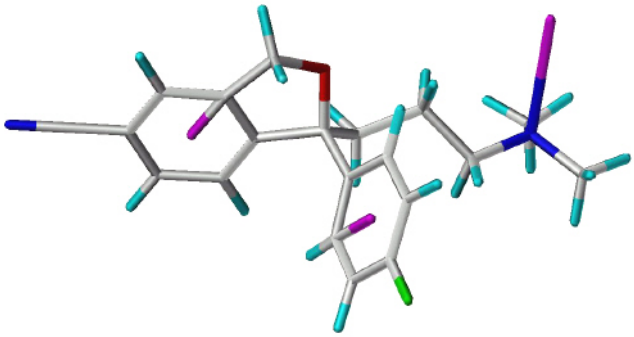
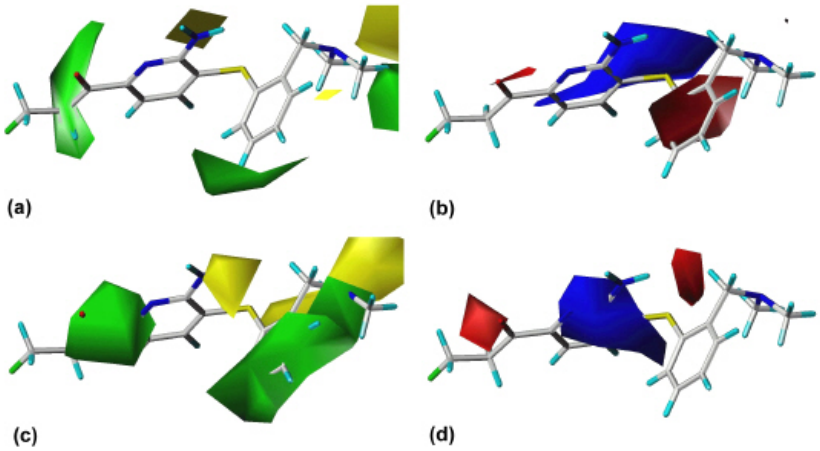


Vth CONVERSATORY on MEDICINAL CHEMISTRY

LUBLIN

13-15.09.2012



Polish Society of Medicinal Chemistry

Medical University of Lublinie

Marshal of Lublin Vojevodship

Chair and Department of Synthesis and Chemical Technology of Pharmaceutical Substances
Faculty of Pharmacy
Medical University of Lublin
Author: dr hab., prof. UM Dariusz Matosiuk

Lublin, 2012

Scientific Comeettee:

Prof. dr hab. Andrzej Bojarski

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Prof. dr hab. Bożenna Gutkowska

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Prof. dr hab. Grażyna Biała

Dr hab. Krzysztof Jóźwiak

Dr hab. Monika Wujec

oraz

dr Monika Aletańska-Kozak

dr Marzena Rządkowska

dr Elzbieta Szacoń

mgr Marcin Hus

mgr Daniel Kupryciuk

mgr Tomasz Wróbel

Conversatory Roster

Thursday, 13.09.2012

15.00-17.00 – Rejestration of Participants

17.00-17.15 - Opening Ceremony

Prof. dr hab. Dariusz Matosiuk , Head of the Organizing Commeettee, Vice-Rector for Scientific Afairs
Representative of hM Rector of Medical University of Lublinie Prof. dr hab. Andrzeja Drop;

17.15-18.00 – Inaugural Lecture

Prof.Irwing. W. Wainer, NIG/NIH, Bethesda, USA
„ Digging deeper into the ketamine paradigm: How archeological drug discovery is leading to new treatments for pain and depression."

18.30-22.00 – Get-together Party

Friday, 14.09.2012

9.00-11.00 – Ist lecture session – TEAM Session p. I

Session moderators – prof. dr hab. Katarzyna Kieć-Kononowicz
prof. dr hab. Dariusz Matosiuk

L-1
Prof. Hugo Arias,Northstate University, Elk Grove, California, USA
„ Novel positive allosteric modulators of the human α7 nicotinic acetylcholine receptor."

L-2
Prof. Lawrence Tall, Torrey Pines Institute for Molecular Studies, Port St. Lucie, Florida, USA
„ NOP, the other opiate receptor: a new target for drug abuse and pain medications."

L-3
Prof. Giuseppe Canazza, Universita degli Studi di Modena,Italy
„ 5-Arylbenzothiadiazine type compounds as positive allosteric modulators of AMPA/Kainate receptors."

L-4
Prof. Krzysztof Jóźwiak, Medical University, Lublin, Poland
„ Development of new derivatives of fenoterol as potential ligands of the β2 adrenergic receptor with novel therapeutic perspectives."

11.00-11.30 – Coffee break

11.30-13.30 – Communications – TEAM Session p. II.

Session moderators – prof. dr hab. Barbara Malawska
prof. dr hab. Krzysztof Jóźwiak

C-1
MSc.Ewelina Rutkowska,Medical University, Lublin, Poland
„ Molecular simulations of interactions between stereoisomers of β_2-agonists and sulfotransferase isoenzymes."

C-2
MSc.Karolina Pająk,Medical University, Lublin, Poland
„ Binding affinities of fenoterol derivatives to the agonist conformation of β_2-adrenergic receptor determined using [^{3}H]-(R,R')-4-methoxyfenoterol as the marker ligand."

C-3

MSc.Katarzyna Targowska-Duda,Medical University, Lublin, Poland

„ Importance of the nicotinic receptors inhibition in the therapeutic action of antidepressants."

C-4

Dr Anita Płazińska,Medical University, Lublin, Poland

„ Interactions between fenoterol and β_2 adrenergic receptor: the stereoselective binding and the ligand dissociation/association profiles. "

C-5

Mgr Martyna Krzykawska, Jagiellonian University, Kraków, Poland

„ Effects of bacteriochlorin based photodynamic therapy on tumor physiology."

C-6

Prof. dr hab. Grzegorz Grynkiewicz, Pharmaceutical Institut, Warszawa, Poland

„ Plant phenolics as drug leads."

13.30-14.30 – Lunch

14.30-16.00 – IInd lecture session – TEAM Session p. III

Session moderators – prof. dr hab. Krzysztof Walczyński
prof. dr hab. Marek Główka

L-5

Dr Manuela Bartolini, Universita degli Studie di Bologna, Italy

„ Acetylcholineesterase-based analytical tool for drug screening."

L-6

Dr hab. Danuta Siluk,Medical University,Gdańsk, Poland

„ HPLC-APCI-MS/MS assay for the simultaneous determination of tocopherols and tocotrienols in human serum."

L-7

Dr Anna Więckowska,CMUJ, Kraków, Poland

„ α-Aryl substituted Fosmidomycin analogues as inhibitors of Mycobacterium tuberculosis 1-deoxy-D-xylulose-5-phosphate reductoisomerase."

16.00-16.30 – Coffee break

16.30-17.30 – Poster session and poster oral presentations

Session moderators – dr hab. Anna Bielawska
dr hab. Andrzej Bojarski

PP-1

Dr hab., prof. UMK Konrad Misiura, CMUMK, Bydgoszcz, Poand

„ Synthesis of the novel 2,4-disubstituted 1,3- thiazoles as potential antifungal agents."

PP-2

MSc Krzysztof Klimkiewicz, Jagiellonian University, Kraków, Poland

„ Development of a 3D cell model to study inflammation and photosensitization processes of the melanoma."

PP-3

MSc Maciej Serda, Silesia University, Katowice, Poland

„ Thiosemicarbazones as anti-cancer iron chelators."

PP-4

MSc Daniel Szulczyk, Medical Uniwersity, Warszawa, Poland

„ Synthesis, structure and pharmacological evaluation of selected tryptamine thiourea derivatives."

PP-5

MSc Rafał Urniaż, Medical University, Lublin, Poland

„ X-ray crystallographic structures as a source of alignment in 3D-QSAR studies of positive allosteric modulators of AMPA receptor."

PP-6

MSc Piotr Drączkowski, Medical University, Lublin, Poland

„ Characterization of acetylcholinesterase interaction with inhibitors."

Sutherday, 15.09.2012

9.00-11.00 – IIIrd lecturesession – Structure vs Activity

Session moderators – prof. dr hab. Janina Karolak-Wojciechowska
prof. dr hab. Grzegorz Grynkiewicz

L-8

Prof. dr hab. Marek Główka, Technical University, Łódź, Poland

„Geometry of long-chain aryl-piperazines from x-ray studies."

L-9

Prof. dr hab. Wiesław Szeja, Technical University, Gliwice, Poland

" Chemical glycosylation of secondary plant metabolites as an effective modifier of their biological activity."

L-10

Dr hab.Jadwiga Turło, Medical University, Warszawa, Poland

„ Tacrolimus vs. Ascomycin – problems with the biosynthesis upscaling."

L-11

Dr Robert Musioł, University of Silesia, Katowice, Poland

„ Quinolines as fragments in design of HIV integrase inhibitors."

11.00-11.30 – Coffee break

11.30-13.30 – Communications – New Trends

Session moderators – prof. dr hab. Bożenna Gutkowska
prof.dr hab. Wiesław Szeja

C-7

MSc Dawid Warszycki, PAS Pharmacology Institut, Kraków, Poland

„ Development of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 2. Privileged fragments."

C-8

Dr Wojciech Płaziński, PAS Catalysis Insitut, Kraków, Poland

„ The 'order-to disorder' conformational transition in CD44 protein: an umbrella sampling analysis."

C-9

MSc Iwona Masłowska-Lipowicz, Medical University, Łódź, Poland

„ Non-imidazole histamine H_3-antagonists - synthesis and preliminary pharmacological investigation of new 1-(substituted)methyl-4-hydroxypiperidine derivatives."

C-10

MSc Marek Staszewski, Medical University, Łódź, Poland

„ Synthesis and preliminary pharmacological investigation of new 1-phenoxyalkyl-4-[(N,N-disubstitutedamino)alkyl]piperazines and N-substituted-N-[ω-(ω-phenoxy-alkylpiperazin-1-yl)alkyl]guanidines as non-imidazole histamine H_3-antagonists."

C-11

Dr Ewa Tykarska, Medical University Medyczny, Poznań, Poland

„ Complexes of glycyrrhizic acid and its salts with biologically active compounds. Their structures and potential applications in drug form technology."

C-12

MSc Agnieszka Gornowicz, Medical University, Białystok, Poland

„ Combined Therapy of Monoclonal Antibody Against MUC-1 and Berenil Complexes of Platinum(II) in Breast Cancer Cell Line MCF-7."

C-13

Dr Maria Kasprzak, Medical University, Łódź, Poland

„ Structure and biological activity of two ruthenium(II) compounds with flavanone-based ligands."

C-14

MSc Monika Lepiarczyk, Medical University, Białystok, Poland

„ Biological studies of novel derivatives of isoquinolines alkaloids in MCF-7 and MDA-MB-231 human breast cancer cells."

13.30-14.30 – Lunch

14.30-15.30 – Poster session and poster oral presentations

Session moderators – dr hab. Monika Wujec
prof.dr hab. Krzysztof Bielawski

PP-7

Stud. Magdalena Bochniak, Medical University, Lublin, Poland

„ Modelling the interactions between glycogen synthase kinase 3β and its inhibitors: Dilema of choice of the most appropriate scoring function while investigating the drug-receptor interactions using the technique of molecular docking. Case study over selected gsk-3β1 inhibitors."

PP-8

MSc Wioletta Cieślik-Kowalczyk, University of Silesia, Katowice, Poland

„ Synthesis and antifungal activity of new quinoline derivatives."

PP-9

MSc Adam Truchlewski, Technical Unvwersity, Łódź, Poland

„ Influence of ortho-substituents of phenyl in aroyldithiocarbazoic acids on their conformation."

PP-10

MSc Ewa Otrebska, CMUJ, Kraków

„ 5- Arylidene derivatives of hydantoin – new hope of inhibiting bacterial multidrug resistance."

PP-11

MSc Anna Mrozek-Wilczkiewicz, University of Silesia, Katowice, Poland

„ Iron chelators – antiproliferative activity and application in photodynamic therapy."

17.30-23.00 – Farewell Evening

List of the posters:

PP-1 Konrad Misiura, Dr hab., prof. UMK
Synthesis of the novel 2,4-disubstituted 1,3- thiazoles as potential antifungal agents.

PP-2 Klimkiewicz Krzysztof, MSc
Development of a 3D cell model to study inflammation and photosensitization processes of the melanoma.

PP-3 Serda Maciej, MSc
Thiosemicarbazones as anti-cancer iron chelators.

PP-4 Szulczyk Daniel, MSc
Synthesis, structure and pharmacological evaluation of selected tryptamine thiourea derivatives.

PP-5 Urniaż Rafał, MSc
X-ray crystallographic structures as a source of alignment in 3D-QSAR studies of positive allosteric modulators of AMPA receptor.

PP-6 Drączkowski Piotr, MSc
Characterization of acetylcholinesterase interaction with inhibitors.

PP-7 Bochniak Magdalena, Stud.
Modelling the interactions between glycogen synthase kinase 3β and its inhibitors: Dilema of choice of the most appropriate scoring function while investigating the drug-receptor interactions using the technique of molecular docking.
Case study over selected gsk-3β1 inhibitors.

PP-8 Cieślik-Kowalczyk Wioletta, MSc
Synthesis and antifungal activity of new quinoline derivatives.

PP-9 Truchlewski Adam, MSc
Influence of ortho-substituents of phenyl in aroyldithiocarbazoic acids on their conformation.

PP-10 Otrebska Ewa, MSc
5- Arylidene derivatives of hydantoin – new hope of inhibiting bacterial multidrug resistance.

PP-11 Mrozek-Wilczkiewicz Anna, MSc
Iron chelators – antiproliferative activity and application in photodynamic therapy.

P-1 Aletańska-Kozak Monika, Dr
Synthesis of oligopeptides using an automated microwave synthesizer.

P-2 Bajda Marek, Dr
Determination of lipophilicity of γ-butyrolactone derivatives with anticonvulsant and analgesic activity using micellar electrokinetic chromatography.

P-3 Baran Marzena, MSc
Synthesis, alpha 1-adrenoceptor activity and anty/mutagenicity of novel arylpiperazine derivatives of benzimidazole and pyridone.

P-4 Baran Marzena, MSc
The evaluation of lipophilicity of benzimidazole and pyridone derivatives.

P-5 Bielawski Krzysztof, Prof. dr hab.
Cytotoxic activity of novel analogues of bis(2-chloroethyl)amine.

P-6 Bielenica Anna, Dr
Synthesis of thiourea derivatives of 4-amino-4H-1,2,4-triazole.

P-7 Bukowczan Jerzy, MSc
Synthesis of 1,2,3-triazoles from 4-amino-3-quinolinesulfonamides with the propargyl group.

P-8 Byrtus Hanna, Dr
Synthesis and anticonvulsant activity of new 4-arylpiperazinylalkyl derivatives of 5-isopropyl-5-phenyl-imidazolidine-2,4-diones.

P-9 Canale Vittorio, MSc
A new application for the Pipecolic linker for the synthesis on solid-support: arylsulfonamide derivatives with potential CNS activity.

P-10 Chłoń-Rzepa Grażyna, Dr
Synthesis, analgesic and antiinflammatory activity of new 1,3-dimethyl-3,7-dihydropurine-2,6-dione derivatives.

P-11 Czarnomysy Robert, MSc
Pro-apoptotic effect of Pt_2(3-ethylpyridine)$_4$(berenil)$_2$ and Pt_2(3-buthylpyridine)$_4$ (berenil)$_2$ in human breast cancer cells.

P-12 Czopek Anna, Dr
An impact of halogen position in 4-phenylpiperazine of LCAPs with spirohydantoins on the 5-HT$_{1A}$/5-HT$_7$ receptor selectivity.

P-13 Dawidowski Maciej, Dr
Secondary amino-acids and ketones as coupling partners in Ugi U-5C-4CR multicomponent reaction. Optimization of conditions and application to synthesis of C-4 disubstituted 2,6-diketopiperazines.

P-14 Dela Anna, MSc
Influence of the length of the linker between arylidene hydantoin and phenylpiperazine moieties on their affinity for α_1-adrenoreceptors.

P-15 Francik Renata, Dr
Stability and antioxidant capacity rating of selected nutricosmetics.

P-16 Gomółka Anna E., mgr
Synthesis of novel azaindole derivatives of pyrido[1,2-*c*]pyrimidine with potential antidepressant activity.

P-17 Gośliński Tomasz, dr hab.
Physical-chemical studies on the newly synthesized porphyrazines possessing peripheral 2,5-dimethylpyrrol-1-yl and dimethylamino groups.

P-18 Grychowska Agnieszka, MSc
An influence of aryloxy-/arylthio-ethyl fragment on 5-HT$_{1A}$/5-HT$_7$ receptor selectivity in a group of quinolinesulfonamide derivatives of aryloxyethyl- and arylthioethyl-piperidines.

P-19 Gunia Agnieszka, MSc
Evaluation of compounds 488 and 530 as potential antiepileptic and/or analgesic agents.

P-20 Handzlik Jadwiga, Dr
Studies on anticancer properties of novel heterocyclic compounds with hydantoin motives.

P-21 Ignasik Michalina, MSc
Novel dual binding site cholinesterase inhibitors with indole moiety.

P-22 Jakubowska Anna, MSc
Determination of the absolute configuration of stereoisomers of 8-*tert*-butyl-2-hydroxy-7-methoxy-8-methyl-9-oxa-6-azaspiro[4.5]dec-6-en-10-one by NMR spectroscopy their methoxyphenylacetic acid esters.

P-23 Kamińska Katarzyna, MSc
Influence of chloro substitution in 6-aryl moiety of 4-(4-methylpiperazino)-1,3,5-triazine-2-amines on histamine H_4 receptor affinity.

P-24 Kamiński Krzysztof, Dr
Synthesis of new propionamide and butanamide derivatives of phtahlimide, and its saturated cyclohexane analogs as potential anticonvulsants.

P-25 Karcz Tadeusz, MSc
Pharmacological characterization of fluorescently tagged human adenosine A_{2A} receptors.

P-26 Kieć-Kononowicz Katarzyna, Prof. Dr Hab.
Analgesic activity of tricyclic annelated derivatives of xanthines with affinity at adenosine receptors.

P-27 Kowalczyk Paula, MSc
Search for new GABA-uptake inhibitors among the derivatives of alpha-substituted *N*-benzylamides of gamma-hydroxybutyric acid.

P-28 Kruk Joanna, MSc
Synthesis of pyrazolo[4,3-e]benzotriazole derivatives of potential anticancer activity.

P-29 Kryjewski Michał, MSc
Synthesis and photochemical properties of unsymmetrical phthalocyanine bearing two 1-adamantylsulfanyl groups at adjacent peripheral positions.

P-30 Kubowicz Paulina, MSc
Liver fraction applications for *in vitro* ADME studies.

P-31 Kujawski Jacek, MSc
New pyrazole derivatives with potential biological activity: theoretical studies on complexation of small-molecule ligands.

P-32 Kurczab Rafał, MSc
The novel approach in structure-based 3D pharmacophore model generation. An application to searching for 5-HT_6R selectivity hypothesis.

P-33 Kurczyk Agata, MSc
Development of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 1. Machine learning.

P-34 Latacz Gniewomir, Dr
The study of H_3 histamine receptor ligand DL77 metabolism using recombinant isoforms of cytochrome P450.

P-35 Liana Piotr, MSc
Chemopreventive properties of 3-ethyl-3-methyl succinic imide derivatives.

P-36 Lijewski Łukasz, stud.
Review of the latest achievements in pharmaceutical formulations of photosensitizers applied in photodynamic therapy.

P-37 Lijewski Sebastian, MSc
Physical-, photochemical properties and biological activity of novel porphyrazine modified with nitroimidazolylbutylsulfanyl substituents.

P-38 Malawska Barbara, Prof. Dr Hab.
Synthesis of novel cyclic analogs of □-aminobutyric acid as potential anticonvulsant agents.

P-39 Marciniec Krzysztof, Dr
Determination of the lipophilicity parameters logk_w and log*P* of azinesulfonamides by reversed-phase high performance chromatography.

P-40 Matys Anna, MSc
Role of chemical modifications within aromatic hydantoins for their abilities to inhibit multidrug resistance mechanisms in selected Gram-positive and Gram-negative bacteria.

P-41 Mazurkiewicz Jakub, MSc
Amine derivatives of hydantoins as novel inhibitors of drug-efflux system in antibiotic resistant bacteria.

P-42 Misiura Konrad, dr hab., prof. UMK
Synthesis and physicochemical properties of new urea prodrugs for melanocyte-directed enzyme prodrug therapy.

P-43 Mojzych Mariusz, Dr
Synthesis and biological evaluation of new sulfonamides derivatives of pyrazolo[4,3-*e*][1,2,4]triazine.

P-44 Morak-Młodawska Beata, Dr
New dipirydothiazine derivatives – potential inhibitors of dopaminergic and serotoninergic receptors.

P-45 Morak-Młodawska Beata, Dr
Aza- and diazaphenothiazines derivatives with antioxidant activity.

P-46 Obniska Jolanta, Dr Hab.
Synthesis and anticonvulsant activity of new N-benzyl-3,3-disubstituted-pyrrolidine-2,5-diones.

P-47 Pachuta-Stec Anna, Dr
Antimicrobial activity of some derivatives of 1,2,4-triazoline-5-thione.

P-48 Pachuta-Stec Anna, Dr
Antimycobacterial activity of new *N*-(substitutedthioureido)aminobicyclo dicarboximide and 3,4-disubstituted 1,2,4-triazolino-5-thione.

P-49 Pająk Karolina, MSc
Binding affinities of fenoterol derivatives to the β_2-adrenoceptor Y308A mutant in relation to the wild type data.

P-50 Paprocka Renata, MSc
Synthesis and Biological Activity of New 1,2,4-Triazole Derivatives.

P-51 Pisklak Dariusz M., Dr
A simple computational methodology used to distinguish between agonists and antagonists of the estrogen receptor.

P-52 Piskorz Jarosław, MSc
Tribenzoporphyrazine possessing annulated styryldiazepine ring as potential photosensitizer for photodynamic therapy.

P-53 Pitucha Monika, Dr
The activity *in vitro* of novel *N*-ethyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide against *Haemophilus* spp. planktonic.

P-54 Pitucha Monika, Dr
Evaluation of antioxidant properties of some novel semicarbazide, triazole and pyrazole derivatives.

P-55 Pluta Ewelina, MSc
Docking of thiopurine derivatives to human serum albumin and binding site analysis with Molegro Virtual Docker.

P-56 Płazińska Anita, dr
Molecular dynamics simulations of structural and thermodynamic properties of fenoterol stereoisomers and β_2 adrenergic receptor.

P-57 Powroźnik Beata, MSc
Vibrio harveyi test in the evaluation mutagenic and antimutagenic activity of anticonvulsant compounds.

P-58 Rataj Krzysztof, MSc
Mutation mining: automated extraction of mutation data from scientific publications.

P-59 Redzicka Aleksandra, Dr
Synthesis and antibacterial activity of pyrrolo[3,4-c]pyrrole derivatives.

P-60 Rodzik Aleksandra, MSc
Synthesis and α_1-adrenoceptor affinities of novel arylpiperazine 5-(spiro)aromatic derivatives of hydantoin.

P-61 Różański Jakub, Dr
ADBO: source of new quaternary ammonium salts.

P-62 Satała Grzegorz, MSc
Cooperative properties of zinc binding to 5-HT7 receptor – pilot studies.

P-63 Ściepura Mateusz, MSc
Synthesis, physical and chemical properties of porphyrazines possessing peripheral benzylsulfanyl substituents.

P-64 Smusz Sabina, MSc
Machine learning method as a tool for searching new 5-HT_6 ligands in fingerprint-based consensus experiment.

P-65 Sobotta Łukasz, MSc
Pitavastatin, its photodecomposition products and their biological activity.

P-66 Sochacka Jolanta, Dr
Characterization of the 6-Thioguanine–serum albumin complex by molecular docking approach.

P-67 Sochacka Jolanta, Dr
Effect of molecular descriptors on the binding affinity of thiopurine derivatives to serum albumin.

P-68 Sochacka-Ćwikła Aleksandra, MSc
Biological studies of new isoxazole derivatives with potential immunorestoring activity.

P-69 Sroka-Bartnicka Anna, Dr
The application of ftir and raman spectroscopy in imaging biological materials.

P-70 Stefański Tomasz, MSc
Synthesis of thiocarbamate and thiophenol stilbene derivatives as potential anticancer agents.

P-71 Struga Marta, Dr hab.
Synthesis, structure and microbiological properties of thiourea analogues of 1,3-thiazole.

P-72 Szacoń Elżbieta, Dr
New 1-(1-arylimidazolidine-2-ylidene)-3-substituted ureas derivatives with potential pharmacological activity.

P-73 Szacoń Elżbieta, Dr
Synthesis and evaluation of antiviral activity of new 1-substituted-3-(4-halogenebenzyl)ureas.

P-74 Szafrański Przemysław, MSc
Application of immobilised *Pseudomonas cepacia* lipase for the stereoselective synthesis of azidoalcohols.

P-75 Szczesio Małgorzata, Dr
The crystal structures of gramicidin complexes.

P-76 Szczesio Małgorzata, Dr
Comparative X-ray structural studies on olanzapine.

P-77 Szczółko Wojciech, MSc
Synthesis and physical-chemical properties of porphyrazines possessing bulky peripheral 2,5-diarylpyrrol-1-yl substituents.

P-78 Szeleszczuk Łukasz, MSc
Application of the docking programs in estimating the binding affinity to the SHBG protein.

P-79 Szkaradek Natalia, Dr
Preliminary evaluation of antibacterial activity of some new xanthone's derivatives against drug resistant strains of *Helicobacter pylori*.

P-80 Szymańska Ewa, Dr
Synthesis of phenylalanine-based AMPA/KA receptor ligands.

P-81 Szymański Paweł, Dr
Application of computer simulations to the prediction of the biological properties of acetylcholinesterase inhibitors.

P-82 Świątek Piotr, Dr
Synthesis and potent antibacterial activity of new isothiazolo-pyridine derivatives.

P-83 Tarsa Monika, Dr
Study the durability derivative of oxazolo[3,2-a]pirydone.

P-84 Tejchman Waldemar, Dr
Homologs of Epalrestat: synthesis and molecular structure.

P-85 Więcek Małgorzata, Dr
Anticonvulsant properties of some imidazole-based histamine h_3 receptor ligands.

P-86 Witek Jagna, MSc
Selected transmembrane receptors – structures, interactions and binding site analysis.

P-87 Wróbel Martyna, MSc
Synthesis of new pyrrolidine-2,5-diones derivatives with dual SSRI and 5-HT_{1A} activity.

P-88 Wróbel Tomasz, MSc
Synthesis of nitrophenylimidazole derivative.

P-89 Wyrzuc Karolina, MSc
Synthesis and undesirable effects prediction for piperazine benzylideneimidazolone derivatives with expected MDR efflux pump inhibitors properties.

P-90 Zagórska Agnieszka, Dr
Estimation of phospholipophilicity of arylpiperazinylalkyl derivatives of imidazo[2,1-*f*]theophylline.

P-91 Zajdel Paweł, Dr
The multiobjective based design, synthesis and evaluation of the arylsulfonamide/amide derivatives of arylxyethyl- and arylthioethyl piperidines and pyrrolidines as a novel class of potent 5-HT_7 receptor antagonists.

P-92 Zajdel Paweł, Dr
Long-chain arylpiperazine derivatives with cyclic amino acid amide fragments as potential 5-HT_7 receptor ligands.

P-93 Żesławska Ewa, Dr
Influence of different solvents on forming hydrogen bonds in crystal structures of ellagic acid.

P-94 Mordalski Stefan, MSc
Influence of different solvents on forming hydrogen bonds in crystal structures of ellagic acid.

P-95 Bochniak Magdalena, Stud.
Modelling the interactions between glycogen synthase kinase and its inhibitors.

P-96 Kuder Kamil J., Dr
Modulation of the lipophillicity of novel tricyclic annelated theophylline derivatives obtained as adenosine receptors ligands.

P-97 Bartuzi Damian, MSc
Identification of hypothetical allosteric binding sites in human μ-opioid receptor.

P-98 Warszycki Dawid, MSc
Development of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 2. Privileged fragments. (see C7)

P-99 Gielara-Korzańska Agnieszka, MSc
Synthesis and the crystal structure of (E)-methyl(4-(2,3,4-trimethoxystyryl)phenyl) sulfane.

P-100 Łazarenkow Andrzej, MSc
Influence of hydrazone derivatives of chromones on proliferation and migration properties of MCF-7 cell line.

P-101 Fabijańska Małgorzata, MSc
Structural and biological studies of *trans*-platinum(II) complex with 3-aminoflavone.

P-102 Tykarska Ewa, Dr
Glycyrrhizic and glycyrrhetic acids in Medicine and Pharmacy.

P-103 Stefanowicz Jacek, Dr
Synthesis of thiocarbamate and thiophenol stilbene derivatives as potential anticancer agents.

LECTURES


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IL-1

Digging Deeper into the Ketamine Paradigm: How Archeological Drug Discovery is Leading to New Treatments for Pain and Depression.

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Ketamine, (R,S)-Ket, is a chiral phencyclidine derivative developed as an anesthetic agent. Ket is extensively transformed into norketamine, norKet, dehydronorKet, DHNK, and a series of hydroxynorketamines including (2S,6S)-HNK and (2R,6R)-HNK. Initial studies demonstrated that the CNS activities associated with general anesthesia and recovery were produced by Ket and norKet, while (2S,6S;2R,6R)-HNK was inactive, and Ket was identified as an anesthetic agent, norKet as the "active" metabolite and (2S,6S;2R,6R)-HNK was an inactive metabolite. When subsequent studies indicated that Ket and norKet inhibited the NMDA receptor, this activity became the accepted explanation of the pharmacological effects, and forms the "Ketamine Paradigm". However, while this approach may be valid for anesthetic dosing of Ket, it is not applicable to sub-anesthetic dosing. Low dose Ket is currently used in the treatment of neuropathic and acute pain and depression. Indeed, studies in our laboratory have shown that plasma concentrations of DHNK, (2S,5S;2R,5R)-HNK, and (2S,5R;2R,5S)-HNK were associated with lower psychotomimetic or dissociative side effects. Thus the observed clinical responses produced by sub-anesthetic doses of Ket may be due to unexplored pharmacological activities of Ket downstream metabolites.

This presentation describes our re-investigation of the Ketamine Paradigm and determination of the pharmacological activities of Ket downstream metabolites. The results of these studies indicate that DHNK and (2S,6S)-HNK are potent inhibitors of neuronal nicotinic acetylcholine receptors (nAChRs), in particular the $\alpha 7$-nAChR and $\alpha 3\beta 4$-nAChR subtypes. Inhibition of these nAChRs results in the inhibition of serine racemase and a decrease in intracellular D-serine, a key co-agonist of the NMDA receptor. This effect and other intracellular signaling cascades are consistent with the clinical effects of the low dose Ket and with data from a metabolomic study in bipolar depression patients treated with low dose Ket.

The results indicate that DHNK and (2S,6S)-HNK may be effective therapeutic agents and a new class of serine racemase inhibitors for use in a broad range of CNS disorders. The potential development of pro-drugs to deliver these agents will also be illustrated.

L-1

Novel Positive Allosteric Modulators of the Human α7 Nicotinic Acetylcholine Receptor.

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Positive allosteric modulators (PAMs) of α7 nicotinic acetylcholine receptors (AChRs) might be important to develop therapies for Alzheimer's disease, schizophrenia, and depression. In this regard, the pharmacological activity of a series of novel amide derivatives was first characterized on several AChR subtypes by using functional and structural approaches. Subsequently, forced swim test studies were compared between mutant β4-/- and wild-type β4+/+ mice to determine the PAM activity on nicotine-induced antidepressant activity. Ca^{2+} influx results indicate that these compounds are not agonists of the human (h) α4β2, α3β4, α7, and α1β1γδ AChRs; compounds 2-4 are specific PAMs of hα7 AChRs, whereas compounds 1-4, 7, and 12 are noncompetitive antagonists of the other AChRs. Radioligand binding results indicate that these PAMs do not inhibit binding of [^{3}H]methyllycaconitine but enhance binding of [^{3}H]epibatidine to hα7 AChRs, indicating that these compounds do not directly, but allosterically, interact with the hα7 agonist sites. Additional competition binding results indicate that the antagonistic action mediated by these compounds is produced by direct interaction with neither the phencyclidine site in the *Torpedo* AChR ion channel nor the imipramine and the agonist sites in the hα4β2 and hα3β4 AChRs. Molecular dynamics and docking results suggest that the binding site for PAMs 2-4 is mainly located in the inner β-sheet of the α7-α7 interface, ~12 Å from the agonist locus. Two hydrogen bond interactions bridging both (+) and (-) subunit faces are found to be critical for the PAM activity at the hα7 AChR. This is reinforced by the fact that compounds with no (e.g., compound 6-8) or only one (e.g., compound 1) hydrogen bond do not behave as α7 AChR PAMs. In addition, hydrogen bonds are only found on the (+) faces of the hα4β2 and hα3β4 AChR subtypes, and these interactions could be responsible for the observed noncompetitive antagonistic activity (i.e., negative allosteric modulators). Another important difference among AChR subtypes is the size of the PAM locus. Since Tyr is larger than His, the natural mutation of α7-His102 to β2/β4-Tyr104 results in a smaller PAM pocket in the latter subtypes. Thus, we propose that the observed subtype specificity is also elicited by steric hindrance between β2/β4-Tyr104 and the furan ring of compounds 2-4. The importance of the locus size is reinforced by the fact that compounds 9-12, which have larger volumes than PAMs 2-4, do not behave as PAMs. Forced swim test analysis on β4-/- vs β4+/+ mice indicate that the β4 subunit is important for the nicotine-induced antidepressant activity, and that PAMs increase the activity elicited by nicotine in female β4+/+ mice but diminish it in male β4+/+ mice. This result reinforces the idea that gender is an important variability regarding the activity of a drug. Interestingly, PAMs increase the activity elicited by nicotine in mutant mice of both sexes. The receptor specificity elicited by PAMs 2-4 and the modulatory action on nicotine-induced antidepressant activity might be therapeutically important for the treatment of depressed patients with Alzheimer's disease.

NOP, the Other Opiate Receptor: A New Target for Drug Abuse and Pain Medications.

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The NOP receptor (originally called ORL1) is the fourth member of the opioid receptor family. The NOP receptor and its endogenous ligand, nociceptin/orphanin FQ (N/OFQ), are highly expressed in brain regions implicated in pain, drug abuse and anxiety, as well as other locations. Although N/OFQ is in the opioid peptide family, when administered i.c.v. it blocks opiate analgesia, blocks opiate tolerance development, and blocks opiate reward. Despite these "anti-opiate" actions, when given intrathecally, N/OFQ has analgesic activity, like morphine. Small molecule NOP receptor agonists have been synthesized. Agonists are anxiolytic but have no analgesic activity when administered systemically to rodents. We have designed "mixed" NOP/mu agonists with the intention of developing compounds that maintain the opiate analgesic activity, but with reduced side effects, due the NOP agonist activity, as well as NOP agonists that could be used as drug abuse medications. Our two prototype compounds are SR14150 and SR16835. SR14150 is a NOP partial agonist/mu partial agonist. It has mu-mediated antinociceptive activity in the tail flick test in mice but does not appear rewarding in the conditioned place paradigm (CPP). This compound is not able to attenuate the CPP induced by morphine. SR14150, or similar compounds, have potential as non-addicting analgesics. SR16835 is a more selective full agonist at the NOP receptor. This compound does not have antinociceptive activity on its own, in the tail flick test. Although not effective for treating acute pain, SR16835 is able to attenuate mechanical allodynia when mice are in chronic pain due to Spinal Nerve Ligation. In addition, when administered prior to morphine, SR16835 blocks the acquisition of morphine CPP. This compound may therefore have potential as a drug abuse treatment medication, as well as a medication for chronic, neuropathic pain. The NOP receptor appears to be an excellent target for medications development.

L-3

5-Arylbenzothiadiazine Type Compounds as Positive Allosteric Modulators of AMPA/Kainate Receptors.

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L-glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system (CNS) and its signal transduction is mediated by ionotropic and metabotropic receptors [1]. Different studies suggest that ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAr) is involved in learning processes and in memory establishment [2]. The therapeutic potential of compounds able to activate AMPAr has led to the search for new AMPAr positive modulators [3,4]. Among these, one of the most investigated chemical class of compounds are benzothiadiazine derivatives such as cyclothiadiazide (CTZ), (±)7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (IDRA21) and (±) 8-chloro-2,3,5,6-tetrahydro-3,6-dimethyl-pyrrolo[1,2,3-de]-1,2,4-benzothiadiazine 1,1-dioxide that are able to inhibit desensitization of AMPAr potentiating ionotropic glutamatergic neurotransmission [5-7]. IDRA21, the first benzothiadiazine effective in increasing learning and memory performance in behavioral tests, represents an important lead compound since it is able to cross the blood-brain barrier [8]. Basing on crystallographic data of the benzothiadiazines binding mode in the S1S2 GluA2 dimer interface, a set of 5-aryl-2,3-dihydrobenzothiadiazine type compounds, using IDRA21 as lead compounds, has been designed and synthesized. Hence, 5-aryl-7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide derivatives were prepared by a synthetic route based on the Pd-catalyzed Suzuki-Miyaura coupling reaction. Electrophysiological results suggested that 5-heteroaryl substituents on benzothiadiazine core like 3-furanyl and 3-thiophenyl dramatically enhance the activity as positive modulators of AMPAr respect to IDRA21 and cyclothiazide. Moreover mouse brain microdialysis studies have suggested that 7-chloro-5-(3-furyl)-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide crosses blood brain barrier after intraperitoneal injection. Biological results have been rationalized by a computational docking simulation that it has currently employed to design new AMPAr positive allosteric modulators.

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L-4

Development of New Derivatives of Fenoterol as Potential Ligands of the β2 Adrenergic Receptor with Novel Therapeutic Perspectives.

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Fenoterol (FEN), a β2-AR selective agonist is used in therapy of asthma as a racemic mixture of (R,R)- and (S,S)- isomers. Rational drug design approach were used to develop a number of FEN derivatives modified at the aminoalkyl tail in various stereoisomeric forms [1,2]. The compounds were tested in β2-AR binding studies using two different radioligands, [^{3}H]-CGP- 12177 and [^{3}H]-(R,R)-4-methoxyFEN what evidenced significantly different binding affinities towards inactive and active forms of the receptor, respectively [3]. Parallel Van't Hoff analyses of binding data showed that the thermodynamics of complex formation highly depends of the stereochemistry of FEN and a marker ligand used [3,4].

The compounds were also widely characterized for functional activities using induced cAMP accumulation measurement, cardiomyocyte contractility assay and proliferation inhibition tests for a number of cancer cell lines. The results suggested that stereochemistry of a molecule affected the coupling properties of the receptor to different G proteins upon agonist binding. In cardiomyocyte contractility studies, the addition of pertussis toxin has no effect on the activity of (R,R)-FEN, (R,R)-4-methoxyFEN and (R,R)-4-aminoFEN, indicating that the receptor selectively couples Gs protein signaling upon binding of these compounds [4]. Conversely, pertussis toxin significantly affected cellular effects elicited by (R,R)-1-naphtylFEN and (R,R)-4-methoxy-1-naphtylFEN, showing that binding of these derivatives activate the receptor to forms able to couple Gs and Gi proteins. Molecular modeling simulations of binding to the β2-AR models linked this difference with dichotomous interactions of derivatives with Y308 residue of β2-AR model. Subsequent studies on Y308A mutant of β2-AR confirmed that binding affinities of Gs selective FEN derivatives are significantly reduced comparing to the β2-AR WT data, while Y308A mutation did not affect affinities for the group of derivatives eliciting both Gs and Gi signaling patterns.

The overall data demonstrate that stereochemistry and chemical constitution of a FEN derivative influence the magnitude of binding affinity, thermodynamics of local ligand - receptor interactions and the global mechanism of β2-AR activation. Even a small change of ligand stereoconfiguration allows observation of biased agonism in respect to Gs or Gi intracellular signaling. This medicinal chemistry project opens new perspectives for developing novel potent and highly selective β2-AR agonists. For example, (R,R)-FEN and (R,R)-4-methoxyFEN is currently clinically tested for treatment of congestive heart failure, (R,R)-ethylFEN derivatives emerges as potent inhibitors of mitogenesis of brain tumor cells in *in vitro* studies.

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L-5

Acetylcholinesterase-Based Analytical Tool for Drug Screening.

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Human acetylcholinesterase (AChE) is a widely studied target enzyme in the development of therapeutic agents for the treatment of Alzheimer's disease (AD). AChE inhibitors have been for a long time the only drugs available on the market for the symptomatic treatment of this widespread pathology and still represent 75% (3 out of 4) of marketed drugs. The interest on AChE inhibitors has evolved in last two decades after AChE's peripheral binding site (PAS) was hypothesised to promote the deposition of the neurotoxic β-amyloid peptide (Aβ) [1]. Therefore, in the light of this non cholinergic activity, new AChE inhibitors, able to prevent the interaction between AChE's PAS and Aβ, have been designed and investigated.
Miniaturization and automation of the screening system will greatly implement the drug discovery process for AD, in which a large number of new chemical entities needs to be screened for affinity towards a specific target, such as AChE.
On the light of these premises, in this talk, two different approaches to the development of screening tools for the investigation of compounds able to bind to the CAS (catalytic anionic site) and PAS of human AChE will be presented.
In particular, first, the development and application of AChE-based enzyme reactors for the automated and rapid screening of inhibitors at the CAS will be described. These bioreactors have been obtained by the covalent immobilization of the target enzyme on a suitable monolithic chromatographic material, inserted into a HPLC system and used for the evaluation of the inhibitory activity and mechanism of action of new ChE inhibitors [2.3].
Then, the initial development of a new AChE-based fluorescence sensing surface for the identification of PAS binders through propidium displacement studies will be presented. The initial selection of the suitable multilayered material, and of the optimal pattern thickness required to maximize fluorescence signal and maintain chemical stability will be also discussed. Then, the selective immobilization of recombinant human AChE on the SiO_2 architectures with optimal geometry and chemistry will be shown. Thanks to the combined use of atomic force microscopy and CLSM it was demonstrated that the enzyme distribution selectively matched with the initial SiO_2 features (independently from their shapes and dimensions). In the optimal design, the AChE-based biosensing surface showed an efficient fluorescence emission after labelling with propidium, a selective fluorescent probe of the peripheral binding site of the AChE [4].

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L-6

HPLC-APCI-MS/MS Assay for the Simultaneous Determination of Tocopherols and Tocotrienols in Human Serum.

