another active compound with better safety results was *R,S*-2*N*-(2,4-dimethylphenoxy)ethoxyethyl]aminopropan-1-ol with $ED_{50}$=27.11 (22.38-32.83) mg/kg b.w. (MES, mice, *i.p.*) and $TD_{50}$=108.91 (101.81-116.50) mg/kg b.w. (rotarod, mice, *i.p.*).

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**P104**

# The study of metabolic stability of 1-[3-(4-*tert*-butylphenoxy)-propyl]piperidine (DL-76), the histamine $H_3R$ antagonist using *in silico*, *in vitro* and *in vivo* methods

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Histamine (HA) plays a key role in functioning of central and peripheral tissues. It is one of the important local chemical mediators and neurotransmitters found in human body and acts by four histamine receptors ($H_1R$ – $H_4R$). Antagonists/inverse agonists for $H_3R$ are currently in advanced stages of clinical development for a broad spectrum of neurodegenerative diseases [1, 2].

1-[3-(4-*tert*-Butylphenoxy)propyl]piperidine (DL-76) is synthesized in our team the histamine $H_3R$ antagonist. The main goal of the presented work was to determine the structures of metabolites of compound DL-76, with using *in silico, in vitro* and *in vivo* methods. For this purpose, two derivatives, which could be treated as reference compounds of potential metabolites of DL-76 were synthesized. Their structures were chosen based on the previous DL-76 metabolism studies conducted at the Faculty of Pharmacy in Jagiellonian University [3]. The compounds were prepared according to the methods described in the literature including own modifications in some cases.

The study on metabolism *in silico* was performed by MetaSite4 software. Then, the metabolic stability of DL-76 was evaluated *in vitro* using human and rat liver microsomes. Metabolism was also examined *in vivo* by analyzing the rat's plasma and urine after DL-76 intragastric or intravenous administration. The LC / MS / MS technique was used to identify the structures of obtained *in vitro* and *in vivo* metabolites and to compare them to the synthesized compounds.

In conducted biological assays we confirmed the presence of both synthesized metabolites of DL-76. Furthermore, we found that the rate and direction of metabolism depends on the investigated species *(in vitro*: human/rat) and the route of administration of test compound *(in vivo*: i.v./ i.g.).

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**P105**

# Significant activity of chalcogen-containing compounds against *Staphylococcus aureus* strains

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*Staphylococcus aureus* is a dangerous pathogen, able to cause a multitude of human illnesses. It is predominantly responsible for minor skin and soft tissue infections but also more serious invasive syndromes such as pneumonia, severe sepsis and endocarditis. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing problem worldwide and accounts for 40-60 % of all nosocomial *S. aureus* infections [1]. As the bacteria acquire resistance to practically all antimicrobials introduced into clinical use, including last-resort antibiotic vancomycin, diseases caused by these resistant strains are usually difficult to treat. Consequently, due to the limited therapeutic options, its appearance in hospitals has become one of the most serious public health concerns. Estimates indicate that mortality rate from multidrug-resistant *S.aureus* bacteremia, despite improvements in medical care, continues to be 20–30% in the developed world [2]. To make matters worse, while for a long time MRSA infections were restricted to clinical units, in the recent decade MRSA strains have emerged in the community in individuals without any history of hospitalization [3]. Because of a relatively rapid acquisition of antibiotic resistance of MRSA and its expeditiously spread throughout the world, continuous efforts on discovery of lead compounds as well as development of alternative therapies to ensure effective antistaphylococcal treatment are highly required.

During the last decade selenium and tellurium-based compounds have attracted reasonable attention due to their interesting biological effects. As an example, it can be highlighted their strong antioxidant, immunomodulatory and chemoprotective activity [4]. Moreover, our previous reports indicated that inorganic tellurite salt is able to significantly enhance the antimicrobial activity of known antibiotics against highly resistant isolates of *S.aureus* [5]. In view of these results, we paid our attention to organic selenium and tellurium-containing molecules to examine if they have similar effect on *S.aureus* pathogen. Although none of the organochalcogens show synergistic activity in combination with employed in the study antibiotics, we discovered that four of the compounds have potent antimicrobial activity against both, the reference strain *Staphylococcus aureus* ATTC 25923 and the clinical strain MRSA HEMSA 5 (Fig. 1). Moreover, our findings revealed that the molecules tested are responsible for generation of cytotoxic reactive oxygen species (ROS) release by bacteria, which can be related to their antibacterial mechanism of action. We believe that our findings will contribute to identify new tools for the treatment of highly problematic infections caused by multidrug-resistant MRSA strains.

