POLSKIE TOWARZYSTWO CHEMII MEDYCZNEJ

PAN Komitet Terapii i Nauk o Leku

Książka abstraktów

14-16 września 2023

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SCIENTIFIC PROGRAM

THURSDAY 14.09.2023

15.00 – 17.00 Registration Hotel Victoria

17.00 – 17.10 Opening Ceremony

17.10 – 18.10 Inaugural Lecture (IL) Hotel Victoria

Marco L. Lolli, University of Turin, Turin, Italy

"Human dihydroorotate dehydrogenase (hDHODH) as drug target: who is going to win the hDHODH golden rush?"

18.10 – 18.40 Memories of the Professor Franciszek Sączewski

Anita Kornicka, Medical University of Gdańsk

18.40 Concert

19.30 Welcome reception

FRIDAY, 15.09.2023 Hotel Victoria

9.30 – 10.15 Lectures L1 (45')

10.15 – 11.30 Communications C1 – C3 (20')

Session moderators: Katarzyna Kieć-Kononowicz & Dariusz Matosiuk

L1

Manuela Jörg, Monash University, Melbourne, Victoria, Australia

„Development of novel bivalent chemical probes to interrogate human biology"

C1

Katarzyna J. Malawska, The University of Tokyo, Tokyo, Japan

„Bioconjugation of Au_{25} Nanocluster to Monoclonal Antibody at Tryptophan"

C2

Vittorio Canale, Jagiellonian University Medical College, Kraków, Poland

„Medicinal mechanochemistry, an emerging strategy for a sustainable synthesis of biologically active compounds"

C3

Mirosław Danch, ABL&E-JASCO Polska Sp. z o.o.

11.30 – 12.00 Coffee break

12.00 – 14.00 Communications C4 – C8 (20')

Session moderators: Barbara Malawska & Tomasz Gośliński

C4

Bartłomiej Rogalewicz, Lodz University of Technology, Łódz, Poland

„Physicochemical characterization and anticancer activity of the new imipramine based Co(II), Pd(II) and Mn(II) compounds"

C5

Agnese C. Pippione, University of Turin, Turin, Italy

„New AKR1C3 inhibitors to target prostate cancer"

C6

Magdalena Wujak, Colegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland

„Uncovering new anti-tumor mechanisms of different generation tyrosine kinase inhibitors: focus on ATP-adenosine pathway in lung cancer"

C7

Greta Klejborowska, University of Antwerp, Wilrijk, Belgium

„Inhibiting ferroptosis: design, synthesis and biological evaluation of novel lipophilic radical traps"

C8

Andrzej Polski, Ryvu Therapeutics S.A., Kraków, Poland

„Formulations for Preclinical Studies: An Introduction and Case Studies"

14.00 – 15.30 Lunch

15.30 – 16.00 Poster oral presentations PP1 – PP5 (10')

Session moderators: Jadwiga Handzlik & Paweł Zajdel

PP1

Kamil Piska, Jagiellonian University Medical College, Kraków, Poland

„The Role of Carbonyl Reduction of Anthracyclines in Cancer Resistance"

PP2

Łukasz Popiołek, Medical University of Lublin, Lublin, Poland

„Substituted benzenesulfonyl hydrazones: synthesis and in vitro bioactivity study"

PP3

Michał Jastrzębski, Medical University of Lublin, Lublin, Poland

„Deciphering the multi-target mechanism of action of D2AAK1 for potential treatment of neurodegenerative diseases"

PP4

Przemysław Zaręba, Cracow University of Technology, Kraków, Poland

„Sulfonamide derivatives of cyclic arylguanidines as a new scaffold for 5-HT_6R ligand design"

PP5

Angelika Grudzińska, Medical University of Lublin, Lublin, Poland

„D2AAK5 and its derivatives as ligands for serotonin receptors”

16.00 – 17.30 Poster session

SATURDAY, 16.09.2023 Hotel Victoria

9.00-10.30 Communications C9 – C12 (20’)

Session moderators: Anna Bielawska & Krzysztof Bielawski

C9

Liwia Lebelt, Medical University of Lodz, Łódź, Poland

„The phosphonate analogues of amino (hydroxy) carboxylic acid with potential biological activity”

C10

Aleksandra Trocha, Medical University of Lodz, Łódź, Poland

„New aminoalkylphosphonates with potential biological activity”

C11

Damian Kułaga, Cracow University of Technology, Kraków, Poland

„The impact of structural modifications of the compound DK-PR6 on its affinity to the 5-HT_7 receptor and the evaluation of metabolic stability using mouse liver microsomes”

C12

Anita Płazińska, Medical University of Lublin, Lublin, Poland

„Understanding the pharmacology of the β2-adrenergic receptor: Comprehensive toxicological and molecular docking analyses of beta-mimetics and beta-blockers in zebrafish”

10.30-11.30 Lecture L2

Session moderators: Monika Wujec & Marcin Mączyński

*Roman Lesyk***,** *Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*

„Thiazole/thiazolidinone-based derivatives. 20-year experience in the search for new biologically active molecules”

11.30 – 12.10 Coffee break

12.10 – 14.00 Communications C13 – C17 (20’)

Session moderators: Dorota Piotrowska & Marcin Kołaczkowski

C13

Dorota Chełminiak-Dutkiewicz, Nicolaus Copernicus University of Toruń, Toruń, Poland

„New materials based on polysaccharides with natural active substances for potential wound dressing applications”

C14

Angelika Becht, Lodz University of Technology, Łódź, Poland

„Selection of protein fragments important in the regeneration of hard-to-heal wounds (DFU)”

C15

Krzesimir Ciura, Medical University of Gdansk, Gdańsk, Poland

„Application of bio-chromatographic methods to evaluate drug candidates”

C16

Sylwia Mroszczyk, Medical University of Lublin, Lublin, Poland

„Facts and myths about italian Dolce - Vita - interdisciplinary about coffee”

C17

Paweł Śliwa, Cracow University of Technology, Kraków, Poland

„Dynamics and binding energy of antagonists and agonists to the dopamine D4 receptor”

14.00 – 15.30 Lunch

15.30 – 17.00 Communication C18 and Poster oral presentations PP6 – PP10 (10’)

Session moderators: Beata Morak-Młodawska & Łukasz Popiołek

C18

Zbigniew Leśnikowski, Institute of Medical Biology PAS, Łódź, Poland

„National Library of Chemical Compounds”

PP6

Kinga Mylkie, Nicolaus Copernicus University of Toruń, Toruń, Poland

„Magnetic nanoparticles coated with chitosan with free dihydroxyboryl groups for fast binding of glycoproteins”

PP7

Aleksander Smolarkiewicz-Wyczachowski, Nicolaus Copernicus University of Toruń, Toruń, Poland

„Hemoglobin-based nanomaterials as potential drug carriers in Photodynamic Therapy (PDT)”

PP8

Sara Janowska, Medical University of Lublin, Lublin, Poland

“Synthesis and Antimicrobial Activity of New Mannich Bases”

PP9

Krzysztof Romaniuk, Lodz University of Technology, Łódź, Poland

„Synthesis and characterization of substrates for the preparation of long-acting insulin analogs. Triazine condensing reagents in the synthesis of insulin analogs”

PP10

Monika Pitucha, Medical University of Lublin, Lublin, Poland

„Application of HPLC and FTIR methods to evaluate immobilization of a new antimicrobial substance on a biomaterial”

17.00 Closing of the conference and award ceremony

20.00 – 24.00 Get-together Party (Hotel Victoria)

Inaugural Lecture

Human dihydroorotate dehydrogenase (hDHODH) as drug target: who is going to win the hDHODH golden rush?

Marco L. Lolli[a]

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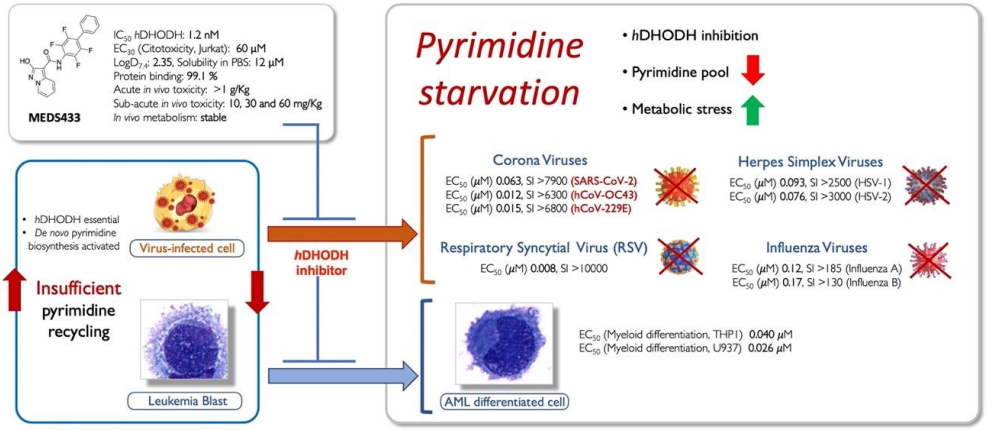
At the end of 2016, the connection between Acute Myelogenous Leukemia (AML) and *dihydroorotate dehydrogenase* (*h*DHODH), a key enzyme in *de novo* pyrimidine biosynthesis, generated considerable interest from the pharmaceutical industry as a possible new therapeutic opportunity for this *unmet clinical need*. Since the COVID-19 outbreak, the use of *h*DHODH inhibitors as *Host Targeting Antivirals* (HTA) became one of the most promising therapeutic options for COVID-19 treatment as well as other pandemic outbreaks. In 2023, the discovery of the *h*DHODH role in blocking *ferroptosis* in solid tumors cells open other scenarios also in these fields.

In this occasion, the program active since 2010 at the University of Turin dedicated to design innovative *h*DHODH inhibitors will be fully presented. By using an innovative bioisosteric approach supported by structure-based techniques, **MEDS433**, a potent *h*DHODH inhibitor (IC_{50} = 1.2 nM) was discovered. **MEDS433** is able to induce myeloid differentiation in AML cell lines (THP1 and U937) in the low nM range (EC_{50} = 40 and 26 nM), superior to the AML phase I/II *lead brequinar* (EC_{50} = 249 nM (THP1) and 189 nM (U937)). By leading the cell into *pyrimidine starvation*, **MEDS433** inhibits the *in vitro* replication of a large panel of viruses, with EC_{50} always in the low nM range. On SARS-CoV-2, the replication is inhibited at EC_{50} = 63 nM, being **MEDS433** five folds superior of the antiviral *Molnupiravir* (EC_{50} = 300 nM), recently approved for COVID-19.

Beside detailing the **MEDS433** design&SAR, PK, ADME, toxicity (acute/subacute on different species) as well as the *in vivo* efficacy in different AML models (leukemic xenograft and IV (mouse, IP, PO)), its synthetic technological transfer (8 g batches, purity > 98.5 %) is also presented. To reinforce the scenario, the pathway that allowed the discovery of the *backup compound* **MEDS700** (EC_{50} = 17 nM, THP1), is also presented. All these studies, most of them still unpublished, are directed to open the incoming **MEDS433** certified



preclinical studies necessary for prepare its Phase I clinical trial for AML.

Moving to the conclusion the conference, the clinical scenario that involve *h*DHODH inhibitors will be detailed. In particular, the most recent strategies investigated for overcame possible *h*DHODH resistance at clinical level will be presented. This final step will try to answer the title question: *who is going to win the hDHODH golden rush?*

References

[1] Sainas S.; Lolli M.L. *et al J. Med. Chem.* **2022**, 65 (19), 12701-12724. [2] Houshmand, M.; Lolli, M. *et al Cell Death and Disease* **2022**, 13 (6), 576. [3] Gaidano, V.; Lolli, M.L. *et al Cancer* **2021**, 13, (5), 1-22. [4] Sainas S., Lolli M.L. *et al J. Med. Chem* **2021**, 64 (9), 5404–5428. [5] A. Calistri, Lolli M.L. *et al Microorganisms* **2021**, *9*, 1731; [6] A. Luganini, Lolli M.L. *et al Antiviral Res.* **2021**, *189*, 105057.

Lectures L1 – L2

L1

Development of novel bivalent chemical probes to interrogate human biology

Manuela Jörg

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Drug discovery programs are typically characterised by a high attrition rate, whilst clinically approved drugs are often associated with severe side effects.[1] The inadequate fundamental understanding of the complexity of chemical reactions that underly human biology has been largely attributed to the disappointing progress in drug development.[1-2] Daily, many billion chemical reactions are performed in our body for it to work effectively. The human body is a complex system, where atoms and molecules play a central role in maintaining the function of our body, including our ability to sleep, breath, move or communicate. Consequently, there is a crucial need to improve our knowledge of human biology on a molecular level. Our research aims to significantly contribute closing this knowledge gap by developing chemical probes that can be used as tools to study important biological processes.

Specifically, we are interested in the design of novel bivalent probes to study protein degradation, receptor dimerisation and antibody delivery systems.[3-4] These concepts are of high importance to the drug discovery community, yet our understanding of these systems on a molecular level remains limited and is haltering the development of more effective and innovative drugs that benefit patients. Therefore, there is a need to develop innovative bivalent probes to dissect the detailed mechanism underlying these systems. Innovative chemical probes are desperately needed to advance chemical biology strategies with increased biological relevance, that overcome the limitations of artificial systems (e.g. approaches relying on tagged or fusion constructs) that are commonly used.[5-6]

[1] Parasrampuria D. A. et. al. *AAPS J.* 20 (2018), *20*, 46.

[2] Editorial “Mechanism matters”, Nature Medicine, 2010, *16*, 347.

[3] K. J. Gregory, M. Jörg *Purinergic Signal.* (4) 2022, 395–398.

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L2

Thiazole/thiazolidinone-based derivatives. 20-year experience in the search for new biologically active molecules.

Roman Lesyk [a], Serhii Holota [a], Andrii Lozynskyi [a], Anna Bielawska [b], Krzysztof Bielawski [b]

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Thiazole/thiazolidinone derivatives belong to the traditionally well-known class of biologically active molecules. Among the 4-thiazolidinones ligands of biological macromolecular targets involved in biochemical processes of tumor growth were identified, namely: inhibitors of the interaction of anti-apoptotic proteins Bcl-XL and BH3, tumor necrosis inhibitors, inhibitors of double-specific tyrosine phosphatases JSP-1, etc. Due to the integrated use of innovative technologies, it was found that thiazoles/thiazolidinones show a high affinity for PPAR receptors and UDP-MurNAc/L-Ala ligase, which led to the creation of potential anti-inflammatory, hypoglycemic, cardiovascular, and antimicrobial agents on different stages of clinical trials.

The design and development of biologically active 4-thiazolidinone-based derivatives is our long-term scientific project. The synthetic strategy of our scientific team involves the chemical modification of the thiazole/thiazolidinone core at positions 2, 3, 4, and 5, utilizing various types of reactions (Knoevenagel condensation, [2+3]- and [3+3]-cyclocondensations, *hetero*-Diels-Alder reaction, Michael additions, etc.). This approach allowed us to yield over 8000 novel heterocyclic derivatives. Among the obtained molecules, we have studied chemically diverse compounds, including thiopyrano[2,3-*d*]thiazoles, thiazolidinone-pyrazole/benzothiazole hybrids, 5-ene-4-thiazolidinones with varied substituents, isothiocoumarin-3-carboxylic acids, thiazolo[4,5-b]pyridines, etc. The different activity types have been studied (anti-inflammatory, antimicrobial, anticonvulsant, choleretic, etc.). However, despite perspective results observed across several testing areas, the main focus was the evaluation of the anticancer, antibacterial (antiprotozoal), and antiviral activities as the field with the highest unmet medical needs and highest predicted success rates. Thus, more than 3,000 biological assays have been conducted, which allowed us to identify about 500 hit-compounds with anticancer, antimycobacterial, antiviral, antitrypanosomal, antileishmanial, antifibrotic and anticonvulsant activities. The screening research and in-depth studies have led to the following conclusions: Leukemia cell lines exhibited high sensitivity to 4-thiazolidinones and related heterocyclic systems. The antitumor activity of the tested compounds was confirmed to be apoptosis-dependent. Anticancer properties of 4-thiazolidinone and thiopyranothiazole derivatives via PPARγ receptors modulation, inhibition tubulin polymerization and topoisomerase II were experimentally established.

The broad scope of synthesized derivatives opens up new avenues for developing pharmacologically active molecules with diverse applications in medicinal chemistry. Exploring their biological potential may pave the way for the discovery of novel therapeutics and contribute to advancements in drug research and development.

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Communications C1 – C18

C1

Bioconjugation of Au_{25} Nanocluster to Monoclonal Antibody at Tryptophan

Katarzyna Joanna Malawska[a], *Shinjiro Takano*[b], *Kounosuke Oisaki*[c], *Haruaki Yanagisawa*[d], *Masahide Kikkawa*[d], *Tatsuya Tsukuda*[b], *Motomu Kanai*[a]

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Cryogenic Electron Microscopy (Cryo-EM) is a dynamic field of structural biology, but despite many advances, the images obtained by cryo microscopes remain extremely noisy. One way to tackle this problem is to apply labelling with heavy metal particles such as colloidal gold. Recent years saw a surge in reports on special types of particles called gold nanoclusters. Compared to colloidal gold, their synthesis requires special skills, but they are more stable, their core is smaller, and they can be covalently attached to proteins which makes them excellent candidates for high- resolution electron probing of proteins. Bioconjugation of larger gold nanoclusters such as Au_{71}, Au_{102}, and Au_{144} with the aim to create an antibody-based label for Cryo-EM has already been described in the literature. Reported methods mostly relied on time- consuming genetic manipulation on protein to install additional cysteine residue, whose free thiol would serve as an actual bioconjugation handle.

In this presentation, the first example of bioconjugation of an Au_{25} gold nanocluster to protein, without genetic manipulation is reported. The novelty of the method is the application of mild tryptophan-selective bioconjugation protocol which was fine-tuned since the first report[1] to make it compatible with gold nanoclusters. Currently, easier-to-handle keto-ABNOH derivatives can be applied in the reaction instead of keto-ABNO radicals, under aqueous neutral buffered conditions and strain- promoted alkyne-azide cycloaddition is employed to secure cluster on protein. The utility of the method is demonstrated on whole monoclonal antibody trastuzumab. Successful separation of the Au_{25} conjugate and comparison with the conjugate synthesized by conventional lysine-selective bioconjugation under Cryo-EM are also presented[2].

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C2

Medicinal mechanochemistry, an emerging strategy for a sustainable synthesis of biologically active compounds

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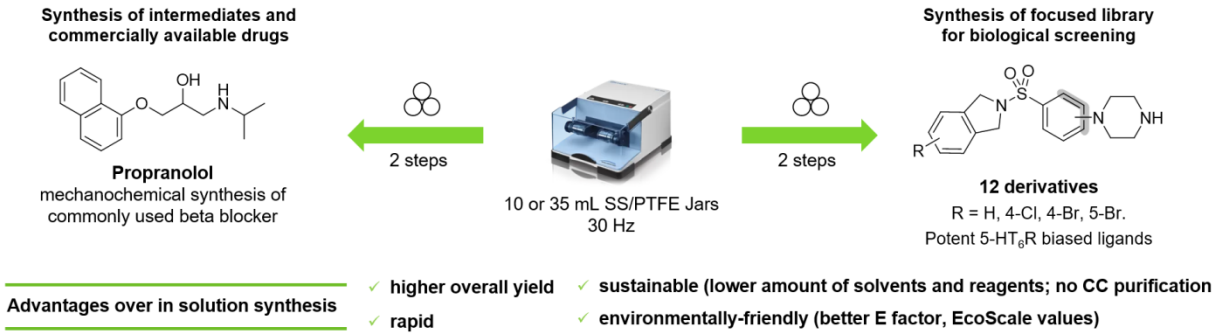
In the last decades, mechanochemistry has been recognized as a widely accepted alternative for the synthesis of organic compounds [1]. The growing interest in this solid-state approach has been pursued because of its methodological advantages over classical thermal methods and to address the need for sustainable development goals. A substantial number of organic transformations to synthesize active pharmaceutical ingredients (APIs) have been successfully adapted for mechanochemical or biocatalytic mechanochemical protocols [2]. This trend led to coining of the term “medicinal mechanochemistry” [3], to highlight the ongoing evolution of solid-state processes from a screening technique in pharmaceutical materials science to a sustainable methodology to produce pharmaceuticals.
Continuing our efforts on the development of greener chemical procedures [4], we have demonstrated the versatility of the medicinal mechanochemical approach for the synthesis of drugs (i.e. propranolol) and lead structure for preclinical evaluation or alternatively for generating focused libraries of compounds for biological screening.



Compared to the classical in-solution approach, the developed multistep mechanochemical protocols improved the overall yield, reduced the reaction time, and decreased the use of toxic reagents and organic solvents. All synthesized intermediates and final compounds were isolated in high purity by simple extraction and/or crystallization without the need for column chromatographic purification. Furthermore, we reported on additional advantages of mechanochemistry over thermal methods in terms of different chemical reactivity and controlled chemo selectivity opening the possibility to obtain unexpected products. The presented results confirmed that the use of mechanochemistry enhances the efficiency and sustainability of chemical transformations for the synthesis of biologically active compounds and could facilitate the cross-collaboration between academia and industry to boost progress and possibilities for greener preclinical development programs and manufacturing methods.

Acknowledgment: The project was supported by National Science Center, Poland grant no 2020/39/B/NZ7/01494 and 2021/05/X/NZ7/01847 and by Jagiellonian University Medical College Statutory Activity N42/DBS/000274.

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C3

Mirosław Danch, ABL&E-JASCO Poland Sp. Z o. o.

C4

Physicochemical characterization and anticancer activity of the new imipramine based Co(II), Pd(II) and Mn(II) compounds

Bartłomiej Rogalewicz [a], Tomasz Sierański [a], Małgorzata Szczesio [a], Andrzej Olczak [a], Agnieszka Korga-Plewko [b], Magdalena Iwan [c], Mariola Michalczuk [b], Agnieszka Czylkowska [a]

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Metal-based materials have long attracted the scientists' attention all around the world due to their unique properties and a vast variety of possible applications. Some of the most important ones comprise areas like medicine [1], electronics [2], catalysis [3], fluorescent or sorption and storage materials [4]. Metal salts or organic molecules alone often don't exhibit as prominent properties as when used in combination. Coordination and organometallic chemistry are currently some of the promising strategies in fighting cancer, which nowadays is undoubtedly one of the deadliest civilization diseases. The combination of biologically active organic molecules and different metal cations allows for obtaining outstanding anticancer activity.

In this study, three new imipramine-based compounds, namely: $(HIMP)_2CoCl_4$, $(HIMP)_2Pd_2Cl_6$ and $(HIMP)_2MnCl_4$ (HIMP = protonated imipramine) were synthesized in a single-step reaction between imipramine hydrochloride and metal(II) chlorides. The physicochemical properties of all three compounds were thoroughly investigated using appropriate analytical techniques: single crystal and powder X-Ray diffraction analysis, elemental analysis, UV-Vis and FTIR spectroscopies, fluorescence measurements and TG-DTG analysis. The investigations proved the high purity of the compounds and the isostructurality of $(HIMP)_2CoCl_4$ and $(HIMP)_2MnCl_4$ compounds. Of all compounds, only $(HIMP)_2MnCl_4$ exhibited fluorescent properties. In the last step, the anticancer activity of all three compounds was evaluated against MKN-74, PANC-1, SCOV-3, MDA-MB-231, MDA-MB-468, A-375 and SK-MEL-28 cell lines. Cytotoxicity evaluation revealed that the incorporation of the active metal ions significantly increased the imipramine activity against all tested cancer cell lines without significant toxicity against normal fibroblasts.

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C5

New AKR1C3 inhibitors to target prostate cancer

Agnese C. Pippione[a], Chiara Vigato[a], Iole Mannella[a], Stefano Sainas[a], Laura Bertarini[a], Sandra Kovachka[b], Barbara Rolando[a], Tomasz Wrobel[c], Osman A. Mirza[d], Karla Frydenvang[d], Francesca Spyrakis[a], Simona Oliaro-Bosso[a], Marco L. Lolli[a] and Donatella Boschi[a]

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Since 2017 our group has developed several molecules able to inhibit the steroidogenic enzyme aldo-keto reductase 1C3 (AKR1C3), that is considered an attractive target in Castration Resistant Prostate Cancer (CRPC): AKR1C3 catalyses some key steps of biosynthesis of androgens testosterone and DHT and, at the same time, it is implicated in resistance to several anticancer drugs.[1]
We recently report three series of AKR1C3 inhibitors containing hydrolated azoles, derived from modulation of the not selective inhibitor flufenamic acid.[2] Here, we describe the bioisosteric approach used to discover the *hit compounds* and how, combining crystallographic experiments and *in silico* guided design, we finally obtained very potent AKR1C3 inhibitors with notable activity against CRPC models.
We also investigated the effects of the best AKR1C3 inhibitors in combination with a drug currently used for the clinical treatment of CRPC, abiraterone (CYP17A1 inhibitor), for whose AKR1C3 enzyme has a critical mechanism of resistance. The obtained results of this combination study showed enhanced effects, suggesting the effectiveness of the combination therapy with these elective drugs to increase their efficacy.
In silico design, synthesis, enzymatic inhibition and biological evaluation against tumoral and non-tumoral cells of the new series of AKR1C3 inhibitors are here described and discussed.

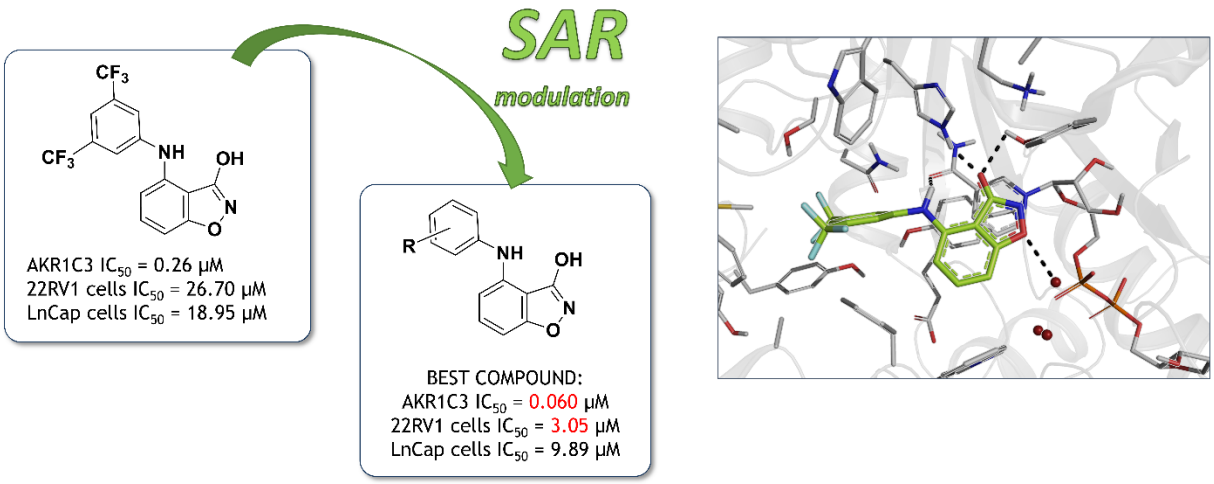

Figure 1. Design of new AKR1C3 inhibitors with benzoisoxazole core.

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C6

Uncovering new anti-tumor mechanisms of different generation tyrosine kinase inhibitors: focus on ATP-adenosine pathway in lung cancer

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Extracellular adenosine triphosphate (eATP) and adenosine (eAdo) are highly concentrated in the tumor microenvironment (TME) and function as signaling molecules in the purinergic signaling system. The concentration of these molecules is regulated by various nucleotide metabolizing ecto-enzymes, including nucleoside triphosphate diphosphohydrolases (in particular, CD39/NTPDase 1 and NTPDase 2), 5'-nucleotidase (CD73), and phosphotransferases involved in the eATP resynthesis (for instance, adenylate kinase 1, AK1). The sequential conversion of eATP into eAdo by CD39 and CD73 hydrolases has a critical impact on tumor progression and immunosuppression. The increasing evidence indicates that modulation of eATP and eAdo concentrations in the TME by targeting the expression and/or activity of nucleotide metabolizing ecto-enzymes may be a novel strategy to combat cancer. For instance, the CD39/CD73 axis is now extensively studied as a promising therapeutic target for reshaping the antitumor immunity [1]. Molecular targeted therapy using tyrosine kinase inhibitors (TKIs) has greatly advanced the treatment for non-small cell lung cancer (NSCLC). Most of TKIs inhibit the cellular signaling of multiple targets, leading either to additional beneficial effects or unwanted side effects. Hence, it is of great importance to explore the landscape of TKI modulatory effects on cancer cell biology [2]. In our work, we first compared the expression pattern of genes encoding CD73, NTPDases 1 and 2, and AK1 in NSCLC cell line A549 after the single treatment with TKIs belonging to different molecular target classes and generations (gefitinib, afatinib, osimertinib, nintedanib, and sorafenib). For the selected TKIs, we next investigated what are the functional implications of the identified gene expression alterations. For this purpose, we studied extracellular ADP and ATP metabolism and the activity of purinergic system ecto-enzymes of TKI-treated cancer cells, with a particular interest in ATP degradation/resynthesis as well as adenosine production.