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Breast cancer is one of the leading causes of mortality in women. Recent studies, both clinical and pre-clinical, have indicated that vitamin E can play a protective role in breast cancer. Vitamin E naturally occurs in four forms: alpha-, beta-, gamma- and delta-tocopherols and four corresponding unsaturated analogues, tocotrienols. Tocopherols and tocotrienols are antioxidants and are believed to play a preventive role in diseases associated with oxidative stress including cancer. A majority of conducted studies have been mostly focused on the role of alpha-tocopherol, thought to be the most biologically important form of vitamin E in breast cancer. However, observational studies have failed to consistently support the theory that alpha-tocopherol provides a protection against this disease.
The primary aim of the study was development of a rapid assay for tocopherols and tocotrienols simultaneous determination in human serum with the use of reversed phase high performance liquid chromatography coupled with tandem mass spectrometry (RP-HPLC-MS/MS). The mass spectrometry analysis was carried out by the use of Agilent Technologies system (Palo Alto, CA) 1200 LC with atmospheric pressure chemical ionization interface and Agilent Technology 6430 triple quadrupole. The sample preparation was performed with the use of solid-phase extraction technique.
The separation of four tocopherols and three tocotrienols was successfully achieved with Cosmosil 2.5 πNAP column. LC-MS/MS parameters were optimized for the future analysis of vitamin E constituents in human serum. The chromatographic run was carried out isocratically with mobile phase composed of methanol : water (90:10; v/v) over 13.5 min at a flow rate 0.5 ml/min.
The results proved the assay to be sensitive, precise, accurate and specific indicating that it can be applied to the simultaneous determination of tocopherols and tocotrienols in human serum.

L-7

α-Aryl Substituted Fosmidomycin Analogues as Inhibitors of *Mycobacterium tuberculosis* 1-Deoxy-D-xylulose-5-phosphate Reductoisomerase.

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Worldwide, tuberculosis remains the most common and important infectious disease regarding both morbidity and mortality. The World Health Organization has estimated that a third of the world's population today is infected with the pathogenic microorganism *Mycobacterium tuberculosis* (*Mtb*), and that 1.7 million people die of tuberculosis every year [1].

Due to the lack of effectiveness of available medication and its serious side effects, the search for new drugs, preferably active against new targets is urgent today. 1-Deoxy-D-xylulose-5-phosptate reductoisomerase (DXR) is an enzyme that lately was proven to be essential for *in vitro* growth of *Mtb* [2], moreover it does not exist in humans what makes it an interesting target for new and potentially selective antimycobacterial agents. DXR inhibitor, fosmidomycin, is currently in phase III trials in combination with clindamycin, for the treatment of malaria. Unfortunately it does not affect the growth or viability of *Mtb* bacteria even though it inhibits *Mtb* DXR with an IC_{50} of 80 nM. This is because of the lack of uptake through the highly complex mycobacterial cell wall which is probably due to the inherent polar and charged nature of this class of phosphonic acid/hydroxamic acid inhibitors. Therefore it would be of interest to explore analogues of fosmidomycin with modified hydrophobic/hydrophilic properties as potential antimycobacterial drugs. Numerous analogues of fosmidomycin with improved physicochemical properties have been reported as *P. falciparum* DXR inhibitors, among them α-(3,4-dichlorophenyl)-fosmidomycin [3] which became a starting point for this study.

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L-8

Geometry of Long-Chain Aryl-Piperazines from X-ray studies.

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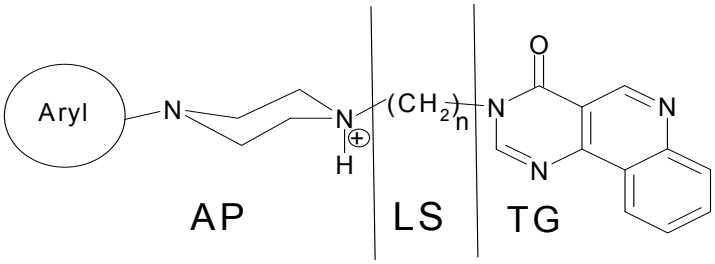
Malfunction of the serotonin system also manifests in mental disorders, including depression. Therefore the structures of the serotonin receptors (14 types known) and their ligands are of interest.
X-Ray crystallography is one of the best tools used for determination of their structures, despite common reservation that a "frozen" molecular structure observed in a crystal state is unrelated to the conformation of the ligand having minimum energy. This is true for the singular case but not for a large set of data. Besides, the "biologically active" conformation is not necessary that of minimum energy. It is well known that molecules may (and sometimes must) undergo significant conformational changes while interacting with a receptor. The last but not least important issue is that some characteristic interactions seen in crystals for a larger group of similar compounds do tell us something about receptor-ligand relations.
While studying a group of pyrimido[5,4-c]quinolin-4(*3H*)-ones with a long-chain arylpiperazine moiety [1], we determined the crystal structures of almost twenty of them in form of hydrochlorides. So the ligands were studied in the protonated forms, which are dominant in the physiological environment, probably being also the pharmacologically active one. The compounds studied were synthesized by Dr W. Lewgowd from Medical University of Łódź.
A year ago we presented here a preliminary report on the subject, paying attention mostly to the linker, being the most flexible part of LCAPs. Now it is time for another part, the Aryl-Piperazine moiety.
In the Cambridge Structural Database there are over 400 N-arylpiperazines, over hundred of which have all molecular features of LCAPs, i.e. the planar terminal group (TG) joined to the piperazine N atom with a chain called a linker or spacer (LS). However, these structures represent an accidental set of structures of unknown activity, seldom related or studied in a systematic way. Therefore our set of ligands of known activity, which differ only in the aryl substitution and in the number of methylene units in the linker, are very valuable.
The analysis of all accessible crystal data on LCAPs allowed us to draw several observations concerning preferential structural features, including the piperazine ring conformation, interactions of alkyl substituted N atom of the piperazine ring with serotonin receptors, orientation (axial/equatorial) of the aryl ring and its twist.



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L-9

Tacrolimus vs. Ascomycin – Problems with the Biosynthesis Upscaling.

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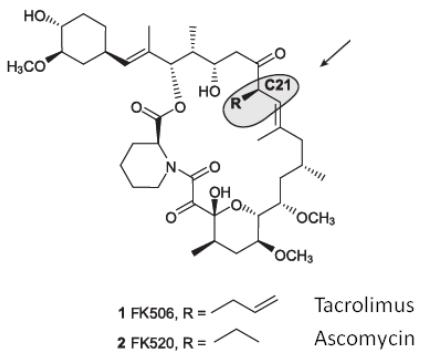

Tacrolimus (FK506) and its structural analog ascomycin (FK520) are secondary metabolites belonging to a class of macrolactone compounds of great medicinal importance. Both are products of fermentation of *Streptomyces tsukubaensis.* Tacrolimus is currently registered for use as immunosuppressants after organ transplantation as well as for treatment of inflammatory skin diseases and eczema (1-3). The elevated price of tacrolimus-containing formulations is caused mainly by a low productivity of microbial strains employed for the biosynthesis. The main objective of our research was therefore to optimize the medium composition to enhance the productivity of tacrolimus in *S.tsukubaensis* strain. According to our assumption, the pyridine and piperidine derivative could be precursors of the macrolide or promoters of the strain growth. The piperidine- and pyridine- derivative tested as the cultivation medium supplements enhance the productivity of tacrolimus in *S. tsukubaensis* strain 3 to 7-fold. The test cultures in the described above experiments were cultivated in the shake flasks of 200 ml working volume. By the LC MS analysis we have determined, that the participation percent of ascomycin, the byproduct of the biosynthesis, in the crude macrolide fraction isolated from the cultivation broth was equal 20-30%. The next stage of our research was the upscaling of the biosynthesis of tacrolimus to the working volume of 7-8 L. The test cultures were cultivated in two types of the fermenters: 8L BioTec and 7 L Infors fermenter, under the same conditions (medium composition, aeration, temperature, agitation). The only one important difference between the construction of the BioTec and Infors fermenter was the diameter / height ratio, much higher for the BioTec fermenter. The upscaling of the process did not change the productivity of macrolides in both fermenters. We have observed, however, significant difference in the ascomycin participation percent in the crude macrolide extracts isolated from two types of fermenters. It was over two times higher for BioTec fermenter (50-70%) than for Infors (17-27%). A much higher concentration of ascomycin was recorded in the post-cultivation medium, than inside the cells. For tacrolimus this relationship was inverted. The unexpected increase of the ascomycin productivity in one type of the tested fermenters may be explained by the higher concentration of oxygen in the cultivation broth, caused by its free diffusion through the surface of the cultivation medium. That most likely affects the biosynthesis of the five-carbon extender unit leading to the allyl group at position C21 of FK506 (4, 5).

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Acknowledgement:
This work was supported by grant NN 405302036 of the Polish Ministry of Science and Higher Education.

L-10

Chemical Glycosylation of Secondary Plant Metabolites as an Effective Modifier of Their Biological Activity.

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Approximately half of the existing drugs are derived from (or inspied by) natural products, while recently obtained large synthetic combinatorial libraries fail to deliver experimentally validated new drug candidates. Secondary metabolites, successfully exploited in medicinal chemistry as pharmacological models or drug leads, frequently contain in their structure a glycosidic element, seemingly indispensable for their biological activity [1]. At a dawn of glycobiology and glycomic era we learn to appreciate molecular recognition mechanisms of carbohydrates on a biopolimer level (which govern majority of the vital cell sociology phenomena) [2], but we are still mystified by functions performed by a single monosaccharide moiety in a low molecular weight ligand. Chemical glycosylation is a useful tool applied in medical chemistry in modification of complex compounds isolated from natural sources [3]. In our experience, addition of a glycosyl residues to pharmacophoric scaffold can be very useful for creating diversity of structure and function in many classes of medicinally useful compounds, but efficient and stereoselective glycosylation of complex aglycones remains difficult, particularly in scale up. Screening of several methods of chemical glycosylation on secondary plant metabolite genistein and its derivatives will be discussed, with focus on application of hex-1-enitols (glycals) as glycosyl donors.
Results of a research program, consisting of chemical derivatization of genistein, molecular modeling and biological activity studies of new derivatives aimed at proposing new potential anticancer compounds will be discussed. Regio- and stereoselective synthesis of O-glycosides and glycoconjugate derivatives of 2,3-unsaturated mono- and disaccharides of genistein, followed by biological screening *in vitro* of new derivatives using cancer cell lines (Hct 116 +/+p53, Hct 116 -/-p53, Ht 29, AGS, LNCaP, PC3, DU 145, A549) indicated a number of compounds of increased potency, actually more effectively inhibiting cancer cell growth in comparison to the parent compound, genistein [4]. The assumed biomolecular mechanism was partially based on the genistein molecular targets, i.e. tyrosine kinases, however, for some derivatives also a new mechanism associated with microtubules, was found [4]. The described group of compounds are important objects for the structure - activity relationship studies because of at least two reasons: formerly none of the genistein derivatives with the spacious group added at C7 of genistein was reported to inhibit tyrosine kinases more efficiently than genistein, microtubules appeared to be new promising target for isoflavonoid derivatives.

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L-11

Quinolines as Fragments in Design of HIV Integrase Inhibitors.

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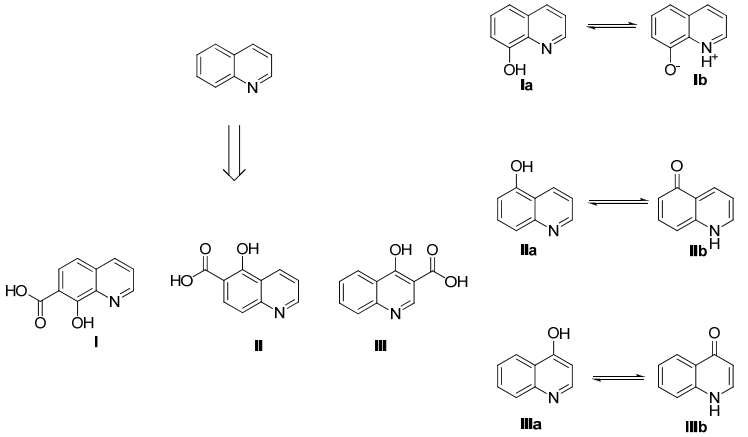
Our struggling with human immunodeficiency virus gives us particularly comprehensive view on drug design process. During last 20 years literally each possible approach was used from HTS, de novo modeling to fragment based design. Among these the privileged structures as building blocks offers particularly interesting approach of high cost-effective design. In 1990 Johnson and Maggiora formulated the similarity principle, which states that structurally related compounds display similar biological activity [1]. Although multiple exceptions to this theory are known, this essential rule still underlies the art of molecular design. Moreover, in some cases it is feasible to identify common molecular fragments, so-called *privileged motifs*, which ease ligand binding to an individual receptor or particular receptor family. The term privileged structures was first introduced in 1988 by Evans and co-workers in their search for cholecystokinin (CCK-A) receptor antagonists derived from the natural product asperlicin[2]. Later this definition was updated by Patchett and Nargund [3]. Since then, several reviews deal with the concept of privileged motifs and numerous molecular fragments have been described as privileged, e.g. benzazepinone, diphenylmethane or quinoline. The last one appears frequently also among HIV integrase inhibitors described within the 25 year history of the developing of the antiviral therapy against AIDS. Elvitegravir or Fz-41 are examples of such compounds being currently under clinical tests. Simple statistical approach to this task would lead to somewhat flat conclusion that quinoline is really privileged structure while the analyze of structures will help to find the prerequisites lying beneath.



Most of recently reported nonpeptidic small molecule HIV integrase inhibitors contain (di)ketoacid DKA moiety. The same is apparent for quinoline based structures where DKA may be masked in several ways (Fig). The substitution pattern of quinoline affect their abilities to form stable tautomers and protomers. This is essential for the way of fulfillment the DKA functionality in specific arrangement of fragments.

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ORAL PRESENTATIONS
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Molecular Insight into the Interactions of β_2-Adrenergic Receptor Agonists and Sulfotransferase Enzymes.

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Sulfotransferases are enzymes that catalyze the transfer of a sulfo group from a donor molecule (3'-phosphoadenosine-5'-phosphosulfate, PAPS) to an acceptor amine, sugar, phenol or alcohol. Cytosolic SULTs are key phase II metabolizing agents involved in the clearance of small endogenous and exogenous compounds, e.g. neurotransmitters, drugs, steroid hormones, dietary carcinogens and proteins. Sulfo group transfer might be predominantly regarded as a detoxification pathway or an activation pathway depending on a substrate molecule.
The most extensive group of the human cytosolic SULTs, the SULT1A family primarily sulfonate endogenous catecholamines such as dopamine, adrenaline, noradrenaline. Moreover this family is considered to sulfonate fenoterol stereoisomers [1]. Recent study shows that among SULTs, three isoforms – 1A1, 1A3 and 1E1 are responsible for methoxyfenoterol metabolism and structurally similar compounds [2,3].
Based on solved crystal structures of three SULT isoforms we aim at characterization of binding site interactions and molecular aspects of β_2-adrenergic receptor agonists metabolism. This study might contribute to the investigation of compounds susceptibility to sulfonation and substrate stereospecificity of these enzymes.

Acknowledgement
The work is supported by the found from Foundation for Polish Science (TEAM Programme 2009-4/5) and using the equipment purchased within the Project „The equipment of innovative laboratories doing research on new medicines used in therapy of civilization and neoplastic diseases" within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

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C-2

Binding Affinities of Fenoterol Derivatives to the Agonist Conformation of β_2-Adrenergic Receptor Determined Using [^{3}H]-(R,R')-4-Methoxyfenoterol as the Marker Ligand.

Lawrence Toll [1], Karolina Pajak [2], Anita Plazinska [2], Krzysztof Jozwiak [2], Lucita Jimenez [3]; Joseph A. Kozocas [3], Mary J. Tanga [3], James E. Bupp [3], Irving W. Wainer [4]

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The β_2-adrenergic receptor (β_2-AR) is a member of G-protein coupled receptors (GPCRs), the largest family of membrane proteins in the human genome and group of important targets for drug discovery. The activation of the β_2-AR is a dynamic process including the agonist-induced conformational changes leading to G-protein activation, which attenuate GPCR-mediated signaling. Although it is well established that β_2-AR bind ligands in multiple conformations, the most often employed marker radioligands in affinity determination, such as (-)-3-[^{125}I]iodocyanopindolol, [^{125}I]iodopindolol, [^{3}H]dihydroalprenolol or [^{3}H]CGP-12177 are nonselective β-AR antagonists, then only a portion of the binding interactions can be explored.

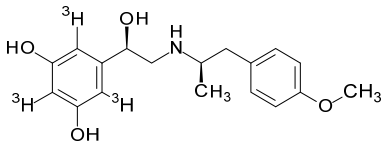

Fig. Structure of [^{3}H]-(R,R')-4-methoxyfenoterol

To probe the agonist conformation of the β_2-AR, we developed a new radioligand, tritium labeled derivative of (R,R')-4-Methoxyfenoterol [1], a potent and selective β_2-AR agonist with high therapeutic potential in the treatment of congestive heart failure [2]. Results of radioligand binding studies showed that [^{3}H](R,R')-4-methoxyfenoterol binds with high affinity to an agonist conformation of the receptor, which represents approximately 25% of the total β_2-AR population as determined with the antagonist [^{3}H]CGP-12177. The series of fenoterol analogs tested in this study has considerably higher affinity for the agonist conformation of the receptor, and K_i values determined for fenoterol derivatives model much better the pharmacological effects of the β_2-AR elicited by these ligands regarding cAMP accumulation.

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Acknowledgement
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C-3

Importance of the Nicotinic Receptors Inhibition in the Therapeutic Action of Antidepressants.

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Nicotinic acetylcholine receptor (nAChR) is an archetypical member of the Cys-loop Ligand Gated Ion Channels superfamily. It is a pentameric assembly of separate membrane embedded proteins (subunits) oriented around a centrally located pore permeable of cations. Neuronal subtypes of nAChR poses a promising target for treatment of many neurological disorders. One of them is a depression, common mental disorder and one of the leading causes of disability and morbidity in the world, affecting ~20% of the U.S. population [1]. Over the past thirty years, several groups reported on the nicotinic receptor inhibitory actions of classic tricyclic antidepressants [2, 3] and later studies characterized more selective monoamine reuptake inhibitors (e.g. selective serotonin reuptake inhibitors [4]) or atypical antidepressants (e.g. bupropion [5]) as nAChRs inhibitors.
The aim of our studies was to present the molecular modeling interactions between nAChR models and common used antidepressants which facilitate understanding of the binding affinities reported for competition binding assays of radioligands and studied compounds to nicotinic receptors. We developed a panel of interdisciplinary methods to characterize the interactions of studied compounds with different subtypes of nAChR. Homology models representing channel domains of the most common nAChR neuronal subtypes were used. These models were employed in docking simulations where several pharmacological groups of antidepressants were probed for interactions with the receptor binding domain. The simulated energy of interactions and the description type of the interactions were used to characterize the binding of ligand molecule to the receptor. In addition, the selectivity between different nicotinic receptor subtypes and the interactions with other ligands, especially overlapping of the ligands in the binding site were taken into account. Furthermore, the molecular modeling results were correlated with experimental data determined by the structural and functional studies characterizing binding affinities for antidepressants. Finally, our work indicated how computational molecular modeling methods can support the research in pharmacology and medicinal chemistry fields.

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C-4

Interactions of between fenoterol and β_2 adrenergic receptor: the stereoselective binding and the ligand dissociation/association profiles

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Fenoterol is a selective agonist of β_2 adrenergic receptor (β_2-AR). In the present study we used docking procedures for the synthesized stereoisomers of fenoterol and its derivatives (*N* = 72) using active (Ac_β_2-AR) and inactive (In_β_2 AR) molecular models of β_2AR (PDB: 3SN6 and 2RH1, respectively). Further, molecular dynamics (MD) simulations were performed for all possible β_2-AR/stereoisomer of fenoterol complexes. Molecular modeling allows to speculate on the basis of stereoselective binding of ligands to β_2-AR. The results indicated that there were significant stereochemistry-based differences in the binding affinities. The relative free energy changes accompanying the binding of fenoterol stereoisomers to β_2-AR were obtained via the thermodynamic integration protocol. The relative order of fenoterol stereoisomers affinities to In_β_2-AR is following: (*R*,*R*) > (*R*,*S*) > (*S*,*R*) > (*S*,*S*); this fact and the order of magnitude of the differences between free energy changes corresponding to the particular stereoisomers (several kJ/mol) remain in a good agreement with the experimental data. Further, some attempts were made to interpret these values in terms of molecular features of the studied systems. The analogous results for Ac_β_2-AR do not reflect the binding stereochemistry. In second part of the study, we used the steered MD simulations to describe the binding/unbinding process of (*R*,*R*)-fenoterol to/from β_2-AR (for both In_β_2-AR and Ac_β_2-AR states). The results indicate that there exists secondary binding pocket in the extracellular region of the receptor. In addition, we discuss the importance for the agonists exit/entry process of non-conserved charged residues and conserved aromatic interactions shared by the entry extracellular chanel.

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The work is supported by the found from Foundation for Polish Science (TEAM Programme 2009-4/5) and using the equipment purchased within the Project „The equipment of innovative laboratories doing research on new medicines used in therapy of civilization and neoplastic diseases" within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

C-5

Effects of Bacteriochlorin Based Photodynamic Therapy on Tumor Physiology.

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Synthetized bacteriochlorin are interesting for PDT [1] because of their strong absorption in the "phototherapeutic window", long triplet lifetimes and low fluorescence quantum yields [2].
S-91 Cloudman *melanoma* and Levis Lung *carcinoma* tumors were grown in the legs of DBA/2 and C_{57}bl mice. When the tumor reached 3-4 mm of mean diameter, bacteriochlorin was administered (2-4 mg/kg BW i.v.) and tumors were treated with 100 J/cm^2 of light (λ=750 nm) either 15 min or 72 h later. The pO_2 was monitored before treatment, 0.25, 3 h and every 24 h after PDT for 6 days. Neutrophilis, macrophages, vasculature and morphology of tumors were estimated immunohistochemically before and 0.25, 3, 24, 48, 96 h after treatment. Structure and function of the vasculature were investigated with Laser Doppler Perfusion Imaging (**LDPI**) and VEVO 2100 ultrasonograph with Doppler mode.
Strong immune response and vasculature destruction was observed after both PDT protocols. However, different kinetics and intensity of the effects were seen. Changes in blood perfusion and loss of vessel density in the tumors treated with vessel targeting PDT is stronger than in cellular targeting PDT. The increase in perfusion observed 5-10 days after PDT in both protocols display different intensity/kinetics and might result from PDT caused inflammation, observed in treated legs. Moreover, strong neutrophil infiltration was detected immediately after vessels targeting PDT and strong macrophage infiltration after anti-cellular treatment. Oxygenation level in the tumor tissue decreased immediately after light irradiation.
Bacteriochlorin PDT stimulates the local immune response, activating neutrophil and macrophages infiltration. PDT cause vasculature destruction and changes of blood perfusion and oxygen partial pressure in tumors .

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This work was supported by grants from the Era Chemistry Project (60303, The National Centre for Research and Development) and Jagiellonian University (BMN 5/2012) and NCN). We would like to thank Dr H. Swartz (EPR Center for The Study Of Viable Systems at Darmouth Medical School) for providing LiPc. The EPR spectra were analysed using the fitting software developed by dr Ben Williams and Tom Mathews from Dartmouth Medical School.

C-6

Plant phenolics as drug leads.

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Secondary metabolites (SM) of higher plants played crucial role in creating the contemporary drugs repository and they continue to provide inspiration for design of modern drug leads. Apart from providing plants with versatile enviromental survival and adapting strategies, SMs also play important role in human nutrition as dietary constituents with proven protective properties against inflammatory and degenerative processes. These health promoting properties are particularly well demonstrated in abundant category of plant phenolics, to which among others phenolic acids, flavonoids, stilbenes and lignans belong [1]. Phenolic compounds of plant origin belong to reactive chemical species and they share certain characteristics, like antioxidant activity, which is considered beneficial for human health; they are seldom pharmacologically inert and last but not least, they are known to share certain metabolic pathways, by which they are metabolized conjugated and excreted, like other xenobiotics, from human body. Biocompatibility of plant phenolics makes them suitable objects for "reverse pharmacology" – both: mental process and logistic procedure, in which demonstrably safe and efficacious pharmacological agent is studied to discover molecular mechanism of its biological action [2]. In this context, selected phenolics: catechin, curcumin, resveratrol, sylibin, are taken as examples of SMs which have obvious medicinal potential, but did not make it as registered drugs [1,2]. Their roles as drug leads will be discussed in some details.

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C-7

Development of Multistep Ligand-Based Virtual Screening Cascade Methodology in a Search for Novel HIV-1 Integrase Inhibitors: 2. Privileged Fragments.

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HIV integrase which is essential in the virus replication cycle and has no homologue among human enzymes [1], became an important target for drug development more than twenty years ago. Nevertheless, progress has been hampered by the lack of assays suitable for high throughput screening. Thus, a real breakthrough was only observed in 2007 with the introduction of the first integrase inhibitor, raltegravir, into treatment.
Crystal structure for HIV-1 integrase is already known and thus, both techniques commonly used in VS campaigns (structure and ligand-based) could be developed. Here we introduced a multistep ligand-based screening cascade because it is suggested that ligand-based methods outperform structure-based in true positives identification [2]. Our strategy consists of two sequential modules: machine learning-based (ML-based) and privileged fragments-based (PF-based).
The PF module is a weight-based scoring function which rates presence of particular molecular fragments, previously recognized as privileged, in screened compounds. Mentioned fragments are defined as structural subunits specially effective in distinguishing active compounds from inactives. PFs were extracted by using MI-DSE formalism [3] on thirteen unique training sets. Finally, the prepared module was applied as standalone or as a second-step in a multistep VS experiment. The test set was composed from 450 actives (not used for the module development) and 16200 DUD [4] decoys generated by an in-house script. The developed module achieved AUC = 0.760 and more than 40-fold enrichment in a single-step experiment. Overall, two-step VS campaign, where PF-based module was a second-step, reached more than 200-fold enrichment of actives/inactives ratio.

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This presentation is a continuation of poster entitled ‘Developed of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 1. Machine learning’.

C-8

The 'order-to disorder' conformational transition in CD44 protein: an umbrella sampling analysis.

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CD44 protein is a major cell surface receptor for hyaluronan (HA). Recent experimental studies [1] revealed that there exist two distinct conformational states of CD44: the rearrangement of the β-strands in the extended lobe (residues 143–148) and disorder of the structure in the following C-terminal region (residues 153–169) may occurr upon HA binding leading to the 'partially disordered' (PD) structure. The PD state differes from the structures found during X-ray diffraction (XRD) studies (i.e. 'ordered', O) state [2]. The PD state exibit greater affinity toward HA in comparison to the O one [3]. Further, it apprears that both the PD and O states exist even in absence of HA. As the previous molecular modeling investigation [4] indicated that the two XRD-originating conformational forms of the CD44 are not responsible for the varying affinity of CD44 to HA, the present study is focused on the O-PD transition. The umbrella sampling procedure was applied in order to obtain the free energy profile corresponding to the initial state of the O-PD transition in CD44 (wild-type). Additionally, the analogical transition was investigated in the Y161A mutant of CD44, as the experimental measurements suggest that such modification leads to the significant discrimination of the O state occurence [3]. The results include the identification of the residues playing a crucial role in the O-PD transition and the types of interactions relevant for maintaining the O state.

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C-9

Non-imidazole Histamine H_3-Antagonists - Synthesis and Preliminary Pharmacological Investigation of New 1-(substituted)methyl-4-hydroxypiperidine Derivatives.

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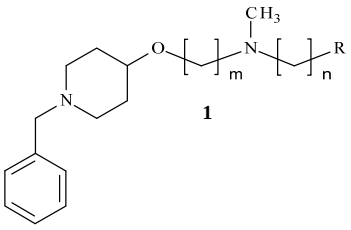
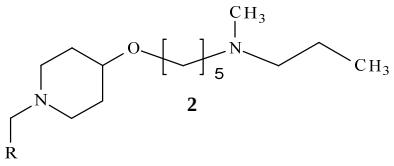
Background
The cloning of the H_3 receptor has provided fresh impetus to the development of drug-like ligands of this receptor. During the years following number of H_3 antagonists belonging to different chemical classes, subsequently divided between classical imidazole-based and non-imidazole series have been described; the imidazole derivatives were considered to be less attractive for pharmacokinetic as well as for toxicological reasons. The successful replacement of the imidazole moiety with piperidine and other basic tertiary amines was demonstrated with a variety of analogs. As might have been expected the effect of replacement of the imidazole by basic tertiary amines affected H_3 inhibitor potency to different degree, depending on the chemical series. Based on literature data it may be concluded that compounds carrying on piperidine ring are more likely to be successful in ethereal analogs than in the other series.

Methods
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-benzyl-4-hydroxypiperidines **1** and 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)- pentyloxy]piperidines **2** as H_3 histamine receptor antagonists.





Results and Conclusion
The presented 1-benzyl-4-hydroxypiperidine **1** and 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)-pentyloxy]piperidine **2**derivatives all possess, moderate to pronounced H_3-receptor antagonist activity. All compounds of 1-benzyl-4-hydroxypiperidine series [1], showed weak to moderate H_3-receptor antagonist potency. The highest potency for these homologous series is seen in the compound with the *N*-methyl-*N*-propylaminopentyloxy substituent. This derivative was used as a new lead compound for further structural modification of phenyl moiety. Therefore, a series of 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)pentyloxy]piperidines was synthesized and pharmacological evaluated. The benzo ring was replaced by chromanyl, chromanonyl, benzofuranyl, indenyl and napthanyl moiety. For this series the highest affinity possessed derivatives of 1-substitutedmetyl-4-[5-(N-methyl-N-propylamino)pentyloxy]piperidine carrying on benzofuranyl subsituents.

This work was supported by the Polish State Committee for Scientific Research, Grant No. 502-13-410

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C-10

Synthesis and Preliminary Pharmacological Investigation of New 1-Phenoxyalkyl-4-[(*N*,*N*-disubstitutedamino)alkyl]piperazines and N-Substituted-N-[ω-(ω-phenoxy-alkylpiperazin-1-yl)alkyl]guanidines as Non-Imidazole Histamine H_3-Antagonists.

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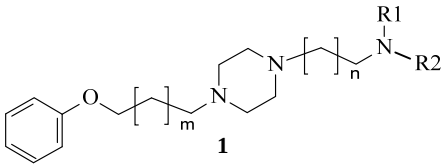
Background

The initial development of potent H_3 receptor antagonists in the early 1980s focused on extensive modification of the natural ligand histamine, and resulted in a series of very potent imidazole-containing H_3 antagonists. In most cases, antagonists of H_3 histamine receptor carrying on an imidazole ring have been proved to be undevelopable as therapeutic agents for humans due to a number of liabilities, including cytochrome P450 inhibition, low affinity for human compared with rat H_3 receptors, lack of selectivity, or suboptimal brain penetration. For this reason, efforts have been directed toward the discovery of H_3 antagonists without an imidazole moiety. Since 1994, when the marine natural product aplysamine-1 was patented as a weak H_3 histamine receptor antagonist (gpH_3R pA_2=2.4 μM), diamine-based ligands, containing the characteristic aminopropoxyphenyl structural pharmacophor, have become an important chemical class of H_3 histamine receptor antagonists. This motif has been repeated in a number of different series of compounds from several laboratories.

One of the first known histamine H_3 receptor antagonists was a guanidine derivative - impromidine which was preliminary proved to be a potent agonist at H_2 receptor, only a small number of compounds containing this structure element are described. However, it has appeared that the guanidine derivatives, both into imidazole and non-imidazole class, are potent and selective histamine H_3 receptor antagonists.

Methods

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-phenoxyalkyl-4-[(*N*,*N*-disubstitutedamino)alkyl]piperazines **1** and N-(4-phenoxyalkyl- piperazin-1-yl)alkylguanidines **2.** as H_3 histamine receptor antagonists.



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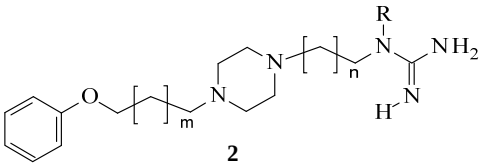

2

Results and Conclusion

The presented 1-phenoxyalkyl-4-[(*N*,*N*-disubstitutedamino)alkyl]piperazine and N-substituted-N-[ω-(ω-phenoxyalkylpiperazin-1-yl)alkyl]guanidine derivatives all possess, moderate to pronounced H_3-receptor antagonist activity.

All compounds of 1-phenoxyalkyl-4-[(*N*,*N*-disubstitutedamino)alkyl]piperazine series [1], independently on the type of substituent at the end of -*N*- group, showed weak to moderate H_3-receptor antagonist potency. In the case of N-substituted-N-[ω-(ω-phenoxyalkylpiperazin-1-yl)alkyl]guanidine series [2] the highest affinity possessed derivatives of N-4-substitutedbenzyl-N-[4-(7-phenoxyheptylpiperazin-1-yl)butyl]guanidine carrying on strong electron withdrawing subsituents at position 4 in the benzo ring.

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C-11

Complexes of Glycyrrhizic Acid and its Salts with Biologically Active Compounds. Their Structures and Potential Applications in Drug Form Technology.

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Medicinal plants are important sources of pharmacologically active substances. Glycyrrhizic acid (GA) [Fig. 1] is a principal, biologically active component of licorice herb (Glycyrrhiza genus) occurring in its natural form as a mixture of potassium, calcium and magnesium salts[1]. It has been used for medicinal purposes since ancient times. The medical studies in humans, animals and *in vitro* experiments revealed a remarkable therapeutic properties of glycyrrhizic acid and its chemically modified derivatives[2,3]. Recently, the interest of this natural compound increased because of its ability to form water-soluble complexes with hydrophobic molecules[4,5]. The effect of GA on the chemical stability, aqueous solubility and bioavailability of drugs is of constant search as well as the possibility of using this compound for drug delivery[6].

Better understanding of divers properties of glycyrrhizic acid would require a better knowledge of its molecular and supramolecular structural features but the structural information on GA is missing in the scientific literature. To get insight into molecular structure and supramolecular organization of this important natural product an effort was made to obtain glycyrrhizic acid and its salts in the form of single crystals to carry out X-ray structural analyses. These studies revealed a specific aggregation mode, similar for GA and its salts where, analogously to other drug carriers, the hydrophilic and hydrophobic parts of the molecules are separated. The results also indicate a plausible reason for similarity in some of physico-chemical properties between GA and its salts. Moreover, the crystal structures revealed a system of intersecting channels in the GA host matrix accommodating cations and divers solvent molecules. As shown by the structures of the GA salt complexes with biologically active compounds, the solvent molecules in the channels can be replaced by small drug molecules.

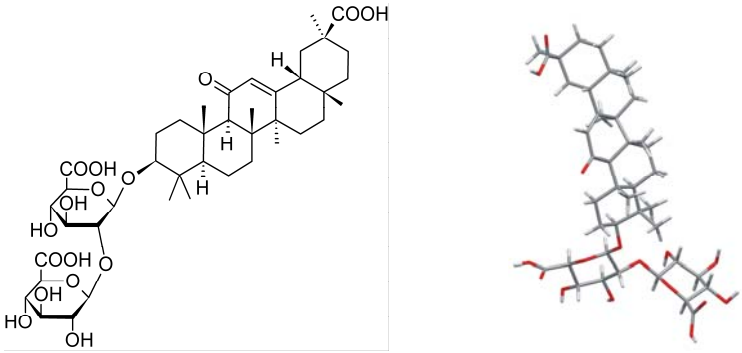

Fig. 1. The GA molecule consists of a lipophilic triterpenoid aglycon conjugated with a hydrophilic disaccharide containing β(1→2) linked D-glucuronic acids.

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C-12

Combined Therapy of Monoclonal Antibody Against MUC-1 and Berenil Complexes of Platinum(II) in Breast Cancer Cell Line MCF-7.

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Mucins are glycoproteins, which are synthesized by the epithelial and glandular cells of the gastrointestinal tract, respiratory tract and in the reproductive system. One of the mucin is MUC1- a highly glycosylated type I transmebrane glycoprotein that is aberrantly over-expressed on the cell surface of multiple carcinomas of epithelial origin, particularly in breast cancer cells. Differences between MUC1 found in normal cells and in cancer's cells are both qualitative and quantitative.
MUC1 is expressed only on the top surface of the normal epithelial cells, whereas in cancer cells, this polarity is lost, which causes the appearance of MUC1 on the whole surface of the cell membrane. This may affect the mutual adhesion between adjacent cells, and between cells and extracellular matrix proteins. This facilitates tumor cell migration and metastasis. The increased expression of MUC1 in breast cancer cells makes MUC1 an attractive therapeutic target.
The aim of this study was to evaluate the effect of a monoclonal antibody against MUC1 on the cytotoxicity of berenil complexes of platinum(II) in breast cancer cell line (MCF-7). Cell viability was performed according to the method of Carmichael et al. [1] using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The proliferative response (DNA synthesis) was assessed by [3H]thymidine incorporation. Collagen synthesis assay was measured by incorporation of a radioactive precursor 5-[3H]-proline into proteins.
The results allowed to conclude that monotherapy with anti-MUC1 monoclonal antibody or berenil complexes of platinum(II) resulted in higher cytotoxicity of breast cancer cells (MCF-7) compared to the reference compound, cisplatin. However, combination therapy involving anti-MUC1 monoclonal antibody and berenil complexes of platinum (II) caused a higher decrease in survival of cancer cells, inhibited biosynthesis DNA and collagen compared to monotherapy. These studies strongly suggest that the antibody used together with chemotherapeutic agent had a better cytotoxic effect against cancer cells and reduced the dosage of the drug. It might be a novel strategy for targeting human breast cancers.

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C-13

Structure and Biological Activity of Two Ruthenium(II) Compounds with Flavanone-Based Ligands.

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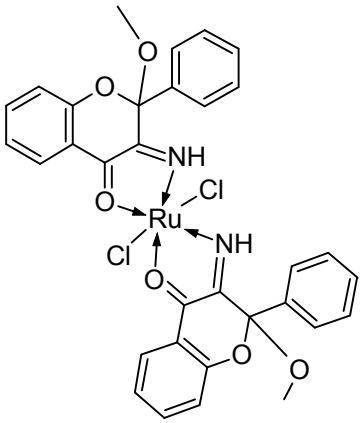
Ruthenium coordination compounds might in future become an alternative to platinum-based anticancer drugs. Thus, there is an increasing interest in chemistry and biological activity of ruthenium complexes.
Two complexes of ruthenium(II) have been synthesized, using 3-aminoflavone as a ligand and hydrated ruthenium(III) chloride as a source of the metal ion. During the synthesis redox reactions have occurred, giving the final products: *cis*-dichloridobis(3-imino-2-methoxyflavanone) ruthenium(II) or Ru-134 (A) and *cis*-dichloridobis(3-imino-2-ethoxyflavanone) ruthenium(II) or Ru-138 (b), depending of the reaction milieu[1]. Both compounds display good antiproliferative and proapoptotic properties, when compared with cisplatin in vitro, towards cancer cell lines. Moreover they overcome resistance to cisplatin in a resistant cancer cell line [1,2].



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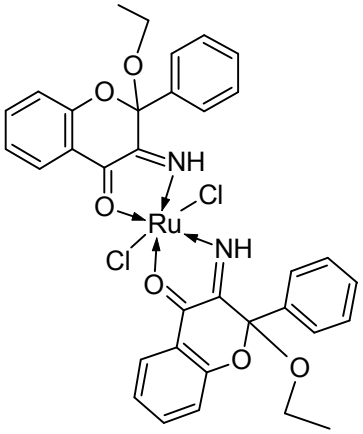

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This work was supported partly by the Polish Ministry of Science and Higher Education grant No. 1823/B/P01/2008/35, the Medical University of Lodz grant statute 503-3016-2 and the UE European Social Funds grant No. 505-07-050/WFARM/RNSD/09.

C-14

Biological Studies of Novel Derivatives of Isoquinolines Alkaloids in MCF-7 and MDA-MB-231 Human Breast Cancer Cells.

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Bioactive alkaloids occupy an important position in applied chemistry and play an indispensable role in medicinal chemistry. Amongst them, isoquinoline alkaloids and their derivatives represent an important class of molecules for their broad range of clinical and pharmacological utility. In view of their extensive occurrence in various plant species and significantly low toxicities, prospective development and use of these alkaloids as effective anticancer agents are matters of great current interest.
The aim of this study was search and evaluation of mechanism of anticancer activity of novel derivatives of isoquinolines alkaloids (RC-6, RC-27, GD-18, RC-108, RC-77, RC-71, RC-105, RC-390, RC-173). A number of novel derivatives of isoquinolines alkaloid were synthesized and examined for cytotoxicity in MCF-7 and MDA-MB-231 human breast cancer cell cultures.
The viability of MCF-7 and MDA-MB-231 breast cancer cells was measured by the method of Carmichael using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). We have found that novel derivatives of isoquinolines alkaloids decreased the number of viable cells in both estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Novel derivatives of isoquinolines alkaloids in MCF-7 and MDA-MB-231 cells proved to be more potent than etoposide. The results of this study show that the analyzed compounds exert significant inhibitory effects on the viability of breast cancer cells. The most active derivative was compound RC-77. After 24 hours of incubation in MCF-7 and MDA-MB-231 breast cancer cells IC_{59} value was 30μM and 23μM for RC-77 and above 100 μM for etoposide, respectively.
Evaluation of inhibition of [^{3}H]thymidine incorporation into DNA in both MCF-7 and MDA-MB-231 breast cancer cells demonstrated that these compounds were more active than etoposide. The concentrations of etoposide needed to inhibit [^{3}H]thymidine incorporation into DNA by 50% (IC_{50}) were significantly higher than novel derivatives of isoquinolines alkaloids. The higher activity showed compounds RC-77 and RC-108 in MCF-7 cells and compounds RC-27 and RC-77 in MDA-MB-231 cells. The profiles of DNA synthesis obtained were similar between MCF-7 and MDA-MB-231. All of the tested compounds showed concentration dependent activity, yet with different potency. Treatment of the cells revealed that these compounds inhibited DNA synthesis and irreversibly inhibited the proliferative activity of the cells.
Evaluation of the cytotoxicity of a novel derivatives of alkaloids isoquinolines employing a MTT assay and inhibition of [3H]thymidine incorporation into DNA in MCF-7 and MDA-MB-231 human breast cancer cells demonstrated that the novel derivatives of isoquinolines alkaloids were more potent anti-proliferative agent than etoposide.
All these data emphasize the potential usefulness of these derivatives of isoquinolines alkaloids as anticancer agents. Therefore, some of the isoquinoline alkaloids indicating the high activity on both assays may be potentially valuable cancer chemopreventive agents.

ORAL PRESENTATIONS
POSTERS


BRUKER


Spectro-Lab


JASCO


POLYGEN

PP-1

Synthesis of the Novel 2,4-Disubstituted 1,3-Thiazoles as Potential Antifungal Agents.

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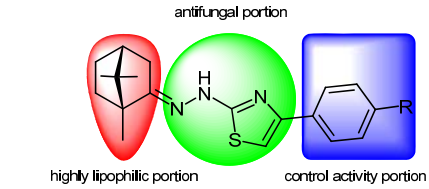
In the recent years, azoles and their derivatives have been widely studied in medicinal chemistry due to their various biological activities such as anti-bacterial [1], anti-fungal [2], anti-tumor [3], anti-HIV [4] and human monoamine oxidase inhibitors [5]. Many biologically active compounds, such as Fluconazole, Bleomycine, Tiazofurin or Ritonavir are examples of azole moiety bearing drugs. This class of drugs can inhibit the fungal cytochrome P450 enzyme 14α-demethylase, preventing the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols [6].
The *in vitro* activity of terpenes has been evaluated against *C. albicans* biofilms and they appear to be promising candidates to either treat or reduce the incidence of device-associated infections [7]. The menthyl derived compounds were the most active, inhibiting biofilm formation by several strains of *S. aureus* with IC_{50} values in the mid to low micromolar range [8].
These results prompted us to investigate several novel highly lipophilic camphor based 2,4-disubstituted 1,3-thiazoles with different substituents in the *para*-position in phenyl ring as potential antifungal agents which through increased lipophilicity could furnish better therapeutic activity. These compounds are evaluated for their antifungal activity against *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and fluconazole-resistant *C. albicans* strains.