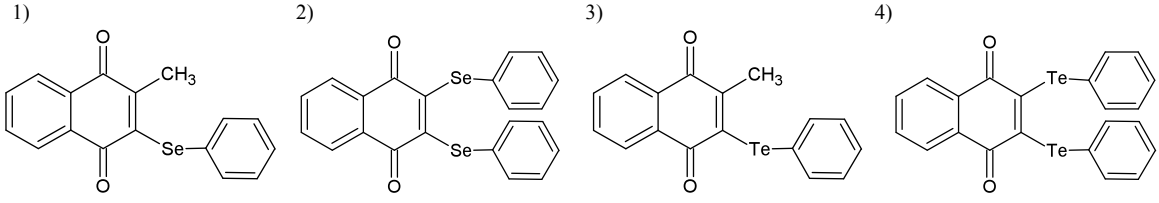


Fig. 1: Structures of the selenium and tellurium compounds employed in the studies.

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**P106**

# Molecular dynamics symulations of adsorption of organic dyes on single walled carbon nanotube

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We performed extensive molecular dynamics simulations of the effect of pH on the co-adsorption of organic dyes with anti-cancer drug doxorubicyn on CNT surface. We considered two different dyes ie. bromothymol blue (BTB) and neutral red (NR). BTB is a sulfonphthaleine dye whit numerous applications in biological and medical fields. It is not only sensitive to pH, but is also used in sensor applications for detecting pesticides, CO2 and ammonia [1]. Due to strong pH-dependent color change near the neutral pH region neutral red (NR) is used as an intracellular pH indicator. NR is also very useful biological probe and has been widely used for various purposes in many biological systems [2]. In solution these two dyes exist in two different protolytic forms. DOX is a member of anthractcline class of chemotherapeutic agents used for the treatment of many common human cancers [3].

We found that adsorption of the considered dyes and drug on nanotubes surface depends strongly on pH conditions. Namely, BTB molecules in acidic pH tend to form densly structure attached to the nanotubes surfaces. DOX molecules incorporate into this structure and avoid adsorption directly on the CNT surfaces. Change of pH toward more alkaline, that is varying concentration of neutral and deprotonated forms of BTB, leads to an increase of electrostatic repulsions between BTB molecules, which in turn causes desorption of almost all BTB and DOX molecules from the CNT surface.

In the case of NR molecules, the situation is different. Namely, in neutral pH we observe partial adsorption of NR and DOX molecules on the CNT surface, while in acidic pH the NR and DOX molecules preferentially located in the bulk. Thus, this phenomenon may be utilized (after some fine tuning of system parameters) to realize the pH controlled drug release in endosomes or tumor tissue.

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**P107**

# Search for chemosensitizers of bacterial MDR efflux pumps among novel arylpiperazine derivatives with carboxylic acid at position 3

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Bacterial multidrug resistance (MDR) is a major clinical and public worldwide health problem.. A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant Gram-negative bacteria. [2] Efflux pump inhibitors are one of the mechanisms that are widely spread among resistant bacteria. A main strategy to circumvent the MDR is to co-administer efflux pump inhibitors (EPIs), independent compounds which are able to block efflux action of protein transporters of a drug. The search for new EPIs, which are promising therapeutic agents is still a main topic of medicinal chemistry.[1] Previous studies indicated that amphiphilic character of the compounds has a great influence on EPI's activity. Thus, a series of arylpiperazine derivatives with carboxylic acid at position 3 will be evaluated on their EPI properties in clinical strains of Gram negative bacteria overproducing efflux pumps and MRSA strains as well. Furthemore, simultaneous evaluation of ADME/Tox properties of compounds is the following step, which is in great importance in the way to search for new drugs.

Taking this into account, a series of 12 arylpiperazine hydantoin derivatives with a carboxylic terminate fragment at position 3 were synthesized as potential EPIs. Elected compounds were investigated on their EPIs action in MDR Gram negative bacteria. Whole series was evaluated in silico on their "drugability", including physicochemical properties (clogP, logS, TPSA) and toxic effects (service OSIRIS). These studies showed low risk of mutagenic and tumorigenic effects for all compounds. One compound seems to have toxic effect on reproduction. Noticeable dependence of length of linkers between piperazine and hydantoin on *drug-like* score was found. The lowest *drug-like* score was observed for the derivative with the longest linker whereas their amphiphilic properties seem to be favorable for bacterial MDR EPIs- action.

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**P108**

# Synthesis of biopolymers coated magnetic nanoparticles and mesoporous carbon materials as a potential sorbents for NSAIDs

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Nonsteroidal anti-inflammatory drugs have a high impact on population and large popularity which can be ascribed to their availability (most prominent members of this group are available over the counter). Moreover, they are frequently used in veterinary medicine for domestic animals. These facts together with the NSAIDs administration in chronic diseases cause their high and still growing disposal. A discharge of therapeutic agents in effluents from production facilities, hospitals and private households, improper disposal of unused drugs, and direct discharge of veterinary medicines lead to the contamination of surface water, ground water and, eventually, drinking water, where they become a potential danger to humans. Moreover, one need to take into account also their metabolites and the products of degradation and photodegradation under the various conditions. Various methods of removing pharmaceuticals from wastewater have been proposed so far: biodegradation, hydrolysis, chemical oxidation or adsorption. Nonetheless, not all of them allow to get rid of not only the drugs but also their metabolites that can be more harmful than the original species. In this respect, the particular hopes can be reposed in adsorption.