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C7

Inhibiting ferroptosis: design, synthesis and biological evaluation of novel lipophilic radical traps

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Ferroptosis was conceptualized in 2012 by Dixon *et al.* [1]. It is a non-apoptotic cell death characterized by peroxidation of polyunsaturated fatty acyl phospholipids (PUFA-PLs), the availability of redox-active iron and the loss of lipid peroxide repair capacity by the phospholipid hydroperoxidase GPX4 [2]. Ferroptosis plays an important regulatory role in the occurrence and development of many diseases [3,4]. Thus, ferroptosis inhibitors may have therapeutic potential in the treatment of ischemia-reperfusion injury, kidney injury, and cardiac diseases as well as in the transplantation applications. In addition, ferroptosis has been linked to neurodegenerative diseases such as Alzheimer's and Parkinson's, in which lipid peroxidation, elevated level of ROS, glutathione deficiency, and iron homeostasis are important features [5]. Different strategies aiming to halt ferroptosis have been identified, including the use of iron chelators, radical trapping antioxidants (RTAs), lipoxygenase inhibitors, deuterated lipids and ACSL4 inhibitors [6].
The Augustyns' and Vanden Berghe's research groups focused on the development of radical trapping antioxidants [7–9]. Over the last few years, several generations of ferrostatins have been synthesized, among which molecule UAMC-3203 [8,9] showed increased metabolic stability, solubility, high potency in animal models and demonstrated no toxicity in mice after daily administration over four weeks. Herein, we showcase the latest findings from evaluating our candidate lead compound, UAMC-3203, as well as the design, synthesis, and biological evaluation of a novel series of lipophilic radical-trapping antioxidants aimed at enhancing membrane permeability.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie Grant Agreement N°101065370 (NeuroFerro).

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C8

Formulations for Preclinical Studies: An Introduction and Case Studies

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The discovery and development of new drugs constitute a highly intricate process with significant challenges. Despite increased investments in research and development, the number of approved new drugs has not shown a proportional rise, while the attrition rate of drug candidates has increased. This phenomenon can be attributed, in part, to the difficulty in identifying formulations that effectively translate from preclinical to clinical stages, largely due to a lack of accurate predictions concerning therapeutic and toxicological responses in the preclinical phases.

This presentation aims to shed light on the critical role of preformulation studies in drug development. We will delve into case studies that exemplify the importance of various formulation strategies, including pH adjustment, reference formulation modifications, and oil-based formulations.

One of the challenges faced during early discovery is the integration of formulation scientists in the lead candidate selection process. Insufficient understanding of the interplay between the physiological system and the formulation has contributed to the failure to incorporate the right expertise at the appropriate stages, leading to suboptimal clinical outcomes.

A pivotal factor influencing the success of preclinical studies is the seamless collaboration and integration between formulation and discovery scientists. Proper communication and cooperation between these two domains are paramount in achieving favorable results in clinical trials.

Furthermore, we will explore the impact of regulatory requirements on formulations. Adherence to these standards is crucial but can also affect the timeline and cost associated with progressing a drug candidate.

Through these case studies, we will demonstrate how optimizing formulations for preclinical studies can significantly impact the overall success of drug development. By illustrating real-world examples of pH adjustment, reference formulation modifications, and oil-based formulations, we aim to provide insights and best practices that can benefit drug development endeavors.

In conclusion, this presentation will serve as a comprehensive introduction to the topic of formulations for preclinical studies, highlighting the significance of this critical phase in drug development. The case studies presented will underscore the importance of collaboration, understanding regulatory guidelines, and the successful integration of formulation scientists in early discovery. Emphasizing these aspects will foster greater success rates in drug development, ultimately advancing the translation of promising preclinical formulations into successful clinical candidates[1].

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C9

The phosphonate analogues of amino (hydroxy) carboxylic acid with potential biological activity

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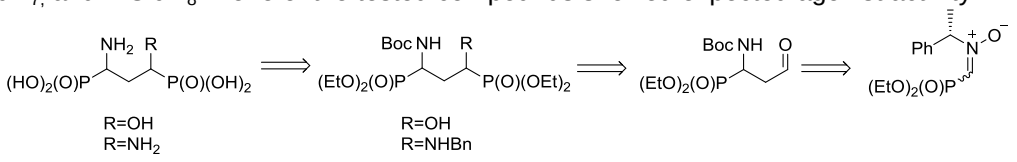
Glutamic acid is the main neurotransmitter in the central nervous system. The changes in its activity are related to disorders such as depression, Alzheimer's, and Parkinson's diseases. The biological activity of L-glutamic acid derivatives has become an inspiration to design new compounds functionalized with a hydrophilic hydroxyl or amino group in the γ position Phosphonic groups [$P(O)(OH)_2$] have been introduced instead of the carboxyl functions. The phosphonic acid moiety may compete with a substrate having a carboxyl group for the active site of an enzyme or other cellular receptor. The introduction of additional amino and hydroxyl functional groups may change the physicochemical properties of these compounds and cause significant changes in the interaction with receptors. Since the designed compounds have two stereogenic centers, the synthesis of all possible stereoisomers should be considered, four enantiomers of (1-amino-3-hydroxypropane-1,3-diyl)diphosphonic acid and three enantiomers of (1,3-diaminopropane-1,3-diyl)diphosphonic acid.

The precursors for the synthesis of functionalized diphosphonic acids were enantiomerically pure diethyl (*S*) or (*R*)-(1-amino-3-oxopropyl)phosphonates, which have been converted into isomeric tetraethyl (1-amino-3-hydroxypropane-1,3-diyl)diphosphonates by Abramov's reaction with diethyl phosphite, and into tetraethyl (1,3-diaminopropane-1,3-diyl)diphosphonates by the Kabachnik-Fields reaction with triethyl phosphite and benzylamine. The obtained diastereoisomers of respective diphosphonates have been hydrolyzed to the corresponding diphosphonic acids. Enantiomerically pure diethyl (1-amino-3-oxopropyl)phosphonates have been obtained from (*S*)-*N*-(1-phenylethyl)-*C*-(diethoxyphosphoryl)nitrone according to the methodology previously developed in our research group. An alternative pathway for the synthesis of (*S*)-*N*-(1-phenylethyl)-*C*-(diethoxyphosphoryl)nitrone was developed using the oxidation of the appropriate amine with oxone. Determination of the absolute configuration at C3 in (1-amino-3-hydroxypropane-1,3-diyl)diphosphonic esters and (1,3-diaminopropane-1,3-diyl)diphosphonic esters required their transformation into the respectively protected derivatives, and then analysis of ^{31}P NMR and ^{1}H NMR spectra, respectively.

Diphosphonic acids were examined for agonist activity towards metabotropic glutamate receptors: $mGluR_4$, $mGluR_7$, and $mGluR_8$. None of the tested compounds showed expected agonist activity.



C10

New aminoalkylphosphonates with potential biological activity

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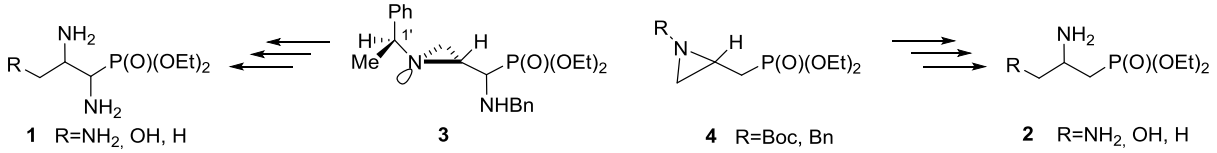
The wide spectrum of biological activity of aminophosphonic acids, their esters, and short phosphonopeptides has become an inspiration to design two series of enantiomeric propylphosphonates **1** and **2** additionally functionalized with amino and hydroxyl groups. The respective aziridinephosphonates **3** and **4** were convenient substrates for the synthesis of designed compounds.

The key step in the synthesis of enantiomerically pure aziridinephosphonates **3** having an amino group in the α position was a one-pot three-component Kabachnik-Fields reaction of respective enantiomeric *N*-(1-phenylethyl)aziridine-2-carboaldehydes with benzylamine and triethyl phosphite leading to the formation of a mixture of two diastereoisomeric aziridinephosphonates **3**. The absolute configuration of a newly generated stereogenic center in both diastereoisomers has been established. On the other hand, aziridinephosphonates **4** without any substituent at α position have been obtained as enantiomerically enriched compounds in a two-step reaction sequence involving aziridine ring formation followed by the introduction of an electro-withdrawing or an electron-donating group on the nitrogen atom.

All enantiomeric aziridinephosphonates **3** and **4** were subjected to the nucleophilic aziridine ring opening reactions leading to the formation of the designed propylphosphonates functionalized with amino and hydroxyl groups at C1, C2 and C3 (the series of compounds **1**) as well as at C2 and C3 positions (the series of derivatives **2**). The influence of the nature of the protection group present at the nitrogen atom on the regioselectivity of the ring-opening reaction in aziridinephosphonates has been examined. Attempts to determine the enantiomeric purity of all new enantiomerically enriched compounds have been undertaken.

The enantiomerically pure aminopropylphosphonates **1** and **2** as well as aziridinephosphonates **3** and **4** were examined for antibacterial, antifungal, antiviral, and cytostatic activities. Aziridinephosphonate (1*S*,2*R*,1′*S*)-**3** showed the highest antibacterial, antiviral, and cytostatic activities. B*iological screening of all* enantiomerically enriched compounds *is currently in progress.*



1 R=NH_2, OH, H **3** **4** R=Boc, Bn **2** R=NH_2, OH, H

C11

The impact of structural modifications of the compound DK-PR6 on its affinity to the 5-HT$_7$ receptor and the evaluation of metabolic stability using mouse liver microsomes

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The 5-HT$_7$ receptor is one of the most extensively studied receptors among the entire range of serotonin receptors. Both agonists and antagonists of 5-HT$_7$R demonstrate biological activity. Inactivating this receptor is primarily associated with the treatment of depression [1] or cognitive disorders [2], while activating the receptor may have an impact in treating neuropathic pain[3]. Recent studies indicate that this receptor may also play a crucial role in the treatment of conditions such as breast cancer [4].

The antagonist DK-PR6 (5-HT$_7$, K_i = 8 nM) represents an intriguing compound as a candidate for *in vivo* studies in depression models. However, due to its low metabolic stability, this compound may not exhibit the desired effects in a living organism. Therefore, DK-PR6 has become a starting point for further optimizations to maintain its pharmacological parameters while enhancing its metabolic stability. Various substitutions were investigated in both the side chain and the indole ring, as well as modifications of 1,3,5-triazine core. It turns out that introducing a morpholine ring into the triazine core or modifying the arylpiperazine side chain caused threefold increase of metabolic stability while pharmacological profile have not changed. For selected compounds, other *in vitro* parameters were evaluated, such as drug-drug interactions, as well as hepatotoxicity and cardiotoxicity in an animal model using *Danio rerio*. All compounds were obtained through an efficient and environmentally friendly synthesis method supported by microwave irradiation.

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C12

Understanding the pharmacology of the β2-adrenergic receptor: comprehensive toxicological and molecular docking analyses of beta-mimetics and beta-blockers in zebrafish

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β_2-adrenergic receptor is a key molecular target that regulates important processes occurring in the human organism. Although over the last decades the zebrafish model has been developed in biomedical research as a complementary one with respect to rodents, the role of fish β_2AR in regulation of pathological and toxicological effects remains to elucidate. Therefore, the present study aimed to clarify the role of β_2ARs with a particular emphasis on the distinct role of subtypes A and B of zebrafish β_2ARs. As model compounds selective β_2AR agonists, (*R*,*R*)-fenoterol ((*R*,*R*)-Fen) and its new derivatives: (*R*,*R*)-4-methoxyfenoterol ((*R*,*R*)-MFen) and (*R*,*R*)-4-methoxy-1-naphtylfenoterol ((*R*,*R*)-MNFen) were tested. We described dose-dependent changes observed after fenoterol exposure in terms of general toxicity and cardiotoxicity responses. Subsequently, to better characterise the role of β_2-adrenergic stimulation in zebrafish, we have performed a series of molecular dockings simulations. Our results indicate that (*R*,*R*)-Fen displays the highest affinity to subtype A of zebrafish β_2AR whereas β_{2A}AR might be involved in pigment depletion. (*R*,*R*)-MFen shows the lowest affinity to zebrafish β_2ARs out of the tested fenoterols which this might be associated with its cardiotoxic effects. (*R,R*)-MNFen has the greatest affinity for zebrafish subtype B β_2AR and modulation of this receptor may be associated with the development of malformations and induction of a negative chronotropic effect [1]. Moreover, we proposed two novel pharmacological models in zebrafish; i.e., epinephrine-dependent heart failure and isoetharine-dependent transparent zebrafish [2]. We provided strong evidence that the zebrafish model constitutes a valuable tool for cardiovascular research. The presented data offer insights into the functional responses of the zebrafish β_2ARs confirming their intraspecies conservation, and support the translation of the zebrafish model in biomedical and pharmacological research.

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C13

New materials based on polysaccharides with natural active substances for potential wound dressing applications

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Wound dressing is an essential tool for wound healing intervention, an important aspect of biomedical materials research. In the past, most wounds were dealt with conventional wound dressings composed of fabric materials. Although, to a certain extent, they can protect the wound from contamination and absorb the wound exudate, they cannot provide an appropriate environment for tissue regeneration. Therefore, advanced dressings such as hydrogels, thin films (membranes), nanofibers, foams, and sponges have been exploited to overcome conventional shortcomings, providing a physical barrier against secondary infection and a compatible physiological environment [1,2].

Polysaccharides make suitable dressing materials because they are components of the extracellular matrix, rendering them excellent biocompatibility and mimicking the extracellular matrix's functions to facilitate wound healing [3]. Thus, polysaccharide-based polymers have great potential and commercial value in wound healing.

In this study, we obtained new materials based on polysaccharides enriched with natural active substances, which can be potentially used as wound dressing materials. Systematical characterizations of the obtained biomaterial were performed, including surface morphology, chemical structure, thermal stability, and mechanical performance. The biological effect of the samples was also assessed, and the results indicated that the material exhibited excellent anti-inflammatory, antioxidant, and antimicrobial properties.

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C14

Selection of protein fragments important in the regeneration of hard-to-heal wounds (DFU)

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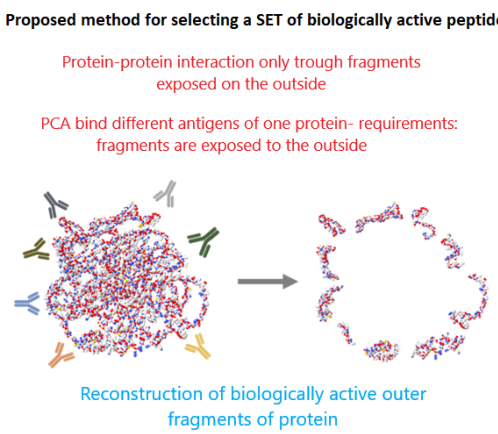
Materials useful in dressing hard-to-heal wounds, including Diabetic Foot Ulcer (DFU), should be consistent with the concept of chronic wound healing - TIME strategy (T - Tissue management; I - Infection or Inflammation; M - Moisture imbalance ; E - Edge of the wound, Epithelium). The goal of TIME is to restore optimal conditions by removing barriers, correcting disorders affecting the lack of healing and enhancing the potential of natural wound healing processes [1-2].

The main goal of the conducted research was to design, synthesize and test the properties of new, biocompatible, multi-component hybrid materials useful in the treatment of hard-to-heal wounds, including diabetic foot syndrome, based on a **set of selected biologically active peptides**, immobilized on a polysaccharide matrix, derived from proteins affecting all stages of the wound healing process. It was assumed that peptide fragments exposed outside the protein should guarantee their biological activity. Libraries of immobilized, non-overlapping peptide fragments covering whole proteins: **IL-6, IL-10, IL-11, IL-13, lactoferrin** and **kappa casein** were synthesized by the automatic SPOT technique with DMT/NMM/TosO$^-$ as a coupling reagent [3]. The dot-blot technique using specific polyclonal antibodies (PCA) allowed for the selection of fragments reproducing the outer sphere of the native protein. Based on the screening tests, **6 fragments of IL-6, 10 fragments of IL-10, 5 fragments of IL-11, 11 fragments of IL-13, 6 fragments of lactoferrin and 13 fragments of kappa casein were selected.** Their resynthesis using the SPPS method allowed to obtain a set of cytokine fragments representing the outer sphere of proteins. Selected fragments of cytokines recreate the outer sphere of native proteins and form stable spatial structures mimicking the conformations of native proteins.



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C15

Application of bio-chromatographic methods to evaluate drug candidates

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Discovering and developing new drugs is an expensive, demanding, and time-consuming process with an uncertain outcomes in clinical trials [1]. Statistically, seven out of eight compounds that started the clinical trial do not meet the safety criteria. Therefore, many researchers focus on developing much cheaper preclinical trials to reduce time and cost significantly. Optimizing lipophilicity is an essential process in drug discovery since it noticeably affects the diffusion of molecules through a biological membrane. Consequently, these physicochemical properties determine pharmacokinetic processes, including absorption, distribution, metabolism, excretion (ADME), and the toxicity of drug candidates.

Nowadays, the detailed experimental protocols proposed by OECD (test no 107 and 117) and several procedures developed for academic and industrial institutions describe methods for lipophilicity assessment. Currently, methods based on solid-liquid partitioning, such as reversed-phase liquid chromatography (RP-LC), are mainly used for lipophilicity estimation. Chromatographic methods owe their popularity to numerous advantages. First, they are reproducible, easy to automate, rapid, and require small amounts of analytes that don't need to be absolutely pure because their impurities are readily separated during the chromatographic process. Consequently, the separation methods have become the primary approach to lipophilicity assessment and easily fit into a high-throughput approach. What is more chromatographic approach is highly repeatable and easily reproducible.

Currently, more biosimilar alternatives to classical lipophilicity are available. The key concept of biomimetic chromatography is the application of high-performance liquid chromatography with stationary phases containing proteins and phospholipids or mobile phases, including micelles or microemulsions.

The main objective of the lecture will be to present the experience and observations of the single-center laboratory regarding the development of new analytical methods and protocols allowing for the determination of the physicochemical properties of xenobiotics. Presented studies will cover not only lipophilicity assessment but also the determination of phospholipids and plasma protein binding [2 -4].

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C16

Facts and myths about italian Dolce - Vita - interdisciplinary about coffee

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Coffee is one of the frequently and favourably consumed beverages in the world, which apart from caffeine, contains valuable chemical compounds such as chlorogenic acid, trigonelline and polyphenols.
The recommended daily intake of caffeine ranges from 200-300 mg per day, which is the equivalent to 5-6 cups of espresso a day. The reasons why people drink coffee are: its unique taste, aroma and increase in energy after its consumption. For some it is a daily ritual, for others it is an obligatory drink during social gatherings. Scientific research has shown that the chlorogenic acid present in coffee reduces blood glucose levels, which results into a reduced risk of type 2 diabetes. In addition, it has been proven that coffee consumption reduces the risk of ischemic strokes, which are one of the main causes of disabilities and deaths among patients. The advantages of consuming coffee also include improving memory and concentration, extending and improving the quality of life, supporting metabolism, increasing exercise tolerance, delaying the onset of neurodegenerative diseases and reducing the risk of dementia. Based on the collected results of meta-analyses, it can be concluded that coffee consumption reduces the risk of hepatocellular carcinoma and, to a small extent, the risk of breast cancer among postmenopausal women. In Poland, hepatocellular carcinoma is diagnosed in about 1,500 patients annually. Men are more often affected than women. Coffee contains valuable chemical compounds that are widely used in medicine. Using it for therapeutic purposes could contribute to the reduction of the treatment cost, as it is available worldwide. However, the dosage of caffeine should be approached diligently, because too much of it can contribute to the appearance of the side effects

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C17

Dynamics and binding energy of antagonists and agonists to the dopamine D4 receptor

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The dopamine D4 receptor (D4R) is a promising therapeutic target in widespread diseases, and the search for new agonists and antagonists appears to be clinically relevant. The binding mechanism to the (R) receptor for antagonists and agonists differs. In the present study, we conducted a comprehensive computational study, extracting key similarities and differences in binding mode, dynamics and binding energies for D4R complexed with agonists and antagonists. The Dynamic Network Analysis (DNA) method was used to investigate the communication pathways between the ligand (L) and the G protein binding site (GBS) of human D4R. Finally, the fragment molecular orbital scheme with pair interaction energy decomposition analysis (FMO/PIEDA) was used to estimate the binding energy of L-R complexes. We found that a strong salt bridge with D3.32 initiates dopamine D4 receptor inhibition. This interaction also occurs with agonist binding, but the conformational change of the receptor to the active state is initiated by interaction with cysteine C3.36. Such a mechanism may arise with agonists unable to form a hydrogen bond with serine S5.46, previously considered crucial in GPCR activation. Energy calculations using the FMO/PIEDA method showed that antagonists interacted with more residues in the receptor binding site than agonists, suggesting they may form relatively more stable complexes. In addition, antagonists were characterized by repulsive interactions with S5.46, which distinguished them from agonists.

C18

National Library of Chemical Compounds

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The infrastructure of the National Library of Chemical Compounds (NLCC, www.pol-openscreen.pl) at IBM PAN was created within the consortium POL-OPENSCREEN - Polish Platform of Screening Infrastructure for Biological Chemistry, which is part of the European consortium EU-OPENSCREEN (https://www.eu - openscreen.eu/). The NLCC infrastructure is financed by the Ministry of Education and Science as one of the undertakings of the Polish Roadmap of Research Infrastructures.

NLCC provides the possibility of depositing chemical compounds synthesized in national laboratories (universities, institutes, research laboratories, etc.) and makes them available to interested entities, both Polish and foreign, for biological screening in order to search for new bioactive compounds, and for other scientific study. NLCC is the first compound bank of this type in the country, providing biologists with access to unique chemical libraries on a non-commercial basis, and chemists with the possibility to test synthesized compounds and generate added value through the wider use of compounds synthesized in Polish laboratories.

The deposited compounds are subjected to initial bioprofiling free of charge (basic physicochemical properties, *in vitro* cytotoxicity) and then made available for screening. The results of bioprofiling and screening tests collected in the NLCC database are available to donating chemists, and to interested scientific institutions and industry on agreed terms that protect the intellectual property of both parties. During the presentation, the NLCC infrastructure, the principles of its functioning and the possibilities of using the Library will be presented.

Acknowledgement: Financial support from the Polish Ministry of Science and Higher Education, project POL-OPENSCREEN DIR/WK/2028/06 and the European Union´s Horizon 2020 project EU-OPENSCREEN DRIVE No. 823893 is acknowledged.

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Poster oral presentations PP1 - PP10

PP1

The Role of Carbonyl Reduction of Anthracyclines in Cancer Resistance

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Anthracyclines are a class of anticancer agents used in the treatment of a variety of cancers, including solid tumors, leukemias, and lymphomas. Because of their significance in cancer therapy, the WHO has registered anthracyclines in the list of essential medicines. However, the therapeutic efficacy of anthracyclines is limited by the development of cancer resistance. One known mechanism of resistance is the intracellular, enzymatic carbonyl reduction of anthracyclines to secondary alcohol metabolites. It has been shown that these metabolites exert decreased anticancer activity. However, this process has been widely characterized only for the commonly used doxorubicin and daunorubicin, while the carbonyl reduction of other anthracyclines is not well known.

In this study, the metabolism of eight anthracyclines was investigated in human liver cytosol. Additionally, molecular docking of anthracyclines with the main cytosolic reductases (carbonyl reductase 1, CBR1; aldoketo reductase 1C3) was performed. To explain the drugs' susceptibility to the reaction, the partial atomic charges of carbonyl oxygen atoms were also calculated. Next, anthracyclines activity was studied in the A549 lung cancer cell line with overexpression of the main cytosolic reductase CBR1 (A549/CBR1). Lung cancer cells were also used to obtain an anthracycline-resistant cell line (A549/R). This cell line was characterized by a high level of ABCB1 (P-glycoprotein) transporter. Using fluorescence microscopy, the ABCB1-dependent efflux of anthracyclines and their metabolites was evaluated with the use of an ABCB1-selective modulator - valspodar. Subsequently, anthracyclines and their metabolites were tested in an ATPase assay with ABCB1 recombinant protein.

The results indicate significant differences in anthracyclines' susceptibility to enzymatic carbonyl reduction. The presence of the hydroxy moiety at the side chain of anthracyclines was found to be a factor affecting the affinity of drugs to enzymatic active centers. CBR1 was also found to exert a different biological effect on different anthracyclines. Cytotoxicity, accumulation, and apoptosis induction effects of anthracyclines which were highly metabolized were more dependent on the intracellular CBR1 level. Metabolites of anthracyclines were determined as substrates more susceptible to the ABCB1 transporter than parental anthracyclines. It suggests the existence of a cross-talk between the process of anthracycline carbonyl reduction and the efflux of metabolites.

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PP2

Substituted benzenesulfonyl hydrazones: synthesis and *in vitro* bioactivity study

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Searching for novel compounds which possess significant antimicrobial activity is nowadays very important due to the increase of number of infections caused by resistant bacterial strains and fungi [1, 2].

Among many classes of organic compounds hydrazones obtained from hydrazides of carboxylic or sulfonic acids play important role as potential antimicrobial agents [3 - 5]. In this study we decided to synthesize and perform *in vitro* antimicrobial activity study of novel 2,4,6-trimethylbenzenesulfonyl hydrazones [6].

New hydrazones were synthesized in single step reaction. The hydrazide of 2,4,6-trimethylbenzenesulfonic acid was subjected to condensation reaction with diverse substituted aromatic aldehydes to obtain novel 2,4,6-trimethylbenzenesulfonyl hydrazones. The chemical structure of all obtained compounds was established on the basis of spectral methods [6].

Newly synthesized substituted benzenesulfonyl hydrazones were tested for *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacterial strains and fungi belonging to *Candida* spp. The *in vitro* bioactivity study confirmed the potential application of few of obtained hydrazones as antimicrobial agents (MIC = 7.81–15.62 μg/mL and MBC = 7.81–15.62 μg/mL). Synthesized compounds displayed antibacterial activity especially against Gram-positive bacterial strains. The bacteria from *Staphylococcus* spp., *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, and *Bacillus* spp. were sensitive to tested compounds [6].

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PP3

Deciphering the multi-target mechanism of action of D2AAK1 for potential treatment of neurodegenerative diseases

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D2AAK1 was discovered in structure-based virtual screening as a multi-target ligand of aminergic GPCRs [1] and a potential antipsychotic [2]. Further studies indicated that it can be repurposed for the treatment of neurodegenerative diseases, in particular Alzheimer's disease as it increases the proliferation of mouse hippocampal neuronal cells (cell line HT-22) and do not increase proliferation of neuroblastoma cells (cell line SH-SY5Y) based on the MTT assay [3]. Importantly, D2AAK1 exhibits pro-cognitive properties in passive avoidance test, modified elevated plus maze test and novel object recognition test in mice models, both after acute and chronic administration [3]. The effect of D2AAK1 on the proliferation of hippocampal cells was also observed *in vivo* [3].

In this work we performed *in vitro* and *in silico* studies to decipher the possible molecular mechanisms underlying the observed activities of D2AAK1. The activity of D2AAK1 was tested *in vitro* against over 100 molecular targets. It turned out that D2AAK1 significantly inhibits AChE, BuChE and MAO-B. PASS and Pharma Expert software indicated that CAMK1 [3] and MAPK kinases can be additional molecular targets underlying D2AAK1 pharmacological properties. Thus, D2AAK1 was docked to the binding site of the above mentioned enzymes using Glide from Schrödinger software to rationalize its activity at the molecular level. In order to confirm the proposed multi-target mechanism of action of D2AAK1, a number of its derivatives were synthesized and tested.

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PP4

Sulfonamide derivatives of cyclic arylguanidines as a new scaffold for 5-HT$_6$R ligand design

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The 5-hydroxytryptamine receptor 6 (5-HT$_6$R) belongs to G$_s$-protein coupled receptor, that activates cAMP formation after agonist stimulation in numerous recombinant systems and primary neurons [1]. 5-HT$_6$R antagonists may find application in the treatment of neurodegenerative diseases, depression and obesity [2]. Recent studies has implicated this receptor in signaling pathways related to brain metastasis in triple-negative breast cancer (TNBC) [3] and the pathogenesis of CNS tumours [4], making it a promising target for the treatment of glioblastomas and advanced TNBC.