R = H, Br, F, OCH_3, CN, NO_2, $NHCOCH_3$, $NHSO_2CH_3$, $NHCOCH_2Cl$, Ph;

The structures of synthesized compounds were proved by spectroscopic (NMR and MS) analyses. The results of antifungal screening reveal that obtained compounds exhibit strong activity against thirty clinical isolates of *Candida* spp.

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PP-2

Development of a 3D Cell Model to Study Inflammation and Photosensitization Processes of the Melanoma.

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Endothelial cells are the first cells that participate to the stroma establishment in tumor development [1]. They take part in angiogenic process, which is the fundamental step in transition of tumors from a dormant state to a malignant one, in two manners. Firstly, vicinal endothelial cells are recruited locally. Secondly, the distant recruitment of bone marrow endothelial precursors occurs [2]. Moreover, recent reports indicate the role of various cells in tumor microenvironment in promotion of these angiogenic processes by secretion of specific chemokines [1].
Melanoma, as highly metastatic and extremely dangerous skin cancer [3], needs to be properly investigated, in terms of tumor angiogenesis. For that reason we have built a 3D cellular model, in which tumor recruitment of endothelial cells and their angiogenesis can be studied *in vitro* using video microscopy. We used model of mouse melanoma (B16F10-GFP-luc cells) grown as spheroids to mimic the *in vivo* tumor and both precursor endothelial cells from mouse embryo (MAgEC - Murine Aorta-gonad-mesonephros Endothelial Cells) and mature endothelial cells from mouse lungs (Lung FVB) to mimic *in vivo* angiogenic processes. Optimized matrix consisting of collagen, methyl cellulose and Matrigel™ was used as migration environment.
Firstly, we have validated this model by demonstration of active recruitment of endothelial cells by B16F10-GFP-luc spheroids. Then the influence of hypoxia, UV irradiation and photosensitization were investigated. An optimized mixture of UVA and UVB was utilized for irradiation. Photosensitization was performed using 5-aminolevulinic acid, which stimulated production of endogenous photosensitizer – protoporphirin IX. We have shown diminishing effect of UV and photosensitization treatments on B16F10-GFP-luc spheroids to recruit endothelial cells. However, proangiogenic influence of hypoxia was recorded, as well as, upregulation of endothelial progenitor cell migration. This effect was significant after photosensitization, but diminished by UV irradiation.
Moreover, production of chemokines was measured in co-cultures of melanoma and endothelial cells to study cell-cell communication. A production of SDF-1, TARC and CX3CL1 proteins was detected and their upregulation by both UV irradiation and photosensitization was shown. Hypoxia also increased their production, especially for photosensitized cells. SDF-1 and TARC production was shown to occur only in presence of endothelial progenitor cells.
In conclusion, the 3 dimensional cellular model using spheroids can be used as reliable model to study tumor biology. In this study we were able to confirm an important role of endothelial progenitor cells in tumor angiogenesis. We have also demonstrated, that in terms of skin cancer, such as melanoma, physiologically occurring hypoxia, and possible UV irradiation and photosensitization have significant influence on this process. Their consideration in development of new approaches to melanoma treatment is recommended.

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PP-3

Thiosemicarbazones as anti-cancer iron chelators

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Iron chelators are commonly used to treat diseases connected with altered iron metabolism, *e.g.* β-thalassaemia major. However, considering the marked anti-proliferative activity of this group of agents, recent investigations have focused on the anti-cancer efficacy of iron chelators. In fact, there are many reports of the anti-proliferative activity of desferrioxamine (DFO), Triapine® and other ligands based on the (thio)urea moiety.

The cytotoxic mechanisms of chelators include: **(1)** the inhibition of cellular iron uptake from the iron-binding protein, transferrin (Tf); **(2)** mobilization of iron from cells;' **(3)** the inhibition of the iron-containing enzyme involved in the rate-limiting step of DNA synthesis, ribonucleotide reductase; and **(4)** the formation of redox-active iron complexes that generate reactive oxygen species (ROS).The latter mechanism is significant, especially in the context of recent reports demonstrating the role of ROS generation in increasing the anti-proliferative activity of chelators against tumor cells.

All compounds were synthesized in microwave reactor (CEM-DISCOVERY®) and the purity of final products was determinated by HPLC. The structures of final compounds were confirmed by NMR spectroscopy and HRMS spectroscopy. The cytotoxicity tests were performed using MTS assay with DFO and Dp44mT as the references. Iron (^{59}Fe) mobilization and iron uptake experiments were carried out using neuroepithelioma cell lines SK-N-MC and HCT116 cells were obtained from the American Type Culture Collection (ATCC; Vanassas, VA, USA).

In this study, a series of novel thiosemicarbazones were designed to contain a quinoline scaffold in order to investigate their effect on iron uptake, iron mobilization and anti-proliferative activity against tumor cell lines. Several QT analogs demonstrated marked chelation efficiency in terms of mobilizing cellular iron and preventing iron uptake from Tf. The anti-proliferative effect of this series of agents showed limited correlation with their ability to promote cellular iron efflux or inhibit iron uptake. Thus, their iron chelating ability only partially explains their anti-proliferative efficacy and suggests that other properties such as their redox activity may be important. The anti-proliferative activity and iron chelation efficacy of several of these agents indicates that further investigation of this class of thiosemicarbazones is certainly worthwhile.

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PP-4

Synthesis, Structure and Pharmacological Evaluation of Selected Tryptamine Thiourea Derivatives.

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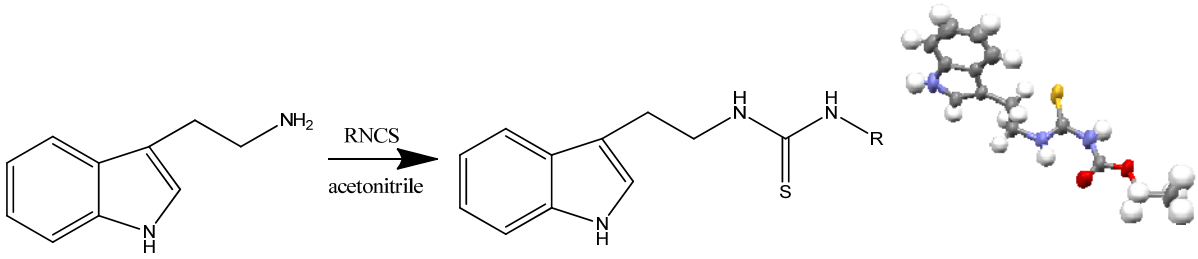
Several thiourea derivatives of tryptamine were synthesized (Scheme 1) and four selected compounds investigated for CNS activity. Structure evaluation was made for obtained crystals of synthesized derivatives.



Scheme 1. General synthesis and example ellipsoid (**TR20**) of obtained crystal structures.

Compounds 1-(2-(1H-indol-3-yl)ethyl)-3-(4-fluorophenyl)thiourea (**TR17**), 1-(2-(1H-indol-3-yl)ethyl)-3-benzylthiourea (**TR10**), 1-(2-(1H-indol-3-yl)ethyl)-3-ethylthiourea (**TR22**) and 1-(2-(1H-indol-3-yl)ethyl)-3-ethoxycarbonylthiourea (**TR20**) were tested for spontaneous activity, amphetamine-induced hyperactivity, changes in body temperature, locomotor , antinociceptive and anticonvulsant activity.
From four analyzed compounds, the 1-(2-(1H-indol-3-yl)ethyl)-3-(4-fluorophenyl)thiourea statistically significantly change locomotor activity of animals, moreover this action was observed when doses 0.05 ED_{50} and 0.025 ED_{50} were administrated. The (**TR10**) and (**TR20**) showed impressive decrease of spontaneous activity but without any statistical importance. Amphetamine-induced hyperactivity was not noticed for all investigated tryptamine derivatives, what is more none of them caused motor coordination disorders.
The results presented in this work, based on literature data, indicate the possible involvement of the serotonergic system in the activity of the thiourea series compounds. 5-HT_2 and/or 5-HT_{1A} receptors are also probably involved, which seems to be confirmed by the inhibition of the “head twitch” response by (**TR10**).

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PP-5

X-ray Crystallographic Structures as a Source of Alignment in 3D-QSAR Studies of Positive Allosteric Modulators of AMPA Receptor.

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Background

It is generally accepted that 3D-QSAR models are alignment sensitive and selection of appropriate overlay function is a "bottleneck" of these methods [1-2]. The basic assumption of pharmacophore theory that compounds with the same scaffold share similar orientation in the protein binding site is not always fulfilled. Structurally similar compounds may assume different modes of binding. The general aim of presented study is to compare predictability of CoMFA models generated for wide range of alignment algorithms and alignment derived from crystallographic studies.

Materials and methods

The ligand binding domain of the AMPA receptor was co-crystallized with almost 40 different allosteric modulators and their high-resolution X-ray structures [3] give an opportunity to sample ligand orientations. Current study collects all of unique ligand poses of congeneric ligands co-crystallized with the binding domain of the AMPAr and aligns them using a protein scaffold. In our consideration of the problem we applied CoMFA modelling to the system known from the fact that structurally similar ligands do not share the same mode of binding (Figure 1).

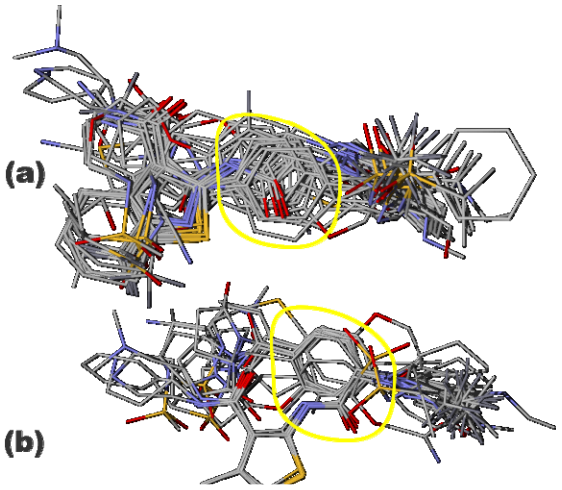

Figure 1. Compounds overlaid with two methods (a) crystal structures receptor alignment and (b) structural alignment.

The benzene ring system (yellow line selection) of the molecules is the main scaffold for alignment in structural alignment while the same ring in receptor alignment shows slight shift of ligands when adapting to the ligand binding domain.

Results

The results show that groups based on receptor alignment have better interpolation to QSAR equation. They more relevantly predict the activity of compounds and have lower prediction errors that the models based on others types of alignment. Although the 3D-QSAR methods are mainly employed when crystal structure is not known, current study underlines that it is the approximation method and the selection of the inappropriate alignment can lead to false conclusions.

Acknowledgements

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PP-6

Characterization of Acetylcholinesterase Interaction with Inhibitors.

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Acetylcholinesterase (AChE, EC 3.1.1.7) plays an important role in neurotransmission. Enzyme rapidly catalyzes the transformation of the active neurotransmitter acetylcholine into the inactive compounds choline and acetic acid. This terminates impulse transmission at the cholinergic synapses. Therefore, controlled and specific inhibition of AChE has therapeutic uses, including Alzheimer's disease (AD) treatment. AD is the most common form of dementia among the elderly and has been estimated to affect 11% of the population between ages 80 - 85 and 24% of the population over age 85 [1].Finally the disease is the fourth leading cause of death in people over 65 years old in western industrialized countries [2]. Most of the available drugs for the management of AD are AChE inhibitors [3]. Therefore, characterization of inhibitors binding to this enzymatic protein and further development of new compounds that will modulate AChA are still an important issue.
In this study we demonstrate the application of isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) to characterize the acetylcholine-inhibitor interactions. Those label-free measurement techniques allow to directly determine thermodynamic and kinetic parameters of different ligands binding to target protein. Obtained results indicate that those methods can be useful in rational designing and screening of new AChE inhibitors. Recent research suggests that SPR can also give information about shifts in conformation of the immobilized AChE caused by ligand binding [4].

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Acknowledgement:
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PP-7

Modelling the Interactions Between Glycogen Synthase Kinase and Its Inhibitors.

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Glycogen Synthase Kinase is a prominent member of the family of protein kinases, broadly investigated and described. It was originally described in the early 1980's as one of five enzymes responsible for the process of phosphorylation of Glycogen Synthase, the rate limiting enzyme in glucose methabolism. In the middle of 1990's the interest in the enzyme substantially increased due to its role in the regulation of the homeosthasis of the organism. GSK-3 is a pluripotent, multitasking protein kinase that serves as a nexus, integrating and suppressing the majority of existing intracellular signaling trails.

The aim of presented work was to explore the molecular interactions between GSK-3β1 and a carefully selected group of its pharmacological inhibitors with different affinity to the enzyme (values micro- and nanomolar). The investigation was conducted by method of molecular docking using GOLD software.

In order to perform the research it was necessary to compile a library of GSK-3β ligands as well as to select an appropriate crystal structure of the protein. The 3D-structures of examined inhibitors were generated with the aid of the latest version of the programme Spartan (Spartan'10). After being drawn, all structures were submit the optimalisation of energy and geometry by both semi-empirical method AM1 and the ab initio method 3-21G. For some of the egzamined molecules the operation of calculating the Density Functionnals was performed (using the B3LYP function).

The generated structures of GSK-3β inhibitors were finally docked into the extracted binding pocket of the crystal encoded 1Q5K obtained from the Protein Data Bank.

Taking into consideration the outcome of the performed analysys allowed indicate the essential residues making up the binding pocket of GSK-3β1`as well as explain the molecular mechanism of interaction: ligand-GSK-3. All in all, the conducted investigation provides useful tips and opens up new directions in designing potent, ATP-competetive inhibitors of GSK-3.

PP-8

Synthesis and Antifungal Activity of New Quinoline Derivatives.

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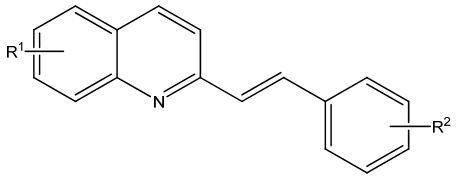
Over the past several decades, significant changes in the epidemiology of fungal infections can be observed. The number and diversity of new threatening fungal infections increased dramatically during this time [1]. There are some reasons of this phenomenon such as growing population of immunocompromised patients and appearing of new, drug resistant fungal strains. Fungal infections caused by common and unusual fungi pathogens are occurring with increasing frequency and result in both significant morbidity and mortality. The majority of these infections are caused by *Candida* spp., *Aspergillus fumigatus* and *Cryptococcus neoformans*. Less common (but emerging) pathogens include *Zygomycetes*, *Fusarium* spp, *Trichosporon beigelii*, *Blastoschizomyces*, *Scedosporium*, *Acremonium* and some dematiaceous fungi [2]. Thus, searching for novel drugs remains to be one of the major challenge for modern science. In spite of, broad arsenal of drugs we have still an urgent need for new, more effective antifungal drugs with less side effects [3].
Quinoline family compounds possess a wide spectrum of biological activities such as antifungal, antineoplastic and herbicidal activity [4]. For this reason quinoline moiety may be regarded as privileged structure - especially valuable for drug design [5]. Simple derivatives of quinoline have a long history as antifungal agents and some of them are still in use [3]. In our approach styrylquinolines were used for their similarity with allylamines, known antifungals. We are exploring the styrylquinoline derivatives as possessing strong antifungal activity, especially derivatives containing 5,7-dichloro-8-hydroxyquinoline were found interesting in our former research.



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PP-9

Influence of *ortho*-Substituents of Phenyl in Aroyldithiocarbazoic Acids on Their Conformation.

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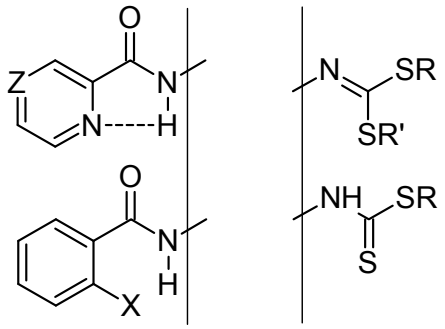
The increasing resistance of *Mycobacterium tuberculosis* to existing agents and the resulting spread of the pathogen, in both developed and developing countries, makes the search for new tuberculostatics an important issue. First aroyldithiocarbazoic acids showing tuberculostatic activity were obtained by professor Foks and coworkers from Department of Organic Chemistry, Medical University of Gdansk [1,2]. Crystal structures of them were studied in our laboratory in hope to reveal the relationship between their molecular structures and activities. Our working hypothesis was that general planarity of the molecules could be a prerequisite for activity [3,4]. The planarity is maintained by conjugations and intramolecular hydrogen bonds, including that between aromatic N atom as an acceptor of 2-pyridine or 2-pyrazine ring and the neighboring NH group (Scheme). Next a study showed that 3,4-dichlorobenzoyl derivatives, in which the aryl cannot form an intramolecular hydrogen bond, are also nearly planar [5]. Surprisingly, despite the twist of the aryl, these compounds also showed tuberculostatic activity.
Comparison of the studied structures with those in the Cambridge Structure Database [6] showed an interesting feature, i.e. a distinct relationship between the *ortho*-substituent type and the twist of the aromatic ring. The sets of compounds suitable for statistical analysis were derivatives with F, Cl, CH_3, OH, OCH_3 in *ortho* position. The biggest twist (average of 52°) of the aromatic ring is present in compounds containing chlorine, due to its large steric hindrance, and the smallest in compounds comprising hydroxyl group (of approximately 6-7°). An intramolecular hydrogen bond with the hydroxyl group as an acceptor resulted in near-planarity of the latter group.



X = H, F, Cl, CH_3, OH, OCH_3

Z = CH, N

R,R' = CH_3, C_2H_5, Bz, …

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PP-10

5- Arylidene Derivatives of Hydantoin – New Hope of Inhibiting Bacterial Multidrug Resistance.

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Today we assist to the re-emergence of infectious diseases involved in more than 15 million deaths each year. One of the reasons for this situation is the increasing resistance of pathogenic bacteria to available antibiotics. The multidrug resistance (MDR) seriously limits treatment of various bacterial diseases (1). Microbial efflux pumps play a key role in MDR strains (2). One of the strategy to combat MDR is search for new chemical compounds that are able to inhibit protein pump system responsible for drug efflux, consequently increase antibiotic concentration in a target cell (3). In this context, a series of new arylidenehydantoin derivatives (Fig. 1) was synthesized and evaluated on their efflux pumps inhibition (EPIs) properties in microbiological assays in strains of *E. aerogenes* with different expression of AcrAB-TolC pump (ATCC and CM64).

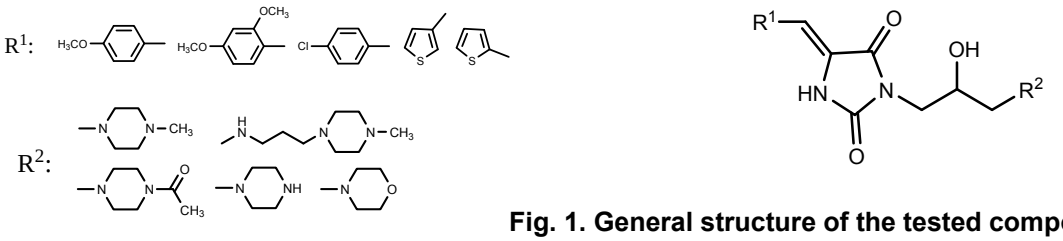

Fig. 1. General structure of the tested compounds.

The series of 9 new derivatives of 5-arylidenehydantoin was obtained within chemical synthesis including Knoevenagel condensation, Mitsunobu reaction, melting with amines, N-deprotection and conversion into hydrochloride forms. New compounds were tested in two types of microbiological assays: (1) tests on direct antibacterial activity, (2) tests of the compounds influence on MIC value of antibiotics. For the most active compounds the direct antibacterial activity, the influence on MIC of antibiotics using different strains EA294 and EA289 as well as their cooperation with the antibiotics (isobolograms) were determined. The real-time efflux test to identify the compounds that act on efflux in EA289 strain by blocking the expelling of fluorescent dye was carried out, too. One compound with p-chlorobenzylidene substituent at position 5 and free piperazine substituent at position 3 displayed ability to increase efficacy of antibiotics in strains over-producing AcrAB-TolC. This compound cooperated with chloramphenicol, doxycycline and nalidixic acid in synergistic way, but additive cooperation was observed in the case of erythromycin. No activity was observed for the tested compounds in the real- time efflux test.

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PP-11

Iron Chelators – Antiproliferative Activity and Application in Photodynamic Therapy.

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Photodynamic therapy (PDT) is now an attractive strategy for treating various ailments, including cancer lesions. From the medical point of view this method of treatment is low invasive and high selectivity relative to normal cells. The essence of PDT is the production of singlet oxygen and free radicals in the reaction of photosensitizer with the light of specific wavelength. In the presence of oxygen two types of reactions are possible, both leading to reactive oxygen species (ROS). These trigger chain reactions in the cell, causing various types of damage and finally the destruction of the tumor [1].

Various photosensitizers are available for use in photodynamic therapy. Most important are prophyrines and chlorines (Photofrin, Foscan), and their precursors as 5-aminolevulinic acid (Levulan). ALA-PDT therapy involves the administration of 5-aminolevulinic acid or its prodrugs, which is metabolized to protoporphyrin IX (PpIX) endogenic photosensitizer. There is marked difference in the accumulation of PpIX in normal and malignant cells, caused by different activities of enzymes involved in the formation of PpIX (porphobilinogen deaminase) and heme (ferrochelatase) [2]. Due to poor tissue penetration, ALA-PDT therapy is mainly used in the treatment of superficial lesions such as actinic keratosis or basal cell carcinoma [3]. Nevertheless the production of PpIX in the tissue is often insufficient to achieve therapeutic concentrations. Low efficacy of the treatment has led to the idea of inclusion of iron chelators to the treatment. The presence of iron chelators allows to increase the intensity of PpIX accumulation in the tumor, by blocking the last steps of heme formation. In our approach novel thiosemicarbazones as highly active iron chelators are combined with ALA-PDT experiments in vitro.

Studied compounds are tridentate metal chelators with high Fe mobilization efficacy and high antiproliferative activity in cancer cells [4]. Their possible mechanism of action includes, generation of reactive oxygen species (ROS) or depleting iron from rapidly proliferating cancer cells [5]. This multi-targeted mechanism of action makes them an especially attractive material for the study of possible application in anticancer therapy.

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POSTERS

Synthesis of Oligopeptides Using an Automated Microwave Synthesizer.

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Searching for biologically active substances led to increased interest in synthetic peptides which are currently used as drugs, cosmetics and industrial raw materials for non-medical use. The main application of new peptide drugs includes treating diabetes, cancer, infertility in women, and comprise anticoagulant or antibacterial, antiviral and antifungal activity. Oligopeptides were obtained using an automated microwave peptide synthesizer. The last step in the synthesis consisted of manual cleavage of the peptide from the resin and removal of protective groups of amino acid side chains.
The resulting compounds were analyzed using IR, MS, 1H NMR i 13C NMR.
The project was developed using the equipment purchased within the Project “The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases” within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

P-2

Determination of Lipophilicity of γ-Butyrolactone Derivatives with Anticonvulsant and Analgesic Activity Using Micellar Electrokinetic Chromatography.

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Among the physicochemical properties, the lipophilicity and acid dissociation constant are the most important ones [1]. Lipophilicity determines the partitioning of a compound between two phases: organic and inorganic, and is usually correlated with its pharmacokinetic properties. To measure lipophilicity, various methods are used. Shake-flask and chromatography are the most common methods for lipophilicity determination of compounds [2]. There are also novel methods, based on capillary electrophoresis such as micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC)[3].

The lipophilicity of 31 γ-butyrolactone derivatives was determined by micellar electrokinetic chromatography with P/ACE MDQ Capillary Electrophoresis System (Beckman). Analyses were carried out in borate-phosphate buffer (pH 7.0, 50 mM + 25 mM) with sodium dodecyl sulfate (50 mM). Fused silica capillary (total length 49 cm, effective 39 cm, i.d. 50 μm, e.d. 375 μm) was used. The separations were performed at 25 °C by application of 10 kV with detection at 220 nm (DAD). Methanol and Sudan III were used as markers for electroosmotic flow and micelles.

Calibration curve was prepared for 10 reference drugs and it enabled to calculate partition coefficient (log P) for novel compounds.

The relationship between experimentally obtained log P coefficients and chemical structures of derivatives was analyzed. Results of MEKC analyses were compared with R_{M0} coefficients from reversed phase thin layer chromatography (RP-TLC) and coefficients calculated by computer programs: Marvin and ChemOffice. The best correlation between MEKC results, RP-TLC and ChemOffice was received.

The relationships between lipophilicity, anticonvulsant activity and toxicity were also analyzed. The statistically significant differences in the average of log P coefficients for group of active and inactive compounds in the MES anticonvulsant test were found.

Proposed method was successfully applied to differentiate and determine the lipophilicity of γ-butyrolactone derivatives. This research showed that MEKC could be applied for analysis of the lipophilic properties of potential drugs.

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Acknowledgements:
The research was supported by Polish Ministry of Science and Higher Education (UJ CM grant No. K/DSC/000082).

Synthesis, alpha 1-Adrenoceptor Activity and Anty/Mutagenicity of Novel Arylpiperazine Derivatives of Benzimidazole and Pyridone.

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A series of different 1,4-substituted piperazine derivatives of benzimidazole and pyridone containing phenyl-, 2-fluorophenyl- or 4-fluorophenylpiperazine moiety were synthesized. These compounds were prepared by condensation of 2-bromobenzimidazole or 6-bromo-2-pyridone with various epoxides [1].
The newly synthesized compounds were evaluated for their *in vitro* affinity to α_1-adrenoceptors in rat cerebral cortex by radioligand binding assays using [^{3}H]prazosin as specific radioligand. All compounds displayed lower affinity for α_1-AR when compared to prazosin, with K_i values 40-700 nM. Furthermore anty/mutagenic properties were assessed for the most active compounds (K_i<150) by using the *Vibrio harveyi* assay [2]. The preliminary results showed that tested compounds did not have mutagenic activity. All compounds demonstrated strong antimutagenic activity (inhibition higher than 40%).

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Acknowledgements
The study was supported by the National Science Centre (Grant No. DEC-2011/01/N/NZ4/01125) and the Medical College of Jagiellonian University (Grant No. K/DSC/000081).
The authors would like to thank prof. G. Węgrzyn from Department of Molecular Biology, University of Gdańsk for strains of *V. harveyi*.

P-4

The Evaluation of Lipophilicity of Benzimidazole and Pyridone Derivatives.

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Lipophilicity is an essential property of molecules whose roles in biological systems are numerous and essential. The hydrophobic interactions of drugs with their receptors, the pharmacokinetic behaviour of drug molecules, toxicological properties and pharmaceutical aspects like solubility are examples of a growing number of topics in which lipophilicity plays an important role [1].
The aim of our study was to estimate the lipophilicity of the newly synthesized derivatives of benzimidazole and pyridone. The lipophilicity was assessed by reversed-phase thin-layer chromatography (aluminium sheets covered with modified silica gel RP-18 F_{254s}, Merck) using acetone as the organic modifier. A linear relationship was found between R_M values and acetone concentrations in the mobile phase. Lipophilicity of the investigated derivatives, expressed by partition coefficient (logP) was also predicted using computer programs (ACD/LogP, Pallas, Marvin, ClogP, ALOGs, milogP). The retention parameter, R_{M0}, was related to theoretical partition coefficients.

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Acknowledgements
The study was supported by the National Science Centre (Grant No. DEC-2011/01/N/NZ4/01125).

P-5

Cytotoxic Activity of Novel Analogues of Bis(2-chloroethyl)amine .

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The alkylating drugs, such as 4-[bis(2-chloroethyl)amino]benzenebutanoic acid (chlorambucil), are in widespread use for the treatment of various cancers. Despite its unparalleled clinical success, as evidenced by the high cure rates of hematological malignancies, chlorambucil-based chemotherapy suffers from major drawbacks. Tumor resistance to the drug, probably the most severe obstacle, is multifactorial in nature and known to be mediated by reduced cellular uptake of the drug, increased levels of detoxifying sulfur nucleophiles in the cytoplasm, enhanced repair or tolerance of drug-DNA adducts, and lack of functional p53 protein. The present study was designed to extend our recent findings relating to amidine analogues of alkylating agents [1-3]. In continuation to the efforts in this direction, it is considered interesting to design and synthesize molecules based on 4-(N,N-bis(2-chloroethyl)aminophenyl)propylamine linked to 5-(4-N-alkylamidinophenyl)-2-furancarboxylic acids moiety by the formation of an amide bond. The introduction of a spacer arm between the carrier and the drug is a strategy widely used to separate the active moiety from the carrier. Ideally, as a conjugate is meant to reach the malignant site, the linkage should be sufficiently stable during circulation in blood stream to maintain its chemical integrity until it reaches its target. However, after uptake into the cells, they should have an intrinsic activity or release the active compound. The DNA binding efficacy and preferred mode of binding of a series of amidine analogues of bis(2-chloroethyl)amine was investigated by ethidium displacement assay using calf thymus DNA, T4 coliphage DNA, poly(dA-dT)$_2$ and poly(dG-dC)$_2$ and by a topoisomerase I/II inhibition assay. Evaluation of the cytotoxicity of these compounds in human breast cancer cells demonstrated that these compounds were more active than melphalan and chlorambucil.

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P-6

Synthesis of Thiourea Derivatives of 4-Amino-4H-1,2,4-Triazole.

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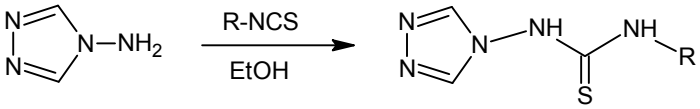
Triazole-based compounds are able to bind with many enzymes and receptors via diverse non-covalent interactions, and therefore display versatile biological properties, including antimicrobial, antitubercular, anticancer, anticonvulsant, anti-inflammatory and analgesic [1]. Literature survey reveals that also thiourea derivatives act as potential pharmacological agents with similar spectrum of activities [2,3], as well as they are selective agonists of the 5-HT_2 receptor [4].
As recently reported, fusing of thiourea fragment with five-membered ring of triazole led to the discovery of potential medicinals with antimicrobial, anti-HIV, antidiabetic and anti-tuberculosis properties [5]. In this work we aimed to combine the heterocyclic 1,2,4-triazole ring with thiourea linker containing various aryl/alkyl substituents.
The title compounds were obtained by a simple one-step condensation, as outlined in the scheme below.



MS and ^{1}H NMR spectra confirmed the identity of the products. The molecular structures of several selected thiourea derivatives were determined by an X-ray crystal structure analysis.
Newly synthesized compounds served as subjects for further structural studies and pharmacological evaluation.

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P-7

Synthesis of 1,2,3-Triazoles from 4-Amino-3-quinolinesulfonamides with the Propargyl Group.

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Barbara Filip-Psurska [2], Jerzy Wietrzyk [2]

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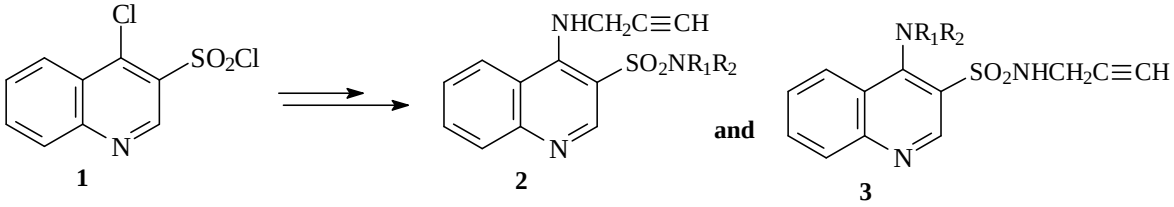
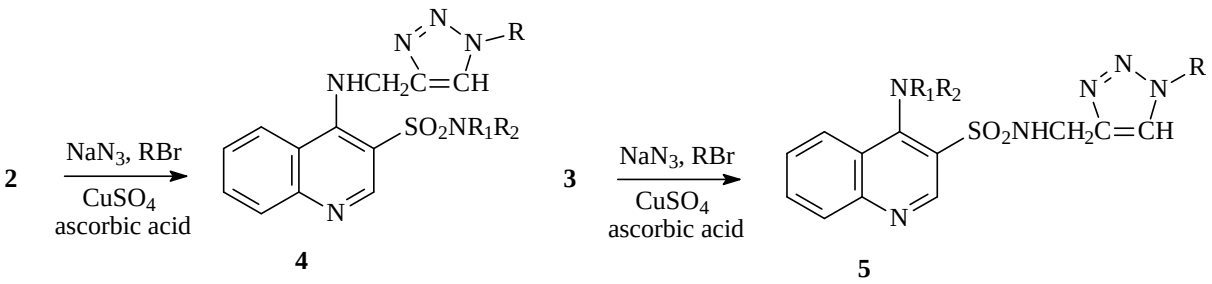
Sulfonamides are a huge group of compounds with various biological activities (antihistamine, antibacterial, antiviral and antineoplastic). The discovery of a new sulfonamide marked as E-7010 in 1992, caused increased interest in this chemical group and led to synthesis of many interesting antineoplastic compounds. Some of them are in advanced stage of clinical trials.
Over a dozen 4-propargylamino-3-quinolinesulfonamides **2** and 4-amino-3-quinoline-*N*-propargylsulfonamides **3** were synthesized lately from 4-chloro-3-quinolinesulfonamide chloride **1**, following the method mentioned below [1]:



Sulfonamides **2** and **3** were tested on neoplastic cells, but their activity is relatively low. That is why a one-pot [2] azide-acetylene addition reaction was performed on selected sulfonamides **2** and **3**. The reaction ran in DMF-water solution, in room temperature for 24 hours, with copper(II) ions and ascorbic acid as catalysts. Compounds **4** and **5** yielded with 77-90%:



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P-8

Synthesis and Anticonvulsant Activity of New 4-Arylpiperazinylalkyl Derivatives of 5-Isopropyl-5-phenyl-imidazolidine-2,4-diones.

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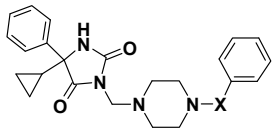
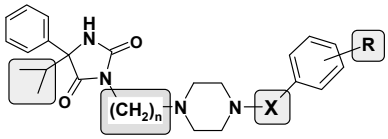
Epilepsy is a frequent disorder that affects about 1% of the general population. At present seizures and epilepsy are assumed to result from an imbalance between excitatory and inhibitory systems in the CNS. Epilepsy treatment is based on pharmacological therapy. Antiepileptic drugs vary in their mechanism of action. Compounds blocking sodium and calcium channels and enhancing GABA-ergic neurotransmission seem to be the most promising candidates. Currently used anticonvulsant drugs may cause many side effects and have no therapeutic effects in up to 25% of patients. That is why new antiepileptic drugs more effective and with a better tolerance profile are sought for. During our studies on new anticonvulsant agents we found that some derivatives of 5-cyclopropyl-5-phenylhydantoin of Mannich base exhibited interesting activity. In this series of compounds the most potent was 3-[(4-benzylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenylhydantoin. It was protective in the maximal electroshok seizure (MES) test in rats with an oral ED_{50} of 13,1 mg/kg, and in the subcutaneous pentylenetetrazole test (scPTZ) ED_{50} of 17,5 mg/kg. [1, 2]



X = $-(CH_2)_0-$ ED_{50} = 5,3 mg/kg (MES)
ED_{50} > 250 mg/kg (scPTZ)
X = $-CH_2-$ ED_{50}= 13,1 mg/kg (MES)
ED_{50}= 17,5 mg/kg (scPTZ)



n = 1, 2, 3 X = $-CH_2-$, $-(CH_2)_0-$
R = H, 3-Cl, 2-F, 4-F, 3-CF_3

As a continuation of our work we have designed and synthesized new 5-isopropyl-5-phenylimidazolidine-2,4-dione ring with arylpiperazinylalkyl moiety. The major modifications of newly synthesized compounds were change of substituent at position 5 of hydantoin and elongation of linker between heterocyclic ring and arylpiperazine fragment, from one to three methylene units.
The investigated compounds were prepared in three step process, in which cyclization of hydantoin ring followed by alkylation and condensation with appropriate arylpierazine moiety, or in Mannich reaction. The compounds obtained were tested for their anticonvulsant activity through the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) in the maximal electroshock (MES) and the pentylenetetrazole (scPTZ) tests.[3]
Of the eighteen new compounds obtained, nine inhibited seizures in the MES test, two showed activity in the scPTZ test. Six compounds prevented convulsions in the MES test, a dose of 100 mg/kg, two compounds were active at a dose of 300 mg/kg. Only Mannich bases a derivative of benzylpiperazine, after 30 min showed anticonvulsant activity in a dose of 30 mg/kg.

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This work was partially supported by CMUJ Research Programme: K/ZDS/003298

P-9

A New Application for the Pipecolic Linker for the Synthesis on Solid-Support: Arylsulfonamide Derivatives with Potential CNS Activity.

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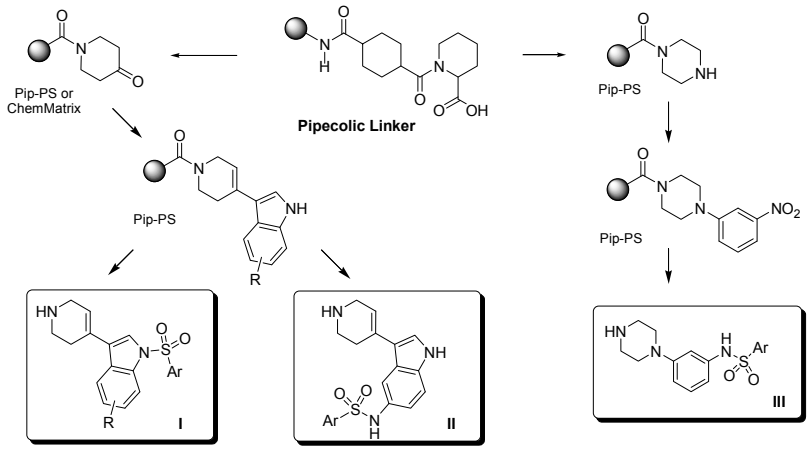
The pipecolic linker (Pip), recently developed in our laboratories, represents a new highly versatile handle for the synthesis on a solid support due to the ease anchoring of primary, secondary and aromatic amines as well as alcohols, thiols and hydrazines [1, 2]. It has been also applied in the N-to-C direction synthesis of peptides, synthesis of cyclic peptides and pseudopeptides, e.g. vinylogous γ-amino acids, urea derived peptides and peptide alcohols [3].
In this study we report on a new application of pipecolic linker for the synthesis of N_1-arylsulfonyl-(1,2,3,6-tetra-hydropyridin-4-yl)-1*H*-indole (set **I**), 5-arylsulfonyl-(1,2,3,6-tetra-hydropyridin-4-yl)-1*H*-indole (set **II**) and 3-arylsulfonyl-phenylpiperazine (set **III**).



The strategy involved an attachment of the cyclic secondary amine (4-piperidone or piperazine) to the Pip-functionalized resin followed by condensation with different indole derivatives (set **I**, **II**) or by Buchwald-Hartwig N-arylation reaction on the piperazine moiety under MW assisted conditions (set **III**). The key point in the synthesis of set **I** derivatives was the sulfonylation in the presence of phosphazene base (BTPP). A critical step in the synthesis of set **II** compounds, reduction of the nitro function, was overcome by using sodium dithionite as reducing agent and potassium carbonate, in biphasis system NMP/H_2O.
Presented solid-phase synthesis strategy allows for generation of combinatorial libraries by extending the variety of substituted sulfonamides in order to develop potential CNS acting agents of pharmacological interest.

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P-10

Synthesis, Analgesic and Antiinflammatory Activity of New 1,3-Dimethyl-3,7-dihydropurine-2,6-dione Derivatives.

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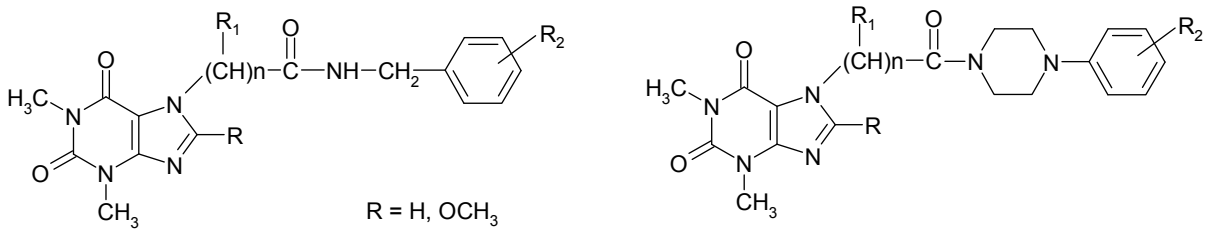
Treatment of acute or chronic pain remains an important medical problem and non-steroidal antiinflammatory drugs (NSAIDs) are still one of the most widely used analgesic agents. Several recent reports indicate that some derivatives of benzoxazolinone, oxazolopyridinone, pyrrole-3,4-dicarboxymide, isothiazolopyridine or dihydrofuranone may be candidates for new analgesic drugs as evidenced in animal models [1-4]. Our interest in this field prompted us to design and synthesize a series of new 8-alkoxy-7-alkyl-1,3-dimethyl-3,7-dihydropurine-2,6-diones with ester and carboxylic terminal groups. These compounds displayed analgesic effect in experimental rodent models of pain (writhing and formalin tests) which was stronger than acetylsalicylic acid used as a reference drug. The tested compounds produced a dose-dependent antinociceptive effect in phase II of formalin test in mice, which represents inflammatory pain. It may suggest that this mechanism of analgesic action is related to the nonsteroidal drugs [5].
As a continuation of our investigation we designed and synthesized new analogues of evaluated compounds with amide group in position 7 of 1,3-dimethyl-3,7-dihydropurine-2,6-dione core. It worth noting that amide group is characteristic for NASID drugs (i.e. benorylate, salicylamide, ethenzamide). For structure-activity relationship study (SAR) some 8-unsubstituted derivatives were also synthesized.



R = H, OCH_3
R_1 = H, CH_3
R_2 = H, 2-OCH_3, 2-CH_3, 2-CF_3, 4-F
n = 1, 3

The structures of the new compounds were confirmed by examination of their ^{1}H-NMR and LC-MS spectra as well as by elemental analyses. The new compounds evaluated in some behavioral models (writhing, formalin and carageenan tests) showed analgesic and antiinflammatory activity stronger than acetylsalicylic acid, indometacin and ketoprofen used as reference drugs. Biological data and SAR studies will be presented.

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This research was partly supported by grant K/ZDS/003299.

P-11

Pro-Apoptotic Effect of Pt_2(3-ethylpyridine)$_4$(berenil)$_2$ and Pt_2(3-buthylpyridine)$_4$(berenil)$_2$ in Human Breast Cancer Cells.