In this work the several types of functionalized biopolymers (chitosan and collagen) and magnetic nanoparticles coated with this macromolecules were synthesized as a potential easy separable material able to interact with NSAIDs and their metabolites. The interactions between prepared materials 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. Additionally, the carbonization of prepared composites should lead to the carbon porous materials with interesting properties that can also serve as sorbents for NSAIDs.

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**P109**

# *N*-Acylbenzenesulfonamides – anticancer activity, metabolic stability and QSAR studies

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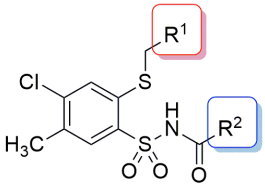
*N*-Acylsulfonamides constitute a class of organic compounds with various types of pharmacological activities such as antibacterial inhibitors for tRNA synthetase, antagonists for Angiotensine II, agents in treatment of Alzheimer's disease, and osteoporosis [1]. The particular attention is focused on *N*-acylsulfonamides as cyclooxygenase [2], tubulin [3] and cyclin-dependent kinases [4] inhibitors. Searching for innovative low-molecular chemotherapeutics we designed a series of new *N*-acylsulfonamides with potential anticancer activity. Cytotoxic *in vitro* tests against human breast (MCF-7), colon (HCT-116) and cervical (HeLa) cancer cell lines were performed at the Department of Biotechnology, UG – MUG. To explain how structural features influence the biological activities the quantitative structure-activity relationship (QSAR) method was applied. Metabolic stability study, one of the parameters characterizing pharmacokinetic properties of a molecule, was performed using pooled human liver microsomes and NADPH.




$R^1$ = alkenyl, alkynyl, carbamoyl, aryl, heteroaryl
$R^2$ = alkyl, alkenyl, aryl

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**P110**

# Structural and molecular docking studies of pyrrolo[3,4-*d*]pyrida-zinone derivatives with analgesic activity

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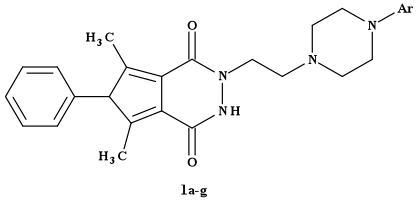
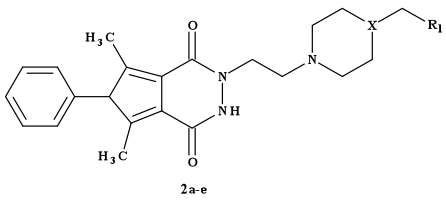
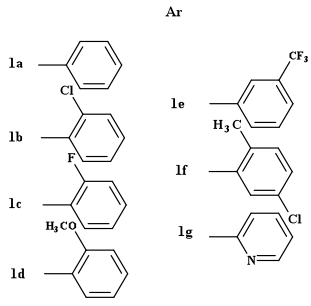
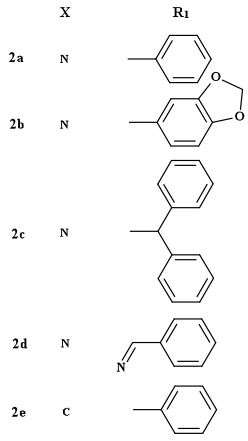
N2-{2-[4-aryl(benzyl)-1-piperazinyl(piperidinyl)]ethyl}pyrrolo[3,4-*d*]pyridazinones exhibited differentiated analgesic activity in the phenylbenzoquinone induced 'writhing' and 'hot plate' test in mice and at radioligand binding assay [1].




To explain the observed discrepancy of biological effects within series of pyrrolo[3,4-*d*]pyridazinone derivatives the structure/analgesic activity relationship analysis was undertaken. The molecular modeling studies using DFT and AM1 methods were undertaken to investigate the conformational preferences of searched derivatives. Moreover, the molecular docking procedure were used to study the interaction of pyrrolo[3,4-d]pyridazinone ligands with the adrenergic and serotoninergic system.

[1] Malinka W., Redzicka A., Jastrzębska-Więcek M. et. al. *Eur. J. Med. Chem.* 46 (2011) 4992–4999.