In our research, we designed a new scaffold of sulfonamide derivatives of cyclic arylguanidines. We obtained a set of low-basic ligands from the group of *N*-(3,4-dihydroquinazolin-2-yl)arylsulfonamides and their long-chain analogs with increased basicity. We developed a fast, eco-friendly method of microwave assisted or sonochemical synthesis, which allowed the construction of a library of over 100 new molecules. The resulting compounds showed a moderately strong affinity for 5-HT$_6$R. For the selected ones, we assessed ADMET parameters *in vitro*. We elucidated the biological activity using molecular modeling methods, including ligand-protein docking and hybrid QM/MM methods.

Acknowledgement

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PP5

D2AAK5 and its derivatives as ligands for serotonin receptors

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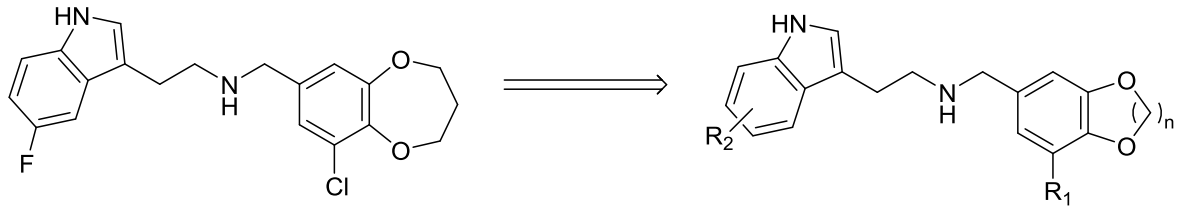
Introduction: Serotonin receptors are considered to be one of the most important molecular targets in the treatment of depression, anxiety disorders and neurodegenerative diseases [1,2]. Blockade of 5-HT_{2A} receptors may increase the therapeutic effectiveness of SSRIs (selective serotonin reuptake inhibitors), examples of which in medicine are risperidone and trazodone. Agonism of 5-HT_{1A} receptors, as in the case of buspirone, may have an anxiolytic effect. Our team designed and synthesized new potential ligands for serotonin receptors.

Aim of the study: The aim of the study was to develop the synthesis of the designed compound D2AAK5, which is a ligand for serotonin 5-HT_{1A} and 5-HT_{2A} receptors [3], and its derivatives (Fig. 1). Another goal was to study the physicochemical properties and determine the receptor profile for D2AAK5 derivatives. Detailed structural and behavioural studies were performed on D2AAK5 to determine the therapeutic profile

Methods: The method of compound synthesis was developed using the Sci-Finder database. NMR, MS, IR spectroscopy was used to identify the synthesized structures. *In-vitro* studies were performed on cell membranes containing cloned human 5-HT_{1A}, 5-HT_{2A} and D_2 receptors. Behavioural studies for D2AAK5 were performed in a mouse model; tests of spontaneous locomotor activity, motor coordination, EMP test, FST test and PA test were performed. Schrödinger software was used in the structural studies.

Results: A synthesis method for D2AAK5 and its derivatives was developed. Compounds D2AAK5 and its derivatives have been shown to be strong ligands for serotonin 5-HT_{1A} and 5-HT_{2A} receptors. Behavioural studies on a mouse model have shown that D2AAK5 may have a positive effect on memory processes. Structural studies of the D2AAK5 compound have clarified the mode of binding between the receptor and the ligand [4].



D2AAK5

Fig 1. D2AAK5 and its development

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PP6

Magnetic nanoparticles coated with chitosan with free dihydroxyboryl groups for fast binding of glycoproteins

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Viruses are the most widespread microorganisms on Earth, the most important feature of which is the ability to infect and cause diseases in all living organisms: animals, plants, as well as microorganisms such as bacteria. Viruses, despite their relatively simple structure, have developed many mechanisms that make it easier for them to use the cell structures of living organisms. For infection to occur, the virus must introduce its genome into the host cell. Surface proteins, otherwise known as envelope glycoproteins, are essential in the process of recognizing cell receptors and virus entry into the cell [1].

Glycoproteins are proteins with covalently attached sugar chains [2]. These proteins are also present in the envelopes of viruses and play a key role in the process of causing infections, e.g. with viruses from the Coronavirinae group, HIV, Ebola, hepatitis C (HCV), Borna, rabies, Epstein-Barr and many others [2]. Diagnostics and the search for new drugs and materials resistant to viruses is still the important trend in medical and material sciences.

Many antiviral active substances are known, but the "universal" one that would effectively block the multiplication of the virus of any origin is still being sought. It is crucial to search for new substances and materials that will be able to inactivate the widest possible number of pathogens in a quick and irreversible way.

The main objective of the research was to obtain chitosan-coated magnetic nanoparticles with highly reactive functional groups capable of effective immobilization of glycoproteins.

The obtained materials are built of a magnetite core coated by a modified polymer layer with dihydroxyboryl groups of boronic acids on its surface. The obtained new polymer material was characterized by structure and surface morphology, and then tested for the ability to bind glycoproteins.

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PP7

Hemoglobin-based nanomaterials as potential drug carriers in Photodynamic Therapy (PDT)

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Photodynamic therapy (PDT) is one of the well-known methods of cancer treatment. It uses the action of light, photosensitizer, and oxygen present in the environment of cancer cells. The act of these three elements leads to the formation of reactive oxygen species capable of destroying cancerous tissues. However, this treatment method has some limitations, such as associated excessive accumulation of the drug in the body, limited target, as well as too low oxygen concentration, which is necessary to cause the photosensitizing effect (it is essential in Photodynamic Therapy). Due to this, new materials are sought that can serve as drug carriers that could eliminate the defects caused by PDT [1].

This study presents the synthesis and characterization of modified chitosan-based magnetic nanoparticles enriched with hemoglobin as potential drug carriers in PDT. The chemical structure studies were based on FTIR infrared spectroscopy and XRD X-ray diffraction results. Surface morphologies were investigated using Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS). The thermal stability of the obtained materials was confirmed by thermogravimetric analysis. We also examined the interaction of the obtained nanomaterials with a photosensitive drug, and an attempt was made to determine the quantum efficiency of singlet oxygen generation. There have been attempts to obtain and characterize protein nanoflowers based on hemoglobin.

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PP8

Synthesis and Antimicrobial Activity of New Mannich Bases

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The Mannich reaction is an effective and widely used method for the synthesis and modification of compounds with potential biological activity. The resulting Mannich bases may contain various types of heterocyclic systems in the structure, which makes this group of molecules an interesting object for further structural modifications. The aim of the research work was to obtain a number of new, not described in the literature, Mannich base compounds with potential antibacterial and antifungal activity.

Target compounds were obtained by reaction of 4-(3-chlorophenyl)-5-(3-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and various piperazine derivatives. The chemical structure of the obtained products was confirmed using ^{1}H and ^{13}C NMR and elemental analysis. The activity of the newly synthesized molecules containing the piperazine moiety was determined against bacteria (Gram-positive: *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus* and *Bacillus subtilis*; Gram-negative: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis*) and yeasts (*Candida glabrata, Candida krusei* and *Candida parapsilosis*). The double dilution test in broth was used in the microbiological tests. In this way, the values of the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) were determined.

The tests showed significant activity against Gram-positive bacteria, mainly staphylococci (8-9) and *Micrococcus* and *Bacillus bacteria* (5-7), as well as selected strains of Gram-negative bacteria, including bacteria from the *Enterobacteriaceae* family (8). At the same time, all tested molecules showed promising fungistatic activity against *Candida spp*. The highest results of fungistatic activity of the compounds were recorded against *C. parapsilosis*, with MIC values of 0.49 µg/ml (8), 0.98 µg/ml (9) and 62.5 µg/ml (5-7). The results obtained in the conducted research work indicate the multidirectional antimicrobial activity of the newly synthesized Mannich bases with a piperazine group in the structure.

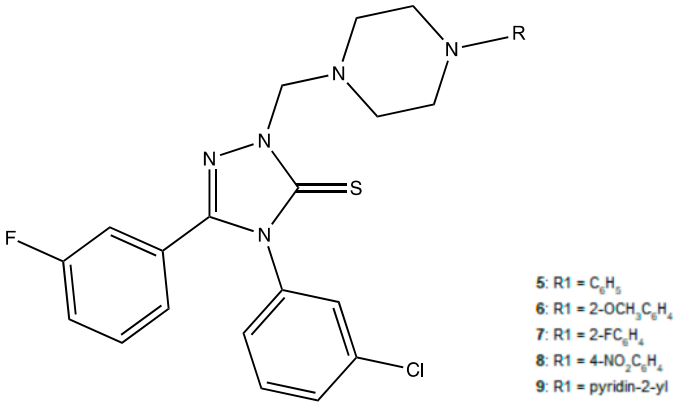

Fig. 1: The structure of obtained compounds

PP9

Synthesis and characterization of substrates for the preparation of long-acting insulin analogs. Triazine condensing reagents in the synthesis of insulin analogs

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Diabetes mellitus is a metabolic disease with a complex and diverse etiology, characterized by chronic hyperglycemia. It is the leading cause of blindness, kidney failure, heart attacks, strokes and lower limb amputations. Diabetes is diagnosed every 10 seconds in the world, and every 6 seconds a person dies from its complications. In Poland, 3 million people suffer from diabetes (9.1% of the population). As much as 90% of cases are related to type 2 diabetes. It is estimated that in 2035 There will be approximately 592 million people with diabetes worldwide.Current data indicate that insulin is life-saving medicine for 30 million people with type one diabetes and many millions with type two diabetes. Since this trend is growing, the above statistics are prompting many research units and pharmaceutical companies to search for new insulin analogs as well as to develop the most efficient methods of producing them. As part of the work conducted at the Institute of Organic Chemistry at the University of Lodz, an efficient method for synthesizing a long-acting insulin analog known as Degludec has been developed. This is the currently available drug Tresiba, but it was necessary to develop synthesis method which is more efficient and more economically viable. The proposed synthetic strategy is based on the use of triazine coupling reagents [1] to obtain the active ester of the Ligand IP13. In the next step, the ester was coupled to the desB30 protein, leading to the expected product (Figure 1).

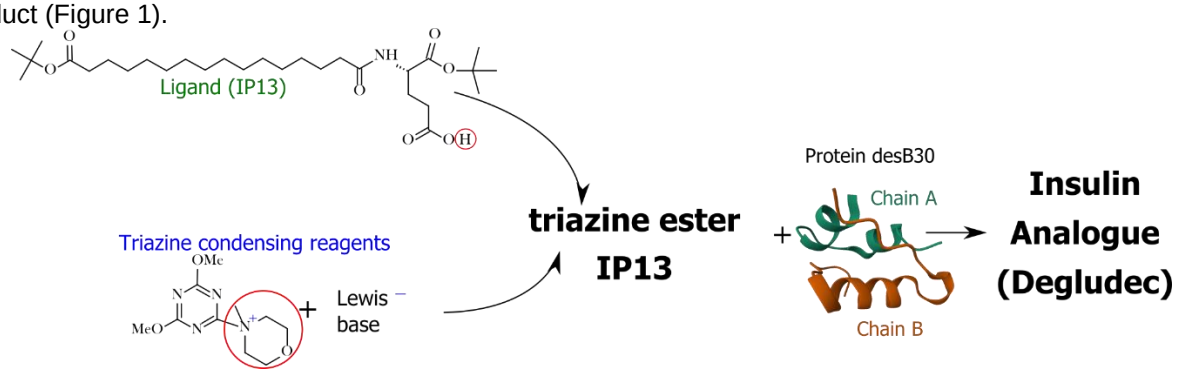

Figure. 1 Insulin analog synthesis strategy - Degludec.

In this study, three different triazine coupling reagents, with differ counter ions, were used. Their application was to test the activity and synthetic potential depending on the difference in structures. Taking advantage of the literature indicating that the in vivo actions of the Degludec analogue are related to its interaction with plasma albumin (HSA) [2], three amides (analogues of Ligand IP13, H_2N-Glu-OtBu amides of stearic, palmitic and 2-ethylhexanoic acids) were obtained and used to synthesize further insulin derivatives. Measurements such as FTIR, NMR and MS confirmed the obtaining of the expected products. In addition, circular dichroism spectroscopy (CD) measurements were made, to study the interaction between human serum albumin (HSA) and respectively: Tresiba, the obtained Degludec analog, and the IP13 Ligand. The results showed that the IP13 Ligand is responsible for binding the Degludec analog to the HSA protein.

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PP10

Application of HPLC and FTIR methods to evaluate immobilization of a new antimicrobial substance on a biomaterial

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In the era of ever-increasing resistance of bacteria to antibiotics, the discovery of new antimicrobial chemotherapeutics and their introduction into medicine is of particular importance. The ideal antimicrobial chemotherapeutic agent should be selective and have appropriate antimicrobial efficacy, and specific pharmacokinetic parameters. An important feature is also the lack of development of microbial resistance to this agent [1].

The aim of the work was to develop a methodology for the evaluation of immobilization of a new antimicrobial chemioterapeutics - a derivative of ethylpyrazole carbothioamide (ePTA) using HPLC and FTIR methods. The substance selected for the study was 3-amino-N-ethyl-5-oxo-4-phenyl-2,5-dihydro-1H-pyrazole-1-carbothioamide with the confirmed antibacterial activity [2]. The biomaterial used for immobilization process was a urological catheter.

The HPLC method with spectrophotometric and fluorescence detection was developed for determining ePTA, evaluating linearity, limits of detection and quantification, stability, selectivity, precision and accuracy. Comparing of parameters of both types of detection the more favorable HPLC-UV methodology was chosen for further research.

The validated HPLC-UV method was applied to assess the degree of binding of ePTA substance with to the modified surface of the urological catheter, obtaining the protection of biomaterial against antimicrobial infections. The presence of the drug on the surface of the catheter was confirmed by the FTIR method, and the antibacterial activity of the bound chemotherapeutic agent was assessed in microbiological tests..

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Posters P1 – P97

P1

Computational attempts aiming to discover novel modulators of the 5-HT$_{2A}$ receptor

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The 5-HT$_{2A}$ receptor belongs to the GPCR superfamily, and plays an important role in numerous physiological functions of the human body [1]. Studies have demonstrated the potential therapeutic benefits of modulators targeting this receptor, including agonists, antagonists, and partial agonists, particularly for the treatment of mental and neurodegenerative disorders [2-4]. Building upon this promising information, a comprehensive campaign was constructed to discover novel ligands for this molecular target, aiming to uncover promising therapeutic possibilities.

This study followed a rational drug discovery paradigms and consisted of two parts. The computational part utilized available structural data on active and inactive 5-HT$_{2A}$ receptor crystals, along with numerous classification algorithms to create robust ligand-based statistical models which examined the structure-activity relationship among currently available 5-HT$_{2A}$ ligands. The computational analysis provided valuable insights into the molecular characteristics required for ligand binding and activity. The second part of the study involved extensive *in vitro* experiments to validate the activity of the identified compounds. These experimental studies confirmed the desired effects of 6 compounds, establishing their potency in modulating the 5-HT$_{2A}$ receptor.

As a result, a collection of novel compounds with unique activity profiles was discovered, offering a promising starting point for the development of potential antipsychotic or antidepressant drugs.

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P2

Optimization of D2AAK1 derivatives for potential treatment of neurodegenerative diseases

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D2AAK1 is a virtual hit which was designed as a multi-target ligand of aminergic GPCRs [1] and a potential antipsychotic [2]. A number of its derivatives were obtained and evaluated as potential antipsychotics [3,4]. It turned out that these compounds can be repurposed for the treatment of neurodegenerative disorders as they increase the proliferation of mouse hippocampal neuronal cells (cell line HT-22) based on the MTT assay [5].

In this work we performed *in silico* studies to select the D2AAK1 derivatives with the most favorable multi-target mechanism of action for the treatment of neurodegenerative diseases, in particular Alzheimer's disease. PASS and Pharma Expert software were used to identify potential molecular targets (CAMK1 and MAPK kinases). Based on *in vitro* results for the virtual hit, D2AAK1, inhibition of AChE, BuChE and MAO-B was also proposed as a potential mechanism of the compounds pharmacological activity. Molecular docking to the binding site of the above mentioned enzymes was carried out using Glide from Schrödinger software. The performed molecular modeling studies enabled to design more potent analogs of D2AAK1 virtual hit.

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P3

Evaluation of *in-vitro* ADME-Tox properties of new inhibitors of mono-ADP- ribosyltransferases

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Research background

Mono-ADP-ribosyltransferases (mono-ARTs) is a subfamily of enzymes that catalyses mono-ADP-ribosylation thus is involved in multiple cellular processes e.g regulation of apoptosis, cell replication, immune response, and neurodevelopment [1]. Therefore, mono-ARTs inhibitors are proposed and investigated as promising and innovative therapeutics for cancer as well as for non-oncological disorders [2]. Selective inhibitor of mono-ARTs - PARP7 (**RBN-2397**) and -PARP14 (**RBN3143**) reached human clinical trials for treatment of patients with solid tumors and atopic dermatitis, respectively Mono-ARTs' inhibitors **OT-32**[3], **OT-76** and **OT-80,** synthesized by the research group of Professor Oriana Tabarrini from University of Perugia (Italy) were tested in vitro to determine their ADME-Tox profile. The research included membrane permeability, cytochrome p450 CYP3A4 inhibition, hepatotoxicity, neurotoxicity and mutagenicity of the compounds.

Methods

Hepatotoxicity and neurotoxicity of the compounds were evaluated using MTS assay and respective cell lines (HepG2 and SHSY-5Y). Permeability was tested using Caco-2 cell line model. Mutagenic properties were evaluated in Ames assay in liquid microplate format using Salmonella typhimurium TA100 strain. Inhibition of CYP3A4 was tested using human recombinant CYP3A4.

Results

The ADME-Tox properties evaluated for compounds **OT-32, OT-76** and **OT-80** resulted in three different profiles. Importantly one of the compounds (**OT-80**) showed very good membrane permeability, low inhibition of CYP3A4, good safety in *in-vitro* models of hepato- and neurotoxicity and no mutagenic properties.

Acknowledgements

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P4

Implications of NOP receptor system in sociability impairments under migraine condition

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Migraine is a neurological disorder characterized by recurring, moderate to severe headaches, affecting a significant portion of the population, particularly females. The exact cause of migraines is not fully understood, but it is believed to involve a combination of genetic and environmental factors. This study aimed to investigate the neurobiological mechanisms underlying sensory and affective aspects of migraine pain, as well as sociability impairments under a migraine-like condition. The nitroglycerin (NTG) migraine model was employed to mimic migraine-like symptoms by inducing vasodilation through systemic administration. NTG administration resulted in social impairments in both male and female mice. We found that a NOP receptor agonist, Ro 64-6198, reduced NTG-induced migraine-like symptoms, including mechanical allodynia. This effect was blocked by a NOP antagonist, SB-612111. In sociability study under migraine, Ro 64-6198 alleviated the social dysfunctions caused by NTG, while SB-612111 partially blocked the NOP agonist effects. Our neuroanatomical studies using TRAP2/Ai9 mice that allow us to identify the activated cells and brain regions, showed that NTG treatment significantly increased the activation in many brain regions including the regions important for migraine pain and social behavior compared to vehicle-treated animals. In addition, these activated regions were significantly inhibited by Ro 64-6198. These findings suggest that the activation of NOP receptors modulates neuronal activity in specific brain regions involved in migraine pain and social behavior. Our brain-wide analysis will provide us with ample information to better understand the relation between the NOP receptor system and sociability under migraine pain.

P5

Synthesis, characterization and fluorescent properties of novel aza-BODIPY derivative chelating boron

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BODIPYs (boron dipyrromethenes) and their analogues – aza-BODIPYs are attracting attention for their fluorescent properties and application in photodynamic therapy (PDT). Photodynamic therapy is a promising therapeutic approach for the treatment of various diseases, including cancer. In PDT, a photosensitizer (PS) is administered to the patient, which accumulates in the targeted tissue. Upon exposure to light of an appropriate wavelength, the PS generates reactive oxygen species causing damage to the cancerous cells.
In recent years, there has been significant progress in the development of new PSs with improved properties for PDT. BODIPY and aza-BODIPY have emerged as a new class of PSs with excellent photophysical properties. These compounds exhibit strong absorption in the red part of the visible spectrum, which may be shifted to the near-infrared (NIR) region. This property is desirable for PDT, as it allows deeper tissue penetration and reduces damage to healthy tissues. [1,2].

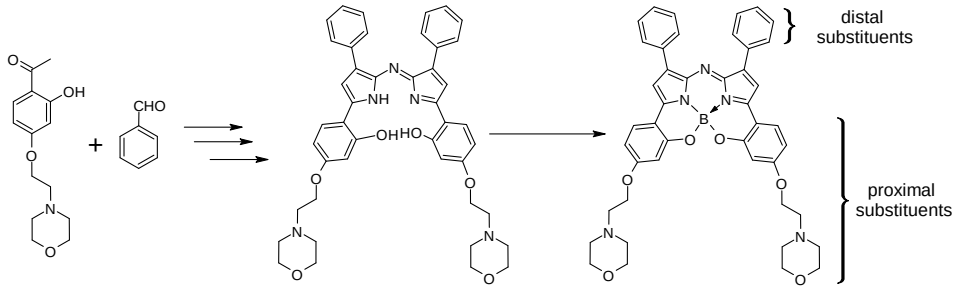

Fig. 1. Synthesis of aza-BODIPY with bulky proximal substituents

Multi-step synthesis yielded azadipyrromethene **1**, which contains hydroxyl groups at the ortho positions of the proximal substituents. Subsequent complexation reaction using boron trifluoride gave **2**, belonging to an aza-BODIPY group. It is characterized by absorption of light in the NIR range (λmax > 750 nm), which is desirable for PDT.

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P6

Novel oxazolo[5,4-*d*]pyrimidine derivatives as potential anticancer drugs targeting inhibition of VEGFR2: their design, synthesis, and *in vitro* biological studies

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The World Health Organization (WHO) is still providing new data indicating a worldwide increase in cancer cases and cancer death. Each year, the Food and Drug Agency (FDA) approves novel anticancer therapies for the treatment of various types of cancer. However, due to the development of resistance against the used drugs and unsatisfactory therapeutic results, there is a constant need to design new, more effective agents. Oxazolo[5,4-*d*]pyrimidine derivatives, mainly due to their structural similarity to naturally occurring purine bases, are crucial in the search for anticancer drugs. It was found that some oxazolo[5,4-*d*]pyrimidines are inhibitors of human vascular endothelial growth factor receptor 2 (VEGFR2), adenosine kinase, Aurora A kinase, Janus kinase 1, Janus kinase 2, and ubiquitin-activating enzymes (E1 enzymes). Oxazolo[5,4-*d*]pyrimidines have also activated the caspase cascade or inhibited angiogenesis [1].

Structures of new series of oxazolo[5,4-*d*]pyrimidines were designed based on molecular modeling and our previous studies of oxazolopyrimidine derivatives, where their anticancer activity was observed [2,3]. Molecular designing included docking to VEGFR2, a receptor through which angiogenesis is controlled. Optimization of the leading compounds caused enhance of their interaction with the hydrophobic allosteric VEGFR2 pocket. The designed derivatives were synthesized using two-step organic synthesis, and then their structures and purity were confirmed by spectral methods and elemental combustion analysis. The cytotoxic activity of novel oxazolo[5,4-*d*]pyrimidines was evaluated on the cultures of normal cells: NHDF (normal human dermal fibroblasts) and three cancer cell lines such as A549 (adenocarcinoma of the lung), HT-29 (adenocarcinoma of the colorectal), A375 (melanoma cell line). Moreover, the lipophilicity measurement of tested oxazolopyrimidines was carried out and their binding ability to human plasma proteins was tested to explain their distribution in an organism (among other absorption, transportation, and final elimination).

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P7

Application of computer-aided drug design approach in search for multi-target-directed ligands blocking PDE4B, PDE8A and TRPA1 with potential application in the pharmacotherapy of asthma and COPD

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The multifactorial nature of diseases provides the logical foundation for the development of an innovative drug design strategy based on multi-target-directed ligands (MTDLs), which gives the potential to broaden the spectrum of therapy and achieve a cumulatively stronger therapeutic effect. This strategy was developed at the beginning of the 21st century and has gained importance in the search for new drugs in recent years. The complicated and complex pathophysiology characterizes asthma and COPD, which are associated with chronic inflammation, bronchospasm and bronchial hyperresponsiveness leading to airway remodelling. The currently available therapies do not address all of these pathological processes, therefore, it is an urgent need to work out a comprehensive solution in the form of rationally designed MTDLs, combining PDE4B and PDE8A inhibition with TRPA1 ion channel blockade [1].

The aim of the study was to develop a complete strategy to search for MTDLs using a computer-aided drug design approach, combining ligand- (LBDD) and structure-based drug design (SBDD) methods.

Research in both approaches was conducted in parallel. In the SBDD procedure, structural models were appropriately prepared and validated for each of the biological targets [2] and then subjected to MD simulations to generate dynophores. The resulting structure-based pharmacophores and structural models itself were applied to perform a two-stage process of virtual screening of a compound library from ZINC15 database: matching to pharmacophore hypotheses followed by docking the compound collections selected in the first stage. In the LBDD approach, empirical regression models predicting the value of pIC_{50} inhibitory activity were developed and used in the second, parallel virtual screening [3]. 38 potential candidates for MTDLs were finally selected, 12 of which were purchased and directed to the *in vitro* activity tests.

The results confirmed the inhibitory activity of 5 compounds against individual biological targets, providing a solid foundation for their further optimization towards multi-target inhibitory activity.

The study was financially supported by the National Science Centre, Poland (grant no. 2020/37/N/NZ7/02365). Calculations were performed partially with the use of computers co-financed by the qLIFE Priority Research Area under the program “Excellence Initiative Research University” at Jagiellonian University and Polish Operating Programme for Intelligent Development POIR4.2 project no. POIR.04.02.00-00-D023/20.

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P8

Prospects of Employing In Situ FTIR Spectroscopy as an Analytical Technique for the Imaging of Chemical Reactions

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In recent years, the demand for ecologically conscious and effective analytical techniques has escalated, propelling the innovation of real-time methods that provide insights into chemical reactions. In situ Fourier Transform Infrared (FTIR) spectroscopy has emerged as an invaluable tool in this context, enabling non-invasive monitoring and characterization of molecular changes. Specifically, the utilization of in situ FTIR spectroscopy offers a versatile approach for studying various chemical reactions, providing researchers with the capability to observe reaction mechanisms, optimize conditions, and enhance reaction pathways.

The versatility of in situ FTIR spectroscopy is accentuated by its diverse applications, encompassing mechanistic elucidation, real-time reaction monitoring, catalyst optimization, and exploration of reaction pathways. Alongside its role in studying chemical reactions, in situ FTIR spectroscopy has showcased its adaptability in quality and quantity control, absorption control for pharmaceutical formulations, evaluation of drug dissolution rates, and ensuring product stability. The ecological implications of this technique align harmoniously with its multifaceted capabilities, further underscoring its significance as a tool for advancing analytical practices.

Our research objectives have centered on investigating and optimizing N-heterocycle synthesis reactions, with a specific focus on crafting 2,3-diphenyl derivatives of quinoxaline and pyrazine scaffolds from 1,2-diketone precursors. The employment of both ex situ and in situ FTIR spectroscopy in this context has facilitated direct visualization of intermediate species formation, final product identification, and purity verification, providing invaluable insights into reaction pathways. Our ongoing endeavors extend to imaging various other types of N-heterocycles, broadening the technique's applicability. Furthermore, our future research directions encompass the integration of microwave (MW) irradiation with in situ FTIR spectroscopy to gain deeper insights into the synergistic effects of these technologies on reaction outcomes.

In conclusion, in situ FTIR spectroscopy offers a promising avenue for advancing ecologically friendly analytical techniques, with the potential to elevate the study and optimization of chemical reactions. Through our exploration of N-heterocycle synthesis reactions and the development of methodologies combining MW irradiation and in situ FTIR, we aim to contribute to a comprehensive understanding of chemical processes and their significance in sustainable chemistry.

P9

Design, Synthesis and Biological Properties of New Tetracyclic Azaphenothiazine Derivatives

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A new method for modifying the structure of tetracyclic quinobenzothiazinium derivatives has been developed, allowing introduction of various substituents at different positions of the benzene ring. The method consists of reacting appropriate aniline derivatives with 5,12-(dimethyl)thioquinantrenediinium bis-chloride. A series of new quinobenzothiazine derivatives was obtained with propyl, allyl, propargyl and benzyl substituents in 9, 10 and 11 positions, respectively.

The structure of the obtained compounds was analyzed by ^{1}H and ^{13}C NMR (HSQC, HMBC) and X-ray analysis. All the compounds were tested against reference strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212, and representatives of multidrug-resistant clinical isolates of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecalis* (VRE). In addition, all the compounds were evaluated in vitro against *Mycobacterium smegmatis* ATCC 700084 and *M. marinum* CAMP 5644. The activities of the compounds were comparable with oxacillin, tetracycline and ciprofloxacin against staphylococcal strains and with rifampicin against both mycobacterial strains. The compounds showed not only the bacteriostatic activity, but bactericidal activity as well. Preliminary in vitro cytotoxicity screening of the novel compounds performed using normal human dermal fibroblasts (NHDF) proved that the tested compounds showed an insignificant cytotoxic effect on human cells (IC50 > 37 μM), making these compounds interesting for further investigation.