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Cisplatin is a well known DNA-damaging agent and the current thinking is that DNA platination is an essential first step in the cytotoxic activity of the drug. However, the mechanism(s) whereby these DNA adducts kill cells is not fully understood. It is known that cisplatin induces two different modes of cell death: apoptosis and necrosis. Modulating intrinsic chemosensitivity or drug resistance by increasing the susceptibility of tumor cells to apoptosis may be important aims in cancer research. The aim of this study was to compare the effect of cisplatin and novel dinuclear platinum (II) complexes with berenil and amine ligands [1-3] on human breast cancer cell line MDA-MB-231. In our study the induction of apoptosis by Pt_2(3-ethylpyridine)$_4$(berenil)$_2$ (Pt10) and Pt_2(3-buthylpyridine)$_4$(berenil)$_2$ (Pt11) in human breast cancer cells was confirmed by several biochemical markers, such as: phosphatidylserine externalization, loss of mitochondrial membrane potential $\Delta\Psi_m$, caspase-3 activity, and DNA degradation. Our experiments carried out with flow cytometry assessment of annexin V binding and fluorescent microscopy assay revealed that Pt10 and Pt11 inhibited the proliferation of MDA-231 cells by increasing the number of apoptotic cells. Exposure to Pt10 and Pt11 resulted decrease of mitochondrial membrane potential $\Delta\Psi_m$ in MDA-MB-231 cells. This paper demonstrates that Pt10 and Pt11 can also induce caspase-3 up-regulation in human tumor cells, raising the possibility that caspase-3 contributes to the cytotoxic effect of these compounds, particularly the promotion of apoptosis. These results demonstrate that Pt10 and Pt11 treatment activate a caspase and mitochondria-mediated apoptotic pathway.

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The author is a scholar of the project „I study, explore, commercialize – the UMB PhD fellowship programme". The project co-financed by the European Union under the European Social Fund.

P-12

An Impact of Halogen Position in 4-Phenylpiperazine of LCAPs with Spirohydantoins on the 5-HT$_{1A}$/5-HT$_7$ Receptor Selectivity.

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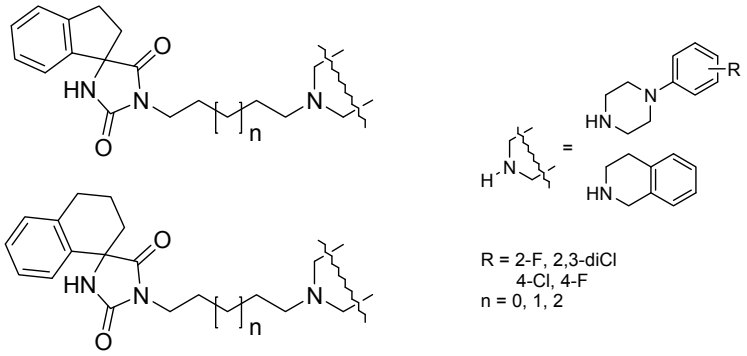
Long-chain arylpiperazines (1-aryl-4-alkylpiperazines, LCAPs) have been extensively studied as 5-HT$_{1A}$ receptor ligands. Due to the structural similarity between the 5-HT$_{1A}$ and 5-HT$_7$ receptors, LCAPs were also extensively modified, in order to identify selective 5-HT$_7$ receptor ligands [1]. Differently substituted, in position 5, hydantoin derivatives of LCAP were previously evaluated in order to study the effect of the amide part on the affinity for 5-HT$_{1A}$ receptors [2].
Continuing our studies, in this work aimed at verification of structural features determining 5-HT$_{1A}$/5-HT$_7$ receptor affinity and selectivity, we selected indene and tetralin substituted spirohydantoins as core imide fragments. Further modification consisted in diversification of halogen position in the phenylpiperazine fragment, and variation of the length of an alkylene spacer (C4–C6). Additionally, we replaced arylpiperazine moiety with its tetrahydroisoquinoline bioisostere.



The tested compounds were obtained in a three-step synthesis starting from cyclisation of spiro-[imidazolidine-4,1'-indene/naphthalene]-2,5-diones from corresponding ketone followed by alkylation with the halogenoalkanes and coupling with differently substituted phenylpiperazines. Herein, we discuss the relationship between structural modifications applied on compounds affinity and selectivity for 5-HT$_{1A}$ and 5-HT$_7$ receptors.

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Acknowledgement: This study was partly supported by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 378437.

P-13

Secondary Amino-Acids and Ketones as Coupling Partners in Ugi U-5C-4CR Multicomponent Reaction. Optimization of Conditions and Application to Synthesis of C-4 Disubstituted 2,6-Diketopiperazines.

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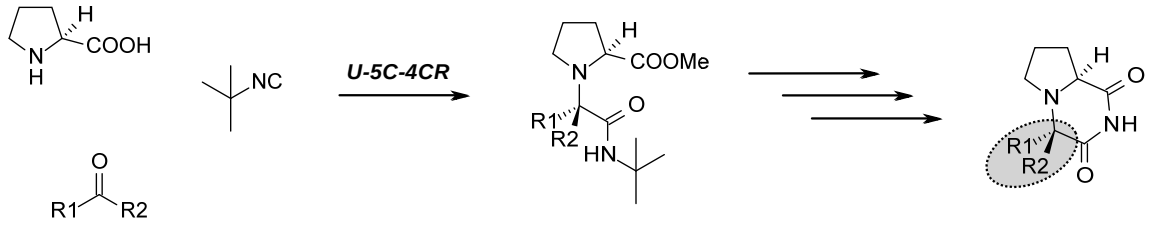
The isocyanide-based multi component reactions (IMCRs) are important tool in modern medicinal chemistry. Due to their preparative simplicity, great efficiency and interesting stereochemical outcome, they are often used to create diversity of small-sized *druglike* molecules for biological screening [1,2]. Recently, we have shown that a variant of popular IMCR, Ugi U-5C-4CR (*Ugi-five-center-four-component reaction*) [3,4] can be efficiently used in a key step of stereospecific synthesis of biologically active bicyclic 2,6-diketopiperazines (2,6-DKPs) [5]. In this strategy, an aromatic aldehyde was coupled with secondary amino-acid to introduce an aromatic substituent in the structure of a final product.
The aim of the presented work was to egzamine the U-5C-4CR condensation of more difficult coupling partners: secondary amino acids and various aliphatic or aryl-aliphatic ketones. The substrate scope, stereochemical outcome of the reaction and its application to the synthesis of C-4 disubstituted 2,6-DKPs was also discussed.



[1] Dömling A.: *Chem. Rev.* **106** (2006), 17.
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P-14

Influence of the Length of the Linker Between Arylidene Hydantoin and Phenylpiperazine Moieties on Their Affinity for α_1-Adrenoreceptors.

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Recently, α_1-ARs have been the subject of intensive research taking into the account their potential role in arrythmias mechanism, especially in ischemic arrhythmia [1]. The α_1-adrenoreceptors are the family of G-protein-coupled seven-transmembrane helix receptors. Studies of receptor binding have shown that the numerous compounds with affinity for α_1-AR contain arylpiperazine moieties. In our previous research several N3-phenylpiperazine derivatives of 5-arylidene hydantoin were obtained [2, 3]. These compounds included hydroxypropyl chain between phenylpiperazine and arylidene hydantoin moieties. They have shown affinity for α_1–ARs in nanomolar range (K_i). In our recent studies, new N3-phenylpiperazine derivatives of 5-arylidenehydantoin were obtained. They have shown high affinity for α_1-ARs. The most active structure (**Fig.1**) was chosen as a lead for the present investigation. In the present work, we designed new modifications of the mono, di, or trimethoxy arylidene hydantoin containing phenylpiperazine ring, modified phenylpiperazine fragment and chain between this two moieties.

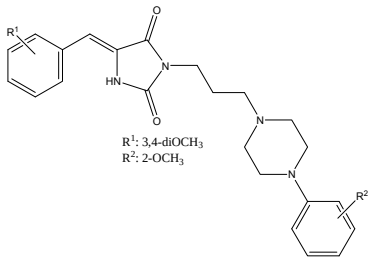

Fig.1

The chemical modyfications were focused on the elongation of the chain between phenylpiperazine and arylidene hydanoin and on the introduction of methoxyl, chloro or fluoro substituents at phenylpiperazine ring.

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

The new hydantoin derivatives were evaluated on their affinity for α_1-adrenoreceptors in radioligand binding assay, using [^{3}H]prazosin as selective radioligand. The affinity was in very wide range (Ki: 25,8nM-1,5μM). SAR-studies demonstrated a profitable influence of methoxy substituent in phenylpiperazine fragment. The affinity for α_1-ARs was significantly better for compounds with shorter chain.

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This work was partly supported by grant: K/ZDS/001915.

P-15

Stability and Antioxidant Capacity Rating of Selected Nutricosmetics.

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Durability of nutricosmetics such as Bellissa Sun Aflofarm, Skrzypovita, and Perfect Skin has been marked with the use of the spectrophotometric method in the UV light. The research has been conducted in ethanol, water, acid (0,1 M HCl) and alkaline (0,1 M NaOH) environments. The use of the spectrophotometric method was possible as the tested compound follows the Lambert-Beer law. Absorbance change correlations in time and degradation speed constants “k” for the tested nutricosmetics have been marked. Antioxidant potential in methanol-acetone and water extracts has been determined with the use of the FRAP method [1, 2]. The reference substance for rating the anti-oxidant properties of the compound was vitamine C.

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P-16

Synthesis of Novel Azaindole Derivatives of Pyrido[1,2-*c*]pyrimidine with Potential Antidepressant Activity.

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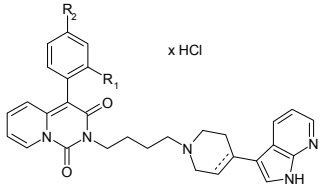
The aim of the research is obtaining a series of novel azaindole derivatives of pyrido[1,2-*c*]pyrimidine in the way of multi-step synthesis, structural analysis and determination of their pharmacological properties. Synthesized compounds were designed on a basis of structural similarities to substances with proven good affinities for 5-HT$_{1A}$ receptors and serotonin transporter protein (SERT), obtained over the last years in the Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw.

Serotonin neurotransmission is involved in mood regulation, instincts, sleep and wakefulness, appetite, libido, thermoregulation, cognition, memory and learning process [1]. Disturbances in the serotonergic system underlie many central nervous system diseases, such as affective disorders (depression, bipolar disorder), schizophrenia and anxiety. Interest in new compounds that affect the regulation of serotonin neurotransmission is due to the increasing number of cases of depression, which according to the World Health Organization affects more than 150 million people worldwide and is predicted to be the leading cause of premature death and disability in 2030 [2].

In the 80's of the twentieth century particular attention has been paid to the role of serotonin in the pathomechanism of affective disorders, which resulted in discovery of selective serotonin reuptake inhibitors (SSRI) – a group of antidepressants that soon dominated the pharmacotherapy of depression. The popularity of SSRIs stemmed mainly from low affinity for histamine, muscarine and adrenergic receptors, which contributes for the favorable profile of side effects and higher therapeutic index. The use of SSRIs allows for the rapid increase in 5-hydroxytryptamine level in the synaptic cleft, but the clinical effects appear after 2-3 weeks of regular administration. Concentration of serotonin in the synaptic cleft is affected not only by the reuptake but also by the activity of 5-HT$_{1A}$ autoreceptors in the raphe nuclei, which control the release of 5-hydroxytryptamine from axon terminals on a basis of a negative feedback - blocking the reuptake of serotonin increases the concentration of the neurotransmitter in the synaptic cleft but also simultaneously activates 5-HT$_{1A}$ autoreceptors in the midbrain raphe nuclei, which leads to opening of potassium channels, membrane hyperpolarization and suppression of serotonin release from axon terminals, weakening the effect of reuptake inhibition [3]. Long-term (several weeks) administration of SSRIs leads to adaptive changes in the brain (including desensitization of 5-HT$_{1A}$ autoreceptors), resulting in a significant increase in serotonin level in the synaptic cleft, responsible for alleviation of symptoms of depression [3]. Linking of 5-HT$_{1A}$ autoreceptors with a delay of SSRI action initiated a search for compounds with dual mechanism of action - SERT protein inhibitors and presynaptic 5-HT$_{1A}$ antagonists.

The presented study involves the synthesis and evaluation of novel SSRI and 5-HT$_{1A}$ ligands of the following structure:



R_1 = H, F, Cl, OCH_3, CH_3
R_2 = H, F, Cl, OCH_3, CH_3

Structural studies (including ^{1}H and ^{13}C NMR, IR, elemental analysis and HRMS) as well as *in vitro* radioligand tests, determining the affinity for serotonin transporter protein (SERT) and 5-HT$_{1A}$ receptors, were carried out for the obtained compounds.

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P-17

Physical-Chemical Studies on the Newly Synthesized Porphyrazines Possessing Peripheral 2,5-Dimethylpyrrol-1-yl and Dimethylamino Groups.

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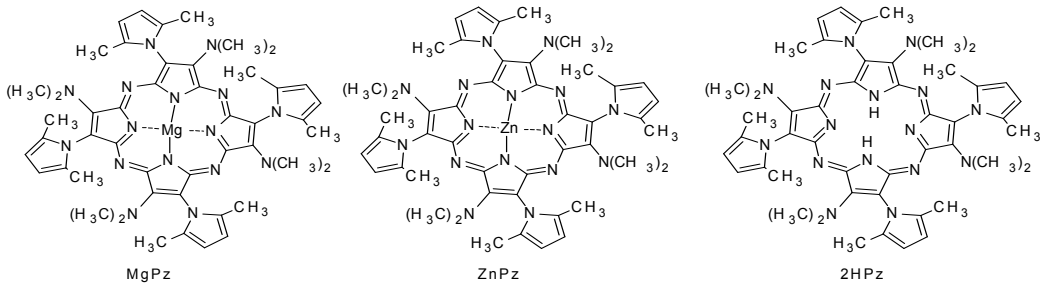
Photodynamic therapy (PDT) is a new promising treatment mainly of cancer, pre-cancerous lesions, bacterial and viral infections. This method involves the use of a photosensitizer, molecular oxygen and light of an appropriate wavelength. All these three agents together cause the cell death. Upon absorption of light of an appropriate wavelength, the photosensitizer is brought to the excited state and in this form it interacts with molecular oxygen yielding singlet oxygen formation. Singlet oxygen can react with many biological components of the cell or tissue and lead to their destruction. For selective treatment after drug injection, pathologic tissue is irradiated only [1].



Three porphyrazines: **MgPz**, **ZnPz** and demetallated form **2HPz** were the subjects of this study [2]. In order to apply a newly synthesized photosensitizer in PDT it is necessary to research its physical-chemical properties, including solubility in various solvents and tendency to form aggregates which tend to reduce the efficacy of singlet oxygen generation. The investigated compounds were studied in 19 solvents: acetonitrile, methanol, ethyl acetate, n-hexane, N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA), cyclohexane, 1,4-dioxane, diethyl ether, tetrahydrofurane (THF), triethylamine, chloroform, dichloromethane, 2-propanol, pyridine, toluene, dimethyl sulfoxide.
According to the experimental data, **MgPz** was found to form aggregates in acetone, diethyl ether, methanol, ethyl acetate and triethylamine, whereas no tendency towards aggregate formation was found in pyridine. **ZnPz** forms aggregates in 2-propanol, DMA and methanol, whereas **2HPz** tends to aggregate in diethyl ether, n-hexane, ethyl acetate and THF.
Additionally, the interactions between solvents and dissolved compounds were analyzed by the Bayliss method. For **MgPz** and **ZnPz** the solvation effects were observed in triethylamine, diethyl ether, ethyl acetate, THF, dichloromethane and pyridine. For **2HPz** the solvation effects were detected in cyclohexane, triethylamine, diethyl ether, THF, dichloromethane. Interestingly, **MgPz** revealed solvation effects in polar environment of 2-propanol, acetone, methanol, DMF, acetonitrile, DMA, whereas **ZnPz** in 2-propanol, methanol, DMF, acetonitrile, DMA. **2HPz** was found to be soluble in acetone, DMF and DMA only, and for this reason no statistical calculation was performed.

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This study was supported by the National Science Centre - grant No. N N404 069440.

P-18

An Influence of Aryloxy-/Arylthio-ethyl Fragment on 5-HT$_{1A}$/5-HT$_7$ Receptor Selectivity in a Group of Quinolinesulfonamide Derivatives of Aryloxyethyl- and Arylthioethyl- Piperidines.

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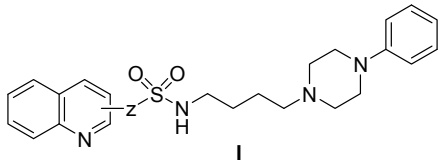
Employing a parallel solid-phase synthesis integrated with virtual combinatorial library design, and followed by multistep virtual screening procedure, allowed us to replace arylpiperazine moieties of LCAP with their flexible biomimetics [1]. In this series of compounds, arylpiperazine moiety was changed into a flexible aryloxy-/arylthio-ethyl fragment, while in the part corresponding to the alkylene spacer of LCAP, different, partially rigidified amines – 3-aminopyrrolidine, 4-aminopiperidine, 4-aminomethylpiperidine were introduced. It was further presented, that the affinity of compounds for the tested monoamine receptors (5-HT$_{1A}$, 5-HT$_6$, 5-HT$_7$, D$_2$) depended upon a kind of a substituent in the aryloxy-/arylthio- ethyl fragments, a distance between the basic nitrogen atom and amide/sulfonamide moiety, and finally a kind of amide/sulfonamide fragment [1].



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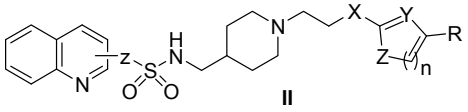

II

X = O, S; Y = CH, N; Z = CH, S;
z = 3, 6, 7, 8; n = 1, 2
Phenyl: Z=Y=CH; n=2

Now we report on the design, synthesis, and biological evaluation for 5-HT$_{1A}$, 5-HT$_6$, and 5-HT$_7$ receptors of a new series of quinolinesulfonamide derivatives of aryl(heteroaryl)oxy-/heteroarylthio-ethyl 4-aminomethylpiperidines. The structural modifications comprised introduction of different quinoline sulfamoyl fragments, to further extend exploration in a group of azinesulfonamides (**I**), variation of different substituents in the phenyl ring of aryloxy-/arylthio- ethyl fragment, as well as a replacement of the central phenyl ring of the aryloxy-/arylthioethyl fragment with five-membered heteroaromatic system. We chose 4-aminomethylpiperidine central core to maintain a distance between tertiary nitrogen atom and sulfonamide bond, corresponding to the four-methylene linker present in the previously reported azinesulfonamide derivatives of LCAP [2]. In particular, we determined an influence of aryl(heteroaryl)oxy-/heteroarylthio- fragment on 5-HT$_{1A}$ and 5-HT$_7$ receptor binding profile.

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The study was supported by the Funds for Statutory Activity of Jagiellonian University Medical College.

P-19

Evaluation of Compounds 488 and 530 as Potential Antiepileptic and/or Analgesic Agents.

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Background: Pharmacotherapy of epilepsy constitutes a great challenge both in the field of clinical neurology as well as medicinal chemistry. Now available antiepileptic drugs (AEDs) are not sufficient to control the seizures in about one third of patients what may be caused by the development of pharmacoresistance. Searching for new anticonvulsants is focused on achieving not only better activity but also improved properties in terms of safety, tolerability and pharmacokinetics.
Neuropathic pain, which is kind of pain related with functional abnormality of neurons, is still considered an unmet need. There are few drugs registered for this condition and among them one finds well known antiepileptic drugs. It has turned out that mechanism of action of anticonvulsants is very common for drugs revealing analgesic activity. As the two condition have similar pathophysiology many drug candidates for epilepsy are being evaluated for activity in neuropathic pain.
Aims: The aim of the presented work was to design and synthesize compounds which could act as antiepileptic and/or analgesic agents.
Methods: Compounds were synthesized at Department of Bioorganic Chemistry Jagiellonian University Medical College in Krakow. They were design based on previous results The structure and chemical purity were confirmed by means of physicochemical analyzes (^{1}H NMR, IR, elemental analysis, TLC, HPLC, melting point). The pharmacological tests were carried out according to the Antiepileptic Drug Development Program at National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville, USA. Pharmacological tests included:

- maximal electroshock model (MES) – mice *i.p.*; rats *p.o.*;
- subcutaneous pentetrazole (scMet) seizure model – mice *i.p.*; rats *p.o.*;
- neurotoxicity assays (TOX) – mice *i.p.* (rotarod), rats *p.o.*;
- 6 Hz model – mice *i.p.*;
- hippocampal kindling model – rats *i.p.*;
- pilocarpine induced status epilepticus – rats *i.p.*;
- formalin test – mice *i.p.*

Conclusion: Compounds 488 and 530 proved to possess broad pharmacological activity. Further modification of their structure may result in discovering new anticonvulsants or analgesics.

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Project is supported by the European Regional Development Fund through the Innovative Economy Program.

P-20

Studies on Anticancer Properties of Novel Heterocyclic Compounds with Hydantoin Motives.

Jadwiga Handzlik, Dorota Łażewska, Katarzyna Kamińska, Katarzyna Kieć-Kononowicz

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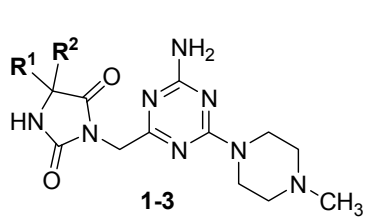
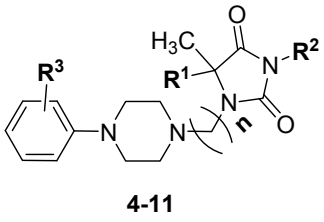
Cancer is a second reason for death, after cardiovascular diseases. It is suspected that more than 1 in 3 people will develop some form of cancer during their lifetime. There is not a single type of cancer but over 200 different types of malignant cells are possible. The available chemotherapeutic agents have distinct mechanisms of action and unfortunately there are not specific, which leads to the many common side effects. The problem is also a developed resistance to the used drug of cancerous cells which are initially suppressed by this specific drug. For this reason cancer chemotherapy may consist of using several drugs in combination for varying lengths of time. That is why there is the strong need to find a good and selective remedies for different types of cancer.
In National Cancer Institute (NCI) Bethesda since 1990 there has been developed anticancer drug discovery program. This project is designed to search for new leading structures showing anticancer activity and there is hope that ongoing studies will help to find useful chemotherapeutic agents [1].



1-3

group I



4-11

group II

R^1, R^2: aryl, benzyl or CH_3
R^3: H, OCH_3, Cl
n = 4-5

As the results of our cooperation with NCI, eleven compounds with imidazolidin-2,4-dione scaffolds (Fig.1) were accepted for a primary pharmacological screening. These compounds were tested in 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney and the obtained results will be presented. Two the most active compounds, belonging to group II (Fig.1), exhibited significant cancer cells growth inhibition. The compounds have been selected for further pharmacological studies.

This work was partly supported by grant No. 501/N-COST/2009/0, COST Action BM0806 (Recent advances in histamine receptor H_4R research) and program K/ZDS/003323.

References
[1] http://dtp.cancer.gov

P-21

Novel Dual Binding Site Cholinesterase Inhibitors with Indole Moiety.

Michalina Ignasik [1], *Marek Bajda* [1], *Michaela Prinz* [2],
Ulrike Holzgrabe [2], *Christoph Sotriffer* [2], *Barbara Malawska* [1]

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[2] *Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Germany*
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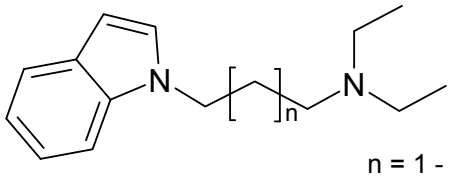
Deficits in the cholinergic system in the brain areas related to memory and learning, brain deposits of amyloid beta (Aβ) peptide and neurofibrillary tangles are considered as the main causes of Alzheimer`s disease (AD). Treatment of AD currently focuses on increasing cholinergic neurotransmission in the brain by cholinesterase inhibitors: donepezil, rivastigmine and galantamine. AD is multifactorial disorder therefore it is important to finding an active structure acting for more than one target [1, 2].
In our previous work, we obtained derivatives of isoindoline-1,3-dione connected by alkyl chain with diethylamine grup. These compounds showed a preferential inhibiting activity against acetylcholinesterase (AChE) and weak Aβ antiaggregation inhibitory activity [3]. As a continuation of this study, using molecular modeling and fragment based methodology, new dual binding site structures of indole connected by alkyl chain with diethylamine have been designed. These compounds were synthesized and evaluated *in vitro* using the spectrophotometric metod of Ellman and fluorometric Thioflavine T test (ThT). The series of compounds showed moderate activity for butyrylcholinesterase (BuChE) IC_{50} = 1,06-24,5 μM and weak activity for AChE. Some of compounds also have ability to inhibit Aβ aggregation.



n = 1 - 6

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P-22

Determination of the Absolute Configuration of Stereoisomers of 8-*tert*-Butyl-2-hydroxy-7-methoxy-8-methyl-9-oxa-6-azaspiro[4.5]dec-6-en-10-one by NMR Spectroscopy of Their Methoxyphenylacetic Acid Esters.

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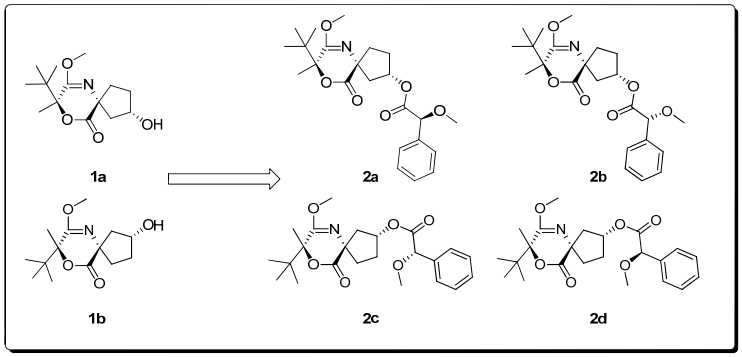
Determination of absolute configuration (AC) of chiral molecules is an important step in any field related to chirality but nowhere is it as critical as in the biological science. The phenomenon of "chiral recognition" - in which the enantiomers of a chiral drug may exhibit differences in biological activity or other processes such as distribution, uptake, and metabolism - makes it a necessity (or requirement) to know the AC not only of the final molecule but as early in the process of development as possible [1-2].
This study is a part of a project aiming the synthesis enantiomers of cyclic α-amino acids. In the course of this study we obtained enantiomers of 8-*tert*-butyl-2-hydroxy-7-methoxy-8-methyl-9-oxa-6-azaspiro[4.5]dec-6-en-10-one (**1**). As a part of our general interest in stereocontrolled organic synthesis, herein we report our efforts towards absolute configuration (AC) determination of compound (**1**). The crucial part of this work was assignment of AC of C-5 carbon atom, the AC of C-2 and C-8 carbon atoms were assignment based on AC of compounds used for the synthesis of **1**. Stereoisomers of 8-*tert*-butyl-2-hydroxy-7-methoxy-8-methyl-9-oxa-6-azaspiro[4.5]dec-6-en-10-one (**1**) were derivatized to esters by the use of enantiomers of methoxyphenylacetic acid. The absolute configuration of the original chiral secondary alcohol was determined based on results derived from the NOESY correlation between the protons of *tert*-butyl group and proper protons of the cyclopentane fragment.



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Acknowledge:
The work was financially supported by the National Science Centre grant no N N405 620938.

P-23

Influence of Chloro Substitution in 6-Aryl Moiety of 4-(4-Methylpiperazino)-1,3,5-triazine-2-amines on Histamine H_4 Receptor Affinity.

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Histamine H4 receptor (H_4R), was discovered and cloned independently by several research teams in 2000 and 2001 [1]. It is primarily located in cells of the immune system, suggesting its participation in (patho)physiological immune responses as well as allergic and inflammatory processes. Therapeutically beneficial effects of H_4R antagonists have been observed in *in vivo* models of related diseases, like asthma, itching, and inflammatory bowel disease [2,3].
The first potent and selective H_4R antagonist – JNJ 7777120 – was disclosed by Jablonowski, et al. in 2003 [4]. In most studies, it became a reference compound and was used to better understanding of the pharmacology and function of H_4R. Since then, many other lead compounds have been described in recent literature [5,6], including *N*-methylpiperazino azine derivatives [7].

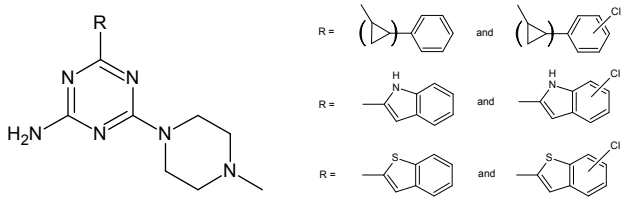

Fig.1. General structure of the synthesized compounds.

In our work, we are investigating new histamine H_4 receptor ligands in the group 1,3,5-triazine derivatives. A series of new structures with different aromatic rings in triazine 6-position (phenyl, benzothienyl and indolyl moieties) was synthesized and then we modified these compounds by introduction of chloro substituents in different positions (Fig.1). All compounds were tested for their affinity to H_4R in radioligand binding assays. In addition, their toxicity and physicochemical properties were examined *in silico*.

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Acknowledgments: Supported by Polish Ministry of Science and Higher Education Grant No. 594-N/COST-2009/0, National Science Center DEC-2011/02/A/NZ4/00031, FP7 EU COST Actions BM0806 and BM1007.

P-24

Synthesis of New Propionamide and Butanamide Derivatives of Phtahlimide, and its Saturated Cyclohexane Analogs as Potential Anticonvulsants.

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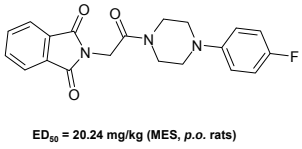
The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority antiepileptic drugs make it difficult to use rational methodologies of discovery based on three-dimensional structure of biological target. Thus the most useful for the design of new anticonvulsants are ligand-based approaches that rely on the use of different pharmacophores established through the analysis of structural characteristics of clinically effective antiepileptic drugs as well as other anticonvulsant active compounds [1]. The two past decades have demonstrated many attempts to identify the structural features of compounds crucial for anticonvulsant activity. As a result it was proved that one of the important core fragments is defined by nitrogen heteroatomic system (imide or lactam), at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system [2, 3].
Taking into consideration the above, in course of developing new anticonvulsant agents our attention has been focused on 1,3-substituted pyrrolidine-2,5-diones. Previous research revealed high anticonvulsant protection among 2-(1,3-dioxoisoindolin-2-yl)-acetamides with differently substituted phenylpiperazines as amide function [4]. The structure of the most active molecule is shown in **Fig**. **1**.



ED_{50} = 20.24 mg/kg (MES, *p.o.* rats)

Fig. 1.

As a continuation of systematic structure - anticonvulsant activity analysis in the current studies we have synthesized a series of analogs of the above compound in which acetamide function has been changed into propionamide or butanamide. This modification let to approximate the structure of amide fragment to observed in Levetiracetam or Brivaracetam, which are the newest antiepileptic drugs. The structures of compounds designed and model drugs are shown in **Fig**. **2**.

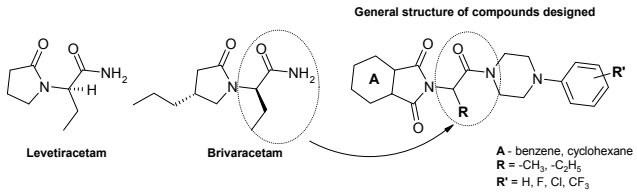


Fig. 2.

The compounds will be evaluated for their anticonvulsant activity and neurotoxic properties within the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Rockville, USA) [5].

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P-25

Pharmacological characterization of fluorescently tagged human adenosine A_{2A} receptors.

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The adenosine receptor (AR) family consists of four G protein-coupled receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. Adenosine A_{2A} receptors (A_{2A} ARs) play an important role in regulating smooth and well-coordinated movement. There is now evidence that A_{2A} AR ligands may provide a novel therapy for the treatment of Parkinson's disease with lower risk of dyskinesias and may exhibit neuroprotective effects. Due to the importance of A_{2A} ARs there is a need of developing the reliable models for investigation of molecular pharmacology of the mentioned receptor [1].

Fluorescence-based methods have recently gained huge interest in biomedical and pharmaceutical research. Fluorescently tagged receptors may be an useful tool for studies of cellular localization and internalization of receptor proteins with use of fluorescence microscopy. Moreover involvement of FRET (Förster Resonance Energy Transfer) techniques enables the investigation of receptor dimerization and receptor-ligand interactions, when appropriate fluorescent ligands are used [2,3].

In current work expression of human adenosine A_{2A} receptor, C-terminally tagged with ECFP, in mammalian cells is described. Both transient and stable tranfection experiments have been performed and fluorescent protein expression was confirmed by means of flow-cytometry and fluorescence microscopy. Receptor protein integrity and functionality was verified in radioligand binding experiments, with use of standard agonist [^{3}H]CGS21680 [4] and antagonist [^{3}H]MSX-2 [5] of adenosine A_{2A} receptor. Measured K_D values were compared with relevant results for non-tagged receptors. Obtained data confirm high expression levels and non-affected binding properties of fluorescently tagged receptor.

Acknowledgement: partly supported by Polish National Science Center, project Harmonia, ID 178522

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P-26

Analgesic Activity of Tricyclic Annelated Derivatives of Xanthines with Affinity at Adenosine Receptors.

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The role of adenosine receptors in nociception is complex and may involve different mechanisms in the central nervous system and in peripheral tissues. In connection with news about the important role of adenosine receptors especially A_{2B} in the process of feeling pain, we designed the synthesis of compounds with expected antagonistic activity on these receptors [1]. An example of this search are novel tricyclic derivatives of xanthine, which were investigated for analgesic activity.
The analgesic activity of newly synthesized substances is generally evaluated first in screening tests using several basic *in vivo* methods. One method is the hot plate test [2], which is a method of testing central analgesic activity of the compounds. The paws of mice are very sensitive to heat at temperatures which are not damaging the skin. The reaction time is prolonged after administration of centrally acting analgesics, whereas peripheral analgesics (COX-inhibitors) generally do not affect the responses.
New tricyclic derivatives of xanthine (**WZ-1**, **WZ-3**, **WZ-4**, **KD-56**, **KD-60**, **KD-114**, **KD-142**, **KD-179**) were evaluated in the hot plate test. The results indicated that three of the xanthine derivatives (**WZ-1**, **WZ-4** and **KD-60**) prolonged the nociceptive reaction time in mice in a dose-dependent manner. The results were statistically significant. The highest analgesic activity was observed for compound **KD-60**. Tested at a dose 100 mg/kg, **KD-60** prolonged the nociceptive reaction time in mice by 104.5%, compared to the control group. While compound **WZ-4**, administered at the same dose, prolonged the nociceptive reaction time in mice by 95.3%. Compound **WZ-1** prolonged the nociceptive reaction time in mice by 44.7% at a dose 100 mg/kg. The performed preliminary tests indicated that three new xanthine derivatives exhibited analgesic effect.
The investigated compounds showed affinity for A_{2B} receptors in the submicromolar to low micromolar concentration range (K_i = 0.59 – 7.2 μM). However, the analgesic effects of the compounds did not correlate with their affinity for adenosine receptors. The explanation of the mechanism of action of these compounds requires further investigations.

Partly supported by Polish National Science Center, project Harmonia ID 178522

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P-27

Search for New GABA-uptake Inhibitors Among Derivatives of alpha-Substituted *N*-Benzylamides of gamma-Hydroxybutyric Acid.

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4-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and GABA re-uptake from the synaptic cleft is one important mechanism in the regulation of GABA activity. Inhibition of the re-uptake of GABA by inhibitors GABA transporters of which four subtypes have been cloned (GAT1-4), enhances GABA activity. This is exemplified by the development of the GAT1 selective drug tiagabine, that is used in the treatment of epilepsy. This constitutes essential proof of concept that the GABA transporters are interesting drug targets in the context of antiepileptic drugs [1].
Taking above into consideration and the interesting results of our earlier studies, a new series of 2-substituted *N*-benzylamides of 4-hydroxybutanoic acid (GHB), whose synthesis is illustrated in Fig.1, was designed and synthesized [2,3]. The structure of compounds designed is based on the 4-hydroxybutyric acid (GHB). In the 2[nd] position of GHB, a 4,4-diphenylbutanylamine or *N,N*-(methyl)(4,4-diphenylbutanylamine was introduced as a part mimicking the biaryl moieties of known for selective GAT1 inhibitors [4].

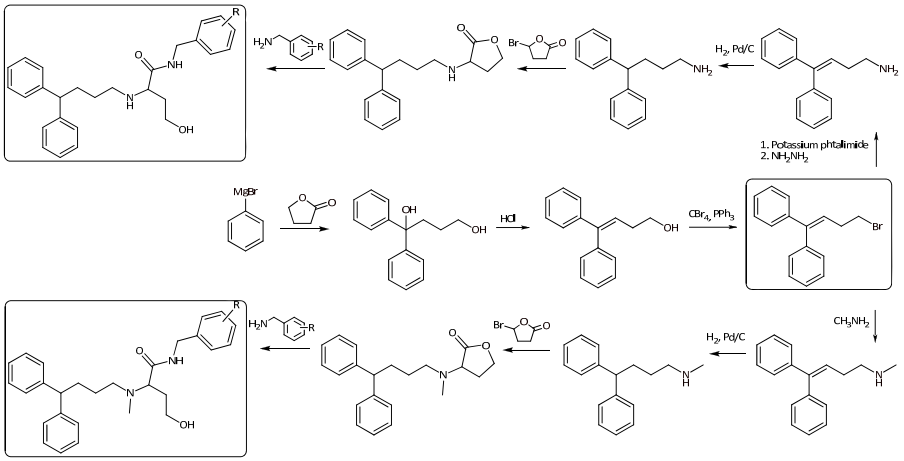

Fig.1. Synthesis of the derivatives of 2-substituted *N*-benzylamides of 4-hydroxybutyric acid; *R: H; 2-Cl; 4-Cl, 4-F, 4-Me*.

The obtained compounds have been tested for the inhibitory potency at the four murine GABA uptake transporters mGAT1 – mGAT4 stably expressed in HEK cell to determine the structure-activity relationship for that class of compounds.

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Acknowledgements
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P-28

Synthesis of Pyrazolo[4,3-e]benzotriazole Derivatives of Potential Anticancer Activity.

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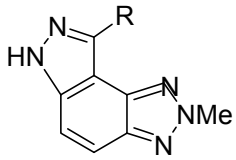
A series of tricyclic pyrazolo[4,3-e] derivatives was obtained through a three-step synthesis that involved:

- Vicarious nucleophilic substitution of hydrogen (*VNS*) using chloromethyl *p*-toluene sulfone or *p*-chlorophenoxyacetonitrile or 1-(phenylsulfonylmethyl)benzotriazole as carbanion precursors and 2-methyl-5-nitro-(*1H*)benzotriazole as a nitro starting material;
- Reduction of the nitro group either catalytic or with tin;
- Diazotization-cyclization of the resulted amine using the solid phase-microwave method.

The final products are currently tested for their cytotoxic activity.



R = Ts, CN, benzotriazol-1-yl

P-29

Synthesis and Photochemical Properties of Unsymmetrical Phthalocyanine Bearing Two 1-Adamantylsulfanyl Groups at Adjacent Peripheral Positions.

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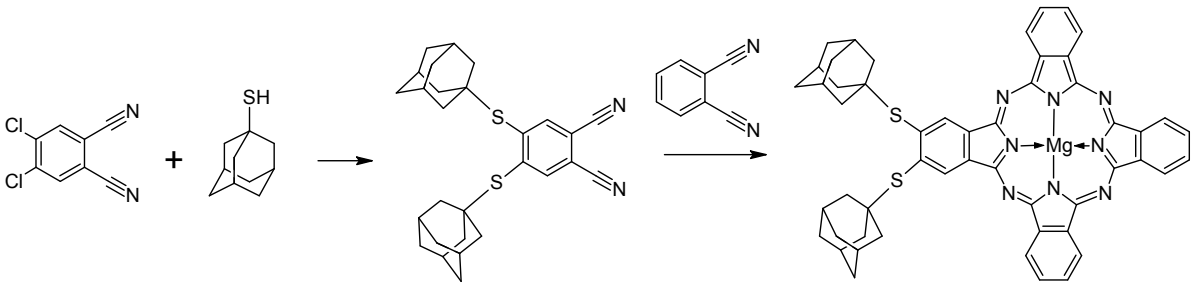
Phthalocyanines are synthetic aza-analogues of naturally occurring porphyrins. They are promising candidates for photodynamic therapy (PDT), which is a novel treatment method of various diseases, especially tumors. PDT requires photosensitizer, which after irradiation with light of an appropriate wavelength generates reactive oxygen species (ROS), particularly singlet oxygen. Damage caused by ROS leads to necrotic and/or apoptotic cell death [1].



Novel unsymmetrical magnesium(II) 2,3-bis(1-adamantylsulfanyl)phthalocyanine **5** was prepared by mixed macrocyclization reaction of two phthalonitriles (phthalonitrile **4** and 4,5-bis(1-adamantylsulfanyl)phthalonitrile **3**) and characterized using UV-vis, MALDI MS and NMR methods. Precursor adamantyl disubstituted phthalonitrile **3** was synthesized from 4,5-dichlorophthalonitrile **1**. Absorption and emission properties of novel phthalocyanine **5** were investigated, including fluorescence quantum yields and fluorescence lifetime determinations and measurements of solvatochromic effects. The quantum yields were found to be as follows: 0.28 in DMF, 0.31 in DMSO and 0.33 in THF. Newly synthesized phthalocyanine can be considered as a potential photosensitizer, as its singlet oxygen quantum yield (Φ_{Δ}) of 0.49 was found both in DMF and DMSO.

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P-30

Liver Fraction Applications for *in vitro* ADME Studies.

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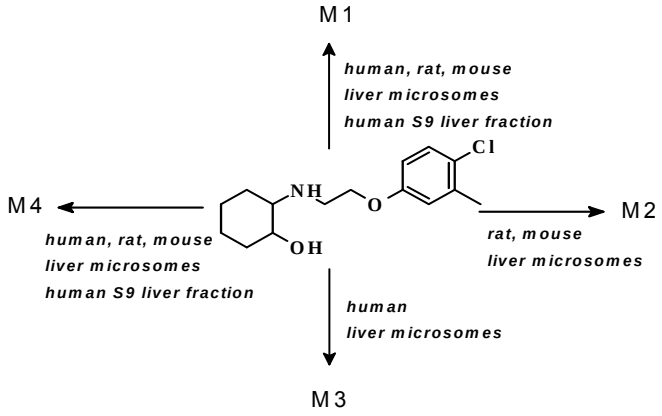
Liver is the most important site of drug metabolism in the body. Its major roles include detoxification of the systemic and portal blood as well as production and secretion of critical blood and biliary components. Approximately 60 % of marketed compounds are cleared by hepatic CYP-mediated metabolism [1].
Liver microsomes are subcellular fractions which contain membrane bound drug metabolizing enzymes. Microsomes can be used to determine the *in vitro* intrinsic clearance of a compound. The use of species-specific microsomes can be used to enable an understanding of interspecies differences in drug metabolism and choose for the preclinical studies the animal with the most similar metabolism of a tested compound to human metabolism. To minimize the effect of interindividual variability microsomes are pooled from multiple donors [2,3].
Liver S9 fraction have been used since 1970s, but not as extensively as microsomes. In contrast to microsomes, S9 fraction contains both microsomal and cytosolic fractions and offer more complete representation of the metabolic profile because they contain both phase I and phase II activities. On the other hand, it contains lower enzyme activity when compared to microsomes [2,3].
The aim of the present study was to analyze the *in vitro* biotransformation of the antiepileptic agent – KP 16. This compound was found to have high anticonvulsant activity against MES test. Human, rat and mouse hepatic microsomal fraction in the presence of an NADPH-generating system as well as human hepatic S9 fraction in the presence of an NADPH were used. M1 and M4 were the metabolites formed in every model. M2 was formed only in mouse and rat liver microsomes assay and M3 only in human liver microsomes model. In human liver S9 fraction assay M1 and M4 were produced.
The analysis of LC-MS/MS spectra allowed us to propose the biotransformation path for KP 16, which is based on oxidation and hydroxylation reaction.