**P111**

# X-ray analysis of *N*-cyclohexyl-2-imino-3-(4-nitrophenyl)imidazoli-dine-1-carboxamide with antiproliferative properties

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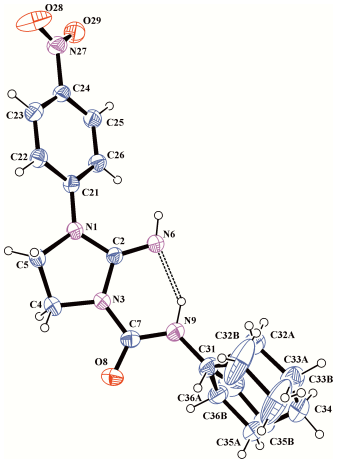
During the course of our investigation of novel urea derivatives with antiproliferative properties [1] we decided to study their ability to influence survival of different microorganisms. This was dictated by the need for protection of the natural microbiological flora. Presented compound *N*-cyclohexyl-2-imino-3-(4-nitrophenyl)imidazolidine-1-carboxamide showed moderate activity against several clinical strains of *Candida albicans*. Its structure was solved by X-ray crystallography which was required for the docking studies to analyse putative binding with 14-*α*-sterol demethylase.




Moreover, detailed X-ray analysis of title compound was performed in order to confirm the synthesis pathway and identification of its tautomeric form in the solid state. The tautomeric equilibrium or the 2-iminoimidazolidinecarboxamide system was investigated using theoretical calculations at DFT/B3LYP/6-311++G(d,p) level in the gas phase and in solutions.

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**P-112**

# Improvement of k-NN entropy estimator with applications in systems biology

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In this work, we investigate efficient estimation of differential entropy for multivariate random variables. We have noticed the biased behaviour of the nearest neighbor entropy estimator in higher dimension and propose bias correction, which yields more accurate k-nn entropy estimates in higher dimensions. In order to demonstrate the accuracy of improvement we calculated the corrected estimator for several families of random variables. For multivariate distributions we considered the case of independent marginals and the dependence structure between the marginal distributions described by Gaussian copula. Presented solution may be particularly useful for high dimensional data, like those analyzed in systems biology field.
To illustrate such application we exploit differential entropy to define robustness of biochemical kinetic models.

Our improved k-NN entropy estimator explore the idea of correcting the density function evaluation near to the boundary of random variable support. This smart idea has been proposed previously by Sricharan et al. [4] for several distributions. However the novelty and usefulness of our approach lies in efficient bias correction of classical k-NN estimator, which is applicable for any data sample (i.e. the knowledge of underlying distribution is not required).

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**P-113**

# Monte Carlo studies of the electroporation process

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Despite the development of experimental techniques, relatively little is known about the molecular mechanisms behind the process of pore formation during electroporation, i.e. the behavior of lipid molecules [1]. To improve our understanding of molecular mechanisms we developed mathematical model based on the Monte Carlo (MC) technique [2, 3]. It allows us to identify electroporation conditions, under which the formation of the pore is feasible, and determine the minimum and maximum pore diameters under various environmental and physico-chemical conditions.

Developed author's model is based on Pink's model, which provides analysis only of the alkyl chains. In author's model also polar lipid heads were taken into account. Due to that medium parameters, such as ionic strength and electric field strenght, can be considered. In author's model the lipid membrane is presented as triangular lattice consisting of two parallel layers, which didn't interact witch each other. Each node is occupied by a hydrocarbon chain, and every two hydrocarbon chains have one polar part - a dipole. Due to the large number of degrees of freedom per molecule in the lipid bilayer, efficient sampling of the configuration space requires their reduction. The Hamiltonian of the studied system, representative of the total energy of the system, consists of four terms: I) the energy of van der Waals interactions $H_{vdW}$, II) the conformational energy $H_{conf}$, III) the energy of electrostatic interactions between the lipid heads $H_{dip}$, and IV) the energy $H_E$ which describes the polar part interactions between the polar parts of lipid molecule (heads) and the electric field $E$.

We have restricted the van der Waals energy to six nearest neighbour lipid chains. Since the value of $H_{vdW}$ depends on the conformation of neighbouring chains, so it makes sense to calculate the sum of $H_{vdW}$ and $H_{conf}$. For the membrane in gel phase $H_{vdW}+H_{conf}$ value, per chain, was equal to $-3.056\cdot10^{-20}$ J whereas for membrane in fluid phase $+3.188\cdot10^{-20}$ J. $H_E$ was calculated under the assumption that only the positive charged end of the polar head (dipole) is mobile and the other end is fixed. The direction of the electric field was assumed to be perpendicular to the membrane. To find the maximum size of stable pores in certain ambient conditions we have performed MC simulations for dipalmitoylphosphatidylcholine (DPPC) membrane in gel and fluid phase, surrounded by 10 and 100 mmol NaCl solution, exposed to the electric field from $10^5$ to $10^8$ V/m. The system was equilibrated for the 200 - steps per node in 320 K (fluid phase), then 1000 steps per site were performed to find the average energy of the studied system. During each computer step each lipid polar head could change its orientation. We have found the relationship between energy of polar heads of lipids creating a pore in a lipid membrane, under the electric field influence, and the pores size. If the electric field intensity is $10^6$V/m potential energy of the studied system is positive and rising with the hypothetical pore diameter. When we added the positive energy of van der Waals interaction of lipid chains, we found that in such conditions the pore is not stable. By increasing the electric intensity to $10^7$ V/m the energy of polar part of a pore is negative from $d_a$=3.68 nm and for smaller pores the energy becomes positive. For larger values of the electric field ($10^8$ V/m) the energy of dipoles is negative and decreases with increasing in pore size. If the considered chains are all in fluid conformation, for which the van der Waals and conformational energy are positive, despite of the negative energy of the polar part, the appearing pore could be stable only in small size. The existence of larger stable pores requires conformational changes of lipid chains toward all-trans conformation.