Moreover, the intermolecular similarity of novel compounds was analyzed in the multidimensional space (mDS) of the structure/property-related in silico descriptors by means of principal component analysis (PCA) and hierarchical clustering analysis (HCA), respectively. The distance-oriented structure/property distribution was related with the experimental lipophilic data.

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P10

Novel CYP17A1 inhibitors and future perspectives in the treatment of castration resistant prostate cancer

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Introduction: Prostate cancer is one of the most common cancers among men worldwide. Enzymes that catalyze the steroidogenesis pathway are promising target for the prostate cancer drug development. Especially northworthy are CYP17A1 and AKR1C3 enzymes. So far, the only clinically used CYP17 inhibitor is a steroidal abiraterone. Recent studies have shown that AKR1C3 is responsible for resistance to abiraterone. One of the current perspectives is focused on the discovery and the evaluation of non-steroidal CYP17A1 inhibitors. The multi-target approach involving dual inhibitors is not well explored and hold promise for better therapeutics. [1,2].

Aim of the study: Designing, synthesis and evaluation of novel non-steroidal CYP17A1 inhibitors and dual CYP17/AKR1C3 inhibitors. SAR analysis and biological studies are also carried out.

Methods: Schrödinger software and GOLD software are used for docking studies. SciFinder is used for exploring synthesis method of the designed compounds. In biological assays, prostate cancer cell lines LNCaP, PC3, DU145 i 22Rv1 are used.

Results: New non-steroidal CYP17 inhibitors have been developed by our team [2, 3]. Analysis of the SAR of the new compounds provided valuable information for further design of this class of compounds. Structural elements of these structures were used to design dual inhibitors against CYP17A1 and AKR1C3. Evaluation of those multi-target hits is underway.

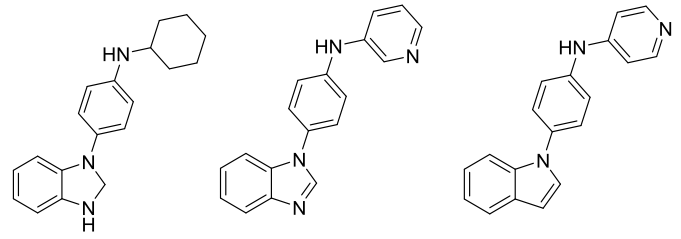

Fig 1. Structures of new CYP17A1 inhibitors.

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[2] Wróbel T. M. et al, *J. Med. Chem.* 66(10) (2023) 6542–6566
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P11

Synthesis, structure and biological activity of novel Cu(II) complexes of *N*-(4,5-dihydro-1*H*-imidazol-2-yl)benzazoles

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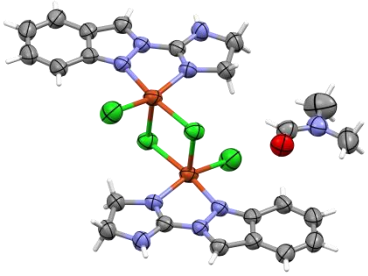
In connection with our research program on synthesis of heterocyclic compounds and their copper(II) complexes with potential anticancer activity [1-2], we have prepared a series of four copper(II) dichlorides bearing *N*-(4,5-dihydro-1*H*-imidazol-2-yl)benzazoles chelating ligands. The synthesized Cu(II)-complexes were characterized by IR, UV-vis spectroscopy, and X-ray crystallography.



Cytotoxicity studies with four human tumor cell lines: A2780, DAN-G, SISO and MCF-7 showed that the most active complexes inhibited the growth of cancer cells with IC_{50} values between 1.25 μM and 25.43 μM. The A2780 ovarian cancer cell line appeared to be the most sensitive of the cell lines to the Cu(II)-complexes (IC_{50} values in the range of 1.25-4.38 μM).

The prepared Cu(II)-complexes were also tested for potential antimicrobial properties on a number of bacterial strains. None of the investigated compounds showed activity against both Gram-positive and Gram-negative bacteria.

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[2] Balewski Ł. et al. *Front. Chem.* 9 (2021) 1-18.

P12

Coordination chemistry as a new way to synthesize compounds with anticancer properties

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The chemistry of coordination compounds is an invaluable field of science that allows for synergy with other disciplines and specialties. Thanks to a wide range of applications, the newly developed coordination compounds dominate both industry and medicine, thus opening the way for further development and improvement in these areas. Within this perspective, the incorporation of metal ions into selected organic scaffolds and the synthesis of various metal-based complexes is the idea on which this proposal is based. Metal ions play an important role in biological processes and in the application of coordination chemistry, which in turn can be a valuable aid in the treatment or diagnosis of diseases [1,2,3]. In view of the above, new, stable coordination compounds of selected d^n electron metals with commercially unavailable 1,2,4-triazole-3-thione derivatives were designed, synthesized and characterized. As a result of the synthesis, fifteen new coordination compounds with Mn(II), Fe(II), Ni(II), Cu(II) and Zn(II) were obtained. The stoichiometric composition of the synthesized complexes was determined by elemental and chemical analysis. The compounds were characterized using selected techniques and research methods. The toxicity of coordination compounds and their biodistribution was assessed by *in silico* methods. By predicting the activity in cell lines, the potential use of the compounds as chemotherapeutic agents was estimated. By using thermal analysis, the thermal stability of solid and volatile compounds as well as intermediate and final pyrolysis products was determined. *In vitro* biological studies on lung (A549) and colon (H29) cancer cell lines confirmed the cytotoxicity of the resulting complexes and free organic ligands.

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P13

Synthesis and cytotoxic activity of a new 4-thiazolidinone derivative against cancer cells

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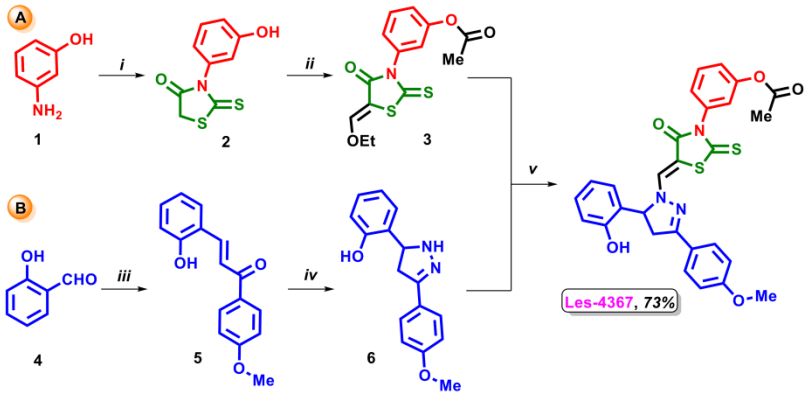
4-thiazolidinone-bearing hybrid molecules possess anticancer activity via various mechanisms of action, such as apoptosis induction, cell cycle arrest, and reactive oxygen species (ROS) induction. This group of compounds includes representants that act as kinase, tubulin, and carbonic anhydrase inhibitors. Recently, we presented the multi-targeting mode of action of 2-{5-[(Z,2Z)-2-chloro-3-(4-nitrophenyl)-2-propenylidene]-4-oxo-2-thioxothiazolidin-3-yl}-3-methylbutanoic acid. We demonstrated that a novel 4-thiazolidinone derivative possesses high cytotoxic and antiproliferative activity in MCF-7 and MDA-MB-231 breast cancer cells. Its molecular mechanism of action is associated with apoptosis induction, where mitochondrial membrane potential decreased, and increased caspase-9 and caspase-8 concentrations were observed. The agent decreased autophagic marker (LC3A, LC3B, and Beclin-1) concentrations in the tested cell lines as well as reduced topoisomerase II concentrations

The aim of the study was synthesis of 4-thiazolidinone-based derivative (Les-4367) and examination of cytotoxic activity against cancer cells. The synthesis of Les-4367 was presented in scheme 1. Preliminary cytotoxic activity studies for Les-4367 were performed on six cancer cell lines—SK-BR-3, HCC1954, MDA-MB-231, HT-29, DLD-1, and AGS. The lowest IC_{50} was detected in human AGS gastric cancer cells [1].



Scheme 1. Synthesis of target 4-thiazolidinone-based derivative Les-4367.

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P14

Unleashing the Potential of LCAPs in conquering *Cryptococcus*: Exploring Novel Compounds with Antifungal Activity

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A year ago, the WHO responded to the growing threat of traditionally neglected fungal infections by developing a Priority Fungal Pathogens List (FPPL). The highest-ranked fungal pathogen in FPPL was *Cryptococcus neoformans (CN)*, which is responsible for causing cryptococcosis, a life-threatening infection primarily affecting immunocompromised individuals, such as those with HIV/AIDS, organ transplant recipients, or individuals undergoing immunosuppressive therapies.[1] The current treatment options for cryptococcosis are restricted to 3 antifungal drugs, which can be used alone or in combination: Amphotericin B (AMB), flucytosine (5-FC) and fluconazole (FLC). However, the therapeutic choices are limited, and challenges with high treatment failure and recurrence rates due to *CN* developing resistance to FLC and 5-FC.[2]

Recently, the concept of hybrid molecules, combining multiple pharmacophore groups in a single framework, emerged. These compounds can potentially inhibit multiple targets, creating bioactive hybrids through this multi-target strategy.[3] In medicinal chemistry, N-aryl piperazines have gained recognition as privileged substructures (PSs). These molecules are considered as a class capable of binding with high affinity to multiple receptors or effector sites.[4]

Considering the above aspects, it was decided to test a selected group of combinations in terms of their antifungal and antibacterial activity. N-alkane-(2/3/4-chlorophenyl)-piperazines ligands were synthesized, with a terminal part containing i.e. phthalimide, benzamide, and sulfonamide moieties. These ligands were obtained based on a method of synthesis in the field of microwave radiation, which is part of the "Green Chemistry" trend. The acquired compounds were subjected to antimicrobial activity screening tests conducted by CO-ADD (UK/Australia) using whole-cell growth inhibition assays against seven different microorganisms: SA, EC, KP, PA, AB, CA, and, most importantly, CN.

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P15

Statin analogs as inhibitors of human adenylate kinase isoenzyme 1

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Statins are the most effective cholesterol-lowering drugs. They also show many pleiotropic effects, including anticancer, cardio- and neuroprotective. Studies on possible interactions between statins and human proteins may provide deeper insight into the pleiotropic and adverse effects of these drugs [1]. Human adenylate kinase has been found to regulate HDL endocytosis, cellular metabolism, cardiovascular and neurodegenerative functions. This enzyme catalyzes the transfer reaction of high-energy β- and γ-phosphate groups between adenine nucleotides according to the reaction: $MgATP^{2-} + AMP^{2-} \leftrightarrow MgADP^{-} + ADP^{3-}$ [2]. Our previous studies showed that statins inhibit the activity of human adenylate kinase isoenzyme 1 (hAK1) in a non-competitive manner [1]. Searching for more effective non-competitive hAK1 inhibitors, we synthesized five statin analogs (Fig. 1) and determined their effect on hAK1 activity.

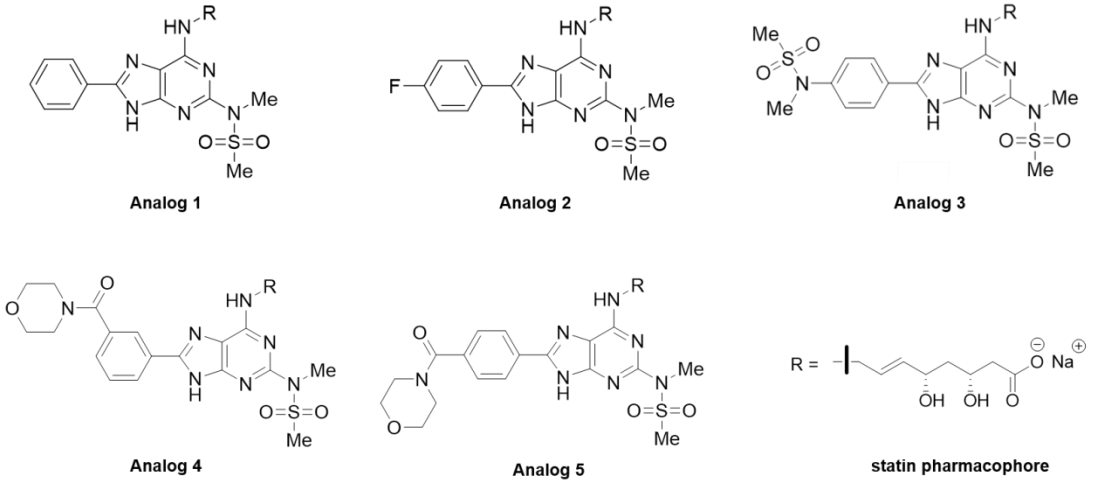

Fig. 1. Statin analogs.

All tested statin analogs showed the ability to inhibit hAK1 in both reaction directions but with different effectiveness. The most effective inhibitor turned out to be analog 4. The IC_{50} value towards ADP synthesis was 198.3 ± 25.6 μM, while towards the synthesis of AMP and ATP the IC_{50} value was 89.31 ± 6.8 μM. The following order of inhibition of hAK1 by statin analogs was observed: A4 > A1 > A2 > > A5 > A3 (ADP synthesis) and A4 > A5 > A2 > A1 > A3 (ATP and AMP synthesis).

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P16

Nanotechnology towards green chemistry - plant extract as a reducer and stabilizer of copper nanoparticles

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Considering the environmental issues associated with the large-scale synthesis of nanoparticles, the green approach is more preferable and promising than conventional methods [1]. The development of a reproducible green method for the synthesis of copper nanoparticles using plant materials seems promising and innovative [1].

The nanoparticles were obtained using copper salts in an aqueous plant extract by a natural method that harmonizes with the assumptions of green chemistry. The methodology of this process is simple, inexpensive and environmentally friendly [2]. The results indicate that the compounds contained in the extracts can act simultaneously as reducers and stabilizers of the resulting nanoparticles, and the additional organic layer allows for the future functionalization of the resulting nanostructures [3]. Nanocopper synthesized using green chemistry has been tested for composition, internal structure, morphology, surface structure and size. The structure of nanomaterials was characterized by the following methods: XRD, TEM, SEM, AFM, UV-Vis, DLS, etc. Physical and chemical studies concerned a wide range of nanoparticles synthesized by the green chemistry method, increasing the probability of obtaining repeatable results. Their particle size and shape can be modified with the use of a reducing agent: the agent used, synthesis time, pH of the solution, the ratio of the reducing agent to the core precursor, etc. The final properties of the obtained nanostructures (size, shape, morphology and homogeneity) depend on the "green" reducer used [3].

The development of nanotechnology in the processes of "green chemistry" will gradually gain an appropriate pace along with technological progress [4]. This fact is recognized as a future-proof and key element in the development of new nanosynthesis processes [3], [4].

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P17

Synthesis and biological activity of mono- and disubstituted 5-methyl-7-phenylpyrido[3,4-*d*]pyridazine derivatives

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Biological studies have shown that compounds containing the pyrido[3,4-*d*]pyridazine ring have a wide spectrum of biological activity [1], therefore the synthesis of new derivatives has been undertaken.

In our previous work [2] we determined a way of synthesizing pyrido[3,4-*d*]pyridazine scaffold. N/O-substituted 5-methyl-7-phenylpyrido[3,4-*d*]pyridazine derivatives were synthesized. As a continuation of our research, a new series of derivatives with a 4-chlorophenyl substituent was prepared. Cyclization of 6-(4-chlorophenyl)-2-methylpyridine-3,4-dicarboxylic acid with acetic anhydride gave the furo[3,4-*c*]pyridine-1,3-dione derivative. Treatment of 6-(4-chlorophenyl)-4-methylfuro[3,4-*c*]pyridine-1,3-dione with hydrazine monohydrate resulted in the rearrangement to the 7-(4-chlorophenyl)-1-hydroxy-5-methylpyrido[3,4-*d*]pyridazin-4(3*H*)-one. The obtained compound was alkylated to the corresponding mono- and disubstituted derivatives of 7-(4-chlorophenyl)-5-methylpyrido[3,4-*d*]pyridazine.

The biological activity assay of the obtained 5-methyl-7-phenylpyrido[3,4-*d*]pyridazine derivatives has been performed. The cytotoxicity of the compounds was determined on regular cell lines L929 (mouse fibroblast) and RPTEC (human renal proximal tubule epithelial cells) and selected tumors: A172 (human glioblastoma derived cells), AGS (Human Caucasian gastric adenocarcinoma). Caco-2 (Human Caucasian colon adenocarcinoma) and HepG2 (Human Caucasian hepatocyte carcinoma) using the Neutral Red Assay Uptake (NR). For compounds which have not manifested toxic effect, the antineoplastic properties were assessed on the A172 and AGS lines by FDA/PI staining flow cytometry (fluorescein diacetate and propidine iodide) and fluorescence microscope observations of Hoechst 33342 stained A172 and AGS cells after exposure to selected compounds.

Moreover, the anti-inflammatory potential of selected compounds was also evaluated. *In vitro* analysis using colorimetric assay for inhibition of COX-1 and COX-2 and *in silico* calculations, looking for potential molecular targets for test compounds and molecular docking of one of them to COX-1 and COX-2 have been checked.

The Neutral Red Uptake assay showed no toxicity of compounds 1-6 to regular cell lines. Combined NR and flow cytometry outcomes indicated that compounds 1, 4, 5 and 6 exhibit potential anti-tumor properties. Microscopic analysis did not allow to assess the type of cell death caused by the tested compounds. Although prediction of molecular targets indicated potential inhibitory properties of the test substances against COX-1 and COX-2, colorimetric test and molecular docking did not prove such effect.

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P18

Synthesis of oligopeptide modulators of tyrosinase

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Peptides occur in the environment, exist in all cell structures, and function as neurotransmitters or peptide and tissue hormones, such as endorphins, insulin, oxytocin, vasopressin, and bradykinin. [1].

Peptides find wide-ranging applications and have piqued the interest of the scientific and industrial communities. They are used, among other fields, in cosmetology, where natural compounds that can be easily absorbed by the body are sought to effectively combat skin aging. Peptides play a crucial role in stimulating collagen and elastin synthesis in the skin, preventing the formation of expression wrinkles, as well as nourishing and transporting oxygen deep into the skin [2]. Furthermore, peptides act as primary modulators, ensuring the proper progression of repair and metabolic reactions. As individuals age, the levels of peptides decrease, and errors during their synthesis can lead to malfunctions in these compounds, which are essential for the functioning of organisms [3].

In solid-phase Fmoc peptide synthesis reactions, the peptide chain is built up step-by-step by attaching one amino acid at a time to the solid support using the Wang resin method. This approach allows for the removal of unnecessary reaction products through washing [4, 5].

The objective of my work was to synthesize four oligopeptides, as described in the literature, that influence the activity of tyrosinase and to determine the optimal reaction conditions. Two of the synthesized oligopeptides underwent chromatographic analysis. However, for the longer chain, impurities appeared in the chromatograms, mainly in the form of shorter oligopeptides. This observation may suggest the necessity of extending the deprotection and acylation reaction time.

To achieve the goal of this work, I utilized the Bill Board device for Distributed Drug Discovery (D3). The Bill Board is a device invented by William L. Scott of Indiana University Purdue University of Indianapolis (IUPUI).

Bill-Board 6-pack Bill-Board Drain Tray Collection Vial Rack

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P19

Anticancer potential of selected diazaphenothiazines

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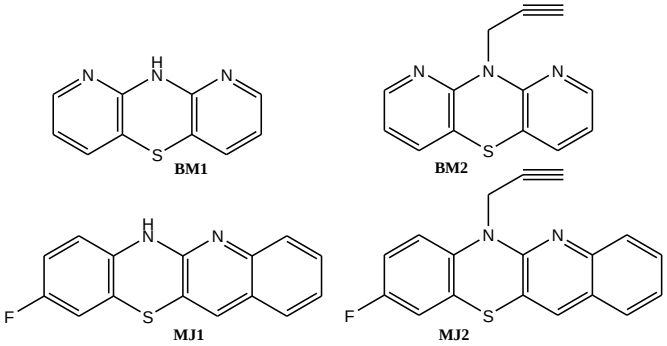
Derivatives of phenothiazines exhibited promising anticancer activity against several cancer cell line (breast, ovarian, lung, colorectal, prostate, leukemia, melanoma, and renal) [1-3]. Among of the azaphenothiazines, 10*H*- and 10-propargyl-1,9-diazaphenothiazines (**BM1**, **BM2**) and 6*H*- and 6-propargyl-9-fluoroquino-benzothiazines (**MJ1**, **MJ2**) have been reported effective in killing breast cancer cells, glioblastoma, melanoma and ovarian cancer cell line instead less toxic towards normal human fibroblast cells [4,5].



The aim of the project was to investigate the effect of selected azaphenothiazine derivatives **BM1**, **BM2**, **MJ1**, **MJ2** on p53-dependent signaling pathway inhibitors. Human colorectal carcinoma lines HCT116 with wild-type p53 protein status, HCT116 with mutated p53 protein and a human erythroleukemic cell line K562 were used in the study. IC_{50} values were determined against selected tumor lines differing in p53 protein status in the MTT experiment. A clonogenic assay was performed to determine the ability to form clonogenic colonies followed by a real-time polymerase chain reaction experiment. These experiments were aimed at determining the relative level of expression of selected genes on cell lines containing two different statuses of p53 protein. The obtained results are highly promising and indicate the anticancer potential of the tested derivatives.

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P20

Potential new mechanism of action of synthetic pegylated curcumin in hypoxic conditions

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Curcumin has long been the interest of medicinal chemists and there exist various modifications to broaden its application in medicine and address its limitations.[1] In this study, we present a series of synthetic curcumin derivatives modified with short polyethylene glycol (PEG) chains and the addition of the BF_2 moiety to the dicarbonyl group. Tested compounds were screened for their cytotoxic activity toward two bladder cancer cell lines - 5637 and SCaBER as well as a noncancerous cell line derived from lung fibroblasts (MRC-5). Cells were tested under normoxic and hypoxic conditions (1% oxygen). Among the tested compounds one in particular did not affect the viability of MRC-5 cells and exerted a stronger cytotoxic effect under hypoxic conditions. Interestingly the flow cytometry studies showed that pegylation did not improve cellular uptake. It did however enhance cytotoxic activity. The preliminary mechanism of action studies indicate that the most promising compound induced G2/M arrest in a dose-dependent manner and increased the expression of stress-related proteins under hypoxic conditions. In summary, the results of the study indicate that PEGylated curcumin is a more potent compound against bladder cancer cell lines than the parent compound, and is worthy of further investigation to clarify its mechanism of anticancer action under hypoxic conditions.

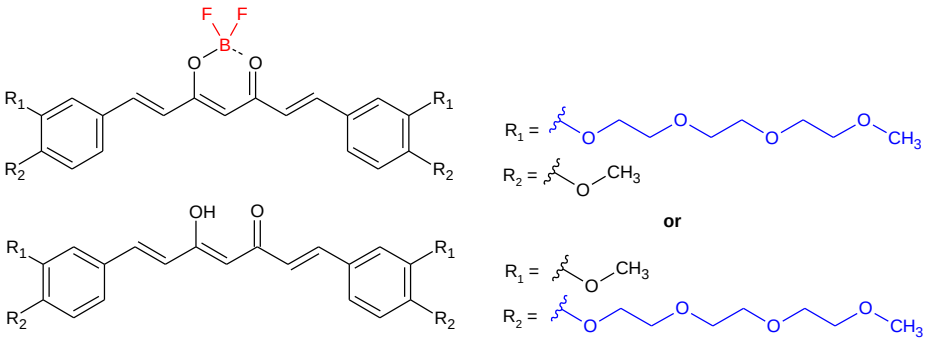

Fig. 1 Structures of pegylated derivatives of curcumin

[1] Tomeh MA, Hadianamrei R, Zhao X. *Int J Mol Sci*. 2019; 20.

This research was funded by National Science Center, grant number 2019/35/B/NZ7/01165.

P21

A novel selenoester as an activator of autophagy in MDA-MB-231 triple-negative breast cancer cells

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The number of new cases of breast cancer increases every year. Only in 2020, the number of newly diagnosed patients with this type of cancer exceeded 2.2 million people. In addition, another fact is also the increasing resistance of cancer cells to standard treatment, which is a significant clinical problem and is becoming a challenge for the modern scientific world. In recent years, compounds containing selenium in their structure have attracted considerable scientific interest. Among a wide group of selenoorganic compounds, selenoesters are particularly active, exhibiting high cytotoxicity at low (even nanomolar) doses, strongly inducing the process of apoptosis and with the potential to break multidrug resistance (MDR) in cancer cells [1,2]. Although apoptosis is the main mechanism responsible for the complete and irreversible destruction of cells, there are several other processes with the same goal, such as autophagy. This process is also known as type II programmed cell death and may enhance the anticancer activity of a compound or have an additional effect on sensitizing cancer cells to used treatment. Regulation of autophagy occurs via several signaling pathways, the main is PI3K/Akt/mTOR. In the case of this pathway, mTOR kinase is an inhibitor of this process, and blocking it has a pro-autophagy effect [2].

Therefore, in the first step, the cytotoxic activity of the compound EDA-71 and cisplatin towards MDA-MB-231 triple-negative breast cancer cells and MCF-10A normal breast epithelial cells was determined using the MTT assay according to the Carmichael method. This was followed by a flow cytometer analysis of autophagy induction and mTOR protein activity. For this purpose, Autophagy Assay, Red kit and fluorochrome-labeled anti-mTOR antibody (Alexa Fluor® 647) were used, respectively.

The obtained results showed that the tested selenoester (EDA-71) has significantly higher anticancer activity than the reference compound (cisplatin). In turn, its cytotoxic effect was weaker against normal cells compared to MDA-MB-231 breast cancer cells. In addition, in the course of further studies, it was observed that EDA-71 has stimulating effects on the autophagy process, which was caused by inhibition of mTOR kinase activity. The above results suggest that the potent anticancer effect may be related to the activation of autophagy.

[1] Radomska D. et al. *Int J Mol Sci* 2021, 22, 1009, doi:10.3390/ijms22031009.

[2] Radomska D. et al. *Cancers* 2022, 14, 4304, doi:10.3390/cancers14174304.

P22

AR71 – histamine H_3 receptor ligand – *in vitro* and *in vivo* evaluation (anti-inflammatory activity, metabolic stability, toxicity, analgesic action)

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Histamine H_3 receptors (H_3R) are, presynaptic receptors, expressed in the central nervous system in the region connected with cognition, sleep, wakefulness and hemostatic regulation. Blocking of these receptors increase the release of histamine itself and other neurotransmitters such as acetylcholine, dopamine, serotonin or noradrenaline. Numerous studies have shown that inhibition of these receptors alleviates memory impairment caused by chronic inflammation associated with neurodegenerative diseases such as Alzheimer's disease.

Our research studies led to obtain the compound **AR71**, [(*E)-3-(3,4,5-trimethoxyphenyl)-1-(4-(3-(piperidin-1-yl)propoxy)phenyl)prop-2-en-1-one*], with high affinity for human H_3R (K_i = 24 nM) and positive anti-inflammatory activity in a model of LPS-induced inflammation in BV2 cells [1]. Continuing this work, we conducted further toxicity studies in Peripheral Blood Mononuclear Cells (PBMCs) and metabolic stability in human hepatic microsomes (HLMs). Furthermore, as chronic and excessive inflammation may lead to sensitization of nerve fibers and intensification of pain sensations, we tested our compound in a neuropathic pain model (Chronic Constriction Injury (CCI) to the sciatic nerve) in the mice.

Our results confirmed further promising anti-inflammatory activity of **AR71** and its low toxicity in PBMCs. Metabolic studies, showed high metabolic stability of this compound (85% remaining of **AR71**) after 120 min of incubation with HLMs. Moreover, **AR71** exhibited analgesic effects at all tested time points at doses 20 mg/kg (as measured in von Frey test), and 10 and 20 mg/kg (in cold plate test) compared to vehicle-treated animals.

Acknowledgements*: This research was partly supported by Jagiellonian University Medical College grant no N42/DBS/00030 (D.Ł.), the National Science Centre, Poland, grant UMO-2019/35/D/NZ7/01042 (K.P.-B.), Medical University of Lodz Statutory Activity No. 503/1-156-06/ 503-11-001 (A.S.).*

[1] Honkisz-Orzechowska, E. et. al. *Inflamm Res.* 72 (2023) 181–194.

P23

Synthesis of dibromo- and tetrabromocurcuminoids with potential antimicrobial activity

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Curcumin is a polyphenolic compound of natural origin. It is the main component of *Curcuma Longa* with therapeutic effects. Curcumin is a compound with a broad spectrum of therapeutic activities (anti-cancer, anti-inflammatory, antioxidant, hepatoprotective) while having low toxicity. Numerous studies report bactericidal and bacteriostatic effects through various mechanisms of action [1].