This work was supported by the Jagiellonian University-Medical College (grant No. K/ZDS/001296).

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P-31

New Pyrazole Derivatives with Potential Biological Activity: Theoretical Studies on Complexation of Small-molecule Ligands.

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A series of indazole derivatives **5** was obtained through a few-step synthesis involving aromatic nucleophilic substitution or *vicarious nucleophilic substitution of hydrogen* (VNS), selective reduction of nitro derivatives **3** to amines **4**, and diazotization-cyclization to fused pyrazole products **5** (**Scheme 1.**).[1] The use of Raney nickel together with hydrazine hydrate as a source of molecular hydrogen allowed for considerable shortening of the hydrogenation time. As the resulted amines **4** were usually insoluble in water, the diazotization was carried out in solid phase using the microwave method (MAOS). The preliminary biological tests showed that some of the final products **5** had cytotoxic activity in cell-based bioassays.[2] The cytotoxicity mechanism may be connected with the compounds ability either to complex metal ions present at the active sites of proteins or to bond with the DNA base pairs. Thus, the simulation of complexation with magnesium ions was performed for compound **5b** (**Fig. 1.**). Moreover, the theoretical calculations (long range DFT- B3LYP/6-31G(d,p), basis set superposition error – BSSE - calculation, NBO 5.0, and QTAIM methods), using the *Gaussian G09* program, as well as *GENNBO5.0*, *AIMAII*, and *AOMix* packages, showed the ability of compound **5b** to form strong and weak hydrogen bonds with cytosine (**Fig. 2.**) and guanine (**Fig. 3.**).

Scheme 1.

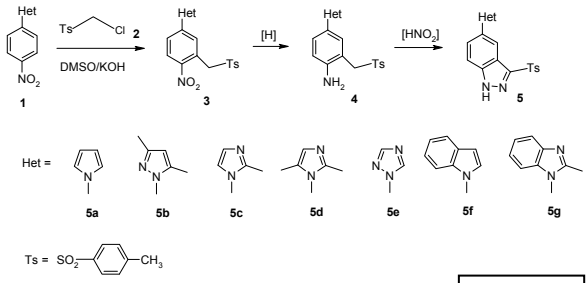


Fig.1.

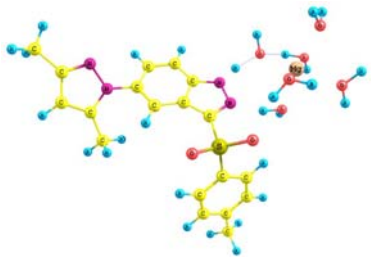


Fig.2.

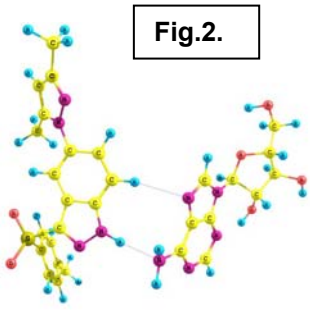
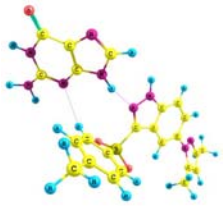


Fig.3.



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Theoretical calculations were supported by PCSS grant No. **92/2011**, polish National Science Centre (NCN) grant No. N N401 642340, and EU scholarship for PhD students (Poznan, WUP, Task 8.2.2. PO KL 2011-2012).

P-32

The Novel Approach in Structure-Based 3D Pharmacophore Model Generation. An Application to Searching for 5-HT$_6$R Selectivity Hypothesis.

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Herein, we present the new strategy in structure-based 3D pharmacophore model generation based on docking of known ligands, and further ligand-receptor complexes analysis using structural interaction fingerprints (SIFts) [1]. To explore the binding site hot-spot amino acids, the cross-docking (Glide SP mode) of the set of around 300 known (structurally diversified) 5-HT$_6$R ligands to a set of homology models was performed. The structure of actives were extracted from ChEMBL 14 database [2] using activity threshold Ki < 300 nM. Parallel, the docked ligand conformations were mapped to a set of pharmacophore features (HBA, HBD, PI, HYD and AR) creating a comprehensive map of spatial distribution of various pharmacophore points in the binding site. The pharmacophore features of the same kind were then clustered, taking distances between all pairs of centroids as a classification criterion. The final pharmacophore map was created from the averaged cluster centroid points, but only those matching crucial amino acids indicated by a parallel SIFts analysis of ligand-receptor complexes. Combinations of three-, four- and five-features were next used to generate a set of pharmacophore hypotheses (Screen Library Protocol, Discovery Studio 2.5).
They were next used to search the best combination of models, by optimization (maximization) of selectivity coefficient between some serotonin receptor subtypes, i.e. 5-HT$_6$/5-HT$_7$ and 5-HT$_6$/5-HT$_{1A}$. For this purpose, the set containing molecules with determined dual affinity values were extracted from ChEMBL 14 database and used to search and test the final pharmacophore combination. To assess the capabilities of proposed algorithm, the comparison between results obtained by optimized combination and existing ligand-based 5-HT$_6$R pharmacophore models [4] will be presented.

Acknowledgements:
This study is supported by project UDA-POIG.01.03.01-12-063/09-00 "Antagonists of 5-HT$_6$ receptor as advanced antipsychotic drugs with pro-cognitive properties" co-financed by European Union from the European Fund of Regional Development (EFRD).

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P-33

Development of Multistep Ligand-Based Virtual Screening Cascade Methodology in a Search for Novel HIV-1 Integrase Inhibitors: 1. Machine Learning.

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HIV integrase which is essential in the virus replication cycle and has no homologue among human enzymes [1], became an important target for drug development more than twenty years ago. Nevertheless, progress has been hampered by the lack of assays suitable for high throughput screening. Thus, a real breakthrough was only observed in 2007 with the introduction of the first integrase inhibitor, raltegravir, into treatment.
Crystal structure for HIV-1 integrase is already known and thus, both techniques commonly used in VS campaigns (structure and ligand-based) could be developed. Here we introduced a multistep ligand-based screening cascade because it is suggested that ligand-based methods outperform structure-based in true positives identification [2]. Our strategy consists of two sequential modules: machine learning-based (ML-based) and privileged fragments-based (PF-based).
ML algorithms could successfully enrich ligand-based VS methodologies. The most common practical ML usage is to classify or prioritize databases of molecules in respect of biological activity or specific ADMET properties. Thus classification can be performed in two different manners: as binary choices for data dividing into two categories or numerical predicting of certain property values. Unsupervised learning tasks are common practice to partition entire dataset in case of difficult or undesired class pre-assignment. Conversely in supervised approaches which require class assignment to carry out a training process. We conducted a comprehensive set of experiments to assess the performance of the various ML methods, e.g. naïve Bayesian classification, nearest neighbors, support vector machines (SVM), random forest and few other less popular algorithms in case of HIV-1 integrase inhibitors classification. Different active (positive instances) to inactive (negative instances) compound ratio was determined and distinct inactivity assumption was made to construct thirteen unique data sets. Compounds were represented using Klekota-Roth fingerprints, which use 4096 SMARTS patterns [3]. Subsequently binary classifiers were trained on each and every composed data set. The performances of the different methods were evaluated via external test sets, which were derived from initial compound ensembles using four different protocols: diverse, populated and random selection and also by implementing LSCO (leave several clusters out) approach. Structures and inhibition data were extracted from ChEMBL [4] database. Our results showed that SVM-based methods, e.g. sequential minimal optimization (SMO) training algorithm, outperformed the other binary ML classifiers.

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This poster presents only first part of a project description and is continued in poster entitled “Developed of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 2. Privileged fragments”.
Agata Kurczyk acknowledges a scholarship from the UPGOW project co-financed by the European Social Fund.

P-34

The Study of H_3 Histamine Receptor Ligand DL77 Metabolism Using Recombinant Isoforms of Cytochrome P450.

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The study of xenobiotic metabolism is a very important part of tests being carried out before distributing the drug to the pharmaceutical market. The use of *in vitro* models for simulating the mammalian metabolism of many molecules of pharmacological importance offers many advantages compared to *in vivo* methods. *In vitro* studies has become a complementary tool to identify metabolites or species-specific metabolic routes and are reliable and efficient alternative to expensive experiments with animals. *In vitro* methods reduce also the number of animal used in the experiments what is very important concerning ethics of animal investigation. Moreover*, in vitro* testing for the role of CYPs in the metabolism of a drug candidate by using recombinant isoforms of cytochrome P450 is now a standard practice in drug discovery and development.
DL77 is an active antagonist of histamine H_3 receptor (ED_{50} = 2,1 ± 0,2 mg/kg per os) with very high affinity (CHO (hH_3R) K_i = 8,4 ± 1,3 nM) and may be useful in treating nervous system diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy and ADHD [1]. So far, we used the fungi *Cunninghamella sp. as an* microbiological model to determine the probable direction of phase I metabolism of compound DL77 in the human body. With the application of these fungi, there were obtained the series of metabolites of DL77 with determined (by LC/MS technique) four different molar masses [2].
The aim of this work was to predict the metabolism of DL 77 using recombinant isoforms 3A4 and 2B6 of cytochrome P450 *in vitro.* The LC/MS analysis of the reaction mixtures confirmed significant differences between used recombinant isoforms of cytochrome P450 and *Cunninghamella* microbiological model and showed the presence of only one metabolite. However, these fungi were reported to metabolize a variety of xenobiotics in the ways that are similar to those in mammalian enzyme system. In the case of DL77 our results showed, that it cannot be considered as an alternative method of biotransformation *in vitro* carried by human isoforms 3A4 and 2B6
Additionally, a computer simulation of metabolism was carried out, with use of MetabolExpert program, for finding possible structures of obtained metabolites after biotransformation.

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Partly supported by grant: K/ZDS/003325

P-35

Chemopreventive Properties of 3-Ethyl-3-methyl Succinic Imide Derivatives.

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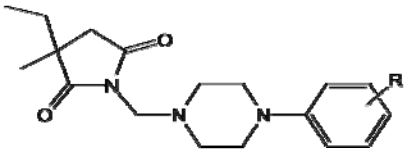
Studies conducted in different researches groups proved that the key molecular event in the process of chemical carcinogenesis is the interaction of the compound with the DNA. Different genetic changes can lead to every possible type of mutation. Thus, mutagenicity assessment plays a crucial role in the safety testing of substances having potential as chemotherapeutic agents. The preliminary in vitro mutagenicity assays present useful screening tools for setting priorities as they are quick and inexpensive way to help to eliminate potentially mutagenic compounds in an early stage of the development process. On the other hand compounds with antimutagenic potential be useful in preventing tumors from being induced or promoted [1].
The present study investigated mutatagenicity and antimutagenicity of seven the 3-ethyl-3-methyl succinic imide derivatives (1-7) which were synthesized and evaluated for their anticonvulsant activity.



Compounds 1-7

Mutagenicity was tested with *Vibrio harveyi* assay employing four *V. harveyi* strains: BB7 (natural isolate), BB7M, BB7X and BB7XM, which are genetic modifications [1]. 4-nitroquinoline-*N*-oxide (NQNO) served as positive controls and references mutagen. Additionally, antimutagenic activity of tested compounds was evaluated using antimutagenicity *V. harveyi* assay procedure [2].
The results of this study showed that compounds 1-7 were non-mutagenic to all the test *V. harveyi* strains. Generally, cpds.1-7 demonstrated relatively strong antimutagenic activity against three strains: BB7, BB7M and BB7X, with exception of compounds 1, 4, 5 and 7, which revealed a moderate inhibition against BB7X strain. The antimutagenic effect of derivatives 1-7 was not observed only against the BB7XM strain. Moreover, most of them have an antimutagenic profile of activity. In fact, antimutagenic studies may be a useful tool in screening for substances which can be used as chemopreventive agents. Thus, antimutagenic substances, like compounds 1-7, might potentially inhibit, delay, or reverse carcinogenesis.

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The project was supported from the Jagiellonian University, Medical College (grant no. K/ZDS/001296) and partially by grant NCN no. N N405298536.

P-36

Physical-, Photochemical Properties and Biological Activity of Novel Porphyrazine Modified with Nitroimidazolylbutylsulfanyl Substituents.

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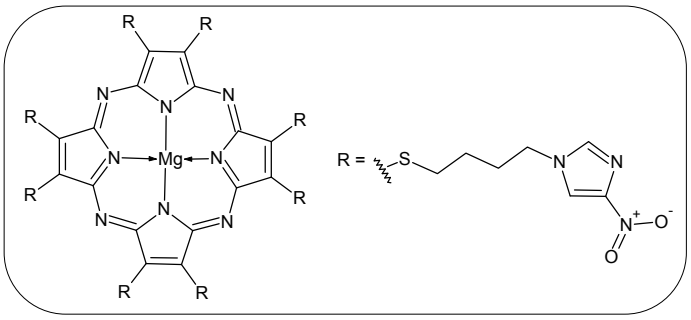
Porphyrazines are macrocyclic, aromatic compounds possessing numerous potential applications in medicine, especially in photodynamic therapy of cancer (PDT) and in photodynamic antimicrobial chemotherapy (PACT) that may represent an alternative treatment for drug resistant organisms [1]. Especially promising are porphyrazines possessing peripheral sulfur atoms in their periphery. Introduction of this heteroatom into macrocyclic periphery proved to adjust desired properties such as improved solubility and singlet oxygen generation ability [2,3]. Nitroimidazoles are compounds well known for possessing anticancer activity in deep hypoxic conditions [4].



Novel, symmetrical magnesium porphyrazine (MgPz) endowed with 4-nitroimidazolylbutylsulfanyl substituents was synthesized, exhaustively characterized using UV-Vis, IR, MS MALDI and various NMR techniques. Finally, it was subjected to advanced photochemical and biological studies.
Potential photosensitizing activity of MgPz was evaluated by measuring its ability to generate singlet oxygen in DMF and DMSO. Additionally, the tendency of this macrocycle to aggregate was analyzed using UV-Vis methods in both DMSO and DMF. Computational methods based on the Density Functional Theory implemented in Gaussian 09 software were employed. IR spectrum of MgPz was predicted and compared with empirical data. Porphyrazine was also examined *in vitro* towards prostate cancer cells PC3 (human prostate adenocarcinoma), LNCaP (human prostate carcinoma) and exhibited moderate anticancer activity.

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The authors acknowledge financial support for the project from the Polish Ministry of Science and Higher Education - grant N401 067238.

P-37

Review of the Latest Achievements in Pharmaceutical Formulations of Photosensitizers Applied in Photodynamic Therapy.

Łukasz Lijewski [1], *Dominik Langer* [1], *Sebastian Lijewski* [1], *Michał Klimont* [1], *Paulina Kordas* [1], *Wojciech Szczołko* [1], *Ágota Kovács* [2], *Tomasz Gośliński* [1]

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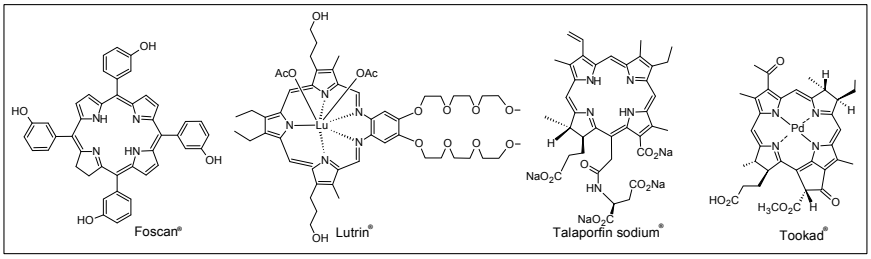
Photodynamic therapy (PDT) is a novel medical approach to treatment of cancer diseases such as neoplasms and dermatological lesions. In PDT photosensitizing agent is administered into the tumor tissue and then activated with light of an appropriate wavelength. Further, energy transfer from a photosensitizer in its excited triplet state to the molecular oxygen generates reactive oxygen species including singlet oxygen causing damage to cancer cells. The cell death occurs rather by apoptosis than necrosis [1].



Nowadays, the most widely used photosensitizer in clinical PDT is Photofrin® which consists of about 60 compounds obtained by chemical processing of hematoporphyrin. 5-Aminolevulinic acid (ALA), benzoporphyrin derivative (BPD), lutetium texaphyrin, temoporfin (mTHPC), tinethyletiopurpurin (SnET2), talaporfin sodium (LS11), Foscan (mTHPC) and Tookad® are other photosensitizers of intensive usage in preclinical and clinical trials [2].
In order to enhance usefulness of photosensitizers in PDT and eliminate their flaws, various formulation approaches have been considered. Solubility of lipophilic photosensitizers can be improved by applying micellar formulations. Their bioavailability may be increased by encapsulation into nanoemulsions of higher selectivity towards breast cancer. Also liposomes have been widely used as carriers for photosensitizers. The liposomal formulation of Foscan (Foslip®) possesses higher efficacy, reduced damage of healthy tissue and toxicity in the absence of light as compared to active pharmaceutical ingredient. Similarly, another liposomal drug verteporfin (Visudyne®) was the first photodynamic drug approved for treatment of age-related macular degeneration (AMD) [3-6].
To date some formulations have been patented and approved for medical treatment, such as Levulan® Kerastick (aminolevulinic acid, DUSA Pharmaceuticals, Tarrytown, NY, USA), Metvix® (aminolevulinic acid methyl ester, Photocure ASA, Oslo, Norway), Hexvix® (aminolevulinic acid hexyl ester, Photocure ASA, Oslo, Norway), Visudyne® (verteporfin, Novartis Corporation, NY, USA), Photofrin® (porfimer sodium, QLT Inc, Vancouver Canada) and Foscan® (temoporfin, Biolitec Pharma, Dublin, Ireland) [7].
Moreover, the nanotechnology achievements seem to affect the development of modern PDT formulations in recent years [8]. It was found that the bioavailability of nanoformulations is high due to the permeability and retention effect (EPR) [9].

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The authors acknowledge financial support for the project from the Polish Ministry of Science and Higher Education - grant N401 067238.

P-38

Synthesis of Novel Cyclic Analogs of γ-Aminobutyric Acid as Potential Anticonvulsant Agents.

Barbara Malawska, Katarzyna Kulig, Katarzyna Szkudlarek, Aleksandra Rak, Maria Daruk

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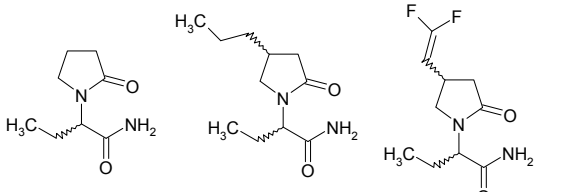
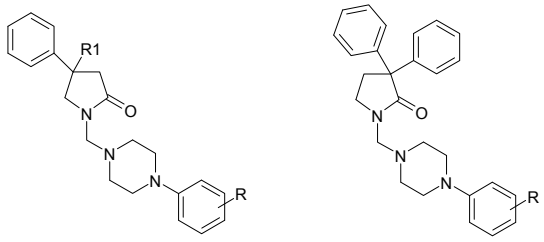
Recently, many studies were carried out for the development of new types of anticonvulsant agents, including γ-aminobutyric acid (GABA) related compounds, and structurally modify compounds of currently used drugs [1]. In the search for new anticonvulsants we undertook a study of pyrrolidin-2-one derivatives a cyclic analogues of GABA. It has been proved that GABA analogues as well as its cyclic derivatives, both influence the gabaergic transmission, which plays a pivotal role in physiological and pathological processes in central nervous system, including epilepsy. In the recent years levetiracetam, derivative of pyrrolodin-2-one has been approved as the antiepileptic drug and other related structures are in the clinical development (brivaracetam, seletracetam).



LEVETIRACETAM BRIVARACETAM SELETRACETAM

This work presents synthesis of several series of 3,3- or 4,4-substituted pyrrolidnin-2-ones with *N*-arylpiperazine-methyl substituent and their pharmacological evaluation.



R = F, Cl, CH_3, O-alkil, R1 = H, Ph

Preliminary anticonvulsant *in vivo* tests of these compounds, i.e. the maximal electroshock test, the subcutaneous metrazole induced seizures and the rotarod neurotoxicity assay in mice were employed.

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Acknowlegements:
Pharmacological dates were provided through the *Antiepileptic Drug Development Program, Epilepsy Branch, the National Institute of Neurological Disorders and Stroke*, Rockville, Maryland USA.
The study was supported by the Jagiellonian University Collegium Medicum Research Programme (K/ZDS/001918).

P-39

Determination of the Lipophilicity Parameters logk_w and log*P* of Azinesulfonamides by Reversed-Phase High Performance Chromatography.

Krzysztof Marciniec [1], *Jolanta Bafeltowska* [2], *Stanisław Boryczka* [1], *Ewa Buszman* [2]

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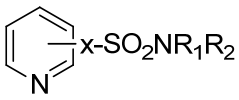
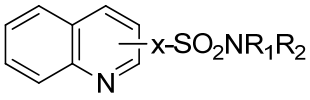
Compounds containing an azinesulfamoyl moiety are of considerable interest since they exibit diverse biological activities with numerous therapeutic applications. They have been shown to inhibit several enzymes as well as modulate the activity of many receptors i.e. Carbonic Anhydrase (CA) [1] or serotonin receptors (5-HT) [2].
The lipophilicity of drugs plays an important role in their biological action. This property determines mainly the fate of drug in the body governing the absorption, distribution, storage and elimination processes. Moreover, lipophilicity is a very important molecular property used in QSAR studies and plays a crucial role in the design of drugs with required biological activity.



1: x = 2, 3, 4;
$R_1 = R_2 = H$;
$R_1 = H$, $R_2 = Me$;
$R_1 = R_2 = Me$



2: x = 2, 3, 4, 5, 6, 7, 8;
$R_1 = R_2 = H$;
$R_1 = H$, $R_2 = Me$;
$R_1 = R_2 = Me$

The aim of this work is to determine the lipophilicity parameters (logk_w and logP_{HPLC}) of positional isomers of pyridine- a quinolinesulfonamides **1** and **2** by the RP HPLC method, to discuss the influence of the substituents and the ring systems on the lipophilicity and compare with the data obtained from computational programs.

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P-40

Role of Chemical Modifications within Aromatic Hydantoins for Their Abilities to Inhibit Multidrug Resistance Mechanisms in Selected Gram-Positive and Gram-Negative Bacteria.

Anna Matys [1] , Ewa Otrębska [1], B. Mastek [1], Jakub Mazurkiewicz [1], Jadwiga Handzlik [1], Ewa Szymańska [1] , Sandrine Alibert-Franco [2], Jean-Marie Pagés [2], Katarzyna Kieć-Kononowicz [1]

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Nowadays, bacteria very often become resistant to more than one class of antibiotics. One of the reasons why multidrug resistant bacteria (MDR) develop antibiotic resistance and why therapy fails is the overexpression of efflux pumps that are responsible for extruding toxic substances including antibiotics outside the bacterial cell [1]. These specific efflux systems have been identified both in Gram- positive and Gram- negative bacteria. One of the methods to combat MDR strains is efflux inhibition using efflux pumps inhibitors (EPIs). Our recent studies show that hydantoins have the ability to inhibit the efflux pump of cancer cells [2] as well as efflux pump systems in strains of Gram- positive and Gram- negative bacteria [3].
The aim of the study was to evaluate a series of 10 compounds for their antibacterial activity and their influence on minimal inhibitory concentration (MIC) value of antibiotics using broth microdilution method. The tested compounds were the derivatives of arylideneimidazolone, 5-arylidenohydantoin and 5-arylhydantoin. As far as Gram-positive bacteria are concerned, the compounds were tested using the clinical strain of *Staphylococcus aureus* MRSA HEMSA 5 overexpressing efflux pumps and the reference strain *Staphylococcus aureus* ATTC 25923. In the case of Gram-negative bacteria two strains of *Enterobacter aerogenes* were used: EA289 - a kanamycin-sensitive derivative of EA27 (an MDR clinical isolate) with the overexpression of the AcrAB-TolC efflux pump and EA294 - a kanamycin resistant derivative of EA289 without the efflux system [4].
The only compound that reduced resistance to oxacillin of the *S. aureus* HEMSA 5 strain was the compound A10 (with p-chlorobenzylidene substituent at position 5 and free piperazine substituent at position 3 joined with the hydantoin ring by 2- hydroxylpropyl linker), which reduced oxacillin resistance over 128-fold. In case of *S. aureus* ATCC 25923, it reduced the MIC of oxacillin only 2-fold. This suggests that the compound A10 may have a potential for therapy of an MRSA infection. In case of *E. aerogenes* three derivatives of arylideneimidazolone (BM-7b, BM-33, BM-34) reduced chloramphenicol resistance of the strain EA289. No activity was observed for strain EA294. This suggests that these compounds could possess EPIs properties. The A10 and JM-1, JM-2, JM-3 compounds were able to slightly reduce chloramphenicol resistance of the two tested strains of *E. aerogenes* so they could act by other inhibitory mechanism as the EA294 strain is deprived of AcrAB but possesses other pumps that could be targeted by the hydantoin derivatives.

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The work was partly supported by grants: 501/N-COST/2009/0 (K/PMN/000031), COST action BM0701 and Polonium (K/PMN/000042) and K/ZDS/003323.

P-41

Amine Derivatives of Hydantoins as Novel Inhibitors of Drug-Efflux System in Antibiotic Resistant Bacteria.

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Despite the development of safe and potent antibiotics serious bacterial infections remain a priority. An increasing concern is the emergence of multi-drug-resistant (MDR) bacteria and their role as opportunistic pathogens. Due to uncontrolled, inappropriate and massive use of antibiotics and chemotherapeutics, the number of drug-resistant strains steadily increases, limiting the effective treatment of many bacterial infections including tuberculosis, as well as fungal diseases or cancer.
Microbial efflux pumps play a key role in MDR strains. The Gram- negative bacteria have evolved specialized tripartite AcrAB-TolC and MexAB-OprM efflux systems that transport molecules from the cytoplasm to the extracellular environment in energy-dependent processes. One of the strategies to combat MDR is blocking the efflux mechanism of bacterial cell by inhibitors [1]. The development of efflux pumps inhibitors (EPIs) could extend the useful lifetime of many antibiotics by improving therapeutic efficacy and by suppressing the emergence of resistant variants that might otherwise arise during treatment. Research carried out in recent years have brought several groups of promising EPIs, like reserpine, verapamil, gemfibrozil, cyclosporine A or PAβN [1].
The present work is a continuation of earlier studies on search for bacterial efflux pumps inhibitors, performed by our group in the Department of Technology and Biotechnology of Drugs CM UJ [2]. Based on the previously obtained results and using structural analogies to the known potent EPI, PAβN, we designed and synthesized a series of compounds belonging to the group of aromatic derivatives of hydantoin. Modelling the structure of PAβN some structural modifications of the hydantoin were performed, namely α- and β-naphthyl fragments were incorporated into 5-position heterocycle ring, while alkylamine chains were introduced as N3-substituents.
The chemical synthesis of compounds consisted of three steps, involving successively: Bucherer-Berg reaction, two-phase N3-alkylation and the Gabriel synthesis. Four final amines were successfully obtained and characterized.
Final compounds were examined for their intrinsic antibacterial activity and the impact of minimum inhibitory concentration for bacterial growth (MIC) to antibiotics such as nalidixic acid, sparfloxacin, chloramphenicol, doxycycline, erythromycin. These tests were conducted on five strains of Gram negative pathogen *E. aerogenes* within the different mechanisms of drug resistance. The greatest activity showed a compound with β-naphthyl at position 5. It reduced the MIC of antibiotics from 4 to 32 times in strains overexpressing AcrAB-TolC pump. Discussion of the results will be presented in details.

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The work was partly supported by the grant Polonium 757/N-Polonium 2010.

P-42

Synthesis And Physicochemical Properties Of New Urea Prodrugs For Melanocyte-Directed Enzyme Prodrug Therapy

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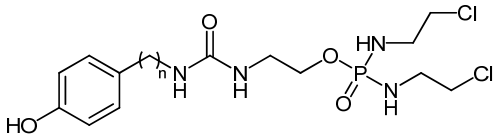
Melanocyte-directed enzyme prodrug therapy (MDEPT) has been proposed as a selective strategy for the treatment of malignant melanoma [1]. This therapy takes advantage of the unique occurrence of over expression of the enzyme tyrosinase gene in melanocytes and melanoma cells [2]. When melanocytes become malignant, the genes responsible for expression of tyrosinase upregulate causing a significant increase in the level of tyrosinase within the cancer cells [3]. Melanoma tissue have a sufficiently high concentration of this enzyme to activate prodrugs. Using tyrosinase as a prodrug-activating enzyme, allowing cancerous cells to locally activate prodrugs, raising anticancer activity without a corresponding increase in overall toxicity and minimization of side effects. There is an assumption that the best substrates for tyrosinase are phenol derivatives, which can be activated by several mechanisms leading to the formation of active form of quinine [4].
The problem to be solved is to design prodrugs that are not undergone activation by cytochrome P450 in the liver to the activation of tyrosinase in melanoma. According to recent studies DNA alkylating agents are promising in the cancer chemotherapy. Among many of the agents isophosphoramide mustard (iPAM) was selected, which is active, cytotoxic metabolite of ifosfamide (IF), a widely used anticancer alkilating drug [5].
Our reaserch concentrated on the synthesis, the selected chemical properties, stability, antitumor activity and toxicity of new compounds, potential prodrug for MDEPT therapy. The aim of research was to find a potential prodrug, which is minimally metabolized by liver enzymes, and eventually will be able to generate reactive quinone metabolites in melanoma cells due to bioactivation by tyrosinase. We designed and synthesized such prodrugs, which contain a unit sensitive to the activation of tyrosinase coupled with isophosphoramide mustard, a DNA alkylating agent, by means of urea group:



n = 0, 2

Prodrugs should have a good stability under phisiological conditions. Solubility and hydrolytical stability of our potential prodrugs was examined. Further studies on the susceptibility to activation of tyrosinase of the obtained compounds are in progress.

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P-43

Synthesis and Biological Evaluation of new Sulfonamides Derivatives of Pyrazolo[4,3-*e*][1,2,4]triazine.

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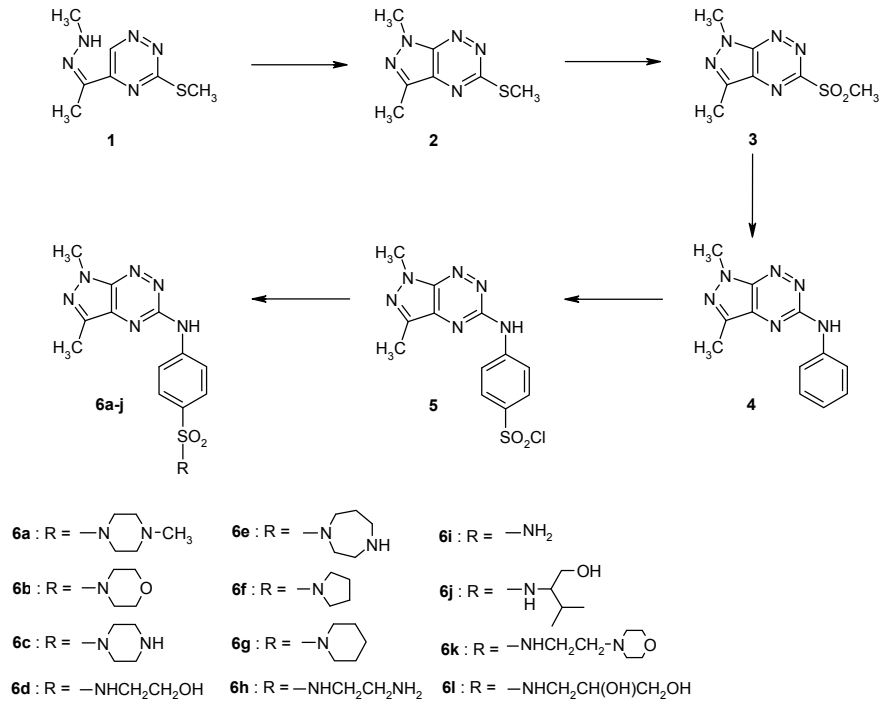
A new series of sulfonamide derivatives of pyrazolo[4,3-*e*][1,2,4]triazine have been synthesized, characterized and evaluated for their biological activity. Some of the new sulfonamides were tested for their in vitro antibacterial activity against the reference strains of aerobic Gram-positive and Gram-negative bacteria. All of these compounds were evaluated for their *in vitro* cytotoxic activity against MDA-MB-231 and MCF-7 human breast cancer cells. The synthesis pathway leading to the title compounds is depicted in scheme.



The preliminary antitumor studies revealed that these agents exhibited moderate antitumor activity in breast cancer cells.

Acknowledgment
This research was supported by Grant No. NN405 092340 from the National Science Centre, Poland.

P-44

New Dipirydothiazine Derivatives – Potential Inhibitors of Dopaminergic and Serotoninergic Receptors.

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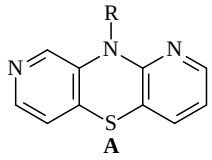
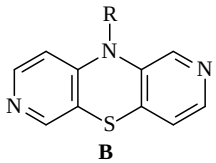
Phenothiazines belong to the oldest, synthetic antipsychotic drugs, which do not have their precursor in the world of natural compounds. They are used as neuroleptics, interact with various receptors in CNS, especially strongly block the dopaminergic receptors. Phenothiazines also inhibit other receptors on neurons in CNS including serotonin, histamine, □-adrenergic or GABA-ergic receptors, however the affinity for dopaminergic receptors is the strongest [1].
In our search we modified the phenothiazine structure with the pyridine rings to form new diazaphenotiazines being 10*H*-1,8-diazaphenothiazine **A** [2] and 10*H*-2,7-diazaphenothiazines **B** [3]. We transformed these compounds to the 10-substituted derivatives possessing dialkylaminoalkyl substituents **1A,B** - **7A,B.**

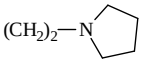


A

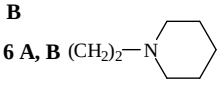


B

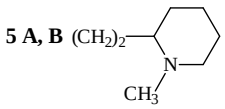
1 A, B $CH_2CH_2CH_2N(CH_3)_2$,
2 A, B $CH_2CH_2N(C_2H_5)_2$,
3 A, B $CH_2CH(CH_3)CH_2N(CH_3)_2$,



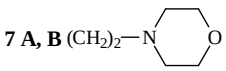
4 A,B



6 A, B



5 A, B



7 A, B

The synthesized compounds **1A,B** - **7A,B** were *in vitro* screened towards monoaminoergic receptors (D_2, 5-HT_{1A}, 5-HT_6, 5-HT_7). The compounds showed lower activity than the neuroleptic phenothiazines: promazine and thioridazine.
It seems that affinity for the monoaminoergic receptors is depended on the conformation of the diazaphenothiazines structures.

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Acknowledgement: The Synthesis were financially supported by The Medical University of Silesia (grant KNW-1-073/P/1/0), Radioligand binding experiments were financially supported by the Norwegian Financial Mechanism as part of the Polish–Norwegian Research Fund, Grant No. PNRF–103–AI-1/07

P-45

Aza- and Diazaphenothiazines Derivatives with Antioxidant Activity.

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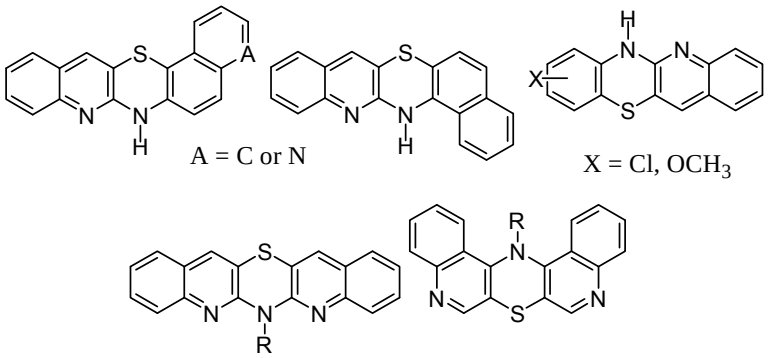
Over the last 10 years selected phenothiazines have been demonstrated to exert a wide range of activities, such as anticancer, antibacterial, antiprotozoic, antiviral, antiprionic and multidrug resistance reversal activity [1]. Phenothiazines can interfere with a variety of cellular processes and potentially with other receptors and channels apart from the dopamine D_2 receptor, their known molecular target as psycotropic drugs [2]. The potency of this class of molecules may vary upon the level of substitution. The nature of substitution has profound influence on the pharmacological actions of these drugs perhaps by modifying their receptor specificity [3].
Bearing in mind the potent antioxidant activity of "classical" phenothiazines, we have previously synthesized a series of 2,7-diazaphenothiazines and have shown them to possess interesting antioxidant activity correlating to some degree with their lipophilicity [4]. Extending this research, we hereby synthesized 11 tetra- and pentacyclic compounds, being aza- or diazaphenothiazine derivatives with various lipophilic substituents in the benzene rings or at the nitrogen atom in the thiazine ring.



Most compounds exhibited a very significant antioxidant activity with IC_{50} values between 1 and 26 μM. Few compounds were inactive. Activity seems to depend on the nature of substitution of the phenothiazine derivative. The theoretically calculated conformation, molecular shape and volume as well as lipophilicity of these compounds were evaluated in relation to their exhibited antioxidant activity.

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P-46

Synthesis and Anticonvulsant Activity of New N-benzyl-3,3-disubstituted-pyrrolidine-2,5-diones.

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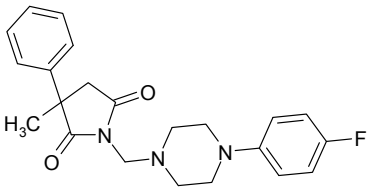
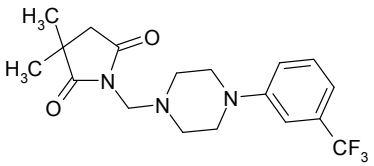
Previous research from our laboratory identified differently substituted pyrrolidine-2,5-diones as targets for new antiepileptic drugs [1-3]. Many of these compounds were effective especially in the maximal electroshock (MES) test, which is known as animal model of human generalized tonic-clonic seizures. The structure - activity relationship (SAR) studies showed high anticonvulsant protection among
N-Mannich bases derived from 3-phenyl-3-metyhyl- and 3,3-dimethyl-pyrrolidine-2,5-diones containing as an amine function 4-phenylpiperazines with electron-withdrawing substituents. The structures of the most active compounds obtained in earlier studies are shown in **Fig**. **1**.



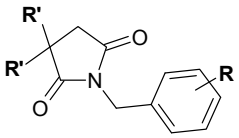
ED$_{50}$ = 7.78 mg/kg (MES, *p.o.* rats)



ED$_{50}$ = 30.0 mg/kg (MES, *p.o.* rats)

Fig. **1**.

As a continuation of systematic structure - anticonvulsant activity analysis in the current studies we have synthesized two series of compounds with 3-phenyl-3-metyhyl- and 3,3-dimethyl-pyrrolidine-2,5-diones as core fragments. These molecules have been designed as analogs of model compounds mentioned above in which 4-phenylpiperazine function have been replaced into benzylamines with electro-withdrawing substituents such as chlorine, fluorine atoms or trifluoromethyl group. The structures of target compounds are shown in **Fig**. **2**.



Compounds **1-16**

R' = CH_3, CH_3
R' = phenyl, CH_3
R = H, F, Cl, CF_3

Fig. **2**.

All the compounds synthesized have been evaluated for anticonvulsant and neurotoxic properties within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, using procedures described elsewhere [4].

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P-47

Antimicrobial Activity of Some Derivatives of 1,2,4-Triazoline-5-thione.

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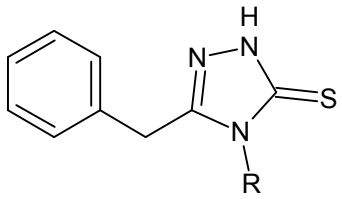
One of the important group in the family of heterocyclic compounds are 1,2,4-triazoles and its derivatives. Compounds containing these structural features have been found to display a wide spectrum of biological activities. Some of them were found to possess anti-inflammatory, antidepressant, antiviral, antifungal and antimicrobial properties. Certain compounds have shown anticonvulsant and antitumour activity.
We present here antimicrobial activity of some derivatives of 3-benzyl-4-substituted-1,2,4-triazoline-5-thione.



1 - 8

R = C_6H_5, 2-$CH_3C_6H_4$, 4-$CH_3C_6H_4$, 2-BrC_6H_4, 4-BrC_6H_4, 2-FC_6H_4, 4-FC_6H_4, C_2H_5

Compounds 1 – 8 were screened for thair *in vitro* antimicrobial potency against a panel of Gram-positive strains (*Staphylococcus aureus* NTCT 4163, *Staphylococcus aureus* 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* 29213, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Micrococcus luteus* ATCC 9341, *Micrococcus luteus* ATCC 10240 and Gram-negative strains (*Escherichia hirae* ATCC 10541, *Escherichia feacalis* ATCC 29212). The assessment of antimicrobial action was performed using the disc-diffusion method (GIZ) and minimal inhibitory concentration (MIC). Minimum inhibitory concentration (MICs) were defined as the lowest concentration of the compounds that inhibited visible growth of microorganism after 18h incubation at 35 °C.
According to our preliminary results among the tested agents only 3-benzyl-(4-bromophenyl)-1,2,4-triazoline-5-thione showed activity against Gram-positive bacteria. The range of value change from 400 to 50 µg/mL. Summing up, 3-benzyl-(4-bromophenyl)-1,2,4-triazoline-5-thione (**5**) may be value for searching new derivatives showing atnimicrococcus activity.

P-48

Antimycobacterial activity of new *N*-(substitutedthioureido)aminobicyclo dicarboximide and 3,4-disubstituted 1,2,4-triazolino-5-thione

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Tuberculosis (TB) is a serious health problem all over the world. This is infections disease caused by mycobacteria usually *Mycobacterium tuberculosis*. Most importantly, one third of people population is infected with latent infection. Moreover, TB is one of the major AIDS-associated infections. According to information from the World Health Organization (WHO), in 2009, 1.7 million people died from TB. During recent years there are new alarming cases of TB caused by multi-drug-resistant strains (MDR). MDR-TB must be treated with second-line drug for a long time, because there are less effective.
Therefore, one possible solution of this problem is to search and synthesize novel antitubercular agents.
Here, we present antituberculosis activity of some novel thiosemicarbazides and 1,2,4-triazoles too. New compounds we screened for their antimycobacterial activity *in vitro* against two strains of mycobacteria: *Mycobacterium smegmatis* and *Mycobacterium* H_{37}*Ra*. Antituberculosis activity of the synthesized compounds were screened using the broth dilution method for determination of the Minimum Inhibitory Concentration (MIC) to inhibit the growth of microorganisms. The MIC was defined as the lowest concentration which inhibited the growth of microorganisms judged by lack of turbidity in the tube.
Based on the preliminary results among the tested agents only two compounds showed moderate activity against the tested mycobacteria. MIC's values of these compounds ranging from 256-512 μg/mL. The antibiotic Streptomycin was used as a standard.