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[2] K. Kubica, 2008, *Comput Biol Chem*., 32(3):163-6
[3] K. Kubica, A. Wrona, M. Żulpo, 2015, *AIP Conf. Proc.* 1648, 220002

**P-114**

# MULTI-SCALE MODELLING OF SPHINGOLIPID METABOLISM IN BREAST CANCER

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Sphingolipids are a class of complex bioactive lipids with a sphingoid base. A notable body of work has been devoted to studying the influence of sphingolipid metabolism on cellular fate: motility, proliferation, differentiation and apoptosis. Indeed, sphingolipids are known to have critical implications for the pathogenesis and treatment of diverse conditions such as cancer, inflammation and neurodegenerative disorders [1]. Despite the recently growing interest in sphingolipid metabolism their role in carcinogenesis is still not fully elucidated. One of the reasons of this situation is lack of adequate computational models and mathematical tools to integrate available different types of data.

Here we present the results of the research aiming to develop a multi-scale model of sphingolipid metabolism in breast cancer. To achieve this goal the detailed kinetic model of sphingolipid metabolism [2] was embedded into the human genome scale metabolic network Recon2 [3]. In this multi-step process our kinetic model (based on Michealis-Menten and Mass Action Law kinetics) was converted into Constraints-base model represented by Recen2 network. Thanks to redefining the reactions from the initial kinetic SL model and careful integration of all added metabolites with Receon2 network, we obtained the genome scale model with realistic flows of metabolites through the analysed pathways. The correctness of the network was checked by the controlling its ability to fulfil the biomass reaction. Metabolites fluxes were analysed using JyMet2.3 software [4] for the Flux Balance Analysis (FBA) [5].

Finally the Shlomi method (iMAT) [6] was used to integrate our extended Recon2 model with transcriptomic data from the METABRIC database [7]. Use of this method allowed us to generate 262 patient specific models of cancer and normal cell metabolism. FBA analysis of these models have shown large differences in metabolite flows through the SL pathway when comparing cancer cells with the normal healthy tissue samples. The most important were observed within the glycosphingolipids and ceremide-1-phosphate synthesis pathways. In further research we plan to check how the observed differences are correlated with the survival of patients. We also plan to extend our analysis to the all 2000 samples stored in METABRIC. Finally we hope to develop new methods of the integration of transcriptomic data with computational metabolic models.

We are sure that this type of computational tools will be of the great importance for the understanding of cancer development. It will also help to find new markers of cancer progression and develop new anticancer drugs and treatment strategies.

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**P-115**

# Functional studies of human and viral methyltransferases involved in the biosynthesis of the RNA cap

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Formation of a "cap" structure in mRNA molecules, which involves RNA methylation of the first two transcribed nucleotides, is an essential process in human cells as well as in many viruses. However, the impact of cap1 and cap2 RNA methylation on physiology of human cells has not been fully elucidated and even the distribution of cap2 methyl groups in human mRNAs is unclear. Our recently published results have led us to identify key differences between human and viral 2′-*O*-methyltransferases (2′-*O*-MTases) - enzymes responsible for these cap methylations [1].

The main line of our current research is focused on the development of small molecule inhibitors of human cap1 MTase, human cap2 MTase, vaccinia virus cap1 MTase VP39, and a cap0/cap1 MTase from flavivirus. Differences between the human and viral enzymes provide the basis for the rational development of cap analogs that selectively block the viral cap MTases without inhibiting the human enzymes.

We present the methodology used in the initial stage development of those inhibitors. Various computational methods (including molecular docking, similarity and pharmacophore searching, machine learning), were applied for virtual screening of large databases of commercially available compounds. Selected, top scoring compounds were tested experimentally both *in vitro* and in human cell cultures (for human MTases).

Developed inhibitors will be used to study the effect of blockage of cap1 or cap2 methylation on human cell physiology, and in particular on the fate of human mRNAs. The inhibitors specific for viral enzymes could be used in the future as a starting point for the development of antiviral drugs.