The main objective of the study is to develop a method for the synthesis of new curcumin derivatives. Modifications of their structure, such as the introduction of bromine into the aromatic ring and the substitution of short alcohol chains, are intended to improve the pharmacodynamic and pharmacokinetic parameters of curcuminoids. In addition, complexes of the derivatives with BF_2 have been obtained, which also show biological activity. The new compounds were obtained by condensation of the corresponding aldehydes with an acetylacetone complex and BF_2. n-Butylamine was used as the catalyst for the reaction, with toluene as the solvent. The decomplexation reaction was then carried out under microwave conditions and curcuminoids with a free keto-enol group were obtained. The reaction was carried out in a mixture of methanol : water (4:1) with sodium oxalate. The structure and purity of the obtained compounds were confirmed using 1D and 2D NMR techniques (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC), absorption maxima in the UV-Vis range were determined using UV-Vis spectroscopy.Six compounds were obtained, of which four were curcuminoid complexes with BF_2, and two were free curcuminoids. The yields of the condensation reaction ranged from 42.57% to 74.6%, while the decomplexation yields ranged from 75% to 89.6%.

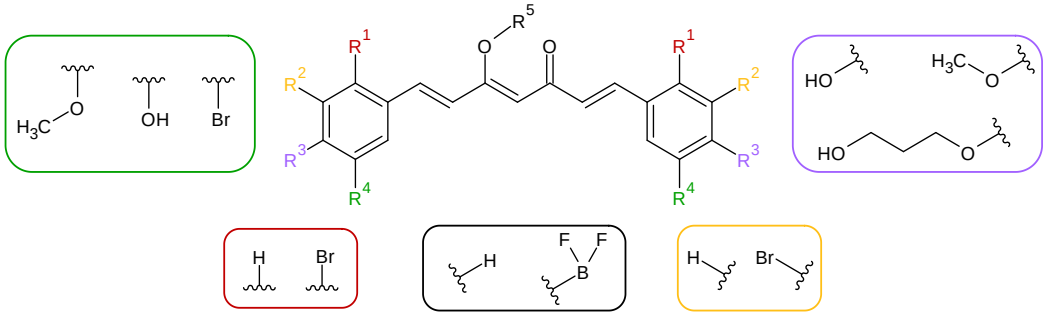

Figure 2 Structures of the synthesized compounds

The selected reactions are a good and efficient way to obtain selected curcuminoids and their complexes with BF_2. Further microbiological studies are planned to determine their antimicrobial activity.

[1] Zheng D, Huang C, Huang H, Zhao Y, Khan MRU, Zhao H, et al. Antibacterial Mechanism of Curcumin: A Review. Chem Biodivers. 2020;17(8).

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P24

Cunninghamella species as an effective tool for bioremediation of selected Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have been identified as a novel class of emerging contaminants (EC) due to their limited removal by wastewater treatment plants and ability to induce physiological effects in humans even at low doses. Moreover, these EC pose a rising threat to marine ecosystems [1,2]. The flexibility of various microorganisms to remove toxic pollutants from the environment creates bioremediation an innovative solution for EC problem [3]. *Cunninghamella* fungi found in soil and plant material are well known for their ability to biotransform various xenobiotics including pollutants [4,5].
The present work aimed at determining selected NSAIDs (ibuprofen, naproxen and indomethacin) removal capabilities of *Cunninghamella* fungi. Within the study, *in vitro* ecotoxicity and mutagenicity of fungal transformation products (FTP) were evaluated.
Biotransformation processes were carried out using three *Cunninghamella* strains (*C. echinulata*, *C. blakesleeana* and *C. elegans*) in liquid medium for 7 days. The progress of biotransformation was monitored using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). FTP ecotoxicity was assessed with the use of the Microtox system, whereas mutagenicity was evaluated with the Ames test.
Different loss of tested pharmaceuticals was observed after incubation with *Cunninghamella* strains. The most efficient removal was observed for ibuprofen as 100% of this compound was biotransformed with all used *Cunninghamella* strains. As regards indomethacin and naproxen, 100% degradation rate was achieved only with one *Cunninghamella* strain, *C. echinulata* and *C. elegans*, respectively. Regarding toxicity data, there was a decrease in the ecotoxicity of the postculture extracts toward *Aliivibrio fischeri* in relation to the parent drugs. Additionally, no mutagenic effects were induced by the postculture extracts in the Ames test.
This study demonstrated that *Cunninghamella* strains have the ability to effectively biodegrade emerging pollutants such as NSAIDs.

[1] Rastogi A. et al. *J. Environ. Manage.* 300 (2021) 113694.
[2] Mezzelani M. et al. *Mar. Environ. Res.* 121 (2016) 31–39.
[3] Saravanan A. et al. *Chemosphere.* 313 (2023) 137323.
[4] Nowak M. et al. *Int. J. Mol. Sci.* 22 (2021) 4360.
[5] Meng M. et al. *Arch. Microbiol*. 205 (2023) 264.

Acknowledgements
The project was supported by the National Science Center Grant No 2020/37/B/NZ7/02546.

P25

Nonclassical phenyl bioisosteres in a series of quinoxaline-2,3-dione derived AMPA/KA antagonists as a method to improve compounds solubility

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Kainate receptors (KAR) belong to the family of ionotropic receptors for the main excitatory neurotransmitter in the central nervous system, glutamate. These transmembrane proteins form neuronal cation channels and may have a function in the pathophysiology of epilepsy, migraine, neuropathic pain, anxiety or schizophrenia. One of the most extensively studied group of competitive KAR antagonists are quinoxaline-2,3-dione derivatives, that have demonstrated neuroprotective and anticonvulsant activity in clinical trials, but due to their unfavorable physicochemical profile and side effects, further clinical trials were discontinued.

The present project is a continuation of our previous study focused on design and synthesis of potent KAR receptor ligands based on the quinoxaline-2,3-dione core [1]. In our search we discovered several analogs, selectively binding to individual KA receptors with various subunit composition, among others – a first selective GluK3 KAR antagonist with submicromolar affinity and unprecedented, more than 400-fold binding preference for GluK3 over other subtypes. Unfortunately, further advanced *ex vivo* and *in vivo* assays have been halted due to low solubility of the investigated compounds.

For this reason, in the present study, a new group of compounds has been designed on the basis of molecular modeling results, based on the assumption that the isolated phenyl ring in the quinoxaline-2,3-dione hydrazide moiety could be effectively replaced by heterocyclic aromatic or saturated groups, which would improve the solubility of the compounds while maintaining the biological activity profile. As part of the project, logS and logP parameters were determined *in silico* for the designed new series, and a chemical synthesis method was developed and optimized. The compounds selected on the basis of *in silico* studies will be synthesized, and their solubility will be determined by experimental methods.

[1] Chałupnik P. et al. *Int J Mol Sci.* 23 (2022), 8797.

The financial support of the Jagiellonian University Statutory Founding (**N42/DBS/000335**) is gratefully acknowledged.

P26

Architecture of the 5-arylideneimidazol-4-one derivatives, potential inhibitors of cancer MDR proteins

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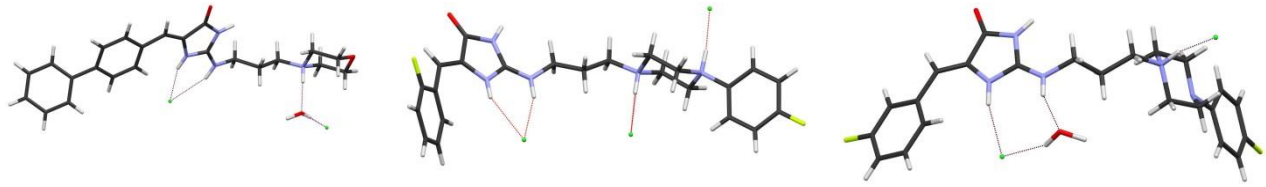
Multidrug resistance (MDR) is a global problem in the treatment of various diseases such as cancer, bacterial, fungal and parasitic infections. An interesting approach in the search for the ways to overcome MDR are so called adjuvants, i.e. the compounds able to block at least one mechanism of resistance. Some previously studied the 2-amine derivatives of 5-arylideneimidazol-4-one showed a potency of adjuvants for antibiotic and chemotherapeutic agents [1,2]. In the search for new adjuvants able to block bacterial and/or cancer multidrug resistance, new derivatives of 2-amineimidazol-4-one were synthesized.

The interest of our group is focused on derivatives of 2-amineimidazol-4-one containing different arylidene substituents at position 5 and morpholine or phenylpiperazine ring connected by the three carbon linker with 2-amine group. We present three new crystal and molecular structures (**I**, **II** and **III**) for compounds investigated on their modulation of MDR efflux pump ABCB1 (P-gp) in T-lymphoma cancer cells.

I **II** **III**



Only compound **I** turned out to act as modulator of the P-gp efflux pump in the accumulation assay performed. The benzylideneimidazol-4-one moiety is the most planar in **I**. The interplanar angle between the planes of the imidazol-4-one ring and aromatic ring of benzylidene moiety is 7.9°, 32.3° and 18.2° for **I**, **II** and **III**, respectively.

[1] A. Kaczor, K. Witek *et al*. *Molecule*s. 24 (2019) 438.

[2] A. Kaczor, K. Witek *et al*. *Int. J. Mol. Sci.*. 22 (2021) 2062.

P27

Trisubstituted triazines as multifunctional TRPA1/PDEs ligands: Synthesis, pharmacological evaluation and molecular modeling study

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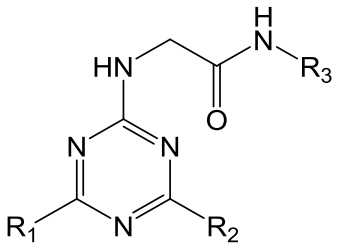
The multi-target-directed ligands (MTDLs) capable of interacting with a number of selected biological targets are the new approach in the treatment of diseases with a complex pathomechanism. Taking into account this promising strategy we have designed and synthesized a new class of trisubstituted triazines combining TRPA1 channel ago/antagonism and multiple PDE inhibitory activity as a likely target for suppression of inflammation and airway hyperactivity in chronic respiratory diseases.



In order to synthesized trisubstituted triazines presented above firstly the 4,6-diaminotriazines were obtained by the consecutive nucleophilic substitution of 2,4,6-trichlorotriazine. In the next step the appropriate intermediate was treated with glycine ethyl ester hydrochloride, then hydrolyzed to appropriate acid and finally coupled with amine to obtain trisubstituted triazine derivative. The *in vitro* assays shoved that the new compounds exert multifunctional ago/antagonistic properties towards human TRPA1 channel and PDE4/PDE7/PDE8 inhibitory activity. Molecular modeling studies confirmed the multitarget-directed activity demonstrated *in vitro*, showing that the binding modes of the selected ligands included crucial interactions for their activity: hydrogen bonds with invariant glutamines and aromatic interactions with the two phenylalanines in the respective PDEs and hydrogen bond with Gln-940 in the TRPA1 ion channel. Pharmacological evaluation of the selected compounds in transforming growth factor type β-induced human lung fibroblast (MRC-5) revealed their limiting effect on cellular responses related to airway remodeling including proliferation and migration. The results of pharmacokinetic study performed for the most active multifunctional ligands showed their favorable serum concentration versus time profile following intraperitoneal administration to mice. In addition, it was vividly distributed to important organs, which makes it suitable for further *in vivo* investigation, e.g. elastase-induced COPD in mice.

This work was supported by the National Science Centre, Poland, funded grant No. 2018/29/B/NZ7/00285.

P28

Advancements in Phthalocyanines and Naphthalocyanines for Biomedical Applications

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Phthalocyanines and naphthalocyanines have emerged as versatile and multifunctional compounds with significant potential in various biomedical applications. This study provides an in-depth review of recent publications focusing on the synthesis, photophysical properties, and therapeutic strategies of these macrocycles in biomedical research.

The synthesis of phthalocyanines and naphthalocyanines with tailored functionalities has been a key area of exploration[1,2]. Researchers have demonstrated remarkable versatility in functionalizing these macrocycles, enabling the development of targeted imaging agents with enhanced biocompatibility and improved cellular uptake[1]. Additionally, efforts have been made to incorporate stimuli-responsive functionalities, facilitating controlled drug release and theranostic applications[3]

The photophysical and photochemical properties of phthalocyanines and naphthalocyanines have shaped their fate in biomedical applications. Investigations into the fluorescence behavior and singlet oxygen generation have been instrumental in designing efficient imaging and photodynamic therapy (PDT) agents. Moreover, the potential of these macrocycles as contrast agents in various imaging modalities, such as fluorescence, photoacoustic, and magnetic resonance imaging, has been explored[4].

In the context of therapeutic strategies, PDT utilizing phthalocyanines and naphthalocyanines as photosensitizers has shown promising results not only in therapy of cancer but in antimicrobial applications as well[5]. Additionally, research has been directed towards employing phthalocyanine and naphthalocyanine-based theranostic agents, allowing simultaneous imaging and therapy for personalized and targeted treatment approaches.

The versatility of phthalocyanines and naphthalocyanines extends beyond PDT and PTT (Photothermal therapy), with potential application in targeted drug delivery systems and biosensing[6]. These macrocycles have been used as carriers for therapeutic payloads, enabling site-specific drug release for enhanced treatment efficacy and reduced side effects[6,7]. Additionally, their incorporation into biosensing platforms has enabled sensitive and selective detection of biomolecules and biologically-relevant analytes.

This presentation provides a comprehensive overview of the recent advancements in the use of phthalocyanines and naphthalocyanines for biomedical applications, emphasizing their synthesis, properties, and possible applications in the biomedical field. By showcasing their versatility in imaging, PDT, PTT, drug delivery, and biosensing, we aim to inspire further research and promote these chemical structures to reach their full potential and to showcase the field for improvement.

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[2]Tedesco, A. C.; Primo, F. L.; Jesus, P. D. C. C. D. Antimicrobial Photodynamic Therapy (APDT) Action Based on Nanostructured Photosensitizers. In *Multifunctional Systems for Combined Delivery, Biosensing and Diagnostics*; Elsevier, **2017**; pp 9–29. [3] Mallidi, S.; Anbil, S.; Bulin, A. L.; Obaid, G.; Ichikawa, M.; Hasan, T. Beyond the Barriers of Light Penetration: Strategies, Perspectives and Possibilities for Photodynamic Therapy. *Theranostics* **2016**, *6* (13), 2458.

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[7]Wei, J. P.; Chen, X. L.; Wang, X. Y.; Li, J. C.; Shi, S. G.; Liu, G.; Zheng, N. F. Polyethylene Glycol Phospholipids Encapsulated Silicon 2,3-Naphthalocyanine Dihydroxide Nanoparticles (SiNcOH-DSPE-PEG(NH2) NPs) for Single NIR Laser Induced Cancer Combination Therapy. *Chin. Chem. Lett.* **2017**, *28* (6), 1290–1299.

Synthesis of functionalized diethyl (pyrrolidin-2-yl) phosphonate and diethyl (5-oxopyrrolidin-2-yl) phosphonate

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Pyrrolidine and pyrrolidinone belong to the important structural units of many pharmacologically active compounds, for example, piracetam **1** and oxiracetam **2** containing pyrrolidinone moiety are used in the treatment of epilepsy and depression (CNS diseases),[1-2] whereas polyhydroxylated derivatives of pyrrolidine **3** and **4** are efficient inhibitors of α-glucosidases.[3] The wide spectrum of pharmacological activity of these classes of compounds has become an inspiration to design new derivatives of pyrrolidine and pyrrolidinone functionalized with the phosphoryl group.

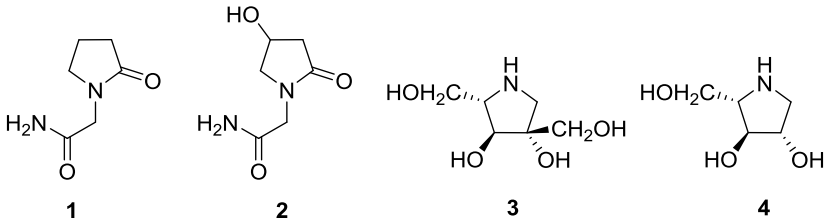

Figure 1 Structures of piracetam **1**, oxiracetm **2** and selected polyhydroxylated derivatives of pyrrolidine **3** and **4**.

Synthesis of pyrrolidinone **5** and pyrrolidine **6** started from the diastereospecific 1,3-dipolar cycloaddition of *N*-benzyl-*C*-(diethoxyphosphoryl)nitrone to the respective alkene (dimethyl maleate or *cis*-1,4-dihydroxybut-2-ene) to give isoxazolidine **7** or **8**. In order to obtain phosphonate **5,** isoxazolidine **7** was subjected to hydrogenolysis, whereas compound **6** was synthesized from isoxazolidine **8** in the reaction sequence involving mesylation and subsequent hydrogenolysis.[4]

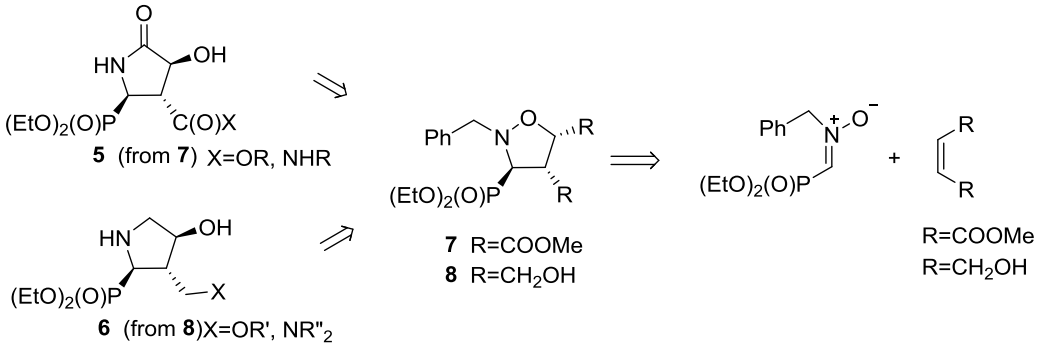

Figure 2 Retrosynthesis of **5** and **6**.

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P30

Synthesis and antimicrobial activity of hydrazones of 2- and 4-iodobenzoic acid

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Hydrazones obtained form hydrazides of carboxylic acids constitute interesting group of organic compounds which possess wide spectrum of application in the synthesis of heterocylic compounds and in coordination chemistry as well as display significant biological activity [1, 2]. The main directions of bioactivity of this group of compounds include their antimicrobial and antitumour activity [1, 2].

This study concerns the synthesis and antimicrobial activity evaluation of hydrazones of 2- and 4-iodobenzoic acid. The compounds were obtained in a two-step synthesis. In the first stage hydrazides of 2- and 4-iodobenzoic acid were obtained by the reaction of appropriate methyl esters with 100% hydazine hydrate. Then hydrazides were subjected to condensation reaction with different substituted aromatic aldehydes. These reactions enabled the synthesis of hydrazones of 2- and 4-iodobenzoic acid. Chemical structure of synthesized compounds was confirmed by infrared spectroscopy (IR) and proton and carbon nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR). In addition, the chemical structure of the obtained substances was confirmed by X-ray diffraction.

All synthesized compounds have been tested under *in vitro* conditions to establish their antimicrobial activity using the broth microdilution method [3]. Standard strains of Gram-positive and Gram-negative bacteria and fungi, representing both pathogenic and opportunistic strains, were used in this research. On the basis of set parameters MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concetrnation) antimicrobial activity of tested substances was established. Some of obtained compounds were chracterized with strong antibacterial activity (MIC < 10 μg/ml), especially against Gram-positive bacterial strains.

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P31

Neuroprotective properties of chalcogen-containing 1,3,5-triazine “hits” found in search for Alzheimer’s disease agents acting via serotonin receptor 5-HT$_6$

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Serotonin receptors 5-HT$_6$ (5-HT$_6$R), due to their unique location in the brain areas responsible for memory and cognition, are an attractive target in search for new drugs against dementia and Alzheimer’s disease (AD). An introduction of chalcogen-containing ether linker into structures of potent 5-HT$_6$R ligands gives possibility for additional neuroprotective properties, thus giving a hope to find new effective therapy against AD. In this study a series of Se-ether derivatives of 1,3,5-triazine (Fig.1) was synthesized and evaluated on the affinity towards 5-HT$_6$R.

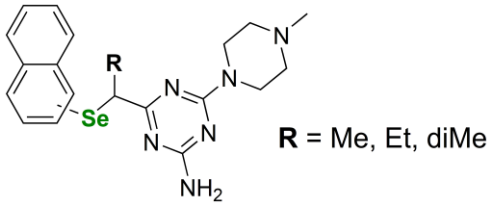

Fig. 1. Structure of the investigated series

The most active 5-HT$_6$R agents (**PPK-32**, **JK-5** and **JK-6**) were investigated on their neurotoxicity and neuroprotective action in neuroblastoma SH-SY5Y cell line, including their influence on selected neurodegeneration/neuroprotection transcription factors. Potential chemical mechanisms of neuroprotective effects were examined. Based on the results obtained, compound **PPK-32** turned out to be especially promising highly potent 5-HT$_6$R agent with beneficial neuroprotective effects - promising to combat the causes, not only the symptoms, of AD.

Partly supported by the National Science Centre (grant UMO-2018/31/B/NZ7/02160)and Jagiellonian University grant no. N42/DBS/000080. Part of the studies was performed within Student Medicinal Chemistry Club (SKN Chemii Medycznej UJ CM w Krakowie).

P32

Synthesis, antiseizure and antinociceptive activity of 3-(benzo[*b*]thiophen-2-yl)- and 3-(benzofuran-2-yl)-pyrrolidine-2,5-dione derivatives

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Epilepsy is recognized as one of the most common neurological disorders just after stroke. It is characterized by multifactorial pathogenesis, which reflects in a very low clinical efficacy of currently available antiseizure drugs (ASDs). It is estimated that up to 30% of epileptic patients do not achieve satisfactory improvement and suffer from drug-resistant epilepsy. Therefore, discovery of more effective and safer medications is still unmet clinical need.[1,2]

Taking into consideration the above facts since many years our efforts have been focused on development of new broad-spectrum antiseizures is group of pyrrolidine-2.5-dione derivatives.[3-6] With an aim of continuing of this innovative studies, we report herein synthesis and biological evaluation of new series of 3-(benzo[b]thiophen-2-yl)- and 3-(benzofuran-2-yl)-pyrrolidine-2,5-diones containing differently substituted piperazine or morpholine moieties as amine function. The results obtained showed that the most potent protection revealed compound **GM-111** whit the following pharmacological properties: ED_{50}=24.1 mg/kg (maximal electroshock test, MES) and ED_{50}=30.9 mg/kg (6 Hz, 32 mA seizure model) after intraperitoneal (*i.p.*) administration to mice. Additionally, no motor impairment was observed in the rotarod test up to dose of 200 mg/kg that yielded in a significant differentiation of active vs. toxic doses. More detailed investigations showed that **GM-111** demonstrated significant analgesic effect in the formalin test, as well as antiallodynic activity in the oxaliplatin-induced neuropathic pain model (in mice, *i.p.*). The preliminary *in vitro* studies confirmed its satisfying safety profile, as **GM-111** in high concentration of 100 μM was devoid of hepatotoxic (HepG2 cell line), neurotoxic (SH-SY5Y call line), and mutagenic (Ames test) properties. The *in vitro* binding studies indicate that the most plausible mechanism of action of **GM-111** relies on the influence on the neuronal voltage-gated sodium channels. In sum, **GM-111** seems to be promising candidate for more detailed preclinical development in epilepsy and pain indications.

The studies were supported by the National Science Centre, Poland grant UMO-2017/25/B/NZ7/01048 and Jegiellonian University Medical College project N42/DBS/000305.

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P33

Microbiological activity of selected thiosemicarbazide derivatives

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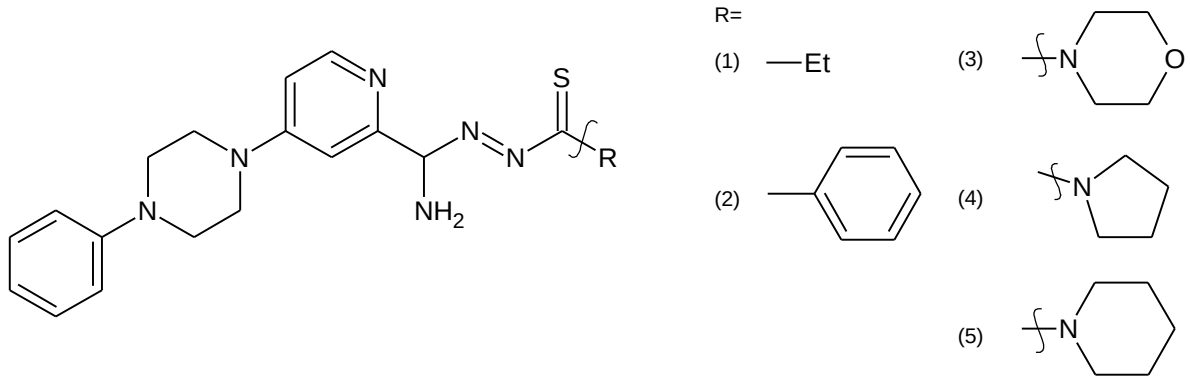
Thiosemicarbazides are popular building blocks used in organic chemistry. Their derivatives have promising anti-tuberculous, anti-bacterial and anti-cancer properties [1–3]. This is an advantage when looking for alternative antibiotics for the already known, drug-resistant bacteria that cause respiratory diseases. The presented thiosemicarbazide compounds in combination with a picolinic acid derivative (Scheme) have promising bacteriostatic properties. All studied compounds show high activity against Gram-positive bacteria.



Scheme - presentation of thiosemicarbazide derivatives.

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P34

Fluorocurcumin derivatives and their nanoformulations using micelles as potential weapon against bladder cancer

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Bladder cancer is responsible for approximately 200,000 deaths annually worldwide, accounting for 2.1% of all cancer deaths. The epidemiology of this disease is a growing problem, despite the development of new anti-cancer therapies [1]. Curcumin has proven anticancer activity. However, its clinical use is limited due to poor bioavailability and solubility. To improve bioactivity of curcumin new curcumin fluoro derivatives were synthesized, but they still have low solubility [2]. The way to modify the bioavailability is a strategy based on the use of carriers, such as micelles, which can contribute to improving pharmacokinetic and pharmacodynamic parameters. The assumptions of the presented project concern: synthesis of curcumin derivatives with higher stability and biological activity, encapsulation of the most active curcuminoid in micelles, and evaluation of the effect of curcuminoid encapsulation on the survival of cancerous bladder cells in an *in vitro* model.

The micelles were prepared using a solubilizer, a crystallization inhibitor, and a matrix-forming polymer reagent called Soluplus®. Characterization of the size of the resulting micelles was performed using the Dynamic Light Scattering (DLS) technique, while encapsulation efficiency (EE) was evaluated using HPLC. The *in vitro* model test was performed for a human bladder cancer line. Cell survival after incubation with the tested compounds was assessed using the MTT assay. The results provided new information on the use of micelles as an effective carrier for phytochemicals, including the possibility of modulating the biological activity and improving the selectivity of curcumin derivatives.

This work was supported by the National Science Center - grant no. 2019/35/B/NZ7/01165.

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P35

A new cinnamic derivatives as potential UV blockers

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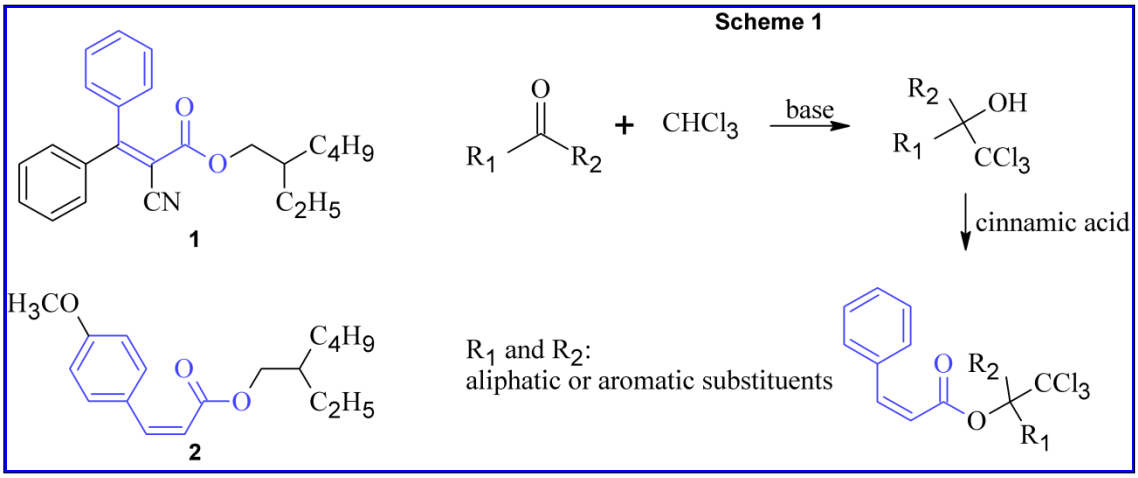
In recent years, sunscreens have caught the attention of many scientists and became quite a hot topic in the science world. It is caused by the fact that climate changes bring more intense UV radiation along with it [1]. Radiation mentioned before does not belong to the visible spectrum, containing waves of length from 100 up to 380 nanometers. Ultraviolet (UV) is significantly harmful for people, causing, among others, skin cancer and being the most common cause of this disease [2]. The importance of organic compounds as UV blockers is immeasurable [3].