P-49

Binding affinities of fenoterol derivatives to the β_2-Adrenoceptor Y308A Mutant in Relation to the Wild Type Data.

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Fenoterol is a long lasting β_2-adrenergic receptor (β_2-AR) selective agonist used in therapy as racemic mixture of (R,R')- and (S,S')-isomers. In our medicinal chemistry project we studied all four steroisomers of fenoterol and several its derivatives modified on aminoalkyl tail [1,2]. Our research showed that these compounds exhibit qualitatively different functional activities in various assays. Cardiomyocyte studies using pertussis toxin indicate that (R,R')-isomers of fenoterol, 4-methoxy-fenoterol and 4-amino-fenoterol activate the β_2-AR to a form which couples selectively to G_s protein while the receptor activated by e.g. 1-naphtyl-fenoterol or 4-methoxy-1-naphtyl-fenoterol is able to couple uniformly both G_s and G_i proteins. Molecular modeling data suggest that hydrogen bond formation between N-alkyl moiety of a ligand with Y308 residue of β_2-AR is a key interaction distinguishing G_s selective derivatives of fenoterol from others, non selective derivatives.
To test this hypothesis *in vitro*, we examined the interaction of the series of fenoterol analogs with the Y308A β_2-AR mutant. Binding affinities for the mutant were determined using membranes from stably transfected HEK-293 cells, with [^{3}H]CGP-12177 as the marker ligand. The Y308A β_2-AR shows significantly reduced affinities for G_s selective derivatives of fenoterol as compared with the wild type-related data, while affinities of non selective analogs were not affected by the mutation.
Fenoterol derivatives appeared important drug candidates for therapy of congestive heart failure or certain brain tumors. Chemical modifications can be used to switch interactions with Y308 residue what seems to be interesting strategy for development of molecules specifically triggering desired cellular downstream effects.

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[2] Jozwiak K., Woo A., Tanga M.J., Toll L., Jimenez L., Kozocas J.A., Plazinska A., Xiao R.P., Wainer I.W.: *Bioorg Med Chem.* **18** (2) (2010), 728-736.

Acknowledgement
This research was supported in part by the Intramural Research Program of the National Institutes of Health National Institute on Aging; by the National Institutes of Health National Institute on Aging [Contract N01-AG31009]; and by the Foundation for Polish Science (TEAM Programme 2009-4/5) using the equipment purchased within the Project „The equipment of innovative laboratories doing research on new medicines used in therapy of civilization and neoplastic diseases" within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

P-50

Synthesis and Biological Activity of New 1,2,4-Triazole Derivatives.

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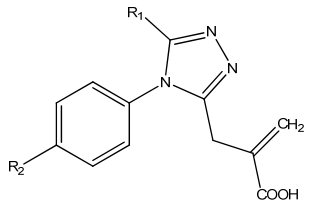
1,2,4-triazole and their derivatives belong to a class of exceptionally active compounds possessing a wide spectrum of biological properties including anti-inflammatory, antifungal, antibacterial, antiviral, analgesic, anticonvulsant and antidepressant activity. Moreover, the derivatives of carboxylic acids (acetic, propionic, benzoic) are used as non-steroidal anti-inflammatory drugs (NSAIDs).
New 1,2,4-triazole derivatives were obtained in reaction of N^3-substituted amidrazones with itaconic anhydride. Structures were confirmed by spectral and elemental analyses and X-ray crystallography.
Potential activities of new compounds were calculated with PASS (Prediction Activity Spectra for Substances) software. Results showed high probability that obtained compounds possess anti-inflammatory, antibacterial and anticoagulant activity.



R_1= -2-C_5H_4N, -4-C_5H_4N, -C_6H_5

R_2= -NO_2, -CH_3, H.

The compounds were evaluated *in vitro* against Gram-negative bacteria *Escherichia coli* strain ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Nocardia corralina*, *Mycobacterium smegmatis*, and *Sarcina lutea* and against pathogenic fungal strain *Candida albicans* to assess their antimicrobial activity. Tested derivatives showed differential activity against tested strains.
Cultured cells (human peripheral blood mononuclear cells - PBMC) were exposed to various concentrations (0.1, 1.0 and 10 μg/mL) of the test compounds for 24 hr. The toxicity of the compounds was determined using the annexin-V/propidium iodide staining procedure. The effect of these compounds on the levels of the proinflammatory cytokine IL-6 and tumor necrosis factor alfa (TNF-α) was also tested. Most derivatives showed immunomodulatory property, some of them possess promising anti-inflammatory activity. Preliminary test showed supression of mitogen-induced (PHA, anty-CD3) proliferation by two derivates.
The influence of the new compounds on clotting times (APTT, PT, TT) will be studied to assess their anticoagulant activity.

[1] Modzelewska-Banachiewicz B., Paprocka R., Mazur L., Sączewski J., Kutkowska J., Stępień D.K., Cyrański M.: *J. Mol. Struct*. **1022** (2012), 211-219.

P-51

A Simple Computational Methodology Used to Distinguish Between Agonists and Antagonists of the Estrogen Receptor.

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In the computer-aided drug design major issue during the early stages of the search for new ligands of the receptor is to determine whether a compound is a potential agonist or antagonist. This is particularly important when there is a need to search in a very large base of active compounds. Our study was based on the paper of T. Nose [1] et al, published in 2009 in the *Toxicology Letters* journal, in which the authors propose a simple computational methodology to distinguish between agonists and antagonists of the estrogen receptor and its application to indicate compounds of potential toxicological significance. The methodology was named agonist /antagonist differential-docking screening (AADS).

The aim of this study was to validate the AADS methodology on a broad set of different ligands of the estrogen receptor and modify the methodology to improve its effectiveness. The proposed modifications have also been validated.

To validate the AADS methodology a base of 106 ligands of estrogen receptor with in-vitro documented activity was selected. According to the proposed methodology, test compounds were docked to 4 crystal structures of the receptor, two of them have been crystallized in the presence of agonists and the other two in the presence of antagonists. Only the ligand binding domain of the estrogen receptor α form was used in docking. Suggested modifications included use of other crystal structures and the new calculation algorithm, based on which the decision whether a compound is an agonist or antagonist of the estrogen receptor was made.

[1] Nose T., Tokunaga T., Shimohigashi Y.: *Tox. Lett.* **191** (2009), 33-39.

P-52

Tribenzoporphyrazine Possessing Annulated Styryldiazepine Ring as Potential Photosensitizer for Photodynamic Therapy.

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Porphyrazines (Pzs), including tribenzoporphyrazines are synthetic analogues of porphyrins. Their structural modifications embrace metal ions inclusion to the macrocyclic core and the peripheral substitutions which result in significant modulations of their electrochemical and optical properties. Peripherally modified Pzs can be substituted in their β positions with sulfur, oxygen and nitrogen residues or possess five-, six- and seven-member rings annulated to both β,β positions [1].
Tribenzoporphyrazines containing annulated diazepine rings were found to exhibit intense absorptions and red-shifted Q-bands towards 700 nm [2,3]. The positions of the Q-band absorptions at longer wavelengths suggest their potential utility as photosensitizers in photodynamic therapy (PDT). It is known that light of longer wavelength is capable to penetrate deeper the irradiated tissue [4].
Tribenzoporphyrazine possessing annulated diazepine ring containing styryl substituent was synthesized according to the lately elaborated methodology [5] and subjected to photochemical studies embracing evaluation of its emission property, photostability and aggregation. Potential photosensitizing activity of tribenzoporphyrazine was evaluated by measuring its ability for singlet oxygen production which is a result of interaction between activated photosensitizer and oxygen. DPBF (1,3-diphenylizobenzofuran) was used as a chemical quencher for singlet oxygen (Fig.).

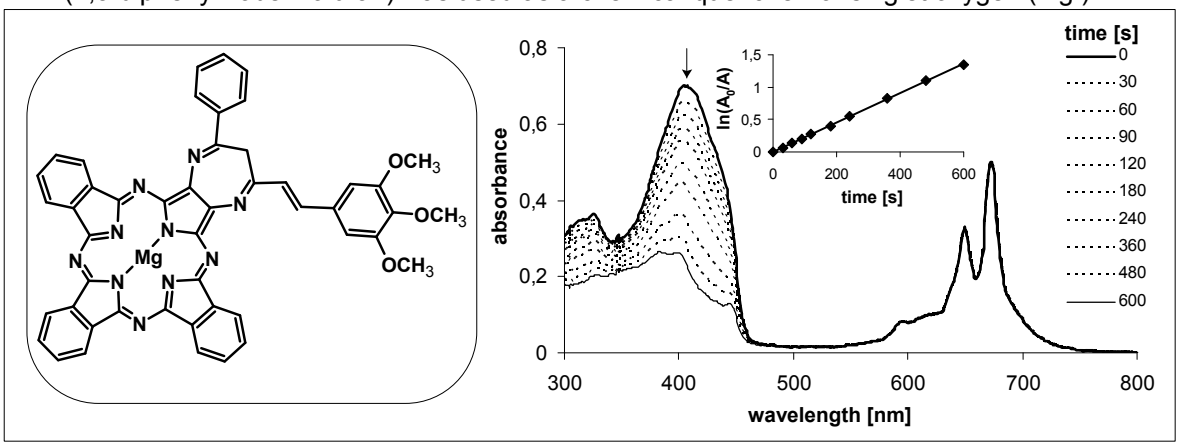

Fig. Changes in the UV–Vis spectrum of DPBF and tribenzoporphyrazine in DMF during irradiation.

[1] Rodriguez-Morgade M.S., Stuzhin P.A.: *J. Porphyr. Phthalocya*. **8** (2004), 1129-1165. [2] Donzello M.P., Ercolani C., Mannina L., Viola E., Bubnova A., Khelevina O., Stuzhin P.A: *Aust. J. Chem*. **61** (2008), 262-272. [3] Piskorz J., Tykarska E., Gdaniec M., Gosliński T., Mielcarek J.: *Inorg. Chem. Commun*. **20** (2012), 13-17. [4] Plaetzer K., Krammer B., Berlanda J., Berr F,. Kiesslich T.: *Lasers Med.* Sci. **24** (2009) 259-268. [5] Goslinski T., Piskorz J., Brudnicki D., White A.J.P., Gdaniec M., Szczolko W., Tykarska E.: *Polyhedron* **30** (2011), 1004–1011.

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Jarosław Piskorz is a scholarship holder within the project “Scholarship support for PH.D. students specializing in majors strategic for Wielkopolska’s development”, Sub-measure 8.2.2 Human Capital Operational Programme, co-financed by European Union under the European Social Fund.

P-53

The Activity *in vitro* of Novel *N*-Ethyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide Against *Haemophilus* spp. Planktonic.

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Nitrogen heterocycles, including pyrazoles, are the important group of natural or synthetic derivatives with a broad spectrum of biological and pharmaceutical activities, e.g. antibacterial, antifungal, anti-inflammatory, analgesic, anticancer and anticonvulsant. Much attention has been paid to pyrazole derivatives due to their wide range of antibacterial activity as a potent and selective inhibitors against DNA gyrase capable of causing bacterial cell death.

In this paper the antibacterial activity *in vitro* of pyrazole derivative *N*-ethyl-3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide against haemophili rods was investigated. It was interesting to check the novel activity *in vitro* of the pyrazole derivative against both pathogenic *H. influenzae* or opportunistic *H. parainfluenzae* in the form of free-floating (planktonic) cells or biofilm-embedded ones.

The rate of growth of haemophili rods in Tripticasein Soy Broth (Biocorp) supplemented with Haemophilus Test Medium Supplement (HTMS SRO158E, Oxoid) with growth factors for haemophili and with or without (control) the tested compound using 96-well polystyrene microplates (NUNC) was measured quantitatively under stationary conditions. Biofilm formation was measured quantitatively by staining with 0.1% crystal violet.

The obtained results show that the title compound was active against both planktonic cells with MIC (minimal inhibitory concentration) = 0.24 - 31.25 µg/mL or biofilm-embedded cells with MBIC (minimal biofilm inhibitory concentration) = 0.24 - ≥31.25 µg/mL. Besides, this compound exhibited activity against ampicillin-resistant *H. influenzae* (MIC = 0.49 – 3.91 µg/mL, MBIC = 0.98 - >31.25 µg/mL) or *H. parainfluenzae* (MIC = 7.82 µg/mL, MBIC = 0.98 – 15.63 µg/mL).

The tested derivative could be considered as an important compound for the rational design of new agents active against both pathogenic H. influenzae or opportunistic H. parainfluenzae, including ampicillin-resistant species. Obtained results allow to expect that the investigated compound will be the starting derivative in the search of antimicrobials with good activity in infections caused by Haemophilus spp.

P-54

Evaluation of Antioxidant Properties of Some Novel Semicarbazide, Triazole and Pyrazole Derivatives.

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Oxidative damages play a significant pathological role in human diseases therefore the antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress. It is known that heterocycles represent an interesting class of compounds possessing a wide spectrum of biological activity. For example, pyrazole derivatives belonged to the third generation on monoamine oxidase inhibitors and bovine serum amine oxidase inhibitors. The triazoles showed urease inhibition and antioxidant activities and pyrroles exhibit antioxidant properties mainly expressed by their capacity to inhibit Fe^{+2} – induced microsomal lipid peroxidation.
Some new heterocyclic derivatives were synthesize in our laboratory by the method described earlier. The semicarbazide derivatives were obtained upon the reaction of carboxylic acid hydrazide with appropriate isocyanate. Cyclization of these compounds in alkaline medium led to obtain triazole derivatives. The pyrazoles were synthesized during the heterocyclization reaction of 1-cyanophenylacetic acid hydrazide with isocyanates.
In the study the antioxidant properties of novel obtained compounds using colorimetric tests was assessed. The principle of the method is based on an ability of tested compounds to reduction of DPPH (1,1-diphenyl-2-picrylhydrazyl) to 1,1-diphenyl-2-picrylhydrazyne. In our investigation, alcohol solution of DPPH was prepared by dissolving 19.92 mg of DPPH (M=394.32g) in 100 ml of methanol. Subsequently the solution was diluted using methanol to reach absorbance of about 0.6 at 517 nm. Tested semicarbazide, triazole and pyrazole derivatives was dissolved to reach following concentrations: 0.5; 1; 5; 10; 20; 50; 100; 250 and 500 µM. The solution with basic level of absorbance was prepared by adding 20 µl of methanol to 160 µl of DPPH solution. In tested probes instead of methanol, methanol solution of compound was added and after 30 min absorbance was measured. In that conditions, the drop of absorbance is proportional to antioxidative force of tested compound. Each test was prepared three times and mean value of absorbance was calculated.
Interestingly, most of tested compounds, in all tested range of concentrations have absorbance above 0.6 (basic level of DPPH); some of rest had absorbance lower than 0.6 but only at high concentrations. It indicates that in these condition most of tested compounds have not revealed any antioxidative properties and rest have only weak. To explain this increase of absorbance the next study aimed to determination of them spectrums are needed.

P-55

Docking of Thiopurine Derivatives to Human Serum Albumin and Binding Site Analysis with Molegro Virtual Docker.

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Binding interaction of 6-Mercaptopurine (6-MP) with human serum albumin (HSA) was investigated by molecular docking. In order to examine the relevance of functional groups of 6-MP to binding, the analogues of 6-MP (2,6-disubsituted 7-methylpurines, Fig. 1), in which the functional groups were modified or added, were employed. Molecular docking studies were performed using the Molegro Virtual Docker (MVD) 2011.5.0.0 computer program. The two dimensional (2D) structures of ligands were obtained using the ChemBioDraw Ultra computer program (Cambridge Soft v.12.0.2.1076). The 2D structures were converted to three dimensional (3D) representations by the use of Chem3DBio Ultra (CambridgeSoft v.12.0.2.1076) software. The 3D structures were energy–minimized using semi-empirical (AM1) method implemented in the same software (Fig. 1). The X-ray structure of HSA (PDB ID: 1AO6) was retrieved from the Protein Data Bank (PDB) [1] and the structure was manually prepared and the protonation state albumin was set to the physiological pH 7.4 (the amino acid residues of His, Arg and Lys were in a protonation state and residues of Asp and Glu were deprotonated). The identification of binding site (cavity) in subdomain IIA of HSA was performed automatically. The ligands as 3D energy-minimized structures were docked one at a time. The obtained complexes were minimized and the binding energy calculated, and binding site interactions for ligands were identified.
6-MP and 2,6-disubsituted 7-methylpurines were found to bind to the site I, but orientation of ligands at the binding site and ascertained interactions were somewhat different. The 6-MP interacted (with binding site through van der Waals and hydrophobic interactions and make four hydrogen bonding interactions (two H-bonds donor and two H-bonds acceptor) and ionic bonding if it was deprotonated at pH 7.4 (Fig. 2). The model binding site based on the structures of the compounds which were bound to it was constructed. A pharmacophore model explained how structurally diverse ligands influenced binding of ligands to a common binding site.

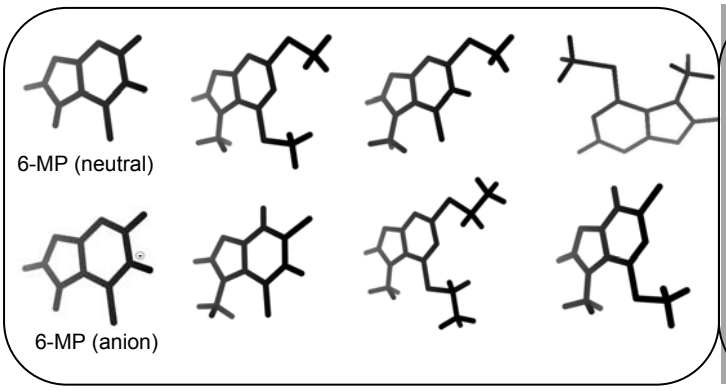

Fig. 1. The 3D energy-minimized structures of 6-MP and 2,6-disubstituted 7-methylpurines

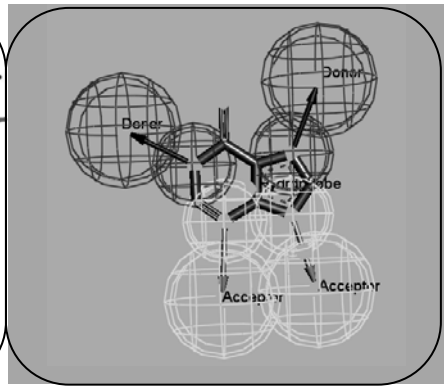

Fig. 2. Possible bonding interactions for 6-MP [2]

[1] http://www.rcsb.org/pdb/explore.do?structureId=1ao6.
[2] Accelrys Software Inc., Discovery Studio Modeling Environment, Release 3.5, San Diego: Accelrys Software Inc., 2012.

P-56

Molecular Dynamics Simulations of Structural and Thermodynamic Properties of Fenoterol Stereoisomers and β_2 Adrenergic Receptor.

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The β_2 adrenergic receptor (β_2-AR) system is one of the best characterized among G-protein coupled receptors. Presented studies concern a long term project aimed at development of new selective agonists of the receptor based on the scaffold of fenoterol molecule (Fig.1) [1, 2].

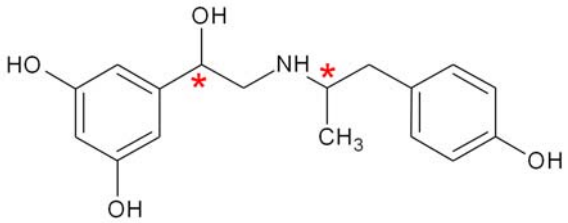

Fig. 1. The structure of fenoterol. The chiral centres are shown by a red-colored stars.

The experimental data demonstrate that not only modification of the leading compound structure (fenoterol) but also the stereochemistry affects interaction with β_2-AR. We observed that stereoisomers of fenoterol influences the magnitude of binding affinity, the thermodynamics of local interaction of ligand within the binding site and the global mechanism of activation of the β_2-AR as evidenced by the Gs/Gi selectivity observation [3, 4]. Generally the (*R*,*R*)-stereoisomer of studied derivatives are the most effective in binding. During the course of these studies, we determined experimentally the binding thermodynamics of the four fenoterol stereoisomers to β_2-AR. The results indicated that there were significant stereochemistry-based differences in the binding mechanisms as this process was entropy-driven when (*R*,*R*)- and (*R*,*S*)-fenoterol were the ligands, while the binding of the (*S*,*R*)- and (*S*,*S*)- isomers was an enthalpy-driven process [4].
In the present study we used molecular dynamics-based methods to calculate the relative free energy changes accompanying the binding of fenoterol stereoisomers to β_2-AR. The thermodynamic integration protocol lead to the results according to which the relative order of ligand affinities is following: (*R*,*R*) > (*R*,*S*) > (*S*,*R*) > (*S*,*S*); this fact and the order of magnitude of the differences between free energy changes corresponding to the particular stereoisomers (several kJ/mol) remain with a good agreement with the experimental data. Further, some attempts were made to interpret these values in terms of molecular features of the studied systems.

Acknowledgement
The work is supported by the found from Foundation for Polish Science (TEAM Programme 2009-4/5) and using the equipment purchased within the Project „The equipment of innovative laboratories doing research on new medicines used in therapy of civilization and neoplastic diseases" within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

[1] Jóźwiak K., Khalid C., et al.: *J. Med Chem*. **50** (12) (2007),2903-2915.
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[4] Jóźwiak K., Toll L., et al.: *Biochem. Pharmacol*. **79** (10) (2010),1610–1615.

P-57

Vibrio harveyi Test in the Evaluation Mutagenic and Antimutagenic Activity of Anticonvulsant Compounds.

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Microbiological mutagenicity tests are an excellent tool to evaluate the mutagenic potential of newly synthesized compounds to be used in therapeutics. Biological methods are based on the observation that substances that are mutagenic to bacteria, also can cause mutations in laboratory animals and even humans.

The aim of this work was to estimate the (anty)mutagenicity of 10 newly synthesized compounds with anticonvulsant activity using the bacteria *Vibrio harveyi* test. The *Vibrio harveyi* mutagenicity assay is mainly used to biodetection mutagenic pollution of the marine environment. It can also use to evaluate the antimutagenic properties of individual compounds. In the test a wild strain of *Vibrio harveyi* BB7 and its genetically modified varieties are used. *V. harveyi* is not pathogenic to humans, and thus it is completely safe to work with. It can be easily cultivated in laboratory conditions. Penetration of mutagens into *V. harveyi* cells is more efficient relative to many other bacteria used in mutagenic assays.

The *V. harveyi* mutagenicity assay is based on the detection of neomycin-resistant mutants on Petri dishes either containing a mutagen in the solid medium or after incubation of bacterial cultures in a liquid medium, in the presence of tested compounds. *V. harveyi* is naturally sensitive to neomycin, but it was demonstrated that mutants resistant to this antibiotic can be easily isolated. The frequency of the appearance of such mutants increases in the presence of mutagens.

The experimental results were estimated by comparing the number of neomycin-resistant colonies mutants grown in the presence of test compound, the number of colonies formed on solid medium with the reference mutagen (NQNO). The experimental results showed that the tested compounds do not cause mutations in any experiment. This means that they can be qualified into further stages of preclinical studies.

The antimutagenic activity of tested compounds was carried out in the modified *V. harveyi* assay. This modification is based on incubation of tested compound, together with the reference mutagen (NQNO) in a culture medium. According to the results obtained, beneficial antimutagenic profiles were observed for all tested compounds. The compounds demonstrated strong (about 50%) and moderate (about 30%) antimutagenic activity.

This study showed that *V. harveyi* test may be a useful tool for compounds safety evaluation.

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P-58

Mutation Mining: Automated Extraction of Mutation Data from Scientific Publications.

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Manual extracting of mutation data from scientific publications is a slow and laborious process. Automated extraction is complicated, since mutations are described in different ways and need to be checked manually. Often, the publications contain data on mutagenesis of other proteins than the target one, rendering standard automated methods helpless. Most available software require manually created mutation databases[1] and do not in fact analyse given publication. Creation of error-free, internet-accessible mutation extracting software would become a major asset in gathering scientific data and database construction. Therefore, we came up with an idea for this task – the Mutation Miner. Mutation Miner software, collaborating with Uniprot[2] and HyperCLDB[3] databases, is the solution for this task. Upon uploading your publication in PDF format, the program uses resourceful algorithms to find both mutations and the target protein, enabling cross-checking of acquired results. Even though still in its alpha-testing phase and few more options are yet to be implemented, Mutation Miner is a useful, time-saving tool.

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P-59

Synthesis and Antibacterial Activity of Pyrrolo[3,4-c]pyrrole Derivatives.

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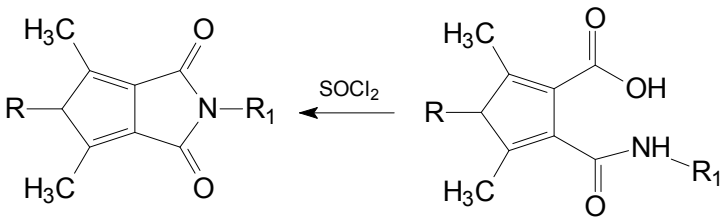
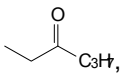
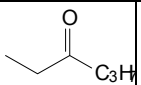
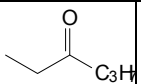
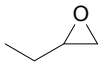
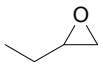
Pyrrolo[3,4-c]pyrrole derivatives possess a various pharmacological activity, like antibacterial [1], antiviral [2] and analgesic [3]. Based on those findings, we decided to obtain a new set of pyrrolo[3,4-c]pyrrole derivatives (1,2,3) and test their activity against *Mycobacterium tuberculosis* ($H_{37}Rv$ species). To synthetize final products, proper anhydrides were transformed into amidoacids and then cyclized in toluene in the presence of thionyl chloride, to obtain imides (1,2,3).
Taking into account that obtained derivatives are instable, and decompose easily in acidic environment. Therefore to describe their pharmacological mode of action, we decided to test main structures (1,2,3) as well as amidoacids used to the synthesis (1a, 2a,3a).



1, 2, 3 **1a, 2a, 3a**

R=C_4H_9, C_6H_5

R_1=C_4H_9,  

Comp.	R	R_1	% Inh.
1	C_4H_9	C_4H_9	55
1a	C_4H_9	C_4H_9	15
2	C_6H_5		44
2a	C_6H_5		23
3	C_4H_9		81
3a	C_6H_5		30

Antimicrobial in vitro test were performed in The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF). Although obtained results (table) have shown that all of synthetized derivatives are active against *Mycobacterium tuberculosis*, the effect is unsatisfying (range between 15-81% (minimal inhibitory concentration, MIC>12,5µg/ml). At the same time semi products, have been significantly less active than corresponding main structures.

[1] Finn J., Yu X.Y., Wang Z.G., Hill J., Keith D., Gallant P., Wendler P.: WO 2000018772.
[2] Lemoine R., Melville C. R., Padilla F., Rotstein D. M., Wanner J.: US 2007191406.
[3] Malinka W., Sieklucka-Dziuba M., Rajtar G., Rubaj A., Kleinrok Z.: *Farmaco* **54** (1999), 390-401.

P-60

Synthesis and α_1-Adrenoceptor Affinities of Novel Arylpiperazine 5-(Spiro)aromatic Derivatives of Hydantoin.

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Arylpiperazines are a popular chemical group in search for biologically active compounds that displays various pharmacological actions such as regulation of blood circulation or central nervous system functions. Recently, some arylpiperazine derivatives were also identified as strong efflux pump modulators in multidrug resistant cancer cells [1, 2] or moderate chemosensitizors of multidrug resistant bacteria [3]. Predominantly, the arylpiperazine moiety is a pharmacophoric fragment of huge population of α_1-adrenoceptor antagonists and ligands for receptors: 5-HT_{1A}, 5-HT_7 as well as dopaminergic receptors D_2 or D_3. Considering the multiple pharmacological action observed for arylpiperazine derivatives, it is in great importance to search for chemical modifications which improve selectivity in this chemical group. Thust, our studies are focused on the chemical modifications in the group of arylpiperazine derivatives of hydantoin. Within our previous works, we examined phenylpiperazine derivatives of 5,5-diphenylhydantoin (phenytoin) on their affinities for α_1-adrenoceptors [4,5]. As a continuation, the present studies are concentrated on two groups (**A** and **B**) of hydantoin arylpiperazine derivatives (Fig. 1) differing within substituents, number and position of their aromatic rings as well as linkers between piperazine and both, hydantoin and aromatic substituents.

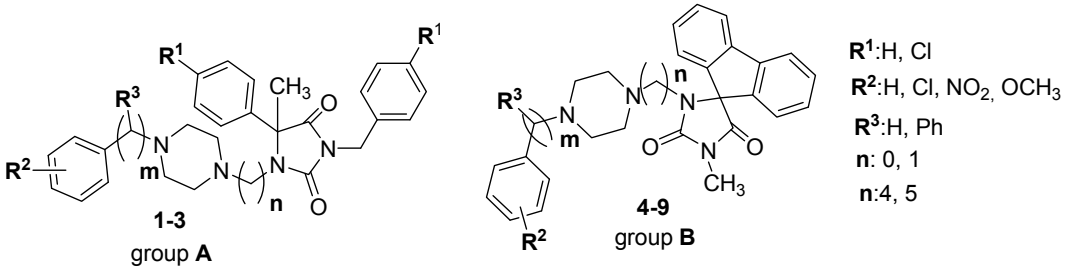

Fig. 1.

A series of 9 new compounds **1-9** (Fig.1) was synthesized using Bucherer-Bergs reaction and two-phase alkylation processes. The compounds were tested on their affinity for α_1-adrenoceptors in radioligand binding assay with ^{3}[H]-prazosin as a selective radioligand. Most of the compounds displayed moderate affinities for α_1-adrenoceptors with Ki in the range of 100-970 nM . The highest activity was observed for phenylpiperazine- and 2-methoxyphenylpiperazine derivatives. A huge decrease of the affinity was observed for diphenylmethylpiperazine derivatives.

[1] Spengler G., et al.: *Anticancer Res*. **30** (2010) 4867-4871. [2] Martins A., et al.: *In Vivo* **26** (2012) 293-297. [3] Handzlik J., et al.: *Eur. J. Med. Chem*. **46** (2011) 5807-5816. [4] Handzlik J., et al.: *Bioorg. Med. Chem*. **16** (2008) 5982-5998. [5] Handzlik J., et al.: *Bioorg. Med. Chem*. **20** (2012) 2290-2303.

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P-61

ADBO: Source of New Quaternary Ammonium Salts.

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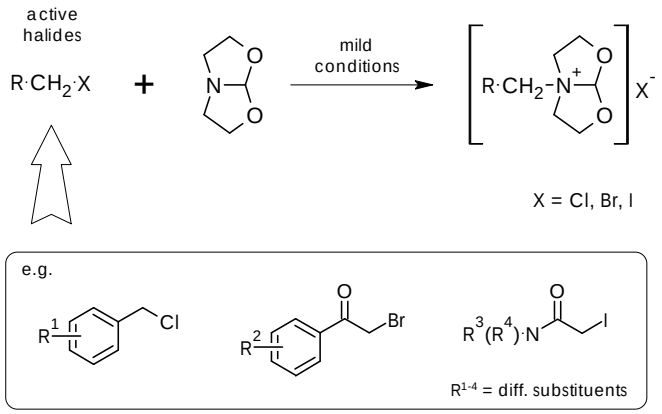
Bicyclic amidoacetals are hygroscopic and highly reactive compounds readily interacted with numerous electro- and nucleophiles [1]. Our principal study on chemical properties of 1-aza-4,6-dioxa-bicyclo[3.3.0]octane has found this structure to react with a number of active halomethylenes (e.g. benzyl or phenacyl halides). A simple synthetic route leads under mild conditions to excellent yields of quaternary ammonium salts consisting tetrahydro[1,3]oxazolo[2,3-b][1,3]oxazol-4-ium moiety (scheme 1). Although, structure of the formula where R = H, X = I (scheme 1) was obtained in 1960 [2] but over the next decades no researches were continued.
We expect new compounds will find commercial applications in chemical, cosmetic or pharmaceutical industry as surfactants, detergents, emulsifiers, softeners, stabilizers, moisturizers, foaming agents, disinfectants, antiseptics, preservatives or phase transfer catalysts.



scheme1

[1] Feinauer R.: *Synthesis* (1971), 16-26.
[2] Meerwein H., Hederich V., Morschel H., Wunderlich K.: *Liebigs Ann. Chem*. **635** (1960), 1-21.

P-62

Cooperative Properties of Zinc Binding to 5-HT7 Receptor – Pilot Studies.

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The main objective is to investigate cooperative properties of zinc ions at 5-HT7 serotonin receptor, to determine whether allosteric regulation of these ions reported at 5-HT1AR [1] is subtype selective, or it can also be detected at other representatives of serotonergic receptor family. It was shown that zinc ions are natural allosteric modulator for several different groups of G-protein coupled receptors (GPCR), such as dopamine D1 and D2, alpha1 and beta2-aderenergic [2], which suggests a more universal character of this mode of zinc ions interaction. Results of our pilot studies seem to support this hypothesis, as a cooperative properties of zinc binding to 5-HT7 receptor has been detected.
Binding of allosteric modulator promotes changes in receptor conformation, which influences the affinity of the endogenous (or other orthosteric) ligand and the formation of ligand-receptor complex. In order to study the mechanism of this interaction, changes in affinity and dissociation rate of orthosteric ligands, radioligand binding methods were used with HEK293 cell lines stably transfected with cDNA vector encoding the human serotonin 5-HT7b receptor and [3H] 5-CT as a radioligand. To the analysis of allosteric reaction results, so-called “allosteric ternary complex model” was adopted.
Both serotonin receptors and zinc ions play important functions in the central nervous system (CNS) but many aspects of their action remains unclear. In recent years, various aspects of GPCR allosterism were intensively studied, because the interaction with the receptor according to other than classical, competitive model of orthosteric ligand binding, creates new possibilities for its regulation. It is even believed, that in a longer research term, it could be an area for the search of the next generation of drugs acting on the CNS.

Acknowledgements
This study is partly supported by project POIG.01.01.02-12-004/09-00 (De-Me-Ter) co-financed by European Union from the European Fund of Regional Development (EFRD);

[1] Ciolek J., Maïga A., Marcon E., Servent D., Gilles N.: *Eur. J. Pharmacol*. **655** (2011), 1-8.
[2] Gregory K.J., Sexton P.M., Christopoulos A.: *Curr. Protoc. Pharmacol*. 2010 Dec; Chapter 1: Unit 1.21.

P-63

Synthesis, Physical and Chemical Properties of Porphyrazines Possessing Peripheral Benzylsulfanyl Substituents.

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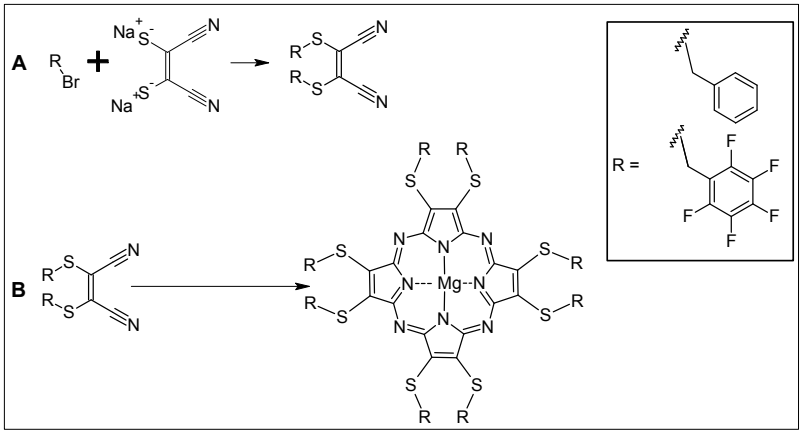
Porphyrazines (Pzs) constitute a large class of porphyrinoids along with phthalocyanines and porphyrins. They are aromatic macrocylic compounds containing four pyrrolic rings bound together by azide groups. Pzs can be easily modified in their peripheral β-positions with various alkyl-, N-, O- and S-groups and annulated aromatic rings. Optical, electrochemical and catalytic properties make them of great interest for photodynamic therapy (photosensitizers), analytical chemistry (chemical sensors), and technology (components of photovoltaic cells) [1]. Lately, the synthesis of novel fluorinated benzylsulfanyl Pzs has been the subject of intensive studies [2].



Reaction of dimercaptomaleonitrile disodium salt with alkylating agents, such as benzyl bromide and pentafluorobenzyl bromide in ethanol led to the corresponding maleonitriles (Figure, A). Maleonitriles were used in the Linstead macrocyclization reaction in n-butanol at reflux under inert atmosphere in the presence of magnesium n-butanolate as a base (Figure, B). Pz macrocycles, including symmetrical magnesium porphyrazine possessing peripheral pentaflurobenzylsulfanyl substituents were isolated and characterized using UV-vis, MS MALDI and NMR techniques (^{1}H, ^{13}C, ^{19}F). Moreover, the photochemical studies, including solvatochromic characterization and an ability to generate singlet oxygen were performed.

[1] Michel S.L.J., Hoffman B.M., Baum S.M., Barrett A.G.M.: Prog. Inorg. Chem. 50 (2001), 473–590.
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This study was supported by the Student Research Grant SBN from the Poznan University of Medical Sciences 502-05-03313427-50671.

P-64

Machine Learning Method as a Tool for Searching New 5-HT$_6$ Ligands in Fingerprint-Based Consensus Experiment.

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The 5-HT$_6$ receptor is a seven transmembrane domain protein, positively coupled to adenylyl cyclase via G$_s$ protein, located almost exclusively in the central nervous system. It seems to be involved in regulation of glutamatergic and cholinergic neuronal activity and is considered to play role in learning and memory, mood control and feeding behaviour [1]. Therefore, it has become a widely explored target for treating cognitive dysfunctions associated with many neuropsychiatric disorders, such as Alzheimer's disease and schizophrenia, depression and anxiety [2].
A number of computational techniques are used in the challenging problem of finding new drug candidates. Among various approaches applied in drug discovery campaigns, virtual screening tools (both ligand- and structure based) can be distinguished [3]. They cover an automatic evaluation of chemical compound libraries in order to identify structures with potential activity towards desired target protein.
Machine learning belong to the group of widely explored methodologies in the field of virtual screening. Major tasks of this artificial intelligence tools are connected with the assignment of objects (in our case: molecules) into classes (here: active or inactive) [4].
In our research, we applied Sequential Minimal Optimization algorithm in the search of new 5-HT$_6$ ligands from the Enamine database [5]. Three different fingerprints were used for molecules representation and consensus prediction was taken as the final answer. Compounds selected according to this procedure are going to be ordered and they will undergo biological evaluation.

Acknowledgements:

This study is supported by project UDA-POIG.01.03.01-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD); http://www.prokog.pl

[1] Wesołowska A.: *Pharmacol. Rep. PR* **62** (2010), 564-577.
[2] Geldenhuys W. J., Van Der Schyf C. J.: *Expert Rev. Neurotherap.* **9** (2009), 1073-1085.
[3] Geppert H., Vogt M., Bajorath J.: *J. Chem. Inf. Model.* **50** (2010), 205-216.
[4] Melville J. L., Burke E. K., Hirst J. D.: *Comb. Chem. High T. Scr.* **12** (2009), 332-343.
[5] www.enamine.net

P-65

Pitavastatin, its Photodecomposition Products and Their Biological Activity.

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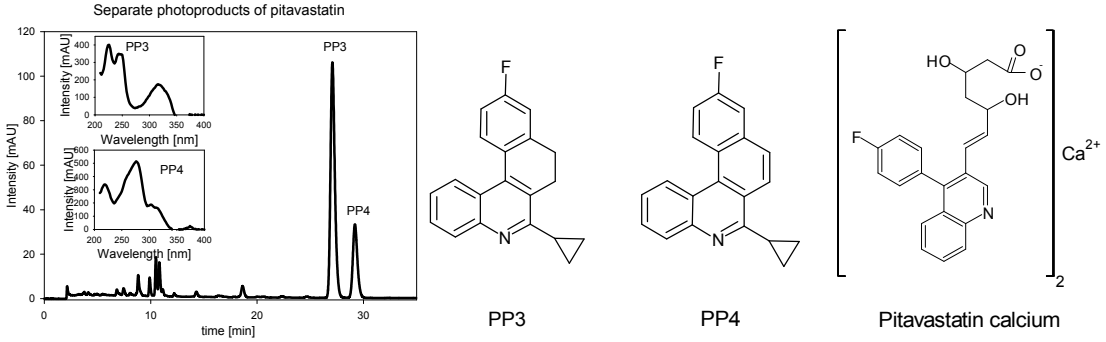
Pitavastatin belongs to an HMG-CoA reductase inhibitor family (statins). It is a synthetic compound with large lipophilic and hydrophilic moieties. Statins are hypocholesterolaemic agents which reduce total LDL fraction in serum and rise the HDL fraction by inhibiting HMG-CoA enzyme. Statins are used commonly in hypercholesterolaemia with cardiovascular disease. Moreover, due to their pleiotropic properties they can be used in osteoporosis, Alzheimer's disease and others. Recent literature has shown the phototoxic potential of fluvastatin in formation of toxic polycylic photoproducts upon irradiation [1].



Pitavastatin was exposed to irradiation with light at 365 nm. HPLC profiles revealed two photoproducts, namely PP3 (6-cyclopropyl-10-fluoro-7,8-dihydrobenzo[k]phenantridine) and PP4 (6-cyclopropyl-10-fluorobenzo[k]phenantridine), which were isolated by the HPLC method. Additionally, biological experiments were carried out to verify the phototoxic ability of the compounds investigated. Tests of cellular phototoxicity, DNA fragmentation, cell cycle analysis, ATP assay, Caspase-3 assay, lipid and protein peroxidation tests in the human keratinocytes NCTC-2544 were performed.
Pitavastatin induced a reduction in viability of NCTC-244 keratinocytes. Photoproducts showed toxic effects in dosage ten times lower than the parent compound. Pitavastatin has a clear phototoxic potential in vitro in a human keratinocyte cell line. Its phototoxicity could be mediated by the formation of photoproducts with high photosensitizing properties. Moreover, we identified plasma membrane as one of the major targets of the Pitavastatin action, which ultimately leads to necrosis as the principal mode of cell death. Additionally, LC-MS assay showed the pitavastatin ability to create toxic PP3 and PP4 upon irradiation of live cells.

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[3] Viola G., Grobelny P., Linardi M. A., Salvador A., Dall'Acqua S., Sobotta Ł., Mielcarek J., Dall'Acqua F., Vedaldi D., Basso G.: *Arch. Toxicol.* **86** (2012), 483–496.

P-66

Characterization of the 6-Thioguanine–Serum Albumin Complex by Molecular Docking Approach.