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**P-116**

# Origin of plastids - still a challenge for phylogenetics

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Primary plastids are photosynthetic, two-membrane organelles characteristic of glaucophytes, red algae and green plants, which cluster together into the Archaeplastida (or Plantae) supergroup, one of several main lineages of Eukaryota. All available data (morphological, molecular, phylogenetic and genomic) unequivocally indicate that plastids evolved from a free living cyanobacterium engulfed by a heterotrophic unicellular eukaryote in a process called primary plastid endosymbiosis. Despite many years of extensive research, the scientific community has not reached a consensus regarding: (i) the number of primary plastid endosymbioses, (ii) the branching order of primary plastid-bearing lineages and (iii) the position of the primary plastid ancestor (or ancestors) among extant cyanobacteria. To answer these questions, we performed phylogenetic analyses based on the concatenated set of 16S and 23S ribosomal rRNA sequences coming from 287 taxa representing large diversity of present cyanonabecteria and plastids. To compute multiple sequence alignments, we used R-Coffee, which is dedicated to RNA sequences and incorporates secondary structure information. In phylogenetic analyses, we applied sophisticated approaches, covarion model assuming that the rates of evolution can change on different branches and/or site-heterogeneous mixture (CAT) model assuming site-specific rates and profiles. Nearly all calculated trees supported: the monophyly of Archaeplastida and glaucophyte-red alga clade in a basal position to green plants, generally with significant confidence. However, the basal position of primary plastids in the cyanobacterial tree was recovered only with the assumption of covarion models mostly with significant confidence. The results indicate that deep emergence of plastids within cyanobacteria obtained by other authors may be a systematic error resulting from various substitution rates, in particular lineages not considered in simpler models. To solve the problem of Archaeplastida classification not only more sequence data are required, especially from underrepresented glaucophytes, but also new phylogenetic methods.

P-118

# Bi-clustering methods in attributes filtering in microarray analysis

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Bi-clustering is a data mining techniques which allows simultaneous clustering of the rows and columns of a two dimensional data matrix. They are used to extract latent information from numerical data matrix. For example, in the analysis of microarray data this techniques can be used to find a subset of genes exhibiting some similarity only in a subset of conditions / patients. This technique belongs to the class of NP-hard problems, and was first presented by Morgan and Sonquist in 1963 [1], than by Hartigan [2](1972), and by Mirkin in 1996 [3]. In the context of bioinformatics problems like gene expression data analysis, the first to use bi-clustering was Cheng and Church [4]. They proposed bi-clustering of result from microarray experiments by introducing the mean square residue in bi-cluster.

Bi-clustering is very well suited for microarray data because it is resistant to their biggest drawback - the size of the data matrix. Even if we have a data matrix consisting of hundreds of experiments and several thousand genes - can be achieved bi-clusters in a finite time. This is done for example through the decomposition of a data matrix into smaller matrices much easier to analyze [5].

In this work we treated bi-clustering algorithms as the only tool for pre-processing analysis of the input data. For example in Eng-Juh Yeoh, at el. [6] publication is gene expression matrix consist of 233 microarray experiments each of which consist of 12070 genes. Each experiment is taken from different leukemia patient. Each patient in test group has one of six subtype of leukemia. Such define data set could have been consist of six bi-clusters each associated with a different kind of disease. Each data analysis in which we have more than 12,000 attributes is very difficult and in many cases impossible. The bi-clustering experiment can select only those that are relevant to the aspect on which we want to focus on.

This is very useful during gene expression data analysis because the vast majority of genes which have been measured has no effect on the studied disease or feature.

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**P-119**

# Biomolecular Feature Explorer - server for validation of protein structures

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**Aleksandra Elżbieta Dawid, e-mail: adawid@chem.uw.edu.pl*