Cinnamic acid derivatives are very promising molecules, displaying a variety of properties, for example antimicrobial and cytostatic [4]. Besides that, they are widely used in sunscreens. Second in terms of popularity cinnamic acid' derivative – octocrylene **1** - and, placed not much lower in rankings, ethylhexyl methoxycinnamate **2** block only UV-B spectrum. It is just a part of threatening radiation.

Trichlorocarbinolesters of cinnamic acid as potentially effective and innovative UV blockers are the subject of our research work (**Scheme 1**). An addition of carboanion to carbonyl group of aliphatic and aromatic ketones were performed to obtain mentioned compounds. The second step involved estrification of cinnamic acid by obtained alcohols. Both reactions were thoroughly validated. The UV absorption capacity of the final esters was assessed, showing their profile similar to the blockers already used.



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P36

Dancing with sterols: can antibiotic-sterol interactions explain the selectivity of aromatic analogues of amphotericin B?

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Aromatic heptaene macrolides (AHMs) were among the first ones to be used for systemic fungal infections. One of the most important heptaene macrolides is amphotericin B (AmB), which contains no sidechain and exhibits 'all-trans' geometry of the heptaene chromophore. On the contrary, the naturally occurring AHMs contain two cis-type (Z) double bonds within their heptaene system, as well as an alkyl-aromatic side chain attached to the macrolactone ring. It is possible to obtain stable all-trans isomers of AHMs by photochemical isomerization, with geometry identical to AmB [1]. The all-trans isomers have shown better selective toxicity

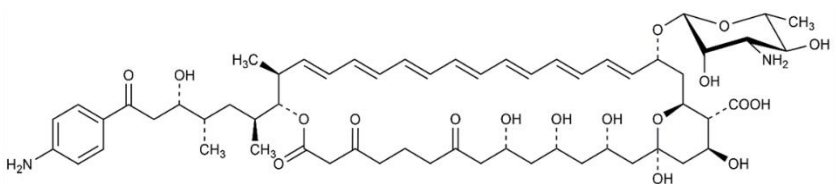

Fig. 2 Structure of iso-Candicidin D most well-known AHM

in vitro than the natural AHMs [2].

The molecular mechanism of action of AHMs and the impact of the isomerization on their biological activity is still not fully understood. Molecular dynamics studies with the use of two-dimensional metadynamics were performed to compare AmB and its aromatic analogues (AHMs) in native and isomeric form. Our goal was to study their preferred orientation while forming a complex with cholesterol or ergosterol molecule, which are the main sterols embedded in mammalian and fungal membranes, respectively. The interactions of the alkyl-aromatic side chain of AHMs with cholesterol- or ergosterol-rich lipid bilayers were also investigated [3].

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The study was supported by the Gdańsk University of Technology under the ARGENTUM—'Excellence Initiative—Research University' program with DEC-47/2021/IDUB/I.3.3 grant.

P37

Exploring the potential of morpholine-modified 1,3,5-triazines as therapeutic agents against colorectal cancer cell lines

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Colorectal cancer (CRC) is the second most common cause of cancer death in the United States. In 2023, approximately 153,020 individuals will be diagnosed with CRC and 52,550 will die for the disease, including 19,550 cases and 3750 deaths in individuals younger than 50 years. [1] Only a few drugs are used in chemotherapy, including 5-fluorouracil (5-FU), folinic acid, oxaliplatin and capecitabine. These cytostatic are often associated with serious side effects however some studies reported that therapy is not beneficial. The greatest hopes now lie in personalized therapy, in which the use of targeted drugs may be safer and more effective throughout the treatment process. [2]

In 2020, a team of scientists led by Wróbel A. et al. presented the results of research on 1,3,5-aminotriazine derivatives, which were tested for activity against the colorectal cancer cell line. In conclusion, the research demonstrated the promising anticancer activity of the novel triazine derivatives, and the most active of the ligands was twice as active as 5-FU. These findings highlight the potential of these compounds as a new class of agents for cancer treatment. [3]

Due to the high mortality rate, low efficacy of cytostatic and the lack of small-molecule targeted drugs, we decided to obtain a new library compounds with high cytotoxic activity against cancer cell lines (SW480 and SW620) and no toxicity against a healthy cell line (CCD841). In the course of the studies, obtained compounds were characterized by high cytotoxicity (IC_{50} below 10 μM for SW480 and SW620) lower than for model cytostatic – 5-fluorouracil. New compounds were obtained according to innovative method of synthesis under solvent-free conditions supported by a microwave irradiation using few drops of water or DMF as a solvent. This approach allowed us to obtain a large library of compounds in a short period up to 2.5 min.

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The project "Programming Excellence - PK XXI 2.0. Development Program of the Cracow University of Technology for 2018-22" co-financed by the European Social Fund Agreement No. POWR.03.05.00-00-z224/17. The project "WAY TO EXCELLENCE - a comprehensive university support program" implemented under the Operational Program Knowledge Education Development 2014-2020 co-financed by the European Social Fund Agreement No. POWR.03.05.00-00-Z214/18

P38

Rapid method for purity determination of AHM antibiotic complexes

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Partricin (also known as aureofacin) is a mixture of antibiotics that belong to the group of aromatic heptaenemacrolides (AHM) and is biosynthesized by*Streptomyces aureofaciens*. It exhibits high antifungal activity but alsois a substrate for the production of meparticin (partricin methyl ester), an active substance used to treat prostatic hyperplasia and eliminate undesirable symptoms of the urinary tract in men. Mepartricin is marketedas Tricandil or Ipertrofan. [1] Another well-known AHM mixture, Candicidin (isolated from *Streptomyces griseus*,) is used against superficial mycosis and against vulvovaginal candidosis.[2]

We have previously shownthat both antibiotic complexesundergo a photochemical isomerization reaction and are partially degradedbysunlight to form stable *all-trans*isomers. These*all-trans*isomers demonstrate different biological propertiesin comparison to their native forms(witha *cis-trans*chromophore structure). [1]Therefore, there is a need to develop a rapid method for determining the purity of AHMs used as drugs, and thus the degree of their photo-conversion to *all-trans* isomers.

In this paper, a chromatographic system and conditions for rapid quantificationof *all-trans*and *cis-trans*isomers of Partricin and Candicin were proposed using the HPLC-DAD-ESI-MS tandem.

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P39

MM-129 a new candidate for an effective and safe drug in the fight against colorectal cancer

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The continuing growth rate of colorectal carcinoma incidence indicates a necessity for intensified research, and forces to seek newer methods for early detection and more ideal treatment for patients suffering from this type of cancer. The purpose of the present study was to examine the anticancer activity and safety profile of 1,2,4-triazine derivative (pyrazolo[4,3-e]tetrazolo[1,5-b][1,2,4]triazine sulfonamide), a novel promising drug candidate against colon cancer. 1,2,4-triazine derivative was assessed for antitumor activity and toxicity through in vitro (DLD-1, HT-29 cells) and in vivo study on Cby.Cg-Foxn1nu/cmdb mice. The mechanistic studies investigated the cellular affinity of new 1,2,4-triazine derivative by measuring levels of intracellular/extracellular signal molecules participating in tumorigenesis. The results indicated that 1,2,4-triazine derivative significantly reduced tumor growth in mice challenged with DLD-1 and HT-29 cells. It exerted the ability to inhibit intracellular molecules promoting tumorigenesis and inducing cell cycle arrest, like Akt, and CDK2. Combined administration of novel 1,2,4-triazine derivative and 5-FU additionally amplified these effects manifest as increase population of cells in G0/G1 phase. No serious adverse events were reported for the use of 1,2,4-triazine derivative, confirming a favorable safety profile for this compound. It was not fatal and toxic to mice at an anticancer effective dose of 10 μmol/kg. These preclinical results suggest that this novel heterofused 1,2,4-triazine derivative, due to high anticancer activity, should be tested in the clinic for treatment of solid tumors, such as colon cancer. It has the potential as a safe and well-tolerated anticancer formulation for future treatment of patients with colon cancer.

P40

Molecular modeling of GPR18 agonists

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The GPR18 receptor, yet still an orphan receptor, belongs to class A, lipid receptors subfamily of GPCR. Involved in the regulation of inflammatory processes, pain physiology, as well as cardiovascular and neurological diseases or glaucoma, constitutes the GPR18 a promising therapeutic target.

The purpose of this study was to investigate the molecular characteristics of possible interactions between selected GPR18 receptor ligands [1,2] and its structure using AlphaFold2 models. The conformational movement of the generated complexes was tracked over time using MD simulations to possibly understand the basis of interactions, through trajectory analysis.

This study was partially financed from the National Science Center grant No. UMO-2021/43/B/NZ7/01938

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P41

Exploration of Novel Peptide Structures with Antitirosinase Activity

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Introduction:

A comprehensive database of peptides was assembled from various publicly available sources and existing peptide databases. This compilation encompassed a wide range of peptide lengths, structures, and sequence.

***Methods*:**

Using a database search method, a subset of peptides with promising antityrosinase activity was identified. Subsequent screening revealed that certain amino acids have the potential to influence the binding pocket of tyrosinase, suggesting their efficacy as effective inhibitors. Based on an extensive literature review, specific amino acids suspected to exert the strongest inhibitory effect on tyrosinase activity were selected. Following this, the selected peptide structures were synthesized on a solid support using Fmoc chemistry.

***Results*:**

The multi-step process of peptide design and synthesis resulted in a set of peptides with different levels of activity against the selected target. Structure-activity relationship (SAR) analysis reveals key structural features that significantly influence the activity of peptides, enabling further refinement of designs.

***Conclusion*:**

Our research shows the effectiveness of a multifaceted approach to identifying the most active peptide structure. The optimized peptides hold great promise for potential therapeutic applications against a selected target, in this case as tyrosinase inhibitors.

Future Directions:

Continuing these findings, our goal is to explore modifications of the identified active peptides to enhance their stability, bioavailability and target specificity. In addition, in vitro studies and toxicity assessments will be undertaken to evaluate their safety profiles and pharmacological potential.

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P42

Thisemicarbazones derived from 4-methyl and 4-methoxypicolinocarbonitriles: synthesis, structure and antimicrobial activity evaluation

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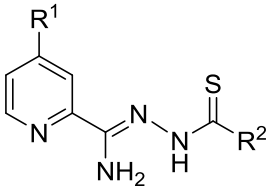
Tuberculosis is caused by intracellular pathogen *M. tuberculosis* [1]. Approximately one third of the world's population is infected, and the number of new cases according to the WHO is 10 million per year, making it one of the ten leading causes of death in the world [2]. Due to drug resistance, new, more effective anti-tuberculosis drugs are sought, which will allow for more effective and faster fight against the bacteria causing this disease. Here we report the synthesis of new thiosemicarnazone derivatives of 4-methyl- and 4-methoxypicolinocarbonitriles (Figure) as the latest result of our studies on the searching of new compound with tuberculostatic activity.



R^1: CH_3, OCH_3
R^2: pyrrolidine, piperidine, morpholine, N,N-dimethylamine

Figure

The first step of the synthesis consisted in the synthesis of methyl dithiocarbazinate, which was later reacted with the corresponding secondary amines: pyrrolidine, piperidine, morpholine or dimethylamine, resulting in cycloalkylaminocarbothiohydrazide compounds. The final thiosemicarbazones were synthesized by reacting these compounds with the corresponding iminoesters. The starting compounds for the synthesis of iminoesters were commercially available 4-chloropicolinonitrile and 4-methylpicolinonitrile. They were simply converted into the designed iminoesters by the action of sodium methanolate or DBU. The structure of the obtained compounds was confirmed by elemental analysis and IR and NMR spectroscopic methods. X-ray crystallography was also performed. The final hydrazones were tested against *M. tuberculosis* strains as well as other bacterial and fungal strains. Here we report the results of these studies, among others SAR analysis.

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P43

Inhibition of MAOB as additional beneficial feature of GPR18 antagonists activity

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Endocannabinoid system (ECS) is composed of endocannabinoid signalling molecules (2-AG) and (AEA) and their G-protein coupled receptors (GPCR) CB1 and CB2. Recently novel CB receptors has been recognized including the orphan GPR18 that is regulated by cannabinoid like molecules and interact with CB system. GPR18 expression has been confirmed in the spleen, thymus, lymphocytes, macrophages, hypothalamus, cerebellum and brain stem. It can regulate a number of neurophysiological processes and that involved in neuroinflammation. As such it became an interesting new target for therapeutic intervention. In our research group as a result of cooperation with prof. Christa Müller and her research group from the University of Bonn, bicyclic imidazole-4-one derivatives were discovered as first synthetic antagonists for GPR18 [1,2]. Their affinity to GPR18 was stated in β-arrestin recruitment assay (vs THC). In the preliminary studies it was stated that such compounds may exert anti-inflammatory (inhibiting MPO) and antioxidant properties [3]. Our latest studies have confirmed that GPR18 antagonists may show human MAOB (hMAO B) inhibitory properties - feature which could be helpful in treating neuroinflammatory states. For hMAO B inhibitory studies two series of compounds were chosen with a general structure shown in **Figure 1**.

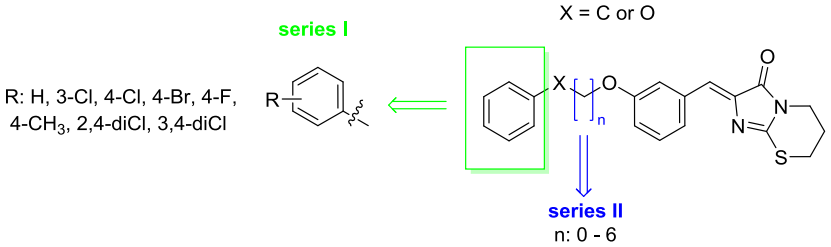

Figure 1. General structure of tested compounds.

Series I - compounds with different substituent/s at the benzyloxy ring and **series II** with a variable carbon linker between the phenyl and benzylidene rings. These compounds were previously obtained and described as ligands of GPR18 receptor with (sub)micromolar affinities. The ability to inhibit hMAO B was determinated by spectrofluorometric method. Most compounds inhibited the activity of this enzyme with IC_{50} values below 400 nM. SAR analysis of pharmacological activity show that among tested compounds are ligands that have both antagonist affinity for GPR18 and hMAO B inhibitory activity in the nanomolar range.

Acknowledgement*:* This research was funded by the National Science Centre, Poland grant based on decision No DEC-2021/43/B/NZ7/0193.

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P44

Are the dopamine receptor ligands useful tool for search an innovative glioblastoma therapy?

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Glioblastoma multiforme (GBM), the most aggressive cancer of central nervous system, maintains the lethal disease with median patient survival 15 months. Despite the huge number of preclinical and clinical studies, the situation has not been changed for 20 years. Unfortunately, GBM cells become resistant for gold standard chemotherapy with temozolomide (TMZ). Such poor perspective makes searching for novel anti-glioblastoma agents extremely urgent.

Interestingly, the well-known D_2R antagonists used in schizophrenia patients turned out to have cytotoxic activity in GBM cells. However, this chemical agents has also high affinity do D_4R. Importantly, the more recent studies with selective D_4R antagonists showed its influence on inhibition of GBM tumour growth in xenograft model in mice, thus suggesting the greater role of D_4R than D_2R in process of GBM growth. However, the research in this field are limited and needs more investigation.

The aim of this study was to verify if antagonism towards dopamine receptors is crucial for an anti-glioblastoma effect in GBM cells or is rather a consequence of interaction with another protein target with similar structural requirements for ligands. Hence, the series of 5-spirofluorenehydantoin derivatives with diverse affinity to dopamine receptors were tested for antitumor activity in two human GBM cell lines (U87MG and A172) and non-tumour cell line as control (human fibroblasts) using cell viability (MTT reduction) and cytotoxicity (LDH release) assays. The three selected compounds, which were further tested in order to evaluate its mechanism of anticancer mechanism, showed much greater cytotoxic activity than TMZ and safety for non-tumour cells. However, the obtained results indicate no correlation between dopamine receptors affinity and antitumor activity, suggesting the contribution of different signalling pathway.

The study was supported by National Science Centre in Poland under the research project no. UMO-2021/43/D/NZ7/00891.

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P45

New xanthone derivatives inhibiting sirtuin 2 (SIRT2) activity

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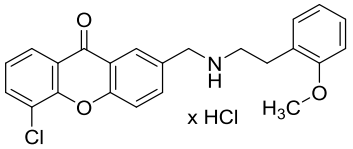
Sirtuins are class III protein deacetylases present in the nucleus (mainly SIRT1, SIRT6 and SIRT7), the cytoplasm (SIRT2) and mitochondria (SIRT3, SIRT4 and SIRT5). By catalyzing enzymatic reactions, sirtuins affect numerous biological processes, which makes them valuable molecular targets for active compounds [1]. Intensive research is carried out, among others, in the search for SIRT1 and SIRT2 modulators with potential utility in the treatment of neurodegenerative diseases and cancer [2]. Among the known modulators of sirtuins' activity there are natural substances, including xanthone derivatives such as gartanin (SIRT1 EC_{50} = 1.79 μM) [3] and γ-mangostin (SIRT1 IC_{50} = 22.4 μM, SIRT2 IC_{50} = 3.8 μM, SIRT3 IC_{50} = 26.8 μM) [4].

Within the presented study, six new amino derivatives of 5-chloroxanthone were designed. The amine moieties included phenylmethanamine, 2-phenylethan-1-amine, *N*-phenylpiperazine or *N*-benzylpiperazine, substituted with one or two methoxy groups in the aromatic ring. The compounds were obtained by means of chemical synthesis (first the heteroaromatic system was obtained by the Ullmann synthesis, followed by bromination of methyl substituent and subsequent aminolysis of the obtained alkyl bromide). All achieved compounds were screened for potential activatory (SIRT1) and inhibitory (SIRT2) properties at a concentration of 50 μM (commissioned study, Eurofins Scientific, CEREP Laboratories, France). Three secondary amines inhibited SIRT2 activity by 82-93% (the highest result obtained for compound **I**, Fig. 1), while the three tertiary amines (piperazine derivatives) showed SIRT2 inhibition of 48-56%. None of the tested compounds significantly increased SIRT1 activity.

The obtained results indicate the possibility of searching for selective SIRT2 inhibitors among the amino derivatives of 5-chloroxanthone, in particular compounds containing secondary amino group.



Compound **I**

SIRT2 inhibition at 50 μM = 93.24%

Fig. 1. Chemical structure and SIRT2 inhibitory activity of Compound **I**.

The research was financially supported by Jagiellonian University Medical College (statutory funds, grant no. N42/DBS/000278).

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P46

Dual piperidine-based histamine H_3 and sigma-1 receptor ligands in the treatment of nociceptive and neuropathic pain

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The treatment of complex, multifactorial diseases by single target-oriented therapies rarely results in good efficacy. For this reason, the approach based on simultaneous modulation of multiple targets' activity captured the interest of the pharmaceutical industry and academia. Importantly, recent studies have shown that some clinically evaluated histamine H_3 receptor antagonists (H_3R) possess additional affinity at sigma-1 receptors (σ_1R), which may play an important role in their pharmacology [1]. Considering the clear relation between H_3R and σ_1R, great effort should be made to develop such ligands for the treatment of various pain conditions. In our study, we decided to combine chemical, biological and computational methods to reveal molecular properties responsible for histamine H_3R and σ_1R selective or dual-target binding of the studied compounds [2]. Next, we designed a series of 16 new ligands and performed their pharmacological characterization using *in vitro* methods. Finally, lead compounds were tested in animal models of nociceptive and neuropathic pain. In a series of novel compounds, we selected three lead structures for further biological evaluation with high affinity at both H_3R and σ_1R. Compound **12** showed a better safety profile than the other two tested compounds, hence we have selected this ligand for further analysis of its analgesic activity. The high potency of **12** in both, formalin- and capsaicin-induced pain indicated that this compound has the potential to attenuate neurogenic pain, regardless of the mechanism of its induction. Finally, we used two different models of neuropathic pain to test the influence of **12** on pain associated with neuronal tissue damage. The obtained results strongly indicate that compound **12** can alleviate both chemotherapy-induced and sciatic nerve damage-driven neuropathic pain. This confirms its broad spectrum of analgesic activity based on the novel molecular mechanism [2].

We are pleased to acknowledge the generous support of the National Science Center, Poland (2020/36/C/NZ7/00284, 2019/35/D/NZ7/01042, 2020/04/X/NZ7/01338).

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P47

Neural circuits regulating pain behaviors in the anterior cingulate cortex

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Pain is an unpleasant sensory experience triggered by noxious stimuli, which provokes essential protective behaviors against life threatening events. Alternations in neural activity of distinct regions of the anterior cingulate cortex (ACC) are associated with psychological and motor functions and play a role in the regulation of pain-related behaviors. Despite ongoing research, our understanding of the mechanism by which the ACC regions assign emotional states to nociceptive information, resulting in the generation of pain aversion at neural circuit and cellular levels, remains poorly understood. Here we investigate the functionality of the neural circuits potentially regulating pain behaviors by focusing on neurons activated by a noxious stimulus in the ACC using c-Fos-2A-iCre (TRAP2) mice combined with neuroanatomy and chemogenetics under a chronic pain condition. By selective inhibition of these activated ACC neurons using hM4Di receptors (an inhibitory DREADD, Designer Receptors Exclusively Activated by Designer Drugs) the experience of affective pain was reduced as measured by the development of conditioned place preference. Interestingly, the ACC neurons that are activated in a chronic pain-dependent manner are responsible for modulating sensory pain. In neuroanatomical analysis using TRAP2/Ai9 (Cre-dependent tdTomato reporter) mice combined with a retrograde tracer, we found a difference in activated ACC circuits in chronic pain versus non-injured animals. Immunohistochemical (IHC) staining of the ACC identified specific neuronal populations activated during a chronic pain condition. These findings provide valuable insights into the ACC circuitries that potentially regulate distinct pain behaviors under chronic pain conditions.

P48

Anti-inflammatory and anti-remodeling effects of two pan-PDE inhibitors in ovalbumin-induced asthma model

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Asthma is a heterogeneous, chronic respiratory disease which pathophysiology involves airway inflammation, hyperresponsiveness, and remodeling. The latest approach to the search for new asthma drugs assumes that inflammation and remodeling should be considered as two very important and parallel processes. In recent years, researchers have been particularly interested in the search for compounds with such dual, anti-inflammatory and anti-fibrotic activity in the group of phosphodiesterase inhibitors.

We designed and synthesized a large group of 7,8-disubstituted derivatives of 1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione. These compounds were characterized as potent pan-PDE inhibitors. We conducted extensive cell-based studies that allowed us to confirm that selected compounds from this group (38 and 145) also possess prominent anti-inflammatory and anti-fibrotic activity *in vitro* [1-4].

Here we provide evidence supporting such dual activity of these compounds in murine model of ovalbumin (OVA)-challenged allergic asthma. 38 and 145 reduced typical features of the OVA-induced airway inflammation including cell infiltration, eosinophil recruitment, Th2 cytokines, as well as, total and OVA-specific IgE. On the other hand, 38 and 145 decreased characteristic for airway remodeling: goblet cell metaplasia, mucus hypersecretion, collagen overproduction and deposition, as well as pro-fibrotic genes expression in airways of allergen challenged mice [5]. Our data suggest that the investigated pan-PDE inhibitors may represent promising, anti-asthmatic drug candidates.

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P49

Multitarget 5-HT$_6$R/FAAH/ChE ligands: the future of Alzheimer's Disease treatment?

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Continued failures in the process of developing a new drug for such complex pathophysiological process as Alzheimer's disease (AD) have increased the popularity of polypharmacological approaches, based on the search for multitarget compounds. Among potential AD therapeutic proteins, in addition to acetyl- and butyrylcholinesterases (AChE and BChE), serotonin 5-HT$_6$ receptors (5-HT$_6$R) have gained tremendous interest in the last decade. past few years. Very recent studies also highlight the importance of regulating the endocannabinoid system (eCB), specifically inhibition of the fatty acid amide hydrolase (FAAH), as extremely relevant in the fight against AD [1]. Therefore, with the support of molecular modelling, a series of twelve 1,3,5-triazine-based derivatives were designed and synthesized, as a potential multifunctional 5-HT$_6$R/FAAH/ChE ligands. New compounds were obtained in four-step synthesis, involving cyclization, *N*-demethylation and *N*-alkylation. Then, biological studies to determine an affinity for the 5-HT$_6$R in radioligand binding assays and an inhibition potency for AChE, BChE and FAAH in enzymatic assays were carried out.
The whole series of triazine compounds displayed significant action on 5-HT$_6$R (pK_i: 6.08-7.59) and FAAH (p*IC50*: 4.78-5.75). Furthermore, compound **KC-11** demonstrated the most promising multitarget action (5-HT$_6$R: pK_i = 7.59; FAAH: p*IC50* = 5.03, AChE: p*IC50* = 5.79, BChE: p*IC50*= 5.00). Finally, SAR analysis results indicated directions of further modifications.

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P50

Antidepressant-like and procognitive properties of metabolically stable PZ-2172, a potent and selective 5-HT_7 inverse agonist at G_s signaling pathway.

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The 5-HT_7 receptor (5-HT_7R) is the latest member of a subfamily of serotonin receptors. 5-HT_7R is coupled to two heterotrimeric G-proteins, G_s and G_{12}. Receptor-mediated G_s activation leads to stimulation of adenylate cyclase, which in turn, results in increasing cAMP levels [1,2]. 5-HT_7R has been considered as a therapeutic target for the treatment of depression and comorbid cognitive dysfunctions [3,4]. Continuing our effort on the development of 5-HT_7R antagonists, a series of novel derivatives has been designed, synthesized and evaluated using *in vitro* and *in vivo* models identifying compound **PZ-2172** as a lead structure.

The affinity of compound **PZ-2172** for 5-HT_7R and selectivity over related serotonin and dopaminergic receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_6 and D_2R) were determined by radioligand binding experiments in HEK-293 cells. The effect on G_s-operated 5-HT_7R constitutive activity was evaluated using their ability to inhibit cAMP production induced by agonist 5-CT (10 nM) in a HEK-293 cells. The metabolic stability of compound **PZ-2172** was assessed in rat liver microsomes (RLM) assay while its preliminary safety profile was evaluated in Hep-G2 cells using the MTT test. Evaluation of the pharmacokinetic profile after i.g. administration of a dose of 3 mg/kg, was performed in Wistar rats. Antidepressant-like and procognitive properties were assessed *in vivo* using the Tail Suspension Test (TST) and the Novel Object Recognition (NOR) Test, respectively.

Compound **PZ-2172** behaves as potent 5-HT_7R inverse agonist at G_s signaling pathway (K_i 5-HT_7 = 3 nM, IC_{50} = 3.2 nM), displaying high selectivity over tested GPCRs (selectivity index > 130). Compound **PZ-2172** is characterized by good *in vitro* metabolic stability, high *in vivo* oral bioavailability, well brain-penetration and low hepatotoxic effect. **PZ-2172** exerted antidepressant-like activity and pro-cognitive properties at the dose of 0.625 and 1 mg/kg (i.p), respectively. Of note, the potency was similar to that of the reference SB-269970.

The promising pharmacological properties, along with favorable drug-likeness profile, justify further development of compound **PZ-2172** as a molecular probe to confirm the role of 5-HT_7 receptor in affective disorders.

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P51

Anticancer activity of a new 4-thiazolidinone derivative with pertuzumab or trastuzumab in AGS cancer cells

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Novel 4-thiazolidinone-bearing hybrid molecules possess well-documented anticancer activity, and together with anti-HER2 antibodies, may represent a promising strategy in treating patients with gastric cancer with confirmed human epidermal growth factor receptor 2 (HER2) expression.

The aim of the study was to examine anticancer activity a new 4-thiazolidinone derivative (Les-4367) in combination with trastuzumab or pertuzumab in human AGS gastric cancer cells. AGS cell viability was tested by MTT assay. The effect of the tested combinations as well as monotherapy on metalloproteinase-2 (MMP-2) and intercellular adhesion molecule 1 (ICAM-1) concentrations were also demonstrated by the ELISA technique.