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The molecular docking simulation was employed to examine the binding mode of 6-Thioguanine (6-TG) with human serum albumin (HSA). 6-TG as neutral and monoanionic form was docked to the site I located in subdomain IIA of HSA. In the experiment warfarin (RWF), phenylbutazone (PhB) and n-butyl *p*-aminobenzoate (ABE) were used as the site markers that bind specifically to site I of HSA; RWF and PhB were used as the markers for warfarin–region and for azapropazone–region binding of drug, respectively, and ABE as a marker which represents binding region Ic located adjacent to the warfarin binding region but apart from that of azapropazone [1]. Molecular docking procedure was performed using the Molegro Virtual Docker (MVD) program [2]. X-ray structure of HSA (PDB ID: 1AO6) [3] was downloaded from the Protein Data Bank (PDB). The structure of HSA and the structures of ligands were manually prepared and the protonation state albumin and ligand were set to the physiological pH 7.4. The residues of His, Arg and Lys were in protonation state, while those of Asp and Glu were deprotonated; 6-TG was in the neutral and monoanionic form, RWF and PhB were fully ionized and ABE was as 100 % non-charged molecule. Before docking of the ligands into the HSA structure, the MVD docking protocols were validated using the two crystal structures of RWF/HSA and PhB/HSA complexes.

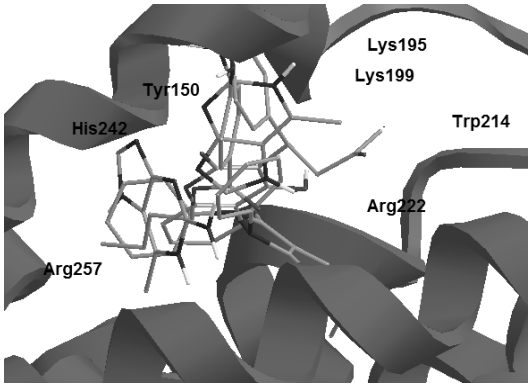

Fig. 1. Top poses of 6-TG, RWF, PhB and ABE in the site I of HSA and important binding site residues around the bound compounds

Based on the findings obtained in the docking study, it is presumed that 6-TG can interact with HSA in subdomain IIA. The potential 6-TG binding site may correspond to partly overlapped warfarin–region and azapropazone–region binding drugs of site I.

[1] Yamasaki K., Maruyama T., Kragh-Hansen U., Otagiri M.: *Biochim. Biophys. Acta* **1295** (1996), 147.
[2] Thomsen T., Christensen M. H.: *J. Med. Chem*. **49** (2006), 3315.
[3] http://www.rcsb.org/pdb/explore.do?structureId=1ao6.

P-67

Effect of Molecular Descriptors on the Binding Affinity of Thiopurine Derivatives to Serum Albumin.

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Thiopurines, synthetic analogues of natural purine bases, are used in cancer chemotherapy. The original syntheses of purine antimetabolites focused on isosteric replacement of oxygen or amino group in the natural purine by sulphur or halogen atom. 6-Mercaptopurine (6-MP) was among the first purine thioanalogues in which the oxygen atom in position 6 of hypoxanthine was replaced with a sulphur.
The physicochemical properties of bioactive compounds decide about its biological activity and play dominant role in several aspects of ADME processes of compounds within human organism. Serum albumin plays important role in the transporting and distribution of numerous compounds, mainly as a complex, to various organs and tissues. The interaction of 6-MP with serum albumin has not been characterized in detail. In order to study how important is the chemical structure of 6-MP for its interaction with serum albumin, some derivatives with S and N7-methylated 6-MP and isomeric thiopurine derivatives with sulfide or thione structure (**1**–**5**) have been synthesized. Principal component (PC) analysis was performed by non-linear mapping and cluster analysis to describe the similarities and differences between investigated thiopurines taking into consideration their chromatographic lipophilicities (R_{M0}, S and φ_0) and calculated physicochemical and structural parameters (MW, MR^{20}, MV^{20}, n^{20}, γ^{20}, d^{20}, log$P$, pK, logS_W, PPB%, HBD and HBA, log$D_{7.4}$).

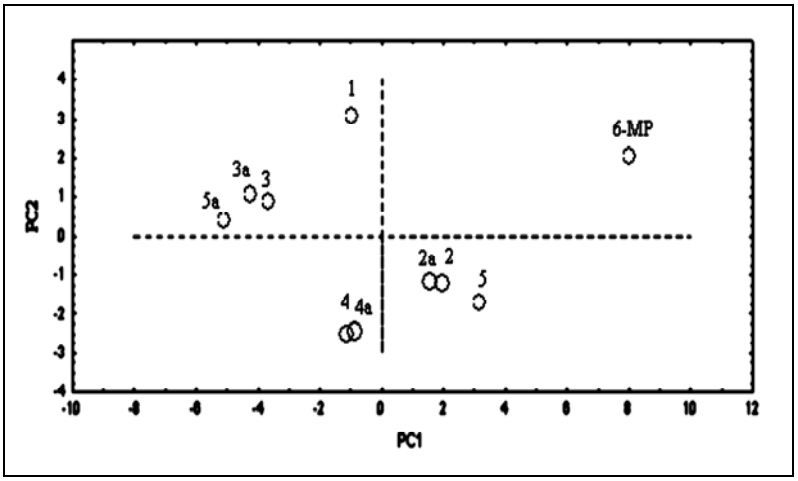

Fig. 1. Similarities and differences between **6-MP** and 6- or 2,6-disubstituted 7-methylpurines (**1**–**5**). Two-dimensional nonlinear map of principal component variables.

The investigated compounds form three separate clusters on the two-dimensional non-linear map of principal component variables (Fig. 1). The first group consists of compounds with chloro atom (**3 and 3a)** and/or thiomethyl group (**1** and **5a**) in purine ring, which are weak bases, the second one comprises compounds with 2- or 6-purinethione structure (**2**, **2a**, **4**, **4a** and **5)**, which are acidic, while **6-MP** can be treated as the third, completely individual group. This suggests that the 6-MP probably may interact with protein in a different mode than other thiopurines.

P-68

Biological Studies of New Isoxazole Derivatives with Potential Immunorestoring Activity.

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[2] Department of Experimental Therapy, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, Wrocław, Poland
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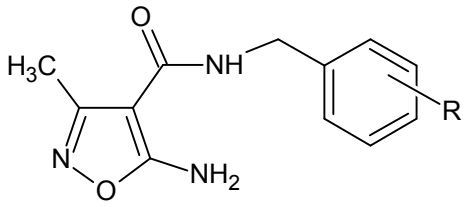
Our research of the synthesis of biologically active isoxazole derivatives led to obtain compounds with immunostimulatory and immunosuppressive activity. These compounds showed immunological activity with low toxicity in various experimental models in mice and human blood cells [1-5]. On the basis of our previous studies we designed and synthesized a new series of substituted benzylamides of 5-amino-3-methylisoxazole-4-carboxylic acid **1**.



1

In our present work the cytotoxicity research of benzylamides of 5-amino-3-methylisoxazole-4-carboxylic acid **1** were performed. We investigated the influence of compounds **1** on the PHA-induced proliferation of human peripheral blood mononuclear cell (PBMC) and the LPS-induced production of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) by human peripheral blood cells. Then the toxicity of benzylamides **1** against human PBMC was tested. In these studies benzylamides **1** demonstrated immunosuppressive and anti-inflammatory activity with low toxicity.

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P-69

The Application of FTIR and Raman Spectroscopy in Imaging Biological Materials.

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Optical microscopy is highly valuable to biomedical research. Fourier Transformed Infared (FTIR) and Raman imaging are complementary to each other and both have been extensively applied to the analyses of biological materials.
The vibrational spectrum of a molecule provides a useful fingerprint for identification of substances without the necessity for external labels (which may perturb the physical properties of the biological model under study). The non-invasive character of the technique as well as the associated chemical information may offer advantages over other imaging techniques such as fluorescence microscopy [1].
The ability of infrared and raman spectroscopy to distinguish and map cancerous and non-cancerous tissue has opened the question of the origin of spectral differences between normal and cancerous cells, normal and tumoral tissue.
These both techniques has been successfully demonstrated as an effective tool for tissue characterization and diagnosis, for characterization of the chemical composition and physical state of bone, to probe the distribution of components within a formulation, to characterize homogeneity of pharmaceutical samples. The vibrational spectroscopy is successfully applied for a manifold of tasks in the whole process of drug discovery and drug development [2].
The aim of the present study is to demonstrate that the imaging has ability to accurately characterize small intestine cancer tissue and distinguish between normal (noncancerous), and tumoral (cancerous) types.

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Acknowledgement
The work is supported by the found from Foundation for Polish Science (TEAM Programme 2009-4/5) and using the equipment purchased within the Project „The equipment of innovative laboratories doing research on new medicines used in therapy of civilization and neoplastic diseases” within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

P-70

Synthesis of Thiocarbamate and Thiophenol Stilbene Derivatives as Potential Anticancer Agents.

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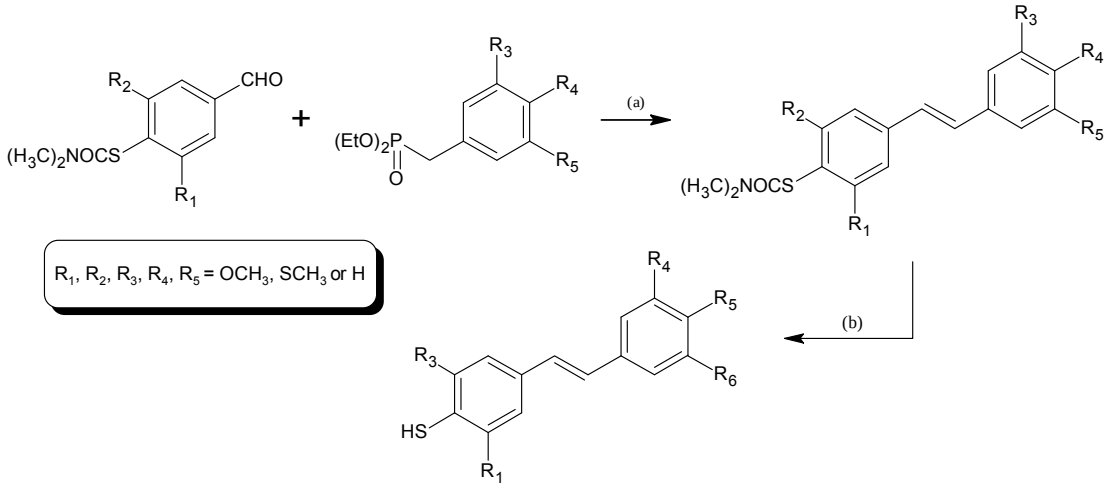
Microtubules, dynamic polymers that occur in eukaryotic cells, play important roles in cell division, motility, transport and signaling. They arise in the process of polymerization of α- and β-tubulin dimers. The compounds interfering tubulin assembly cause alteration of cell cycle parameters leading preferentially to G2/M blockade. The interest in tubulin as a molecular target for anticancer drugs increased significantly since nineties when mitotic inhibitor paclitaxel was introduced into the clinic as a chemotherapeutic agent. Combretastatins are natural *cis*-stilbenes isolated from the bark of African willow tree *Combretum caffrum,* that exhibit cytotoxic properties in cultured cancer cells *in vitro*. Combretastatin A-4 (3'-hydroxy-3,4,4',5-tetramethoxy-*cis*-stilbene; CA-4) is presently one of the best known inhibitors of tubulin polymerisation, and CA-4 phosphate (Zybrestat^{TM}) as a CA-4 prodrug is in the phase III of clinical trials. The search for new natural and synthetic products with tubulin-interactive properties is still continued.



Scheme 1. General synthetic route for synthesis of the thiocarbamate and thiophenol stilbene analogues.
Reagents and conditions: (a) NaH, DMF, rt.; (b) 10% MeOH/KOH, reflux

The aim of this study was to design, synthesize and characterize novel *trans* CA-4 analogues. A series of thiocarbamate stilbene derivatives differing in number and position of methoxy/methylthio substituents was synthesized by the Wittig-Horner reaction of diethyl 3,4,5-trimethoxybenzylphosphonate or 4-methylthiobenzylphosphonate with the corresponding benzaldehydes substituted with N,N-dimethylcarbamoylthio group in DMF using sodium hydride as a base. Subsequent hydrolysis allowed to obtain corresponding thiophenol stilbene derivatives. They were investigated for their activity in a microtubular polymerization assay with the use of purified porcine tubulin purchased from Cytoskeleton Inc. (Denver, CO, USA) according to a protocol recommended by the manufacturer. Polymerization was followed turbidimetrically at 350 nm. Paclitaxel and nocodazole were used as positive stabilizing and destabilizing controls, respectively.

P-71

Synthesis, Structure and Microbiological Properties of Thiourea Analogues of 1,3-Thiazole.

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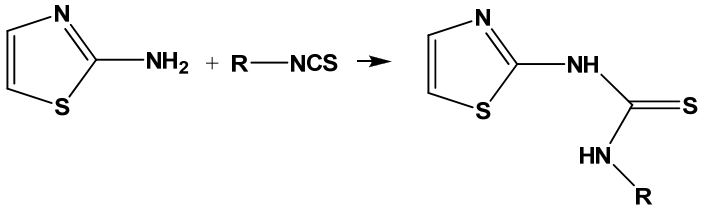
The 1,3-thiazole are an important group of medicinal substances with exibit a wide range of activity, such as antibacterial, fungicidal, anti-inflamatory, antiviral and anticancer.
The title derivatives of 3-(1,3-thiazol-2-yl)thiourea were synthesized as the potential antimicrobial agent.



R = C_6H_5, C_6H_4-p-OCH_3, C_6H_4-p-Cl, C_6H_4-p-CH_3, C_6H_4-p-F, C_6H_4-o-Br, C_6H_4-p-Br, C_6H_4-m-Br, C_6H_4-p-I, C_6H_3-o,p-Cl, CH_2-C_6H_5, CO-C_6H_5, C_6H_{11}, C_6H_4-o-F, C_6H_4-m-F, C_6H_3-o-CH_3-m-Cl, C_6H_3-m-Cl-p-CH_3, C_6H_4-o-Cl, C_6H_4-m-Cl, C_6H_3-m-Cl-p-F, C_6H_4-m-CF_3

These compounds were obtained in the reaction of 1,3-thiazol-2-amine with respective isothiocyanates. The ^{1}H NMR, ^{13}C NMR and MS were used to confirm structures of obtained thiourea derivatives. The molecular structure of selected was determined by an X-ray analysis. All obtained compounds were tested for antibacterial activity against Gram-positive cocci, Gram-negative rods and antifungal activity. Compounds (C_6H_5, C_6H_4-p-OCH_3, C_6H_4-p-F, C_6H_4-o-Br, C_6H_4-m-Br, C_6H_3-o,p-Cl, C_6H_3-o-CH_3-m-Cl, C_6H_3-m-Cl-p-F, C_6H_4-m-CF_3) showed significant inhibition against Gram-positive cocci. MIC value of standard Gram-positive strain was in the range 128 – 2 μg/ml Microbiological evaluation was carried out over 20 standard strains and 30 hospital strains. Selected compounds were examined for cytotoxicity, antitumor, and anti-HIV activity.

P-72

New 1-(1-Arylimidazolidine-2-ylidene)-3-substituted Ureas Derivatives with Potential Pharmacological Activity.

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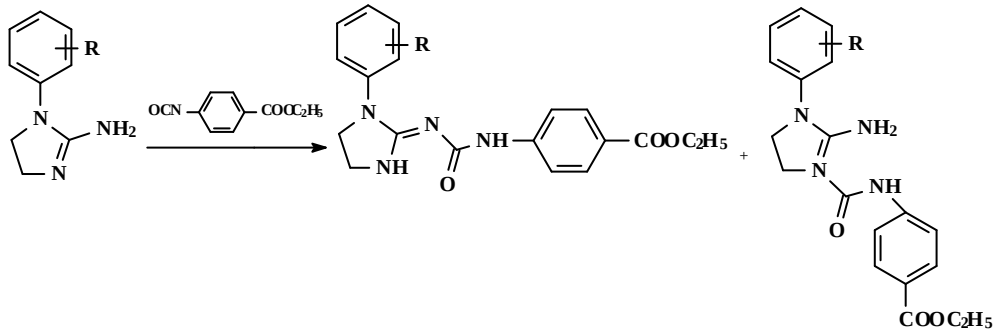
1-Aryl-2-aminoimidazoline were used to synthesize many chain and fused imidazoline derivatives exhibiting pharmacological activity. Compounds bearing the 1-aryl substituted were found to process significant central activity, especially antinociceptive and serotonergic [1-3].
When reserching new compounds with potential pharmacological activity 1-(1-aryimidazolidine-2-ylidene)-3-(4-ethoxycarbonylphenyl) ureas were received.
Novel derivatives were receicved by condensation of 1-aryl-2-aminomidazoliduine with ethyl 4-isocyjanatebenzoate.

Scheme:



The structure of all new compounds was confirmed by elementar analysis, as well by the ^{1}H NMR.

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P-73

Synthesis and Evaluation of Antiviral Activity of New 1-Substituted-3-(4-halogenebenzyl)ureas.

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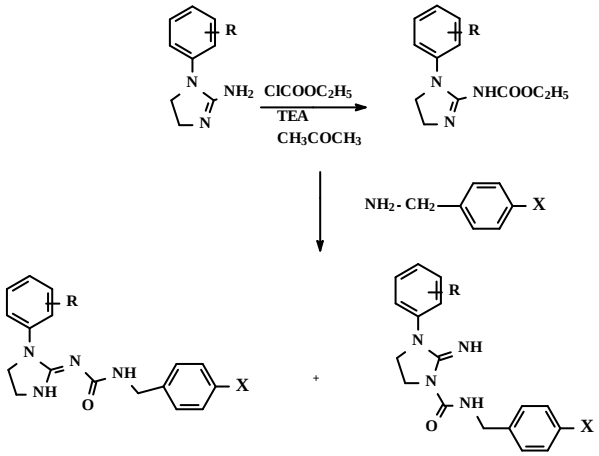
In the recent years at the Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Faculty of Pharmacy, a number of 1-(1-arylimidazolidine-2-ylidene)-3-arylurea was synthesised.
By the reaction of ethyl N-(1-arylimidazolidine-2-ylidene) carbamic acid ester with 4-halogen-benzylamine new derivatives of 1-(1-arylimidazolidine-2-ylidene)-3-(4-halogenebenzyl)ureas were obtained.



Scheme 1. Synthesis of new derivatives of 1-(1-arylimidazolin-2-ylo)-3-(4-halebenzyl)ureas.

In order to assess the cytotoxicity of compounds No. 1, 2, 3, 4, 5 normal human (HEK 293 - human embryonic kidney) and animal (Vero - African green monkey kidney) cell lines 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 5 to 2000 µg/ml. Data obtained after performing the MTT test was used to calculate EC_{50} values which are the measure of cytotoxicity of tested compounds. All experiments were performed in triplicate. The EC_{50} of tested substances ranged from 211,38 – 519,16 µg/ml on HEK 293 and 163,49 – 485,29 µg/ml on Vero cell line. The lowest cytotoxicity towards HEK 293 was observed for compound No. 6 (519,16 µg/ml) and towards Vero for compound No. 1 (485,29 µg/ml). The highest cytotoxicity (EC_{50} = 163,49 µg/ml) was noted when compound No. 5 was tested on Vero cell line. Further studies will involve the assessment of antiviral activity of compounds No. 1, 2, 3, 4, 5 against HSV (HSV-1) and Adenovirus type 5 (AV5).

P-74

Application of immobilised *Pseudomonas cepacia* lipase for the stereoselective synthesis of azidoalcohols.

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Among the enzymes used currently in stereoselective synthesis, lipases take an important place. Cleavage or formation of ester bonds with this class of enzymes allows to easily obtain optically pure chiral alcohols, through enzymatic kinetic resolution (KR) or enantioselective desymmetrization of prochiral compounds (EED). [1]
Lipases allow to conduct the synthetic procedures in mild conditions, which is vital for compounds displaying limited stability, such as azidoalcohols, being the subject of the presented study. Azidoalcohols, earlier used as precursors in aminoalcohol synthesis, have recently gained a novel field of application: as substrates in triazole synthesis in the copper-catalyzed 3+2 Huisgen cycloaddition, the flag reaction of *Click chemistry* strategy, proposed by Sharpless. [2]
In the presented study, *Pseudomonas cepacia,* immobilised on the Immobead 150 carrier, was applied for the stereoselective synthesis of azidoalcohols. In comparison to earlier works using the free enzyme for the same systems, [3; 4] this allowed to obtain better synthetic yield and to limit the overall cost of the process. In the presented study, also the influence of an organic modifier (acetonitrile) on lipase efficacy, was analyzed. The optically pure azidoalcohols, obtained with the elaborated methodology, will be used for further research, as substrates for the synthesis of 1,2,3-triazole systems.

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P-75

The Crystal Structures of Gramicidin Complexes.

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Gramicidin D is a natural mixture of six linear gramicidins produced by *Baccillus brevis* species. It shows antibacterial activity against Gram$^+$ bacteria [1]. The linear gramicidins are pentadecapeptides with modified ends:

HC(O)-X_1-*D*Gly-Ala-*D*Leu-Ala-*D*Val-Val-*D*Val-Trp-*D*Leu-X_{11}-*D*Leu-Trp-*D*Leu-Trp-NH_2COOH

X_1 = Val (Vg) or Ile (Ig)
X_{11} = Trp (gA), Phe (gB) or Tyr (gC)

The gramicidin create a pore (channel) in the membrane, which allows transport of monovalent cations[2]. Helical structure of gramicidin is caused by alternate D-L configurations of its aminoacids. The channels formed are double- or single-stranded type (Fig.) with a diameter between 3 and 4Å.

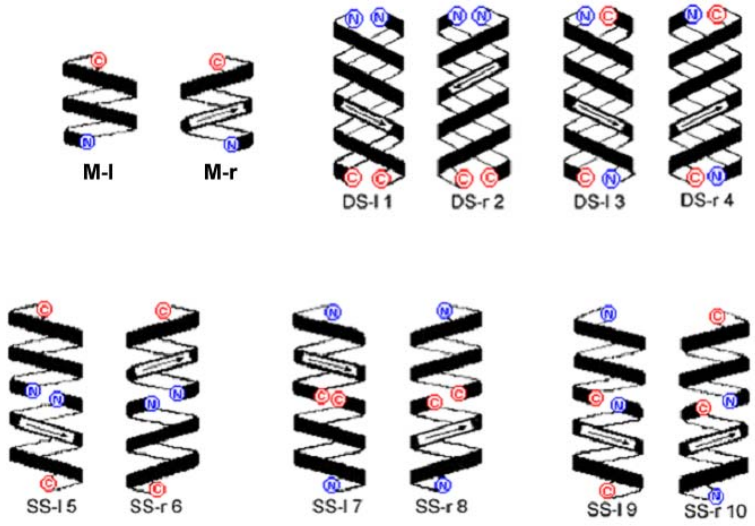

Fig. Single- or double-stranded dimers [3]

We analyzed the structures of five non-stoichiometric gramicidin complexes with NaI, KI, KSCN, RbCl and CsCl. In these structures, large differences in the distributions of cations and waters exist. There is a distinct difference between the distributions of light (K^+ and Na^+) and heavy (Cs^+ and Rb^+) alkali metals.

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P-76

Comparative X-ray Structural Studies on Olanzapine.

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2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-b][1,5]benzodiazepine, $C_{17}H_{20}N_4S$, known as olanzapine, a popular serotonin-dopamine antagonist, is used as a second generation, atypical drug, in the treatment of various psychoses, such as schizophrenia or psychotic depression [1,2]. Nevertheless, the production process of the solid drug is complex because of its ability to create polymorphs and pseudo-polymorphs. The search of the CSD, (ver. 5.31, 2011) [3, 4] revealed 15 olanzapine crystal forms, characterized by single crystal diffraction studies so far: three polymorphs [5, 6, 7], five hydrates [6, 8] and seven other solvates [5, 8, 9, 10, 11]. In order to increase our knowledge about the olanzapine polymorphism, we have studied crystal structures of three new olanzapine solvates with: acetic acid, water-propanone and water-isopropanol. Our interest was to examine their crystal packing and to use this information in conjunction with crystallographic data of other olanzapine forms, to study their isostructurality, which is a useful tool in the deeper understanding of close-packing principles.
Comparing these three new forms, it is possible to conclude that similarly to previously reported structures, they crystallize as monoclinic in two, the most characteristic for olanzapine, space groups: $P2_1/c$ and $C2/c$. The olanzapine molecule consists of three fused rings: benzene, diazepine and thiophene, and also an *N*-methylpiperazine fragment. Geometrical parameters of all olanzapine structures are very similar. The 1,5-diazepine rings have a boat conformation. The benzene and thiophene systems are planar. The piperazine rings adopt a chair conformation with the methyl group assuming an equatorial orientation. Nevertheless, structures consist of different packing and are sustained by diverse strong and weak, intra- and intermolecular hydrogen bonds, as a result of the inclusion of different solvent molecules. On the other hand, all of them exhibit C-H$\cdots\pi$ interactions, between the phenyl ring and the piperazine system. One very characteristic feature of all known solid-state structures of olanzapine is the presence of the olanzapine dimers (centro- or pseudo-centrosymmetric) as the structural building unit. Furthermore, the calculations of isostructurality of all olanzapines reveal that both the unit-cell similarity index [12] and volumetric index [13] are in good agreement. The majority olanzapine structures (~80%) demonstrate good isostructurality with nearly identical packing motifs. Around 20% molecules indicate pronounced resemblance.
Detailed structural analysis of hydrogen bonding, the conformational and crystal packing preferences, including discussion of isostructurality for all known olanzapine forms will be presented at the conference.

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P-77

Synthesis and Physical-Chemical Properties of Porphyrazines Possessing Bulky Peripheral 2,5-Diarylpyrrol-1-yl Substituents.

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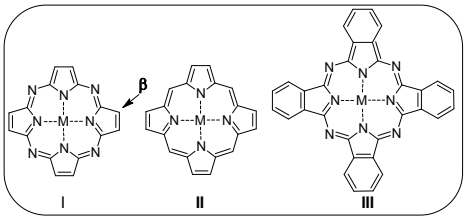
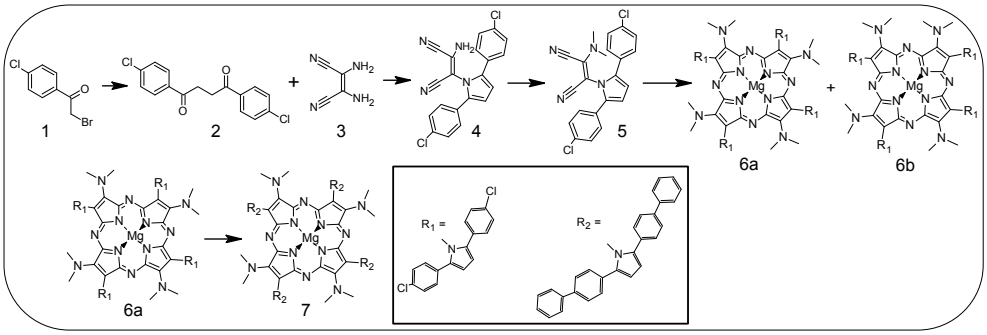
INTRODUCTION

Peripherally functionalized porphyrazines **I** form a distinct class of novel macrocycles, along with the functionalized porphyrins **II** and phthalocyanines **III** (Figure). The porphyrazine (Pz) macrocycle is isoelectronic with porphyrin, but in comparison it has substantially different electronic properties. The first possibility to modify Pz is to substitute the core with metal entities (M), while the second one is connected with peripheral modifications (β). Pzs substituted in their periphery possess many potential applications as photosensitizers in photodynamic therapy and in nanotechnology (sensors, molecular semiconductors and non-linear optical materials) [1].



RESULTS



4'-Chloro-2-bromoacetophenone **1** was used in the Würtz-like type reaction leading to diketon **2** (Scheme) [2]. The Paal-Knorr reaction of diaminomaleonitrile **3** with diketon **2** gave 2-amino-3-[2,5-di(4'-chlorophenyl)-1*H*-pyrrol-1-yl]-(2*Z*)-butene-1,4-dinitrile **4** [3]. Product **4** was subjected to methylation reaction to novel dinitrile **5** which was successfully utilized in the Linstead macrocyclization towards magnesium porphyrazines **6a** and **6b** [4].The purity of both Pzs was confirmed by HPLC and NMR studies. Pz **6a** was used in the Suzuki type reaction with phenylboronic acid to give pz **7**. The purity of **7** was confirmed by HPLC methods.
Moreover, the structures of dinitrile **5** and porphyrazine-Mg complex **6a** were determined by X-ray crystallography. The single crystal analysis shows that **6a** is a symmetrically substituted porphyrazine derivative with 2,5-di(4'-chlorophenyl)pyrrol-1-yl and dimethylamino groups located alternately in the *β*-positions of the macrocycle. The pyrrole substituents are nearly perpendicularly oriented to the porphyrazine core, whereas the dimethylamino groups are situated in the plane of the macrocycle.

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P-78

Application of the Docking Programs in Estimating the Binding Affinity to the SHBG Protein.

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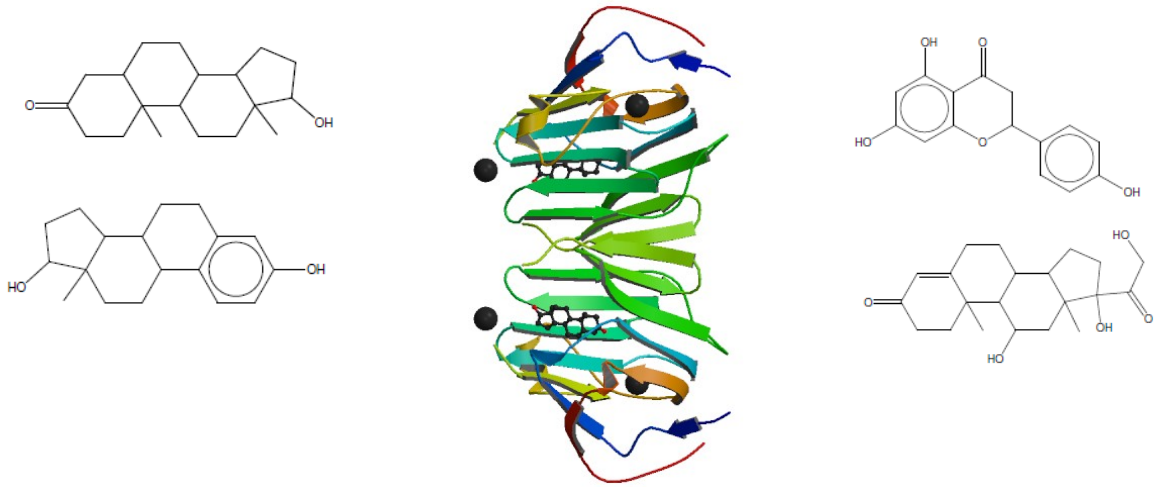
Docking is one of the basic tools in the drug design, used to determine the pose of the compound in the active site and to predict the strength of association or binding affinity to the protein. Validation of those methods can be done by comparing the calculation results with the values obtained experimentally.
SHBG (Sex Hormone Binding Globulin) is a plasma factor responsible for the transport of hydrophobic steroid hormones (among others estrogen and testosterone). Due to the fact that SHBG is a kind of a ligand transport protein (there is no internal activation) it can be a good model for testing the scoring functions used by the docking software in estimating the binding affinity of ligands.



The aim of this study was to determine the accuracy of docking software and to evaluate the results of scoring functions in reproducing the real values of dissociation constants of the complex ligand-receptor.
In this study nine crystal structures of protein SHBG were used for docking and the base of 87 ligands with a specific affinity (in vitro experiments results) to the SHBG protein was tested. Crystallographic structures varied in resolution, ligand present in binding site of the protein and the possible inherency of zinc and magnesium ions. The ligands used in the study differed in the structure and affinity to the SHBG protein (pKa ranging from 4.4 to 9.7).

P-79

Preliminary Evaluation of Antibacterial Activity of Some New Xanthone's Derivatives Against Drug Resistant Strains of *Helicobacter pylori*.

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Searching for the new biologically active structures in the group of xanthone derivatives belongs to main scientific topics at the Department of Bioorganic Chemistry in the collaboration with the Department of Pharmaceutical Microbiology, Faculty of Pharmacy UJ CM. Bibliography confirms wide range of biological activity of both naturally occurring and synthetic xanthone derivatives (e.g. antimalarial [1], antituberculotic [2], antibacterial [3] and antifungal [4]).

Scientific works conducted at the University La Sapienza in Roma proved anti-*Helicobacter pylori* efficacy of some N-substituted 2-oxo-2*H*-1-benzopiran derivatives, revealing structural similarity to xanthone skeleton [5]. Moreover, our synthetic and microbiological research in the group of xanthone derivatives enabled selection of a few compounds with microbiological activity especially against *Mycobacterium tuberculosis* [6] and pathogenic dermatophytes [7]. Results of those investigations constituted subject of our former publications and conference presentations. Those facts, prompted us to undertake searching for new xanthone derivatives with hindering effectiveness against growth of *Helicobacter pylori* strains.

Our interest in this field is also due to worldwide increase of the primary and the secondary resistance of *Helicobacter pylori* strains to the using therapies. Nowadays, the treatment of choice is simultaneous application of proton's pomp blockers or bismuth salts and two from the antibiotics: metronidazole, clarithromycine, amoxycylline and tetracycline. Nevertheless, such a therapy is effective in about 70 % of patients. In the case of failure of *Helicobacter pylori* eradication, the second-line quadruple therapy is used and after all last chance therapy with increased doses. In this light, searching for the new, more effective and less toxic agents seems to be well grounded [8, 9].

Herein we report on preliminary investigation of anti-*Helicobacter pylori* activity of some amine xanthone's derivatives including efficacy against resistant strains collected from hospitalized patients. In addition, antibacterial activity against control strains of *Staphylococcus aureus* ATCC 25 923, *Staphylococcus* aureus MRSA and Escherichia coli ATCC 25922 was estimated. In the first stage of the research we used the qualitative agar disc diffusion method with solutions of test compounds at a concentration of 10 mg/ml. On the basis of the measured inhibition zones of bacteria it was found that all the compounds showed the activity against *Helicobacter pylori* and its lack or the low activity in comparison with other reference strains, other than *H. pylori*. Among the tested structures, majority revealed growth inhibition zones' diameters ≥ 30mm. For the most active compounds quantitative assays were performed to estimate MIC values. We used the quantitative agar disc diffusion method using solutions of six selected compounds at the concentrations of 10mg/ml, 5mg/ml, 1mg/ml, 200µg/ml, 100µg/ml and 50µg / ml. For most strains, MIC value ranged from 100 to 200 µg/ml. The study was a screening test which allowed to draw preliminary conclusions concerning the presence of chemical groups which determine the antimicrobial activity of the compound. An evidence for the effectiveness of synthetic derivatives xanthone's derivatives against *Helicobacter pylori* confirms the significance of further research. In the time of increasing bacterial resistance to currently recommended medications these compounds may be the only effective agents against this pathogen in the future.

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P-80

Synthesis of Phenylalanine-Based AMPA/KA Receptor Ligands.

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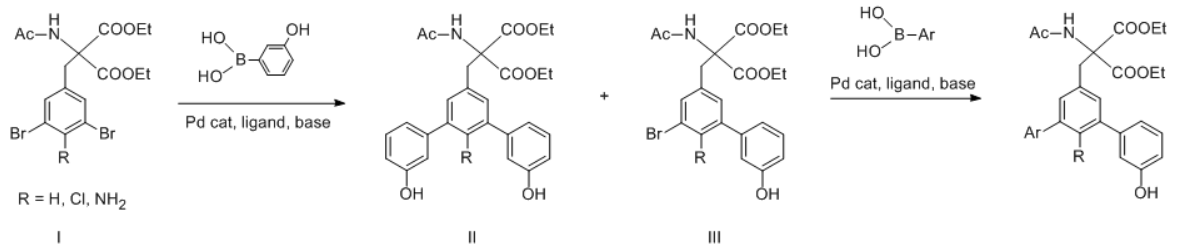
An excessive production of (*S*)-glutamic acid, the major excitatory neurotransmitter in the mammalian central nervous system, appears to constitute a major component in neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. Intervention in glutamatergic neurotransmission has also been proposed to be beneficial in the treatment of epilepsy, pain, stroke, ischemia and certain psychiatric disorders.
The present project is focused on the search for new potent and selective competitive antagonists of an ionotropic subfamily of the glutamate receptors (AMPA, KA) among phenylalanine derivatives. Previously, a group of compounds with the general structure based on the phenylalanine scaffold was designed, synthesized and pharmacologically characterized on both native and cloned receptors [1-3]. Based on these results, a series of molecular docking experiments was performed for a new set of designed phenylalanine derivatives, using as templates available crystal structures of the GluA2 [2] and GluK1 [3] binding cores co-crystallized with various ligands. The most promising compounds, presenting the best docking score function values, were selected to further synthetic studies.
The key step in the synthesis of the target amino acids is the Suzuki reaction (Fig.), leading from 3,5-dibromo derivative **I** to the di- or/and mono-substituted products (**II** and **III**, respectively). In the present work the synthesis and optimization of chemical conditions of the described reaction are reported. The influence of a catalyst, a ligand, a base and a kind of used solvent on time and yield of the reaction as well as ratio **I** : **II** : **III** is studied.



[1] Szymańska E., et al.: *Bioorg. Med. Chem.* **17** (2009), 6390-6401.

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This work was partly supported by Polish Ministry of Science and Higher Education, Grant Nr N N405 303936 and K/ZDS/003324.

P-81

Application of Computer Simulations to the Prediction of the Biological Properties of Acetylcholinesterase Inhibitors.

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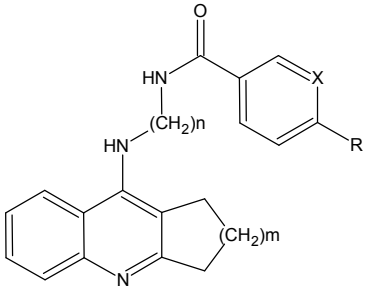
Acetylcholinesterase inhibitors inhibit the enzyme hydrolyzing one of the neurotransmitters - acetylcholine, which stimulates the postsynaptic part of cholinergic neurons. This relationship is used to treat senile dementia such as Alzheimer's disease. This illness is a civilization disorder of the modern world. There are many theories that try to explain the emergence of this disease, one of them is the cholinergic theory which implies that the characteristic clinical symptoms of this disease result from cholinergic dysfunction. [1,2]

The aim of this study was to determine basic ADMET parameters using the software ACD/Percepta. Protein binding, penetration of blood/brain barrier, the toxicity of the Ames test or risk due to the presence of particular pharmacophore groups were defined using appropriate algorithms. The analysis was performed on 32 previously obtained new acetylcholinesterase inhibitors containing tetrahydroacridine or 2,3-dihydro-1H-cyclopenta[b]-quinoline in their structure. Subsequently, on the basis of the results the fundamental relationships between structure and activity in different groups of compounds were determined.

Then, the possible way of binding of various substances to acetylcholinesterase using methods of molecular modeling was showed. Derivatives were characterized by a similar conformation when docked to the enzyme active site. However, during docking to butyrylcholinesterase, we could observe two conformations in the active center. The compounds also showed a dual mechanism of binding to an enzyme – interactions with both the catalytic site and the peripheral site were observed.



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P-82

Synthesis and Potent Antibacterial Activity of New Isothiazolo-Pyridine Derivatives.

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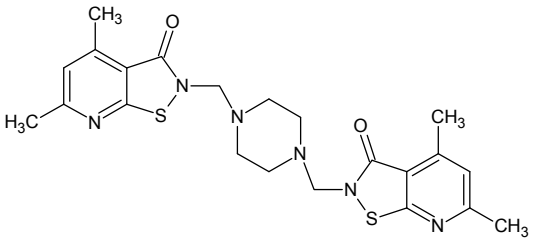
Research of new compounds with potential antibacterial activity are made mainly in group of heterocyclic compounds. A number of papers about biheterocyclic componds like isothiazolopyridine, thiazolopyridine and their analogues (benzo(iso)thiazoles) has been published.
Derivative 1 (containing piperazine as a link between two isothiazolopyridines) has been synthesized. As a comparison, compound 2 (containing aliphatic amine in the middle chain) has been also synthesized. A new derivatives were obtained in Mannich reaction based on previous analouges, synthesized by Hamama [1]. In microbiological test, these compounds have shown higher activity against *Staphylococcus aureus* than Chloramphenicol, used as a reference drug.
It is interesting, how the convertion aryl benzisothiazolone ring to heretoaryl ring in new set of isothiazolopyridine derivatives will impact their antibacterial activity.



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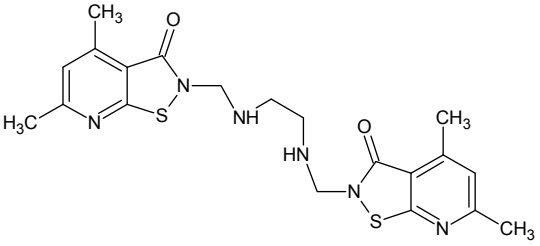

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P-83

Study of the Durability of Derivative of Oxazolo[3,2-a]pirydone.

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The aim of this research was defining influence of pH and temperature on durability of 2-[(4-phenylpiperazin-1-yl]-2,3-dihydro-5H-[1,3]oxazolo[3,2-a]pyridyn-5-one, and introduction analysis of the desintegration of this chemical compound.
The research was introduced in 1-12 range of pH, in temperature 60, 70, 80, 90 and 120 ^{0}C.
In this study, determining of order of reaction, reaction rate constant k, activation energy and half-life were included with using spectrophotometer method in UV light.
UV-VIS spectroscopy could be used, because examined chemical compound fulfilled Beer-Lambert law.
The preliminary analysis of 2-[(4-phenylpiperazin-1-yl]-2,3-dihydro-5H-[1,3]oxazolo[3,2-a]pyridyn-5-one decomposition was done using TLC chromatography, whereas spectroscopic metods (H^{1}NMR, UV) were used to identify products of disintegration.The identyfication of hydrolysis products was also performed using a densitometric method and the HPLC-MS technique.

P-84

Homologs of Epalrestat: Synthesis and Molecular Structure.

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Rhodanine derivatives (2-thioxothiazol-4-one) exhibit a number of interesting biological properties, therefore they are studied intensively for over hundred years [1]. Compounds containing at position N-3 an aryl group exhibit antibacterial [2] and antiviral [3] activities. Some rhodanine derivatives are used as precursors of ligands for complexing gold (I) ions. Such complexes have similar cytotoxic effect to cis-platinum compounds [4]. Derivatives containing at N-3 position carboxy- methylene group and at C-5 position substituted aryl groups show an anti-inflammatory activity [5].

Epalrestat, (5-(2-methyl-3-phenyl-2-propenylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)-acetic acid) is the condensation product of 3-carboxymethylenerhodanine with α-methylcinnamaldehyde. Epalrestat, an aldose reductase inhibitor, has already been approved for clinical use in Japan [6]. The first step in the synthesis of epalrestat and its homologs is to obtain a 3-carboxymethylenerhodanine and its homologs by a known procedure described previously in [7]. In the second step a suitable derivative of rhodanine is condensed with α-methylcinnamaldehyde. The reaction is carried out in an alkaline environment, wherein C-5 carbon atom exibits a nucleophilic activity [8]. In our study we used a new method of condensation of rhodanine derivatives with α-methylcinnamaldehyde in a pyridine solution. After treatment of the intermediate products with hydrochloric acid epalrestat or its homologs were produced with yields of 35 to 45%. Structures of synthetized derivatives were confirmed by IR, ^{1}H-NMR and MS analysis.