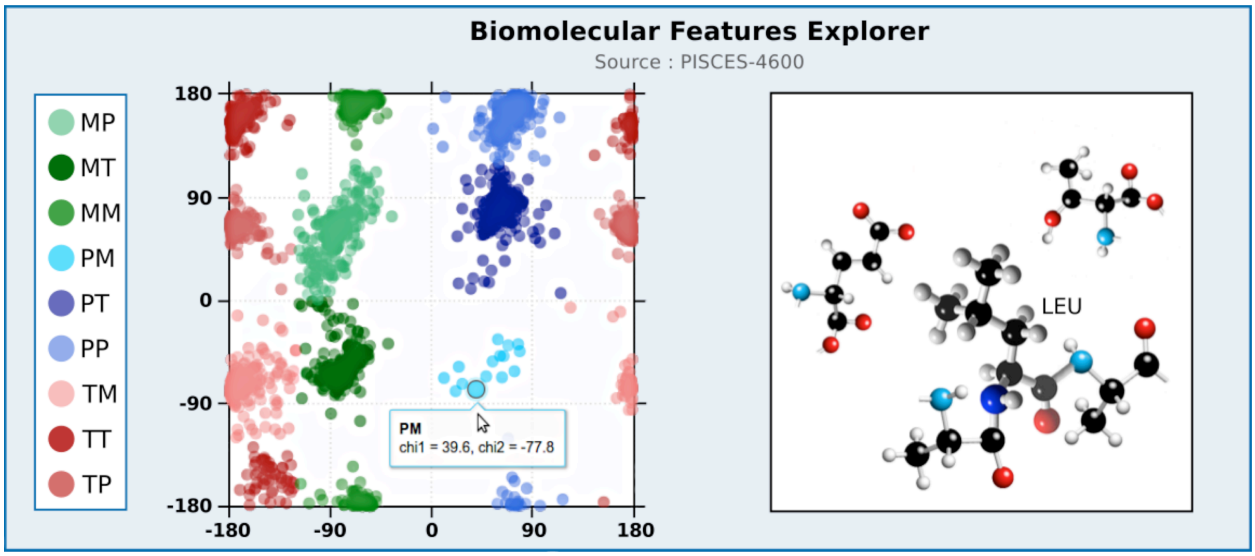


*Figure shows the screenshot of server: left panel: two-dimensional histogram of chi-angles (rotamer type); right panel: three-dimensional structure of each residue and its neighbors.*

Quality control of protein models shows that many of them have errors in the structure. One of the main reasons for these errors is still related to the wrong side chains packing just as bad stereochemistry and other geometric properties of the polypeptide chain. The problem is still exists, in spite of routine optimization of possible conformations by satisfaction of spatial restrains.

Considering the increase in number of high resolution experimental data we decided to carry out a statistical analysis of crucial geometric regularities occuring in the backbone and side chains of the polypeptide. We have prepared a unique, non-redundant set of proteins with high resolution and correct electron density that was used to derive the reference statistics, and optionally can be displayed by the user as a background of the histogram. After loading your own PDB file, you can check the correctness of the backbone based on torsion angles Phi-Psi in the Ramachandran plot and the correctness of the side chain packing in the histogram of any two chi-angle combination. We believe that a large part of wrong stereochemistry errors can be eliminated by the analysis in the local Cartesian coordinates of each amino acids rotamer found in the protein. Our novel approach was to create a library of coarse-grained rotamers defined in the local Cartesian space (each CG virtual pseudo atom representing an amino acid side chain except for carbon Cβ). When we investigate the outliers (cases outside of the range), we can easily detect errors in the experimental data or unique exceptions justified by the specific interactions.

For the first time in addition to the interpretation of the numerical results of the statistical analysis, we have the opportunity to observe in real-time the fragment of protein structure, which relate to specific points on the plot. Therefore we can have a visual analysis and evaluation of each residue in the experimental data: type of rotamer, stereochemistry, spatial restrictions and clashes. You can choose from a variety of filtering options, such as selecting amino acids or secondary structure, display reference statistics as a background of histogram, showing hydrogen bonds and the neighbors of residue away by some distance. Our Biomolecular Features Explorer is available online as a part of Bioshell package (*http://www.bioshell.pl*).

**P-120**

# Coarse-Grained Modeling of Peptide Docking Associated with Large Conformation Transitions of the Binding Protein: Troponin I Fragment–Troponin C System

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Most of the current docking procedures are focused on fine conformational adjustments of assembled complexes and fail to reproduce large-scale protein motion. In this paper, we test a new modeling approach developed to address this problem. CABS-dock is a versatile and efficient tool for modeling the structure, dynamics and interactions of protein complexes. The docking protocol employs a coarse-grained representation of proteins, a simplified model of interactions and advanced protocols for conformational sampling. CABS-dock is one of the very few tools that allow unrestrained docking with large conformational freedom of the receptor. In an example application we modeled the process of complex assembly between two proteins: Troponin C (TnC) and the N-terminal helix of Troponin I (TnI N-helix), which occurs in vivo during muscle contraction. Docking simulations illustrated how the TnC molecule undergoes significant conformational transition on complex formation, a phenomenon that can be modeled only when protein flexibility is properly accounted for. This way our procedure opens up a new possibility for studying mechanisms of protein complex assembly, which may be a supporting tool for rational drug design.

**P-121**

# Merging high-dimensional data at the p-value level as a superior solution to entire data set fusion

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Biological data repositories are continuously growing data collections which require efficient processing techniques to acquire accurate knowledge about the studied processes. The aim of this research was to demonstrate the performance of statistical integration algorithms in comparison to the application of direct data merging techniques.

The study was conducted on two twin data sets obtained in the course of microarray gene expression experiments performed in the search of radiosensitivity biomarkers. The data were normalized and adjusted for batch effects in order to enable their joining. The first experiment provided 60 samples: 30 radiosensitive patients and 30 radioresistant, and the second contained 59 samples: 31 radiosensitive and 28 radioresistant.

We proposed a statistical p-value data integration method, based on weighted Z-score modifications of Stouffer's approach, for combining results obtained in the two studies and juxtaposed them against two restrictive result validation approaches: statistical and non-statistical methods provided within the Arraymining [1] web-service. All three algorithms were compared to the setting where the two sets were merged directly at the data level.