We proved that pertuzumab and trastuzumab were very effective in increasing the sensitivity of AGS gastric cancer cells to novel Les-4367. MMP-2 is considered to be a prognostic marker in patients with gastric cancer. To our knowledge, MMP-2 expression was more frequent in aggressive gastric cancers and related to high cyclooxygenase-2 (COX-2) expression. We proved that Les-4367 combined with pertuzumab was the most effective in reducing MMP-2 concentrations. Intercellular adhesion molecule-1 (ICAM-1) is a cell adhesion molecule and plays the main role in numerous immune responses. Researchers have reported that the increased expression of intercellular adhesion molecule-1 (ICAM-1) in gastric cancer could be related to the aggressive nature of the tumor and has a poor prognostic effect on gastric cancer. In our study, a significant reduction in ICAM-1 concentration in AGS gastric cancer cells was observed after 24 h of incubation with trastuzumab and novel Les-4367. Based on the obtained results, it can be concluded that the combination of a novel 4-thiazolidinone derivative (Les-4367) with anti-HER2 antibodies (trastuzumab and pertuzumab) possesses high anticancer activity against AGS gastric cancer cells and decreases the concentrations of molecules, which are engaged in gastric cancer progression [1].

[1] Gornowicz. et. al.. *Int. J. Mol. Sci.* 24 (2023) 6791.

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P52

(*R*)-AS-1 - the first-in-class antiseizure drug candidate reveals broad-spectrum antiseizure and neuroprotective activity

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Compound **(*R*)-AS-1** has been identified as a first-in-class, selective positive allosteric modulator of the glutamate transporter subtype EAAT2, with good prospects for preclinical and clinical development in epilepsy.[1] Analysis of the expression of EAAT2 by immunoblotting, qRT-PCR and imaging by confocal microscopy data confirmed that **(*R*)-AS-1** does not influence EAAT2 expression, suggesting that chronic administration of the compound does not induce epigenetic modulation of this transporter. The *in vivo* data disclosed herein demonstrates that **(*R*)-AS-1** has a much broader spectrum of antiseizure activity compared to the vast majority of antiseizure drugs currently on the market. **(*R*)-AS-1** was shown to be effective in both acute and chronic seizure models in rodents such as maximal electroshock (MES), 6 Hz (44 mA), corneal kindling, mesial temporal lobe epilepsy, lamotrigine-resistant amygdala kindling, as well as seizures induced by pilocarpine and Theiler's murine encephalomyelitis virus. Notably, **(*R*)-AS-1** also has a favourable safety profile in the rotarod test and the Irwin test. Furthermore, **(*R*)-AS-1** demonstrates neuroprotection under trophic stress, oxygen-glucose deprivation conditions, as well as neurodegeneration induced by 6-hydroxydopamine and pilocarpine suggesting that this compound may be beneficial for the treatment of other neurological disorders associated with glutamate excitotoxicity.

The studies were supported by the National Science Centre, Poland grant No 2022/45/B/NZ7/00598. The authors are appreciative to Epilepsy Therapy Screening Program (ETSP) of the National Institute of Neurological Disorders and Stroke (NINDS, National Institutes of Health, Bethesda, USA), for testing and providing selected antiseizure data for **(*R*)-AS-1**.

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P53

Synthesis and evaluation of anticancer activity of selected ciprofloxacin derivatives

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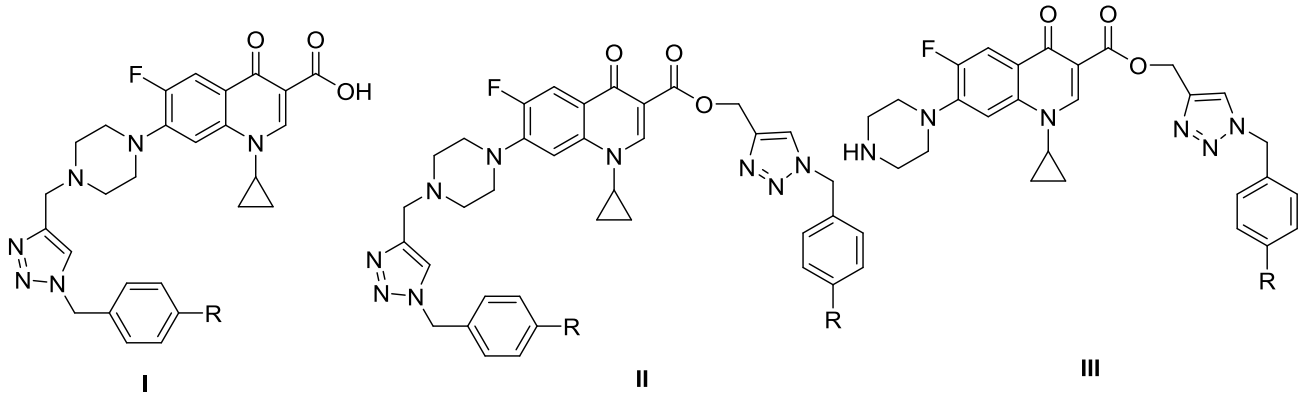
Fluoroquinolones are a group of synthetic broad-spectrum antibiotics against many clinically important pathogens. They show their strong bactericidal properties by inhibiting DNA synthesis by acting on two key bacterial enzymes (topoisomerases): DNA gyrase (topisomerase II) and topoisomerase IV. [1] Ciprofloxacin, a second-generation fluoroquinolone, has a broad spectrum of activity against both gram-positive and gram-negative bacteria and atypical microorganisms. It is used, e.g. in the treatment of urinary, respiratory, and digestive tract infections. Fluoroquinolones have also been shown to be active against eukaryotic topoisomerases and, therefore, may be toxic to tumor cells. Therefore, in recent years there has been an increasing interest in the use of ciprofloxacin derivatives in medicine due to their potential anticancer effect.[2]



I **II** **III**

The aim of the work was to design and synthesis of new ciprofloxacin derivatives containing the 1,4-disubstituted triazole system and showing anticancer activity. New derivatives of general formulas I, II, and III were synthesised using copper(I) ion-catalysed azide-alkyne cycloaddition (CuAAC) protocol. The resulting hybrids of triazole and ciprofloxacin were subjected to preliminary evaluation of their antiproliferative activity. These studies were carried out in melanoma cells (COLO829, G361) and indicate that the new compounds have a high cytotoxic activity against the cell lines.

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P54

New dihydropyrimidine derivatives as antimicrobial agents

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The pyrimidine ring is a distinctive heterocyclic structure with both its presence in the structure of many drug molecules and the different biological effect spectrum reported in many studies. The presence of DNA and RNA bases has made pyrimidine derivatives stand out with their antibacterial, antifungal, antiviral and anticancer chemotherapeutic effect profiles. In the aforementioned therapeutic interventions, one of the targeted approaches to prevent hosts such as bacteria, fungi, viruses and cancer cells from invasively living on a suitable host is the inhibition of DNA/RNA synthesis (1,2). In this study, the synthesis of new compounds containing thiazole or benzothiazole rings carrying the main core of 1,6-dihydropyrimidine were synthesized. The structures of *N*-(thiazol/benzothiazol-2-yl)-2-((5-cyano-6-oxo-4-(pyridin-4-yl)-1,6-dihydropyrimidin-2-yl)thioacetamide derivative compounds (**5a-5o**) were elucidated by ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopies. The antibacterial effects of the compounds on various gram positive and gram negative bacteria; the antifungal effects on some *Candida* species were tested. Among them, 6-nitrobenzothiazole and 6-chlorobenzothiazole containing derivatves exhibited high anticandidal potency whereas non-substituted benzothiazole, 4-nitrophenylthiazole and 4-phenyl-5-methylthiazole including derivativeshigh antibacterial activity. The inhibitory effects of the active derivatives on the DNA-gyrase enzyme will be determined and docking studies will be carried out for determining the binding mode and clarifying structure-activity relationships.

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P55

Synthesis, *in vitro* and *in silico* evaluation of *N*-acylhydrazone derivatives of pyrrolo[3,4-*d*]pyridazinone with dual COX/LOX inhibitory activity

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Secure and effective treatment of pain and various inflammatory disorders is constantly challenging. Although NSAIDs and other painkillers are commonly available, they are sometimes insufficient and can cause dangerous side effects. As reported in our former studies, pyrrolo[3,4-*d*]pyridazinone derivatives act as potent and safe cyclooxygenase inhibitors with a COX-2/COX-1 inhibitory ratio better than meloxicam. Moreover, these compounds revealed satisfactory anti-inflammatory activity in the *in vivo* experiments as well [1, 2]. The studied compounds did not cause injuries in the gastrointestinal tract and decreased the level of such mediators as MPO, PGE_2 and TNF-α. The *N*-acylhydrazone (NAH) moiety is one of the most extensively studied privileged structures in medicinal chemistry nowadays. It is present in a great number of drug candidates with various pharmacological activities. It is worth noticing, that *N*-acylhydrazone derivatives can affect different molecular targets and signalling pathways associated with inflammation [3, 4]. Therefore, we decided to introduce this pharmacophore into new series of pyrrolo[3,4-*d*]pyridazinone derivatives. Herein, we present the synthesis, *in vitro* and *in silico* investigations evaluating the biological and physicochemical properties of title NAH-derivatives. Novel compounds were received with high purity, good yields and did not show cytotoxicity in the MTT assay. Their COX-1, COX-2 and 15-LOX inhibitory activity was estimated using enzymatic tests and molecular docking studies. Title *N*-acylhydrazones appeared to be effective dual COX/LOX inhibitors with promising pharmacokinetic and drug-likeness properties.

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[4] Da Silva, T. F. et al. *Molecules,* 20, 2015, 3067–3088

P56

Studies of human and bovine serum albumin interaction with statin analogs using fluorescence spectroscopy

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Statins belong to the group of lipid-lowering drugs. These compounds are used to treat hypercholesterolemia, ischemic heart disease, or atherosclerotic cardiovascular disease. They are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This enzyme is found in the liver and regulates cholesterol biosynthesis. Statins lower total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglycerides (TG) levels while increasing high-density lipoprotein (HDL) cholesterol levels. An important function of statins is also the inhibition of lipid oxidation and the improvement of coagulation parameters [1]. Moreover, we showed that statins are compounds that effectively inhibit human adenylate kinase isoenzyme 1 (hAK1) [2]. Therefore, in order to find new hAK1 inhibitors we designed and synthesized five statin analogs (Fig. 1). To assess their ability to be distributed in animals and humans, we tested their ability to interact with bovine (BSA) and human (HSA) serum albumin.

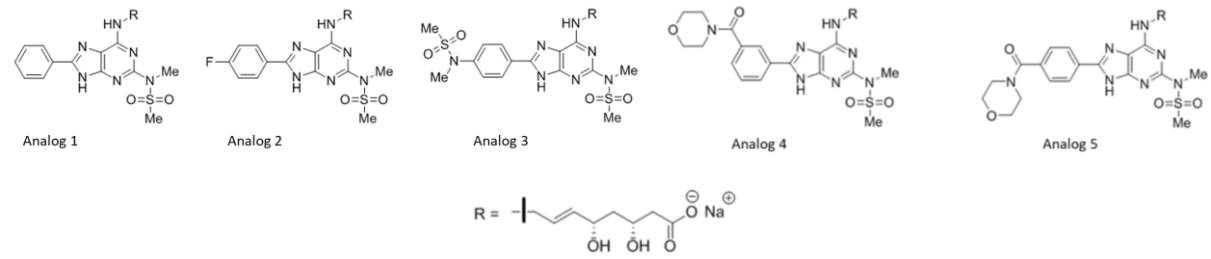

Fig.1. Structural formulas of the studied statin analogues.

Our studies have shown that all tested statin analogs strongly bind to HSA and BSA, as evidenced by a K_b values of 10^4 M^{-1}. The same strong interaction was found in study on the interaction of statins with BSA [3]. The following order of interaction of statin analogs with HSA was observed: analog 3 < analog 2 < analog 5 < analog 1 < analog 4, while with BSA: analog 4 < analog 1 < analog 3 < analog 2 < analog 5. The highest binding constant with HSA was found for analog 4 (Kb = 24.88 ± 5.15) and with BSA for analog 5 (Kb = 15.20 ± 3.77).

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[3] Shi J-H. et. al. J. *Biomol. Struct. Dyn.* 35(7) (2017) 1529-1546.

Acknowledgments: The project was supported by Polish National Science Centre 2017/25/B/ST4/00376.

P57

In vitro study of antioxidant, antigylycation, sugar hydrolysis enzyme inhibitory effect and molecular docking study of angularly condensed diquinothiazines

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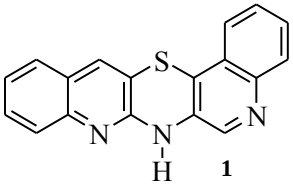
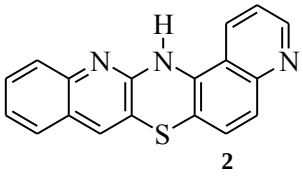
Interest in research on phenothiazine derivatives are still relevant due to their wide range of applications in medicinal chemistry. The modification of the phenothiazine structures with the quinoline ring led to the formation of tetra- and pentacyclic linearly fused quinobenzothiazines and diquinothiazines [1]. Our previous work revealed that pentacyclic azaphenothiazines being naphtoquinothiazines and diquinothiazines (linearly or angularly condensed) have a variable degree of antioxidant activity depending on substitution at the thiazine nitrogen atom and on the type of ring fusion in the azaphenothiazine system. The non-substitution of the thiazine nitrogen atom, and the angular type of ring system fusion promote activity [2].

In this study, angularly condensed diquinothiazines (**1**, **2**, and **3**) were evaluated for antioxidant, antiglycation, sugar hydrolysis enzyme inhibitory effect and molecular *in silico* docking.



1



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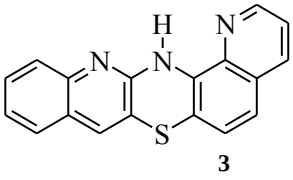

3

Antioxidants were evaluated using DPPH, chelating ion, FRAP and lipid peroxidation. Alpha glucosidase and alpha amylase inhibition was used for sugar hydrolysis enzyme inhibitory activity and antiglycation with BSA-Glucose and BSA-MGO. Insilco docking was done with AutoDock Vina and ADMET properties with pkCSM software. The tested diquinothiazines **1-3** showed free radical scavenging, antiglycation, and inhibition of alpha-glucosidase and alpha-amylase. Insilco docking and pkCSM ADME shows interaction with aldose reductase, glyoxalase 1, receptor AGE, alpha-glucosidase, and alpha-amylase. Antiglycation, alpha-glucosidase, and alpha-amylase inhibition activity of angularly condensed diquinothiazines maybe through deactivation of aldose reductase enzyme activity, activation of glyoxalase 1 pathway, reduction of dicarbonyl stress and oxidative stress.

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P58

Design, synthesis and cytotoxic evaluation of new 1,2,4-triazole-Schiff bases

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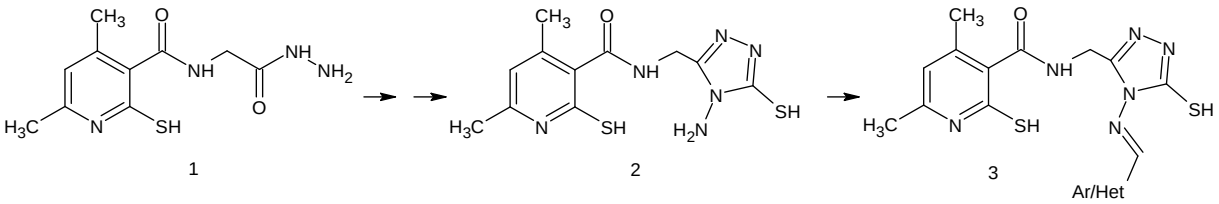
In recent years, 1,2,4-triazole-based compounds have gained broad attention due to their diverse biological activities. It is worth mentioning that several research groups reported the anticancer activity of 4-(arylidene-amino)-4*H*-1,2,4-triazole-3-thione/thiol derivatives [1,2]. In our recently published works, we demonstrated that 4,6-dimethyl-2-sulfanylpyridine derivatives containing differently substituted 1,3,4-oxadiazole moiety exhibited potent cytotoxic activity against various cancer cell lines [3,4]. As a continuation of our research interests in the scope of synthesis and biological evaluation of novel, potential anticancer molecules, we designed and synthesized a series of new hybrid structures of dimethylpyridine core with Schiff bases derived from the 4-amino-4*H*-1,2,4-triazole-3-thiole moiety.



The key intermediate in the present study, 4,6-dimethyl-*N*-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-2-sulfanylpyridine-3-carboxamide (**2**) was obtained in two steps employing salt formation from hydrazide derivative **1**, and cyclization. The synthesis of the title Schiff bases **3** was carried out by the reaction of 4-amino-4*H*-1,2,4-triazole derivative **2** with various aromatic and heteroaromatic aldehydes. HRMS, NMR, and FTIR spectra confirmed the structures of compounds.

All Schiff bases were evaluated for their *in vitro* cytotoxic potency on several human gastrointestinal cancer cells (EPG, Caco-2, LoVo, LoVo/Dx, HT29) and normal colonic epithelial cells (CCD 841 CoN). Some of the compounds exhibited good to significant inhibition of cancer cell viability and possessed a promising safety profile compared to 5-fluorouracil and cisplatin. Further mechanistic studies showed that selected compounds may induce apoptotic cell death through a caspase-dependent mechanism and by regulating the p53-MDM2 signaling pathway.

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P59

Structure and activity of amidrazone derivatives

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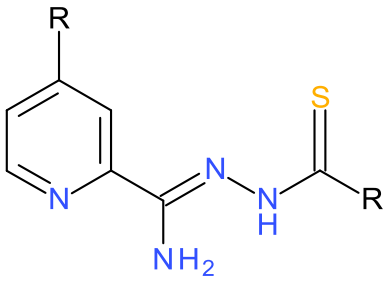
Amidrazone derivatives show a wide spectrum of biological activity, e.g. antibacterial, antifungal, antimalarial, antiviral, anti-inflammatory, analgesic, anticonvulsant and anti-tuberculous activity [1]. In order to determine the relationship between the structure and activity of the tested compounds tests and studies of the compounds were performed. The structure of the tested compounds was determined by diffraction studies, liquid and solid NMR, and ab-initio calculations (Fig. 1). Activity predictions showed antibacterial and anticancer effects.



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P60

Potential role for amino acids analogues of ursolic acid against breast cancer cell lines

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Breast cancer (BC) is an important global health problem [1] and one of the most common types of cancer among women. For this reason, the development of new drugs is imperative, and research is ongoing to understand the difference between healthy cells and cancer ones. One of the most effective methods for obtaining new drugs is the chemical modification of natural compounds with proven high biological activity. An example of such compound is ursolic acid (UA, 3β-hydroxy-urs-12-en-28-oic acid). We designed, synthesized, and characterized a new family of UA derivatives substituted with various amino acids and dipeptides at the C-3 position. The cytotoxicity of all compounds was determined against breast cancer cell lines (MCF-7 and MDA) and DNA synthesis by the inhibition of [^3H]–thymidine incorporation in tested cells. Next, we determined the effect of three most active analogues on the concentrations of: MMP-2, MMP-9, Bax, LC3A, LC3B, Beclin-1, caspase-7, IL-6 and TNF-α. Three most active derivatives showed micromolar IC_{50} values and reduced the concentrations of MMP-2 and MMP-9. The apoptosis pathway associated with an increase in Bax protein and caspase-7 enzyme levels was postulated as a possible mechanism of action for two of these compounds. Third compound increased the release of autophagic markers (LC3A, LC3B) inducing the autophagy pathway. The same compound revealed inhibition of two proinflammatory cytokines (TNF-α and IL-6). The results of our experimental and computational studies, including: *in vitro* anticancer activity, molecular docking, and drug-like properties have shown that the new group of ursolic acid derivatives presented here, has an promising profile and therapeutic potential against triple-negative breast cancer cells, i.e., cancer with poor survival prognosis.

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P61

Development of 3-methoxypropanamide derivatives as effective agents for the treatment epilepsy and neuropathic pain

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Epilepsy is a multifaceted chronic neurological disease, characterized by an enduring (i.e., persisting) predisposition to generate unprovoked seizures and affects people of all ages, races, social classes, and geographical locations.[1] It is characterized by a multifactorial pathogenesis, which often substantially limits clinical efficacy of currently available antiseizure drugs (ASDs). Notably, despite huge advances in epilepsy studies almost 30% of patients with epilepsy have drug-resistant epilepsy (DRE), as they are refractory to medical treatment.[2] It is well known that, DRE is a serious clinical condition that puts the patient at risk of sudden, unexpected death in epilepsy, as well as psychiatric, psychosocial and medical complications, having a profound influence on the overall quality of life.

Therefore, currently one of the promising ways in effective management of multifactorial diseases seems to be the therapy with use of multimodal (multi-target/multi-functional) molecules, which are defined as compounds acting by several and complementary molecular mechanisms simultaneously. Such multi-target molecules are designed most often as hybrid or chimeric congeners, which integrating multiple pharmacophores into a single molecule in order to provide broader and synergic mechanism of action.[3]

Bearing in mind the assumptions of multi-target strategy in the current studies the series of new 3-methoxypropanamide derivatives have been obtained. These compounds were designed combining chemical fragments of potent antiseizures, namely compound **KJ-5** and **KA-93** described in our previous studies[4,5] and lacosamide (a well-known ASD). The obtained molecules showed potent and broad-spectrum activity in the most important seizure models, namely the maximal electroshock (MES) test, the 6 Hz (32 mA) seizure model and notably the 6 Hz (44 mA) model of DRE. The most potent compound **(*R*)-KJ-98** displayed the following pharmacological values: ED_{50}=35.6 mg/kg (MES), ED_{50}=8.4 mg/kg (6 Hz, 32 mA) and ED_{50}=23.9 mg/kg (6 Hz, 44 mA). Additionally **(*R*)-KJ-98** was effective in the *iv*PTZ seizure threshold test and showed favorable pharmacokinetic profile. Moreover, **(*R*)-KJ-98** revealed potent efficacy in the formalin-induced tonic pain, capsaicin-induced pain, oxaliplatin- and streptozotocin-induced peripheral neuropathy. Therefore, the mentioned lead compound seems to be interesting candidate for future preclinical development in epilepsy and pain indications.

The studies were supported by National Science Centre, Poland grant UMO-2021/41/N/NZ7/01181.

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P62

New Approach to Synthesis and 2D NMR Characterization of SSR181507 – the 5-HT1A Receptor Agonist and Dopamine D2 Receptor Partial Agonist with Antipsychotic Activity

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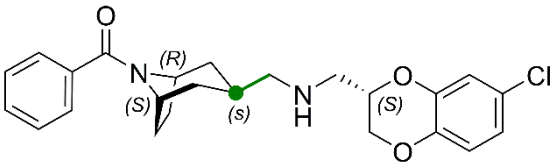
The benzodioxane derivative, SSR181507, possesses high and balanced affinities for D_2-like and 5-HT_{1A} receptors (K_I=0.8, 0.2, and 0.2 nM for human D_2, D_3, and 5-HT_{1A}, respectively) with no appreciable affinity for 5-HT_{2A}, 5-HT_{2C}, adrenergic α_1 and α_2, histaminergic H_1 and muscarinic M_1 receptors. It acts as a full agonist of the 5-HT_{1A} receptor and a partial agonist of the D_2 receptor. This unique pharmacological profile makes it an interesting candidate for an atypical antipsychotic.[1,2]

SSR181507 is quite a complex structure, containing 4 stereogenic centers. It's multi-step synthesis route presented by Sanofi, is complicated and time-consuming. The available information on the synthesis of SSR181507 and its precursors is often ambiguous or even misleading, and provide a very limited analytical characterization of the compounds.[3]



SSR181507

In the course of our studies on tropane derivatives, we developed a facile and scalable 5-step synthesis of SSR181507. The synthesis route starts from commercially available *N*-CBZ-protected (3-exo) 8-azabicyclo[3.2.1]octan-3-yl)methanamine and does not require any catalysts or harsh conditions. A significant value of the presented work is the in-depth analytical characterization of the intermediates and the final product, including 1D and 2D NMR experiments (COSY, NOESY), HRMS and optical rotation.

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P63

Evaluation of Histamine H_3R Antagonists for Anticancer Activity and Cholinesterases Inhibitory Effect

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This study examines the properties of novel guanidines, designed and synthesized as histamine H_3R antagonists/inverse agonists for additional pharmacological targets. We evaluated their potential against two targets *viz*., inhibition of MDA-MB-231, and MCF-7 breast cancer cells viability and inhibition of AChE/BuChE. **ADS10310** showed micromolar cytotoxicity against breast cancer cells, combined with nanomolar affinity at $h$$H_3R$, and may represent a promising target for the development of an alternative method of cancer therapy. Some of the newly-synthesized compounds showed moderate inhibition of BuChE in the single-digit micromolar concentration ranges. H_3R antagonist with additional AChE/BuChE inhibitory effect might improve cognitive functions in Alzheimer's disease. For **ADS10310**, several *in vitro* ADME-Tox parameters were evaluated and indicated that it is a metabolically-stable compound with weak hepatotoxic activity, and can be accepted for further studies.

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P64

Interactions of antitumor unsymmetrical bisacridines, UAs, with C-Myc protooncogene promoter studied by NMR spectroscopy, enhanced by MD techniques

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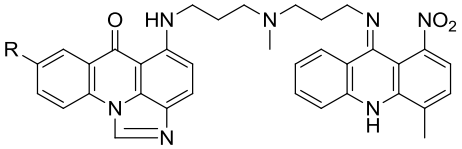
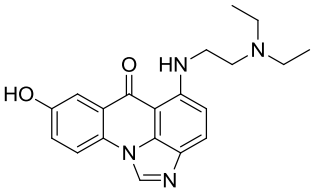
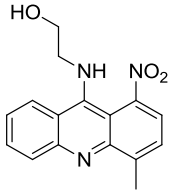
Our team has been extensively studying the molecular mechanism of action of unsymmetrical bisacridine derivatives (UAs) for years. Acridine derivatives are known for their ability to interact with DNA. However, so far, we have been able to show that UAs do not interact with DNA duplexes as originally predicted. Instead, they form well-defined complexes with G-quadruplexes [1]. In this study, the Pu22 sequence, which originates from the promoter regionof C-Myc protooncogene, was selected as a model G-quadruplex due to its important role in the process of carcinogenesis. The interaction of two selected unsymmetrical bisacridine derivatives was studied by NMR spectroscopy. However, it turned out that the direct determination of the three-dimensional structure of the complexes using NOE signals is impossible. Therefore, since they consist of two aromatic systems, the structure of UAs' complexes with G-quadruplexes can be assessed by their monomeric derivatives, C1311 and C1748.





C-1311



C-1748

	C-2045	C-2053
R	OH	H

Previous results indicate that C-1311 formed a complex with Pu22 with 1:2 ratio in such a way that one molecule interacts with the upper tetrad while the other interacts with the lower tetrad. C-1748 weakly interacts with Pu22 in an unspecific manner, due to the fact that 1-nitroacridine ring is not planar and nitro group constitutes major steric hindrance[2]. Based on the NOE signals representing coupling between Pu22 protons and C-1311 protons,the structures of the complexes formed by C-2045 and C-2053 are proposed. In our model of interactions,1-nitroacridine ring is treated as a side chain, which position was studied by molecular dynamics methods, whereas the nature of Pu22 – imidazoacridinone rings interaction was studied by means of molecular modelling, using umbrella sampling method.

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This study was supported by National Science Centre under grant No 2019/33/B/NZ7/02534.

P65

Chemometric optimization of the electrochemical synthesis of hepatic metabolites of selected antifungal drugs

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Fungal infections pose a significant therapeutic challenge, and the use of antifungal drugs continue to rise. Azole antifungals are commonly employed for treating fungal infections, and understanding their metabolism is essential for optimizing therapeutic strategies. Knowledge of drug metabolites plays a vital role in optimizing dosing regimens, evaluating potential toxicity, and predicting drug interactions, thus ultimately enhancing patient safety and therapeutic outcomes. Additionally, studying drug metabolites provides insights into the pharmacokinetics and efficacy of drugs, aiding in rational drug design and personalized medicine approaches.

In recent years, electrochemistry has emerged as a valuable tool for metabolic studies. By mimicking redox reactions in various settings, electrochemical methods aid investigating drug metabolism, offering valuable insights into metabolic pathways and facilitating the identification of metabolites, as well as their semi-preparative and preparative synthesis Furthermore, electrochemistry finds application in environmental research for identifying transformation products, generated under environmental conditions.

In this study, we explore the application of electrochemistry in predicting the metabolism of two selected azole antifungal drugs. By comparing the electrochemically synthesized products with metabolites obtained using biological method, specifically human liver microsomes (HLM), we aim to assess the reliability and effectiveness of electrochemistry in simulating metabolic conditions. The electrochemical experiments were conducted using screen-printed electrodes (SPE), and various parameters including pH, buffer type, and potentials were optimized to mimic the physiological conditions relevant to drug metabolism.

The findings of this study demonstrate the feasibility of using electrochemistry to simulate drug metabolism and predict metabolic products. The selection and optimization of appropriate electrodes enabled a reliable representation of the metabolic processes. Furthermore, the multivariate chemometric analysis (PCA), provided valuable insights into the similarities and differences between the electrochemically generated metabolites and those obtained from HLM, further enhancing our understanding of the electrochemical approach in drug metabolism studies.