The crystal structure of one homolog (5-(2-methyl-3-phenyl-2- propenylidene)-4-oxo-2-thioxo-1,3-thiazolidyn-3-yl)propanoic acid has been determined with use of X-ray diffraction method. The crystals are triclinic, space group $P\bar{1}$. The benzene ring is not coplanar with rhodanine ring, the angle between the planes is 35º. The crystal network in the studied structure is built from homosynthon. Two molecules of investigated compound are connected into a dimer by strong hydrogen bonds O-H···O from carboxyl groups.

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P-85

Anticonvulsant Properties of Some Imidazole-Based Histamine H_3 Receptor Ligands.

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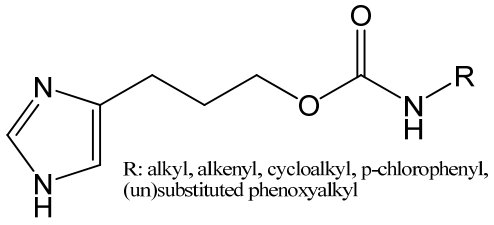
Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures arising from excessive electrical activity in some portion of the brain, is a public health problem, which affects approximately 1% of the worldwide population.
Antiepileptic drugs (AEDs) can influence the inhibitory or excitatory neurotransmitter systems (GABA or glutamic and aspartic acid, respectively), or the ion transport across cell membranes. In the nineties it was demonstrated, that the central histaminergic neuronal system plays an important role in the inhibition of seizure activity. Some studies reported protection by H_3 receptor antagonists in different seizure models including the maximal electroshock (MES) [1], kindling [2], and pentylenetetrazole (PTZ) induced convulsions [3].
In the present study the carbamate derivatives evolved from 3-(1*H*-imidazol-4-yl)propan-1-ol will be investigated on their anticonvulsant properties in pentylenetetrazole (PTZ) induced seizures in Wistar rats, since the histamine H_3 receptor antagonistic activity of these compounds had been previously confirmed *in vitro* and *in vivo* [4].



General structural pattern of investigated compounds

Among investigated compounds only one showed anticonvulsant properties in the doses of 10 and 15 mg/kg. Remaining compounds showed only moderate to lack of activity. A clear correlation between anticonvulsant activity and histamine H_3 receptor affinity could not been observed.

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P-86

Selected Transmembrane Receptors – Structures, Interactions and Binding Site Analysis.

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G-protein couples receptors (GPCRs), sharing consitution of seven transmembrane helices piercing cell membrane, are largely expressed in mammalian organisms. They are involved in a wide variety of physiological processes, such as autonomic nervous system transmission, immunological response, behaviour and mood regulation.
A number of receptors belonging to GPCR family is present in neural tissue and is considered to be involved in learning and memorizing processes. They can be modulated by exogenous compounds, what makes them a common target in drug research. Acquiring information about protein's structure is a basic step to discover interactions involved in ligand binding, which is crucial in design of potential therapeutic compounds. Since structures of proteins with transmembrane domains cannot be easily determined, it is almost impossible to construct their 3D conformation using physical methods. This is why homology modelling is extremely helpful in determining structures of such proteins. Moreover, application of Structural Interaction Fingerprints (SIFts) and averaged SIFt profiles, enables fast and convenient binding site analysis. Such approach allows to determine residues involved in ligand-protein interaction, reveal its type giving insight into binding site proterties.

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Acknowledgments
This study is supported by project UDA-POIG.01.03.01-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD); http://www.prokog.pl

P-87

Synthesis of New Pyrrolidine-2,5-diones Derivatives with Dual SSRI and 5-HT$_{1A}$ activity.

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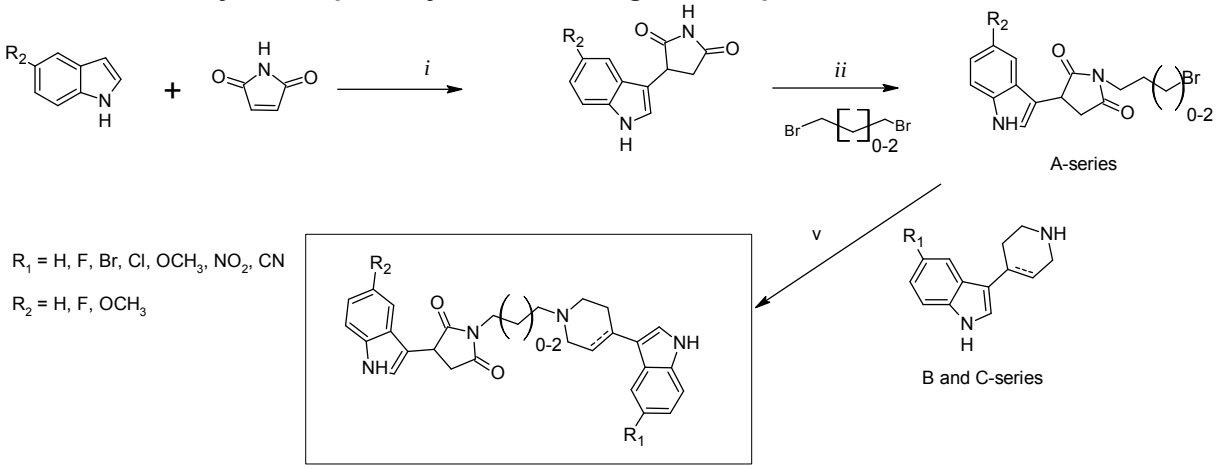
The aim of our research is to synthesise a series of pyrrolidine-2,5-dione derivatives, the compounds with dual binding to 5-HT$_{1A}$ serotonin receptor and SERT, which is a potential new 3[rd] generation SSRI+ antidepressants. Achieving this goal will allow to assess the influence of performed structure modifications on the interaction with certain receptors and SERT. The aim of planned modifications of the leading structure is to receive a compound characterised by a higher degree of affinity to 5-HT$_{1A}$ and SERT than vilazodone [1]. It will enable to verify the hypothesis on a better activity of this group of drugs (SSRI+) than 2[nd] generation drugs (SSRIs) due to reduced latency period.
The synthesis of SSRI+ compounds designed in this studies follows the most recent trends in the search for efficient 3[rd] generation antidepressants. Their chemical structure has been devised in such a way as to obtain a compound with the highest possible affinity to 5-HT$_{1A}$ receptor and SERT transporter. The results of biological *in vitro* and *in vivo* tests have allowed to assess the influence of planned modifications on binding, which in the future can contribute to the discovery of an efficient, new drug.

Scheme 1. The synthesis pathways of the investigated compounds.



Reagents and conditions: (i) CH_3COOH (ii) acetone ,K_2CO_3 (iii) CH_3OH,CH_3ONa (v) CH_3CN, K_2CO_3 , KJ

Three series of synthons were necessary for synthesising the planned compounds: N-bromobutyl, bromopropyl and bromoethyl derivatives of 3-(1H-indole-3-yl)-pyrrolidine-2,5-dione with appropriate substituents at the 1H-indole 5 position (**A-series**) and derivatives of 3-(1,2,3,6-tetrahydropyridine-4-yl)1H-indole (**B-series**) and 3-(piperidine-4-yl)1H-indole (**C-series**) substituted at the 1H-indole 5 position[2-3]. The final designed compounds were obtained according to the methodology developed in Department of Drug Technology and Pharmaceutical Biotechnology.

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P-88

Synthesis of Nitrophenylimidazole Derivative.

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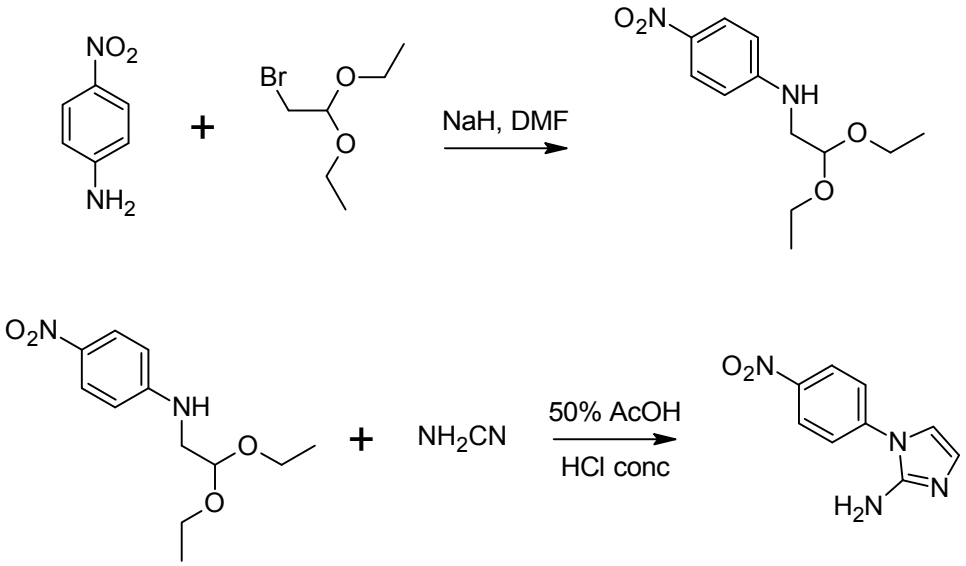
The need for synthesis of 1-(4-nitrophenyl)imidazole-2-amine arose during the pursue of finding novel GSK-3β inhibitors. The title compound was needed as analytical reference standard thus it was required to be of high purity. Several conditions were evaluated and a suitable one is presented in this poster. Final product showed one spot on TLC check and was fully characterized by MS, IR and NMR methods.



The project was developed using the equipment purchased within the Project “The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases” within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

P-89

Synthesis and Undesirable Effects Prediction for Piperazine Benzylideneimidazolone Derivatives with Expected MDR Efflux Pump Inhibitors Properties.

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Multidrug resistance (MDR) has become a factor seriously limiting treatment of various diseases, including anticancer treatment [1], bacterial infections [2] and antifungal therapy [3]. 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. A disadvantage of this strategy is a fact that such reversal agents might simultaneously increase the side effects of antibiotic- or chemotherapy by blocking physiological drug efflux from normal cells. Another problem are various side effects caused by number of EPIs described. Thus, the search for new inhibitors of the efflux pumps and studies on their possible side effects and "drugability" properties has been, and still is, a main topic of medicinal chemistry. Our previous studies were focused on search for new EPIs in group of various hydantoin derivatives. During the studies, piperazine derivatives of arylidenimidazolones were found as the most promising chmosensitizers of resistant Gram negative bacteria *E. aerogenes* that over-produced protein pump AcrAB-TolC. Compound **BM-7** (Fig. 1), found as a most active one in the previous studies, was elected as a lead for further chemical modifications to find compounds with better EPIs activity and low risk of undesirable effects.

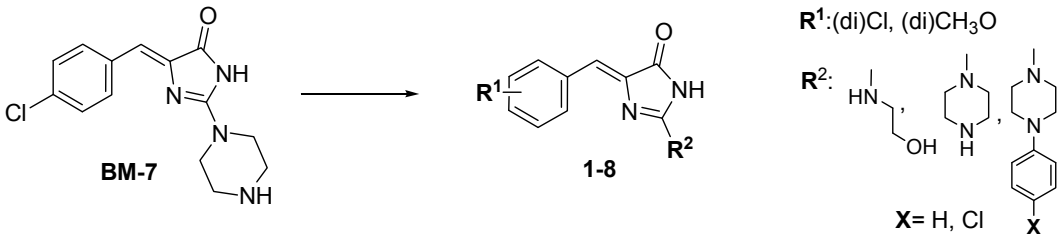

Fig. 1.

The present studies are concentrated on new chemical modifications of the lead **1-10** (Fig.1) and side effects investigations. A series of new compounds was synthesized using 3-4 step synthesis including Knoevenagel condensation, S-methylation, reaction with primary- or secondary amines and N-deprotection. Side effects of the obtained compounds were investigated in silico by the use of program OSIRIS that allowed to predict risk of the imidazolones to cause mutagenic, tumorigenic and reproductive effects as well as their "drugability" properties. In the case of two representative compounds possessing phenylpiperazine fragments, their influence on α_1-adrenergic receptors was expected. The compounds were tested on their affinity for α_1-adrenoceptors in radioligand binding assay with ^{3}[H]-prazosin as a selective radioligand. Results indicated that both compounds have very low affinity for the receptors, what could be a good prognosis for their potential selective EPIs-action.

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P-90

Estimation of Phospholipophilicity of Arylpiperazinylalkyl Derivatives of Imidazo[2,1-*f*]theophylline.

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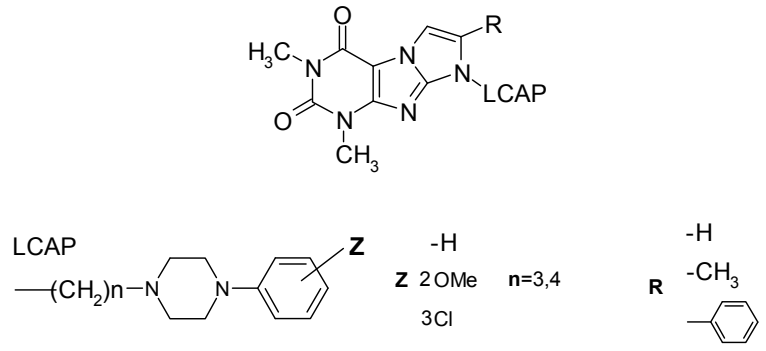
The property-based design has become a new tool in medicinal chemistry [1]. This strategy led to combining optimization of the chemical structure with consideration of potency and molecular properties for absorption, distribution, metabolism, excretion and toxicity (ADMET) [2]. The ADMET parameters can be improved and optimized by chemical modification of structure drug-like molecules. One of the most important physicochemical properties of drug-like molecules is lipophilicity, a key parameter in predicting permeability and one widely used in quantitative structure-activity relationship (QSAR) studies [3].

In our earlier attempt to find new 5-HT$_{1A}$/5-HT$_{2A}$ receptor ligands, a series of the imidazo[2,1-*f*]theophylline derivatives with arylpiperazinylalkyl substituent in N8 position were synthesized. These compounds have been tested in vitro for their 5-HT$_{1A}$ and 5-HT$_{2A}$,receptor affinities and were potent 5-HT$_{1A}$ receptor ligands with K_i within the range of 5,6-96,5nM and demonstrate lack of affinity for 5-HT$_{2A}$ subtype [4].



The aim of this study was to determine the phospholipophilicity of arylpiperazinylalkyl derivatives of imidazo[2,1-f]theophylline using immobilized artificial membrane high-performance liquid chromatography (IAM-HPLC). The performed analysis allowed the calculation of log k_{we} values for each of the tested compounds. Experimental phospholipophilicity data has been compared with the affinity for serotonin receptors (p*K*i). The experimental values were also compared with data calculated by means of software packages.

Results obtained in this study can be used in future for optimizing the drug design and synthesis of new tricyclic theophylline derivatives with optimal physicochemical properties

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The work was supported by grant: K/ZDS/003300

P-91

The Multiobjective Based Design, Synthesis and Evaluation of the Arylsulfonamide/amide Derivatives of Arylxyethyl- and Arylthioethyl Piperidines and Pyrrolidines as a Novel Class of Potent 5-HT$_7$ Receptor Antagonists.

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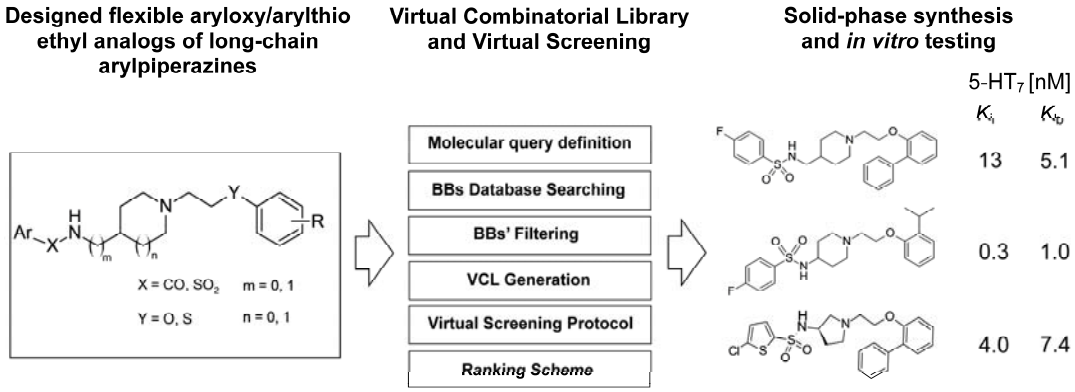
A computational approach involving a combinatorial library design and a multistep virtual screening [1], followed by post-docking filtering and building block ranking within compounds satisfying the desired 5-HT$_7$R binding pattern allowed us to identify critical molecular substructures and provided rationale data for designing 72-member library of the arylamide and arylsulfonamide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines [2]. All compounds were synthesized according to an elaborated parallel solid-phase method and were biologically evaluated for their affinity for 5-HT$_7$. Additionally, the targeted library members were tested for 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ receptors.



Selected compounds of particular interest were examined for their intrinsic activity at 5-HT$_7$R in vitro employing a cAMP assay. The study allowed us to identify compound **68** (4-fluoro-*N*-(1-{2-[(propan-2-yl)phenoxy]ethyl}piperidin-4-yl) benzenesulfonamide) as a potent 5-HT$_7$R ligand (K_i = 0.3 nM) with strong antagonistic properties (K_b = 1 nM) and a 1450-fold selectivity over 5-HT$_{1A}$Rs.

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This study was partly supported by Funds for Statutory Activity of Jagiellonian University Medical College. Radioligand binding experiments were financially supported by the Norwegian Financial Mechanism as part of the Polish-Norwegian Research Fund, Grant No. PNRF–103–AI-1/07.

P-92

Long-Chain Arylpiperazine Derivatives with Cyclic Amino Acid Amide Fragments as Potential 5-HT$_7$ Receptor Ligands.

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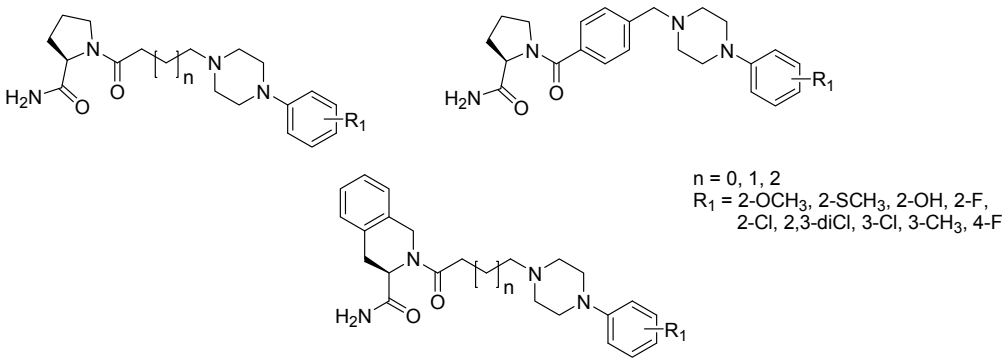
Recently, the 5-HT$_7$ receptor (5-HT$_7$R) has emerged as a new target with a potential for the treatment of psychiatric disorders. It was evidenced that the antidepressant-like effects of well-known atypical antipsychotics amisulpride and aripiprazole are mediated by 5-HT$_7$R antagonism [1]. More recently, it was shown that the 5-HT$_7$Rs may significantly influence cognitive dysfunction and therefore represent a potential therapeutic target for the treatment of memory dysfunction in cognitive disorders (Alzheimer's disease, age-related decline) [2].
As a part of our efforts in identifying selective 5-HT$_7$ receptor ligands with arylpiperazine structure we designed and a series of LCAPs containing amino acid amide fragments (pyrrolidine-2-carboxamide, 1,2,3,4-tetrahydroisoquinoline-3-carboxamide). Herein we present our initial data on design, solid-phase synthesis and biological evaluation of a 48 member library.



Selected library representatives displayed high-to low affinity for 5-HT$_{1A}$ (K_i = 0.2–6307 nM), 5-HT$_7$ (K_i = 18–3134 nM), and D$_2$ (K_i = 25–2892 nM) receptors. Herein, we examine an influence of position and character of a series of electronic and polar substituents and discuss on structural features determining 5-HT$_{1A}$ and 5-HT$_7$ receptor affinity and selectivity.

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This study was partly supported by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 671540.

P-93

Influence of Different Solvents on Forming Hydrogen Bonds in Crystal Structures of Ellagic Acid.

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Ellagic acid (EA), a natural polyphenolic compound, is found in many fruits and vegetables, including raspberries, strawberries and walnuts. Interest in EA has increased during last decade due to its biological properties. On the basis *in vitro* and small-animal models, it was reported that EA, as many other antioxidants, is also an anticancerogenic and antiproliferative agent [1-3]. Recent studies have indicated that EA has also antibacterial, antiplasmodial and chemopreventive activity [4, 5].
EA is considered as a potentially active pharmaceutical, so it is important to study its ability to form different crystalline modifications. Investigation of polymorphism and solvatomorphism in drug molecules is a crucial step in pharmaceutical technology. Pseudopolymorphs exhibit different physicochemical properties which should be considered during drug development [6].
The molecular structure of EA (systematic name: 2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) has been determined earlier in its anhydrous form, by X-ray diffraction analysis [7]. The molecule of EA comprises four hydroxyl groups which exhibit a very good functionality for hydrogen bonding as proton donors. The lactone groups, present in EA molecule, can be involved in hydrogen bonding as proton acceptors.
To study the solvatomorphism in ellagic acid two crystal structures have been determined with the use of X-ray diffraction method. The obtained single crystals of dimethyl sulfoxide solvate and of dimethylformamide solvate belong to $P\bar{1}$ and to $P2_1/c$ space groups, respectively. The packing of the ellagic acid molecules in the crystals of these two solvatomorphs is dominantly controlled by the molecules of solvents, which form different hydrogen bonding patterns. Only one type of the conventional hydrogen bond (O-H···O), formed by the four hydroxyl groups of EA with oxygen atoms of the solvent, is observed for both structures. In the structure of EA_DMSO are observed rings $R_4^4(14)$, in which there are four hydroxyl groups as donors and two oxygen atoms of the solvent as acceptors. The molecules of EA, connected *via* solvent molecules, give the rise to “double chain” throughout the crystal, which can be also called ribbons. In the structure EA_DMF are observed chains $C_2^1(12)$, which form the rings $R_8^4(36)$. These rings are built by two mutually perpendicular pairs of parallel molecules of EA and four molecules of DMF. The weak hydrogen bond $C_{sp}{}^2$-H···O has significant influence on the packing in this crystal structure.

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The equipment used for X-ray data collection was purchased thanks to the financial support of the European Regional Development Fund in the framework of the Polish Innovation Economy Operational Program (contract no. POIG.02.01.00-12-023/08).

P-94

Feature selection for structure based pharmacophore model by means of Structural Interaction Fingerprint and 3D motif.

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Pharmacophore models are a common tool used in experiments of Virtual Screening (VS) aimed for searching active compounds. Among the various methods of developing such molds, the structure based pharmacophore model is of great importance, as it encapsulates the information about the binding site of the target protein.
In this research we present the method of selecting the pharmacophore features of the amino acids forming the binding cleft, which play an important role in accommodating the active compounds. On the basis of Structural Interaction Fingerprints (SIFts) [1], the bitstrings describing ligand – receptor contacts in a formalized manner, the frequently interacting residues are selected, and an ensemble of atoms common for a set of ligand – protein complexes named 3D motif [2] is employed to assign the appropriate pharmacophore features of the binding site.
Such a model of the binding site can be further used in the process of developing the structure based pharmacophore model, or to apply the restrains for the docking experiments.

Acknowledgements
This study is supported by project “Diamentowy Grant” DI 2011 0046 41 financed by Polish Ministry of Science and higher Education.

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P-95

Modelling the Interactions Between Glycogen Synthase Kinase 3β and Its Inhibitors. Dilema of Choice of the Most Appropriate Scoring Function While Investigating the Drug-Receptor Interactions Using the Technique of Molecular Docking. Case Study Over Selected GSK-3β1 Inhibitors.

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A throughout understanding of the major principles that govern the interactions within the intramolecular ligand-protein complexes is of great significance in medicinal chemistry especially in the field of rational drug design. Among the vast range of computational techniques applied in order to investigate them, the method of molecular docking is the paramount.
Unfortunately despite the enormous development of docking over the recent years this method still has some serious drawbacks that ought to be improved. The diversification of generated docking results and lack of consilence in case of their classification by various scoring functions substantially impedes the identification of the real binding mode of a ligand, reflecting the molecule's maximal adjustment within the enzyme.
The presented research was performed with the aim of exploring the molecular interactions between GSK-3β1 and a selected group of its pharmacological inhibitors with different affinity to the enzyme (values micro- and nanomolar). The investigation was conducted by method of molecular docking using GOLD software.
During docking ChemPLP was selected as the main scoring function. The docking was performed with a simultaneous rescoring using GoldScore function.
The analysys revealed the major components of the binding pocket of GSK-3 involved in ligand binding. Needless to emphasize that it perfectly reflected the complexity of problems that modern medicinal chemistry must come across in the field of molecular modeling. Owing to the lack of agreeableness in grading the given solutions by various scoring functions choosing the most adequate one turned out to be the hindrance of utmost importance.
In order to overcome it we performed a QSAR analysys by correlating the IC50 values with the values of the applied scoring functions for best ranked solutions obtained for the molecules egzamined. The linear regression analysys prompted us to select GoldScore as more adequate scoring function for our research. In order to make certain about the selection of the most appropriate scoring function it is advised to expand the investigated group of compounds, alternatively to perform the docking with the utilisation of the described methodology for other available crystal structures of GSK-3.

P-96

Modulation of the Lipophillicity of Novel Tricyclic Annelated Theophylline Derivatives Obtained as Adenosine Receptors Ligands.

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Adenosine is an important regulator for homeostasis of the brain, heart, kidney and other organs. Adenosine interacts with four different G-protein coupled receptors classified as A_1, A_{2A}, A_{2B} and A_3 receptor subtypes. Selective interaction with adenosine receptor (AR) subtypes offers very broad therapeutic potentials including CNS disorders, regulation of electrophysiological properties of heart, immune system and inflammatory diseases, cell growth, asthma, kidney failure and ischemic injuries.
Adenosine receptors' ligands are currently being developed as promising agents for CNS disorders (Parkinson's, Alzheimer's, morbus, ischemia)[1]. The lipophilicity is a parameter regarded as an important factor significantly influencing CNS bioavailability.
Searching for new selective AR ligands, we have synthesized tricyclic derivatives of theophylline with, pyrimidine- annelated ring and substituded with hydoxy(alkyl)phenyl substituent connected directly, or *via* ethylene linker with the structure presented at Fig. 1 (n=0 or 1). Obtained compounds have shown affinity to ARs mainly A_{2A} subtype [2,3].

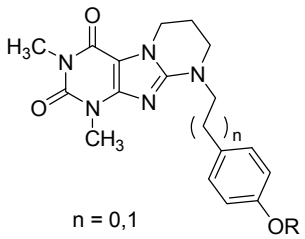

Fig. 1 General structure of novel adenosine receptor ligands

In order to influence on the biological activity of the obtained compounds their physicochemical properties were changed. Introduced were substituents R (via ether linker having: ester, acidic, and aminoalkyl funcitionalities) influencing on their lipophilicity and soulubility. The obtained compounds were bioassayed for the affinity towards A_1, A_{2A}, A_{2B} and A_3 human receptors in *in vitro* binding tests.
In the aim to evaluate their physicochemical properties their lipophilicity was evaluated expressed by R_{M0} values using planar RP-TLC method. The theoretical partition coefficient parameters (logP) were also calculated using computer programs: Marvin and QikProp for Schrödinger [4]. The influence of the lipophilicity parameters (R_{M0}, logP) on their activity K_i was discussed.

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P-97

Identification of Hypothetical Allosteric Binding Sites in hHman μ-Opioid Receptor.

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Receptor proteins are key units responsible for communication of a living organism and it's environment, as well as for it's internal signalling and regulation. Seven-transmembrane spanning receptors constitute one of the most numerous family of proteins, involved in great number of signaling pathways. For this reason, this family is particularly interesting group of potential drug targets. Human μ-opoid receptor is one of the members of this family, involved in pain perception, mood and immune system regulation as well as in respiratory system function. There are well-known effective opioid orthosteric drugs available. Unfortunately, they present serious side effects, e.g drug dependence or respiratory depression. This problem could be solved by design of effective allosteric opioid ligands, with better selectivity towards desired medicinal effect and more favourable therapeutical profile [1].
The aim of the work was to find approximate binding regions of non-typical ligands [2-5] in μ-opioid receptor. Models of the receptor were obtained with the method of homology modelling, using various sets of multiple templates, consisting of crystal structures of mouse inactive-state μ-opioid receptor, turkey β_1 active-state receptor, human β_2 active-state receptor and human active-state adenosine A2a receptor, with use of Modeller 9.10 program. Model populations were initially assessed by screening procedure with limited flexibility docking, with the use of Surflex Screen approach from SybylX 1.3. Models with docking results following theoretical constrains [6] were chosen for series of more detailed flexible dockings, performed with Surflex GeomX.
Analysis of the results have led to the conclusion that there are three hypothetical allosteric pockets to focus on. The choice was based on various criteria, including scoring function results, visual assessment, repeatability of subsequent dockings and repeatability of docking patterns among different ligands from the investigated set. First pocket involves two Lys residues (K235 and K305) on the extracellular part of TM V and TM VI helices, a region known for being involved in GPCR activation [7]. Second pocket contacts second extracellular loop in the area of K211 and T220; probability of binding in this location is supported by analogous location of allosteric pocket in muscarinic M2 receptor [8]. Third site is located in the region of TM II, in the nearness of N129 and Y130. These hypothetical allosteric binding sites can be a starting point for allosteric ligands design and modifications.

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P-98

Development of Multistep Ligand-Based Virtual Screening Cascade Methodology in a Search for Novel HIV-1 Integrase Inhibitors: 2. Privileged Fragments.

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HIV integrase which is essential in the virus replication cycle and has no homologue among human enzymes [1], became an important target for drug development more than twenty years ago. Nevertheless, progress has been hampered by the lack of assays suitable for high throughput screening. Thus, a real breakthrough was only observed in 2007 with the introduction of the first integrase inhibitor, raltegravir, into treatment.
Crystal structure for HIV-1 integrase is already known and thus, both techniques commonly used in VS campaigns (structure and ligand-based) could be developed. Here we introduced a multistep ligand-based screening cascade because it is suggested that ligand-based methods outperform structure-based in true positives identification [2]. Our strategy consists of two sequential modules: machine learning-based (ML-based) and privileged fragments-based (PF-based).
The PF module is a weight-based scoring function which rates presence of particular molecular fragments, previously recognized as privileged, in screened compounds. Mentioned fragments are defined as structural subunits specially effective in distinguishing active compounds from inactives. PFs were extracted by using MI-DSE formalism [3] on thirteen unique training sets. Finally, the prepared module was applied as standalone or as a second-step in a multistep VS experiment. The test set was composed from 450 actives (not used for the module development) and 16200 DUD [4] decoys generated by an in-house script. The developed module achieved AUC = 0.760 and more than 40-fold enrichment in a single-step experiment. Overall, two-step VS campaign, where PF-based module was a second-step, reached more than 200-fold enrichment of actives/inactives ratio.

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This presentation is a continuation of poster entitled ‘Developed of multistep ligand-based virtual screening cascade methodology in a search for novel HIV-1 integrase inhibitors: 1. Machine learning’.

P-99

Synthesis and the Crystal Structure of (E)-methyl(4-(2,3,4-trimethoxystyryl)phenyl)sulfane.

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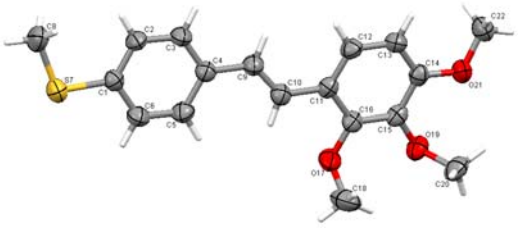
The key synthetic step for the construction of this compound involves the generation o diethyl 4-methylthiobenzyl phosphonate as an intermediate. This was prepared from commercially available 4-methylthiobenzyl alcohol in two steps. First, 4-methylthiobenzyl alcohol was converted to the chloride using $SOCl_2$ in toluene at room temperature. Then, through the Michaelis-Arbuzov reaction of the 4-methylthiobenzyl chloride with triethylphosphite at 130°C we obtained the corresponding phosphonate ester [11]. Final compound was prepared by Wittig-Horner reaction of diethyl 4-methylthiolbenzyl-phosphonate with the commercially available 2,3,4-trimethoxybenzaldehyde in DMF using sodium hydride as a base [2].



The asymmetric unit of (E)-1-(2,3,4-trimethoxyphenyl)-2-(4'-methylthiophenyl)ethene **(I)** contains one molecule. For this compound, the double bond C9-C10 in the conjugated linkage is in the trans configuration (torsion angle C(4)-C(9)-C(10)-C(11) 179.7(2)°. Futhermore, the observed double bond is exactly as theoretical value and the single bonds are shorter (C(4)-C(9) 1.466(3)Å, C(10)-C(11) 1.469(3)Å) than the theoretical values (1.32Å for double bond and 1.51Å for single bonds; [3]) indicating the formation of a weak conjugated π-electron system. The crystal structure is stabilized by this weak C—H···O contacts as well as van der Waals interactions. In the structure of stilbene (**I**), the aromatic rings deviate not significantly from a coplanar arrangement, with a dihedral angle of 6.6(2)° between the planes. Among the three methoxy substituents of aromatic ring, only that at C14 is approximately coplanar with the attached ring; the other two, at C15 and C16, are oriented towards opposite sides of the attached ring [9].

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P-100

Influence of Hydrazone Derivatives of Chromones on Proliferation and Migration Properties of MCF-7 Cell Line.

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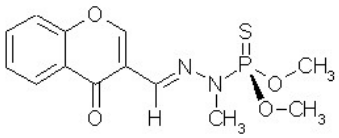
Natural and synthetic compounds of chromone and coumarin have come to the attention of many scientists on the world because of their wide biological activity. The benzo-γ-pyrone derivatives of chromone possess useful properties, like anticancer[1], antiviral[2], antimicrobial[3,4], antifungal[5], antioxidant [6,7] and many others.
Some previously synthesized in Department of Bioinorganic Chemistry benzo-γ-pyrone compounds were evaluated *in vitro* using NALM-6, HL-60 and HL-60 ADR cell lines. The significant cytotoxicity and influence on apoptosis of several studied derivatives were observed [8]. For proliferative and migration-affecting properties towards MCF-7 cell line we selected four hydrazone compounds of chromone:



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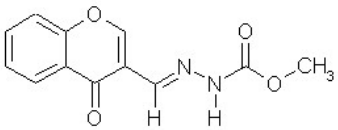

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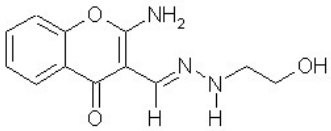

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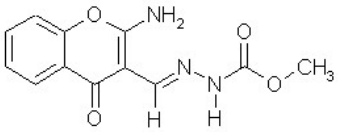

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P-101

Structural and Biological Studies of *trans*-Platinum(II) Complex with 3-Aminoflavone.

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Diamminedichloroplatinum(II) (cisplatin, *cis*-DDP) is one of the most widely administered antitumor drug. However, the use of *cis*-DDP is severely limited because of its serious side effects such as nephrotoxicity, ototoxicity and allergy. Therefore, effort is concentrated on the development of the others platinum compounds with a broader activity spectrum, effectiveness in chemotherapy, but lower toxicity[1].
For many years it was believed that the platinum complexes of *trans* geometry were non-active as antitumor agents because of inactivity *in vivo* and less cytotoxicity *in vitro* than cisplatin. However, since the 1990s scientists discovered numerous biologically active platinum complexes with *trans* geometry and different substitutes instead of ammine groups, e.g. planar amines, iminoethers, aliphatic amines or non-planar heterocyclic ligands [2]. In an attempt to design new antitumor drugs based on transplatin complexes, we synthesized new *trans*-platinum(II) complex, containing 3-aminoflavone as non-leaving ligand (*trans*-$Pt(af)_2Cl_2$, af = 3-aminoflavone.
The aim of our studies is to synthesis a new *trans* platinum(II) complex of 3-aminoflavone) and estimate its cytotoxic and proapoptotic properties. Cisplatin was used as a reference compound. The *in vitro* anticancer activity of the compounds was evaluated against three leukemia cell lines (L1210, L1210R, HL-60).
Trans-Pt(II) complex with 3-aminoflavone was obtained. Pt(II) ions are four-coordinated by two chloride ligands and two nitrogen atoms of 3-aminoflavone. The geometry of the Pt(II) complex is square-planar adopting *trans* configuration. The obtained results indicated that the novel *trans*-platinum(II) complex effectively inhibited cancer cells growth. The tested complex was active against all three leukemic cell lines: L1210, L1210R and HL-60. After 72 h treatment, *trans*-$Pt(af)_2Cl_2$ showed significant activity (IC_{50}<10μM), but lower in comparison to cisplatin. Apoptosis induction was observed after cells treatment with both tested compounds (*trans*-$Pt(af)_2Cl_2$ and cisplatin). After 4 hours incubation and 24 hours postincubation a new *trans* platinum(II) complex with 3-aminoflavone caused apoptosis. Apoptotic cell death involves a series of morphological and biochemical changes, which include phosphatydylserine externalization, collapse of mitochondrial transmembrane potential (Ψm) and activation of caspase-3. Interestingly, *trans*-$Pt(af)_2Cl_2$ was a stronger inducer of apoptosis in contrast to *cis*-DDP despite its lower cytotoxicity [3]. Details of this study will be discussed in the poster session.

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P-102

Glycyrrhizic and Glycyrrhetic Acids in Medicine and Pharmacy.

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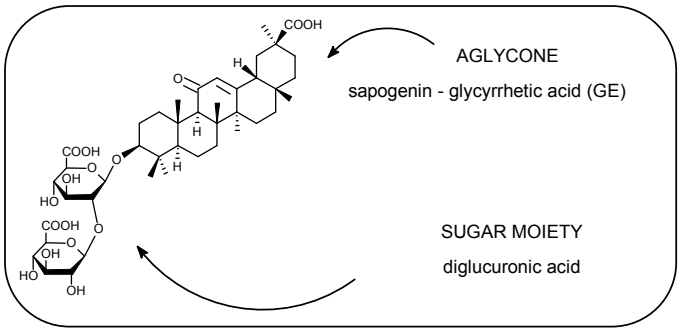
Glycyrrhizic (GA) and glycyrrhetic (GE) acids are the main biologically active compounds of licorice roots (*Glycyrrhiza glabra L., Leguminosae*). GA exists in form of a mixture of potassium, calcium and magnesium salts [1] often called glycyrrhizin. GA is a glycoside that consists of a sugar moiety (glycone) and an aglycone part. The glycone is made of two molecules of glucuronic acid, whereas the aglycone is a sapogenin - glycyrrhetic acid (Figure). The presence of hydrophilic (diglucuronic acid) and hydrophobic (glycyrrhetic acid) fragments causes the self-aggregation of amphiphilic GA molecules and their salts, and further micelle formation in water solution [2]. Lipophilic GE is the active metabolite of GA and its solubility in water is very low (below 0.01 mg/mL), which results in its poor bioavailability.



GA and GE exhibit several biological properties including hepatoprotective, antiviral, immunomodulatory, anti-inflammatory and chemopreventive effects. Because of the variety of pharmacological activities, GA and GE emerge as promising group of compounds for use in therapies of many diseases. Moreover, GA forms complexes with drugs, which increases their solubility, stability, bioavailability and synergistic therapeutic effect is often observed. Recent studies have shown that the use of GA and GE as active substances or excipients in complex medicinal products allows for better control of transport and release of the drug in the body, which increases the effectiveness of therapy [3-6].

GA and GE are very often used in targeted therapies of liver diseases, in prolonged release products on the basis of cyclodextrins, chitosan and co-polymers of lactic and glycolic acids, and also in modern pharmaceutical formulations – micelles, liposomes and quantum dots. Moreover, GA in form of its sodium salt is applied in suppositories. Interestingly, GE exerts soothing, anti-irritant and anti-inflammatory effects in topical applications. GA can also act as a permeability enhancer in gels including sodium diclofenac and carboxymethylcellulose. In transferosomes, GA disodium salt ensures flexibility of the whole structure during its skin penetration. Due to the variety of pharmacological effects GA and GE are promising compounds for therapies of many diseases and both as active substances and excipients in formulations [7-10].

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P-103

Synteza nowych 3β-acyloaminowych pochodnych tropanu o potencjalnym działaniu antypsychotycznym (II)

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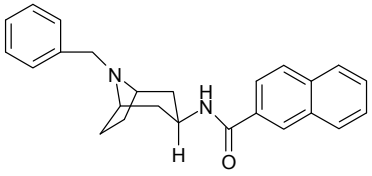
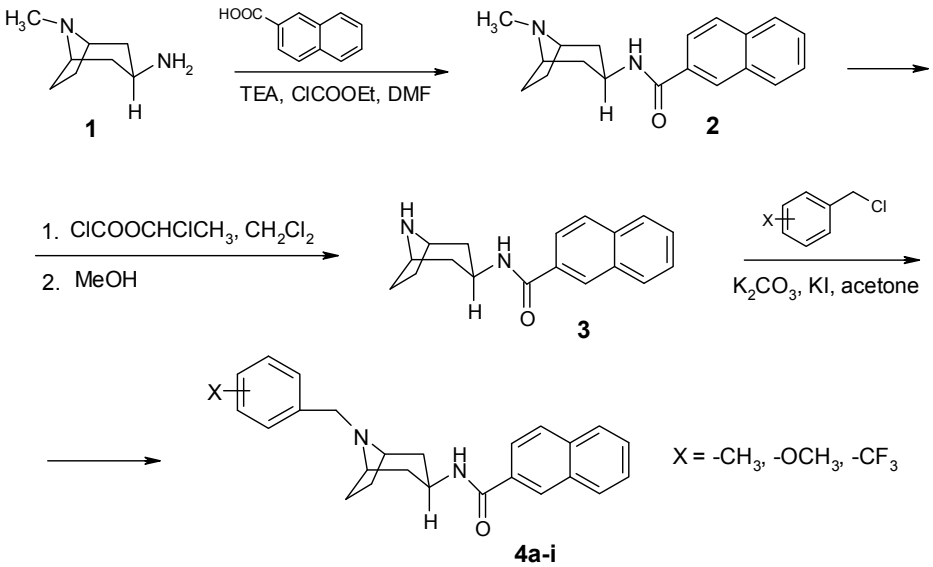
Kontynuując poszukiwania nowych 3β-acyloaminowych pochodnych tropanu o potencjalnym działaniu antypsychotycznym przeprowadziliśmy syntezę szeregu analogów związku **A**.
Związek ten wykazuje bardzo wysokie powinowactwo do receptorów 5-HT_{1A}, 5-$HT_{2A,}$ i D_2. Posiada on również bardzo korzystny indeks Meltzera (p*Ki* 5-HT_{2A}/p*Ki* D_2), który kwalifikuje go do grupy pochodnych o potencjalnym atypowym działaniu antypsychotycznym [1].



A

Syntezę analogów struktury wiodącej **A** z podstawnikami –CH_3, -OCH_3, -CF_3 w pozycjach o-, m- i p-pierścienia fenylowego przedstawiono poniżej. Budowę nowych związków potwierdzono za pomocą widm IR, ^{1}H-NMR i HRMS. Wszystkie otrzymane pochodne, zgodnie z założeniem, są izomerami ekwatorialnymi.



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