The differentially expressed gene lists were then used to build classifiers by means of logistic regression and support vector machines. Leave-one-out cross-validation was used as the classification scheme.

The findings reveal that the incorporation of statistical information via p-value integration yields superior results in terms of biomarker determination for classification models than in the case of simple data integration. This points to the potential of statistical testing outcomes as a relevant solution to the issue of overcoming different data set inconsistencies that are not entirely adjusted in the course of normalization and batch effect reduction. Moreover, among the single experiment validation techniques, p-value integration allows for better performance with regard to the restrictive approaches.

**P-122**

# Comparisons of gene signature construction methods using plots of graph level versus information content of gene ontology terms

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A crucial step in the design of a classification system for gene expression data is the choice of a gene signature (method of construction), which includes a subset of genes with the strongest correlations to the studied molecular phenomena and to the classification problem. There are many approaches to the design of gene signatures published in the literature. Therefore, there is need to develop methodologies of comparisons between these approaches.

We propose a methodology of comparison/evaluation of methods for the construction of gene signatures based on presentation of gene ontologies (GO) of the sets of genes on a plane with axes defined by GO graph level (GL) and ontology term information content (IC). The two parameters, GL and IC are important determinants of ontology terms related to gene signatures. Different methods of construction of gene signatures lead to different locations of their ontology terms on the GL – IC plane, as is shown in the figure below where the experimental gene expression data were analyzed. Dots correspond to ontology terms of the signature obtained by re-annotation using a “classic” enrichment method and asterisks correspond to ontology terms of the signature obtained by using our integrative method. Grey level in the figure below represents the value proportional to the probability density function related to numbers of biological process ontology terms of different classes. We compare several methods of construction of gene signatures using our approach. We discuss relations between accuracies achievable by different classifiers and stabilities of gene signatures, and characteristics of gene signatures on the GL-IC plane.

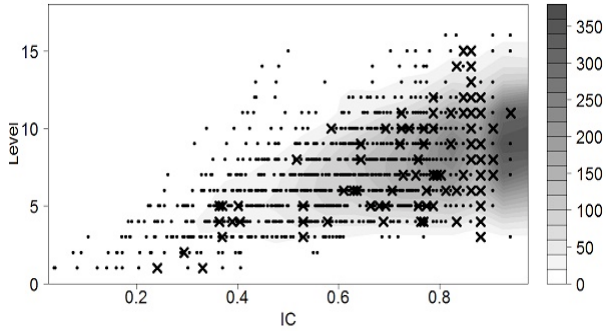


Figure. GL-IC characteristics of two different methods of construction of gene signatures.

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**P-123**

# In search of Cytoplasmic Male Sterility genes in Sugar beet (Beta vulgaris)

Przemysław Gierski, Katarzyna Ogórek, Wojciech Wesołowski, Anna Herda, Wojciech Rymaszewski, Murat Öztürk, Marek Szklarczyk

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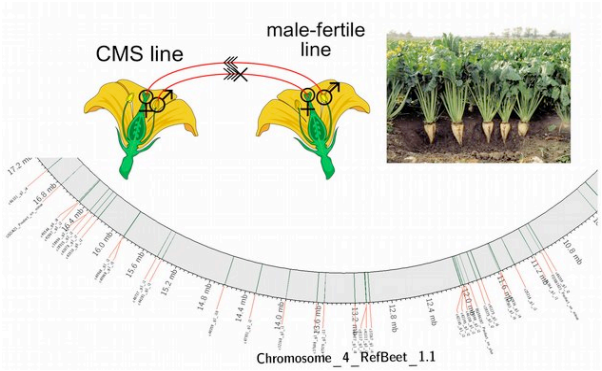


Cytoplasmic male sterility (CMS) is a maternally inherited inability of a plant to produce a functional pollen[1]. CMS is determined by a joint action of sterilizing cytoplasm and specific nuclear genes. The use of CMS lines simplifies the lines crossing, thus remarkably accelerates breeding. It eliminates the need of isolation or castration the flowers before the pollination. In Sugar beet we have CMS lines[2] and also lines with restored fertility. We want to find the gene connected to the restoration of fertility in CMS Sugar beet lines. In this study we have sequenced total mRNA of 6 closely related lines of Sugar beet (*Beta vulgaris*) with different genetic background and fenotype. We have identified genes with different expression levels located closely to previously obtained molecular markers cosegregating with the restorer gene. These genes are now verified using traditional molecular methods on a broader set of plants in order to find the restorer gene. The understanding of the mechanism of CMS in Sugar beet may help us in introducing CMS into new species of plants.

[1] M.R. Hanson, S. Bentolila, *Plant Cell*, 2004, 16, 154-169.
[2] F.V. Owen, *J Agric Res*, 1945, 71, 423-440.