P66

Synthesis and spectral properties of novel heteroleptic zinc(II) aza-dipyrromethene complexes

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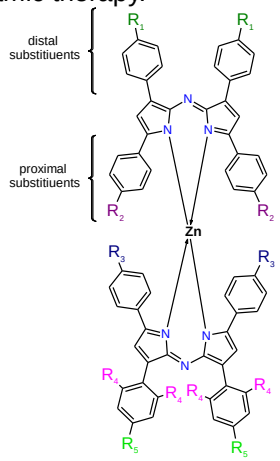
Aza-dipyrromethenes (ADPMs) are a class of synthetic molecules with strong absorption in the visible light and near-IR region [1]. Due to their optical properties, aza-dipyrromethene derivatives are considered as photosensitizers for photodynamic therapy. The most studied ADPM compounds are BF_2^+ chelates, known as aza-BODIPY. ADPMs are also used as chelating ligands for the synthesis of single metal complexes. ADPMs metal complexes are obtained by direct reaction of metal salt and ADPM ligand bearing specific substituents [1,2]. So far, only homoleptic zinc(II) aza-dipyrromethene chelates have been reported.
In this research, both proximally- and distally-substituted ADPMs (bearing hydroxyl, nitro, methyl and 2-(morpholin-4-yl)ethoxy substituents on the phenyl rings) were used as ligands for the synthesis of zinc(II) complexes. Each mixed complexation reaction of two different ligands with zinc(II) acetate resulted in the formation of three different compounds – one heteroleptic (ZnAB) and two homoleptic complexes (ZnA_2, ZnB_2), which were chromatographically separated. Six novel heteroleptic and two novel homoleptic complexes were obtained and characterized. Structure, purity and optical properties were investigated using mass spectrometry, NMR techniques (including 1D and 2D experiments) and UV-VIS spectroscopy. UV-VIS spectra of the complexes were recorded in toluene, chloroform, tetrahydrofuran (THF) and dimethylformamide (DMF). The UV-VIS spectra showed two main bands – in the UV region and long-wavelength part of the visible spectrum. Strong absorption of the red part of visible spectrum is considered optimal for photodynamic therapy.



1 : $R_1 = R_4 = R_5 = H$, $R_2 = R_3 =$
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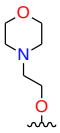
3 : $R_1 = R_3 = H$, $R_4 = R_5 = \mathbf{CH_3}$ $R_2 =$
4 : $R_1 = R_3 = R_4 = H$, $R_5 = \mathbf{OH}$ $R_2 =$
5 : $R_2 = R_3 = R_4 = H$, $R_5 = \mathbf{OH}$ $R_1 =$
6 : $R_2 = R_3 = R_4 = H$, $R_1 = R_5 = \mathbf{NO_2}$
7 : $R_1 = R_3 = R_4 = H$, $R_5 = \mathbf{NO_2}$ $R_2 =$
8 : $R_2 = R_3 = R_4 = H$, $R_5 = \mathbf{NO_2}$ $R_1 =$



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P67

Fail early, fail cheap - ADMETox profiling of compounds with potential activity in chronic respiratory diseases

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The gold standard in drug research is the assessment of safety of a potential drug candidate before determining its clinical effectiveness. Not without a reason, such studies are first performed with use of *in silico* methods as well as *in vitro* models and only later with the use of laboratory animals. Toxicity, undetected early may withdraw a potential candidate from the process of entering the market [1].

The subject of our research was to determine several *in silico* and *in vitro* ADMETox parameters of newly synthesized derivatives of xanthine, benzimidazole and triazine. These compounds were identified as the modulators of TRPA1 channel. It is a calcium-permeable channel, which role is extensively investigated in terms of pain, inflammation and recently in respiratory disorders [2].

„A" for absorption was determined by PAMPA *in vitro* test and the *in silico* model of "Boiled egg" generated by the SwissADME program. "M" for the metabolism of the tested derivatives was simulated by the Metasite 6.0 program. In turn, various cell lines, such as pulmonary fibroblasts, as well as Derek Nexus program were used to test their potential toxicity, i.e. to determine their Tox profile.

Most of the tested derivatives were classified to medium or high permeability category, which proves their probable good bioavailability. In turn, the *in silico* metabolism simulation indicated groups in the structures that are prone to biotransformation. *In vitro* cytotoxicity studies proved, that investigated derivatives are safe in a wide range of tested concentrations (0.5-50 μM). These results were mostly consistent with the *in silico* predictions. However, Derek Nexus has generated a potential hepatotoxicity alert for most of the tested derivatives. Nevertheless, it was not reflected in the in vitro cytotoxicity studies. This example shows that despite the undeniable usefulness of *in silico* tools, there is a need of verifying the results obtained with them, using at least *in vitro* models.

Obtained results are optimistic and encouraging to investigate other ADMETox parameters of our compounds, as well as to plan experiments determining their effectiveness in chronic respiratory diseases.

Research funded by the statutory program of the Jagiellonian University – project number: N42/DBS/000323.

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P68

Application of flow chemistry technology in the development of a synthesis pathway for a potential FLT3 kinase inhibitor with anticancer properties against acute myeloid leukemia (AML)

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The FLT3 kinase belongs to the family of class III receptor tyrosine kinases. It regulates many cellular processes, including hematopoietic cell differentiation, growth, adhesion, motility, and apoptosis.1 Unfortunately, FLT3 kinase genetic aberrations often contribute to the development of acute myeloid leukemia (AML). FLT3 kinase mutations found in approximately 30% of people with acute myeloid leukemia.2 It is aggressive malignancy with few therapeutic options. It leads to the proliferation of abnormally functioning white blood cells in the bone marrow, which reduces the production of erythrocytes. In addition, infection and organ dysfunction can occur.3 Despite the development of several FLT3 inhibitors that have entered clinical trials, there is still huge need for new generation drugs. This is due to the fact that many of the tested medicinal preparations showed limited treatment efficacy and even toxicity.4

Flow chemistry technologies were used to develop the synthesis pathway for a potential FLT3 inhibitor. It is an innovative way of conducting chemical processes based on the flow of reactants in a continuous stream. This technology has many advantages, as it enables the reaction to be carried out above the boiling point of the solvent and provides better control of parameters, mass and heat transfer, and mixing of reagents. In addition, it also enables multi-stage reactions under various conditions, affects the repeatability of reactions, increases the scale of reactions, and ensures greater safety when working with dangerous reagents. However, the most important advantage of the use of the flow system is the reduction of the reaction time to few minutes, which in the classical way lasts several days.5,6 The use of this technology facilitates and accelerates the process of developing a new compound that may prove to be a good candidate for clinical trials.

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P69

Dual inhibitors of GSK-3β and ROCK-1 kinases as new tools to target protein aggregation and neuroinflammation in Alzheimer's disease

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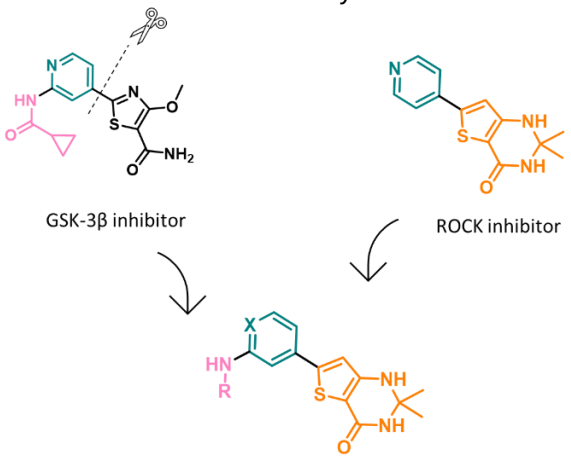
Alzheimer's disease (AD) is the most common type of dementia that affects memory, thinking and behavior. Symptoms eventually grow severe enough to interfere with daily tasks of people suffering from the disease [1]. From molecular perspective AD is characterized by misfolding and accumulation of β-amyloid peptide (Aβ) and tau protein with accompanying neuroinflammation and loss of neurons. It translates into directions in search for new therapies of the disease. Among compounds in Phase 3 clinical trials there are 19% targeting Aβ , 6% thauopathy and 6% immune system-related events what highlights the paramount role of these processes in the pathophysiology of the disease [2].

Glycogen synthase kinase-3β (GSK-3β) and ROCK-1 are kinases that link influence on misfolded proteins and neuroinflammation thus constituting an excellent therapeutic targets in AD. They have been implicated in Aβ plaque formation, hyperphosphorylation of tau protein but also the production of inflamatory markers and apoptotic neuronal cell death. A strong correlation between their effects on AD pathology triggered an idea of combining inhibitory activity against both these kinases in one molecule [3].

As the starting point for the design of novel dual GSK-3β and ROCK-1 inhibitors we chose structures of known, potent inhibitors of GSK-3β and ROCK-1 (Figure 1). We evaluated their pharmacological properties *in vitro* against GSK-3β and ROCK-1 in the Kinase-Glo™ bioluminescent assay. Furthermore, we have evaluated their anti-inflammatory effect in lipopolysaccharide-stimulated BV-2 microglial cells, using IL-6, TNF-α and NO as inflammatory markers.



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P70

Immunomodulatory effects and structure-activity relationship of new diosgenin and tigogenin analogues

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Currently, an increase in diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation is observed worldwide. The main purpose of immunomodulators is to suppress immune response as a treatment for autoimmune diseases. [1]. Additionally, immunostimulant agents also act as an adjuvant to chemotherapy for various diseases [2]. Medicinal plants are important source of the active compounds, often acting as immunomodulators. The development of immunomodulatory and anti-tumor drugs from natural compounds has attracted considerable interest. We designed, synthesized, and characterized a new family of diosgenin and tigogenin derivatives with new structural fragments at the C-3 position. All compounds have been evaluated *in vitro* for their antiproliferative activity against HUVEC and selected cancer cell lines by the MTT assay. Their immunomodulatory properties have been determined by examining the effect on the expression of several cytokine (IL-1, IL-4, IL-10, IL-12, TNF-α) genes by real time RT-PCR. Structure–activity relationship studies indicate that two of the tested compounds exhibit desired effects. They do not induce the gene expression of the pro-inflammatory cytokines (i.e. IL-12, IL-1, TNF-α) but strongly stimulate the expression of anti-inflammatory IL-10 and IL-4. A molecular docking study has revealed a high binding affinity of diosgenin and tigogenin analogues to the active site of the glucocorticoid receptor (GR). The GR is expressed in in the body cells and regulates genes that control, inter alia, the immune response. Promising results of the in vitro immunomodulating activity, molecular docking, and drug-likeness properties of new compounds presented here indicate that these compounds can be used as potential lead compounds for further development and investigation of novel immunomodulatory agents.

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P71

Connection of protoporphyrin IX with PLGA polymer – synthesis, characteristics and prospective application as a drug delivery system for photodynamic therapy

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Porphyrins are macrocyclic compounds with interesting photochemical and photophysical properties, and many of them have already found practical applications in photodynamic therapy (PDT). The basis of PDT is the photodynamic reaction, in which the photosensitizer, after irradiation with light, is activated to its excited form and then transfers energy to the oxygen present in the environment, producing reactive oxygen species that have a high potential to damage cancer cells [1]. The photosensitizer can be attached to or encapsulated in a drug delivery system (DDS) that allows the active pharmaceutical ingredient to be released at the appropriate place in the body or tissue. The development of the DDS approach is related to, among others, the development of drug delivery systems activated with chemical compounds with oxidation-reduction properties.

The aim of the experimental studies was to obtain and characterize polymer micelles based on conjugates of selected porphyrin with cysteamine-modified polymers. For this purpose, the PLGA polymer was modified with cysteamine according to the literature procedure [2], and then combined with selected porphyrin. The resulting conjugate was characterized by spectroscopic methods such as IR and NMR, as well as X-ray diffraction and thermal analysis. SEM microscopy and the Nanosight apparatus were used to characterize polymer nanoparticles. The Microtox® apparatus was applied to determine the acute toxicity of the obtained materials. The release of porphyrin from the polymer was assessed using dithiothreitol (DTT).

The project was financed by the National Science Center, Poland - PRELUDIUM BIS 2, No 2020/39/O/NZ7/00351

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P72

Application of microwave technology in the development of a synthesis pathway for an innovative small-molecule MER kinase inhibitor with high anticancer activity against acute lymphoblastic leukemia (ALL)

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Acute lymphoblastic leukemia (ALL) is the most common malignant cancer in children and is caused by abnormal expression of MER kinase. This kinase belongs to the TAM family of receptor tyrosine kinases that are overexpressed or hyperactive in cancer cells and are therefore an attractive target for cancer treatment.[1,2] At present, there are no registered small chemical molecules capable of inhibiting the selective MER TK receptor, thus justifying the validity of our research leading to the development of new selective small molecule MER kinase inhibitors.[3,4] The design and synthesis of a compound library are time-consuming, expensive, and resource-intensive. Microwave technology allows us to shorten the time of conducting reactions, as well as control the reaction parameters, i.e., temperature and pressure, as well as the speed of heating the reaction mixture.[5] The approach used directly leads to the intensification of the optimization process and allows for a quick evaluation of the process from an economic point of view. Thanks to this approach, producing the right number of a future candidate for clinical trials is easier and faster. Using this technology, several pyrrolopyrimidine and pyrimidine derivatives have been developed with activities against Mer kinase in the nanomolar range.

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P73

Influence of different topology of hydantoin 5-HT$_7$ serotonin receptor ligands on ADMET properties tested *in vitro*

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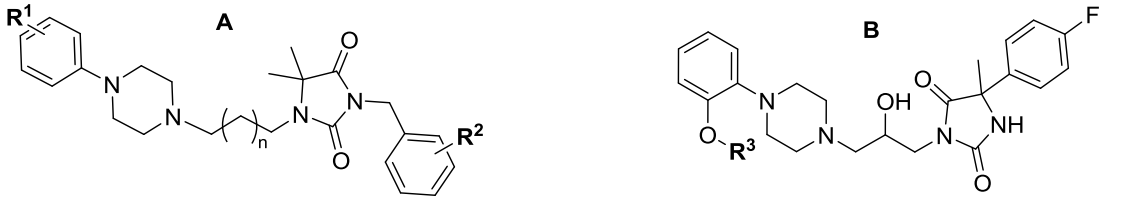
The 5-HT$_7$ serotonin receptor plays a pivotal role in modulating various neurological and neuropsychiatric processes, making it an intriguing target for drug development [1]. Antagonists of 5-HT$_7$R have been extensively studied both in *in vivo* animal studies and *in vitro* experiments and have demonstrated their efficacy in altering behavioral responses, neural plasticity, and neurotransmitter release in a manner that aligns with their potential therapeutic applications including depression, anxiety disorders, schizophrenia, cognitive impairments, and certain neurodegenerative diseases [2]. Hydantoin-based compounds have shown promising activity as antagonists for the 5-HT$_7$ receptor [3], presenting a fertile ground for investigating their diverse topological arrangements and their impact on the AMDET. In this study, we describe the synthesis, molecular modeling and *in vitro* evaluation for a series of arylpiperazines derivatives of hydantoin compounds, divided into two topological groups **A** and **B** (**Scheme 1**), possessing nanomolar affinities towards 5-HT$_7$R.



R^1 = H, 2-MeO, 2-CN, 2,3-Cl, 2,4-Cl

R^2 = 2,4-Cl, 4-Cl; **n** = 1, 2, 3

R^3 = CH_2-naphthyl, isobutyl, $CH_2CH_2N(CH_3)_2$

Scheme 1

In vitro ADMET screening including hepatotoxicity, hERG inhibition, Caco-2 permeability and metabolic stability were conducted to evaluate the pharmacokinetic properties and safety profile of obtained ligands. Compound PPK-31 showed the most promising druglikeness properties; high metabolic stability, lack of potential risk for liver damage, high recovery and acceptable permeability and lowest affinity for the hERG protein. These combined results position PPK-31 as an outstanding candidate for further development, showcasing its potential for therapeutic applications with favorable pharmacokinetic and safety profiles.

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P74

Cinnamic acid derivatives as autophagy and SIRT3 modulators in doxorubicin injured cardiomyocytes

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Doxorubicin (DOX) is anthracycline antibiotic commonly used in the treatment of hematological malignancies and solid tumors[1]. However, its clinical application is limited by dose-dependent and irreversible heart injury, which increases the risk of congestive heart failure. To date, no effective therapy has been introduced to prevented by DOX-induced cardiotoxicity, with the exception of dexrazoxane, which uses is limited. Previously, we proved multidirectional cardioprotective activity of cinnamic acid derivatives (CA), but the detailed mechanism of its action is still unclear[2]. Literature indicates an important role of the SIRT protein family in cardioprotection. Sirtuin 3 (Sirt3), is a mitochondrial deacetylase that regulates activity of proteins involved in apoptosis, autophagy and plays a pivotal role in regulating mitochondrial dynamics and oxidative stress[3].

Here we proposed potential cardioprotective mechanism of CA in DOX-injured cardiomyocytes model. H9c2 rat cardiomyocytes were preincubated in the presence of CA for 3 hours, then DOX was added for next 24 hours to cause injury. Our hypothesis is that cardiomyocyte treatment in the presence of CA, pre-conditioning of Sirt3 protein levels decreases DOX-induced cardiotoxicity. Dexrazoxane was used as reference. Immunofluorescence analysis showed that CA derivatives protected cardiomyocytes by decreasing of SIRT3 level induced by DOX. Furthermore, CA derivatives attenuated the DOX toxicity and reduced apoptosis by downregulating levels of caspase-3 and 7, inhibited autophagy and decreased the ROS level in cardiomyocytes.

Taken together, our results demonstrate for the first time that CA derivatives ameliorates DOX-cardiomyocytes injury- via activating the SIRT3 signaling pathway.

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P75

1,3-Disubstituted Thiourea Derivatives: Promising Candidates for Medicinal Applications with Enhanced Cytotoxic Effects on Cancer Cells

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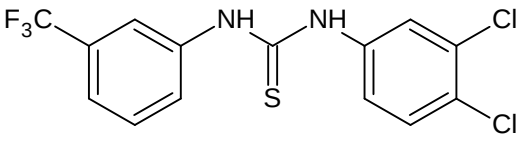
Numerous urea and thiourea derivatives with 1,3-disubstitution have been documented to exhibit antiproliferative effects against both solid tumor cell lines and leukemia. These derivatives have demonstrated a remarkable ability to minimize side effects. Particularly, the derivatives that displayed the highest effectiveness were those containing electron-withdrawing substituents incorporated into the terminal phenyl rings [1,2]. In our previous work we selected from the group of 1,3-disubstituted thiourea derivatives, the most potent compounds: 2 (3,4-dichlrophenyl) and 8 (3-(trifluoromethyl) substituted phenyltioureas.



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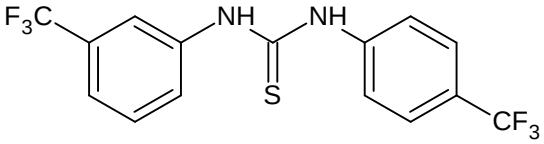

8

1-(3,4-dichlorophenyl)-3-[3-(trifluoromethyl)phenyl]thiourea (**2**)
1-[3-(trifluoromethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]thiourea (**8**)

The present study has demonstrated mechanisms of their anticancer activity against colorectal (SW480 and SW620), prostate (PC3) and leukemia (K-562) cancer cells by targeting key molecular pathways involved in cancer progression. They included caspases activation, NF-κB reduction, VEGF and cell migration inhibition as well as crucial metabolites changes. Moreover, the effectiveness of the studied compounds was also tested on spheroids of cancer cells (3D culture). Results were successfully obtained and visualized using the luminescent, ELISA, “scratch-test” and LC-MS methods, whereas the effect on spheroids was evaluated by using Live-Dead Cell Viability 3D Assay Kit and confocal microscope detection (FV10-ASW 4.2 Olympus). The apoptosis induction was proved by the increase of caspase 3/7 activation at different time intervals (12, 24, 48h), which was most noticeable in colorectal cancer cells, however compound **8** also exhibited an effect on prostate and compound **2** on leukemia cells. Both compounds decreased NF-κB activation and the effect was more pronounced in SW480, PC-3 and K-562 cells. The inhibition of cell invasiveness has been tested by assessing effect of tested phenyltioureas on VEGF level and cell migration. It turned out, that both compounds exhibited anti-metastatic activity by significantly reducing of the amount of VEGF especially in PC3 and K-562 cells as well as by inhibitory effect on cell migration in solid tumours (SW480,SW620,PC3). Moreover, these all processes remained without significant changes in normal cells (HaCaT). The metabolomic analysis after treatment with studied compounds revealed interesting metabolic profile in cells depending on the type of cancer. Therefore in colon and leukemia cells they were primarily changes in carbohydrate, amino acids and pyrimidine metabolites, meanwhile the changes in prostate cancer cells concerned primarily on steroid metabolism. The determination of cell viability based on plasma membrane integrity and esterase activity in 3D cell culture (spheroids) indicated that SW480, SW620 and PC3 cells were highly sensitive to compounds **2** and **8**. The obtained results clearly demonstrate that modified phenyltioureas have cytotoxic and anti–metastatic activity against colon and prostate cancer as well as leukemia. Taken together the structural modifications of thiourea terminal moieties pointed dihalogenophenyl derivative **2** and para-substituted analog **8** gave anticancer potential while leaving them safe for normal cells.

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P76

2,4-dichlorophenoxythiosemicarbazide derivatives as potential antibacterial agent

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The resistance of pathogenic microorganisms to antibiotics is an important and current problem of modern medicine. The rapid evolution of resistant bacteria requires new preventive measures to slow down this process, however, long-term progress cannot be achieved without a good understanding of the mechanisms by which drug resistance is acquired and spread in microbial populations.

The development of new effective antimicrobial drugs is a difficult and long-term task. One of the methods used in research is the modification of compounds that already function as drugs. This strategy involves the redesign of known antibacterial drugs or optimization through various modifications of existing cores in order to improve their pharmacokinetic and pharmacodynamic properties and reduce the toxicity of these compounds. Using high-throughput screening methods, compounds with new or improved mechanisms of antibacterial and anti-mycobacterial activity may be potential candidates for clinical trials.

In search of new compounds that could be antimicrobial preparations, ten newly synthesized 2,4-dichlorophenoxythiosemicarbazide derivatives were tested. The screening of the tested substances was carried out on the following strains: *M. tuberculosis H37Ra*, *E. coli*, *P. aeruginosa*, *S.aureus*, *S.aureus MR3* and *S. epidermidis*. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined for three substances that showed antibacterial activity in the screening test.

Analyzing the dependence of the activity on the structure of the tested 2,4-dichlorophenoxythiosemicarbazide derivatives, it was observed that only derivatives with a fluoro-phenyl, bromo-phenyl and naphthyl substituent possessed antibacterial activity. Moreover, the activity was also affected by the position of the halogen. Only derivatives substituted in position 4 of the phenyl ring were active, while derivatives with halogen in position 2 and 3 showed no antibacterial activity.

Although the antibacterial activity of the newly synthesized 2,4-dichlorophenoxy thiosemicarbazide derivatives is not very high, the synergy with anti-tuberculosis drugs offers the prospect of reducing the standard doses of antibiotics, which would be associated with lower toxicity and limiting side effects.

P77

Synthesis and characterization of hydrazide starch as an potential pH-sensitive drug carrier

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The pH value found in cancer cells is slightly acidic, unlike healthy cells in the body. It seems reasonable to use this difference in the selective transport of cytostatic drugs. The hydrazone bond has the ability to be hydrolyzed at slightly acidic pH. The use of this binding in systems that transport anticancer drugs will result in the release of the active substance only in the affected area without damaging healthy cells, which will significantly contribute to reducing systemic cytotoxicity and improving the patient's comfort of life during therapy [1].

As part of the presented research, starch, which is a biodegradable and biocompatible polymer, was modified in order to create a modern anticancer drug carrier based on a hydrazone bond. A material was obtained that has the ability to bind to the carbonyl group of anticancer drugs. At each stage of the synthesis, the material was characterized in detail by taking FTIR-ATR spectra, thermal analysis, XRD and SEM images. The final stage of the synthesis was the binding of doxorubicin on the surface of the material as a model cytostatic drug. In the next stage of research, the carrier-hydrazone bond-drug system prepared in this way was tested in solutions with different pH values, imitating the environment of a cancer cell, a healthy cell and their subcellular elements, in terms of drug release from the carrier. The obtained results allow us to conclude that the drug in a slightly acidic environment was released from the carrier more than four times more than in a neutral environment.

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P78

Aromatic heptaenes seen in a new light: aromatic analogues of amphotericin B with improved selective toxicity.

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Systemic fungal mycoses constitute a major problem for modern chemotherapy. Fungal microorganisms (*Aspergillus sp., Candida sp.*) are etiological factors which cause many serious, possibly fatal, infectious diseases, especially in case of immunocompromised patients [1]. Macrolide antibiotics (PM) used in clinical treatment exhibit 3 out of 4 basic features of non-existing “ideal” antifungal chemotherapeutic: a fungicidal mode of action, the broadest possible spectrum of antifungal activity encompassing both human pathogenic yeasts and filamentous fungi and a minimal ability to induce specific and multidrug resistance [2]. The most effective antibiotic proposed so far, amphotericin B (AmB), a member of the heptaene macrolide family, is considered as a ‘life-saving drug’ and excels among other antifungal therapeutic agents. In the meantime, PM belonging to the aromatic heptaene subgroup (AHMs) exhibit higher than AmB antifungal in vitro activity (an order of magnitude lower MICs), whereas their ST is not advantageous, due to the high hemotoxicity [3]. Our recent studies have proven that the AHMs, while exposed to a moderate UV-Vis irradiation, undergo photochemical isomerization process [4,5]. The resulting, stable photoisomers exhibit the *all-trans* geometry of the chromophore, identical to the one of AmB. After the photoisomerization process, the presence of the alkyl-aromatic sidechain remains the only major structural difference in comparison to AmB, therefore the *all-trans* AHMs may be regarded as *aromatic analogues of AmB*. Nevertheless, their significantly higher antifungal activity in comparison to the AmB remains uncompromised after the isomerization reaction. Our *in vitro* biological studies have also displayed that the selective toxicity indexes (STIs), defined as antifungal/haemolytic activity ratio, in case of the *all-trans* isoforms were much higher in comparison to the native AHM compounds and the AmB. Therefore, the photoisomerization of AHMs may be regarded as an important first step in the design of potentially better drug(s) than the current standard.

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P79

Acridine derivatives – heavy metal complexes , as an anticancer agents, encapsulated in PEG – coated liposomes drug delivery platforms

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Nowadays, there are more and more scientific studies on liposomes, as novel bioactive products and drug carriers, have been conducted. Research has proven that liposomes not only improve water solubility and control release of bioactive agents but also improve their pharmacological effects. Moreover the surface of liposome can be modified by attaching molecules such as PEG, which expand the range of possible nanocomposites applications and enable active targeting. Incorporating drugs into the structure of liposomes allows reducing their toxicity and increasing compatibility with human body at the same time. Acridines are nitrogen heterocycles, structurally related to anthracene but with nitrogen instead of one of CH groups. They are being extensively researched as potential anti-cancer drugs. These compounds are focused on DNA and DNA-related enzymes (e.g. topoisomerases, telomerases). The presence of donor nitrogen atoms in acridine derivatives makes it possible to coordinate these compounds with metal ions. Metal complexes are widely explored in cancer therapy. Adducts of metal complexes and DNA, enzymes or metalloproteins are formed covalently or non-covalently. It leads to inhibition of cell division. [1] A complex made of metal ion and acridine is expected to serve as a better DNA intercalator.

Three new solid copper(II) coordination compounds with acridine derivatives were synthesized. In the next step obtained metal complexes were encapsulated in PEG-coated liposomes which increased solubility these coordination compounds in water. The potential cytotoxic effects of the novel complexes have been tested against A549 cancer cell line using the MTT assay and standard anticancer drugs (etoposide, 5-fluorouracil, and cisplatin) served as reference compounds The results observed for coordination compounds were better than we expected. In comparison to cis-platinum the results are promising. In the case of the most active compound for the A549 cell line, IC_{50} value gives encouraging results for further research.

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P80

Novel non-basic, selective antagonists of the dopamine D_2 receptor with antipsychotic activity

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The dopamine D_2 receptor is a member of the G protein-coupled receptors (GPCR) family. Due to its wide distribution, in particular in the central nervous system, as well as its involvement in the pathophysiology of many disorders, it constitutes an important drug target in the field of medicinal chemistry. Schizophrenia is one of the most frequently occurring diseases related to the dopaminergic neurotransmission disturbances, in which the D_2 receptor is the main drug target.

In the presented work, we aimed at discovering new selective antagonists of the D_2 receptor with potential antipsychotic activity. Twenty-three compounds were synthesized, based on the scaffold represented by the previously reported virtual hit, D2AAK2 [1]. This compound is an interesting example of a D_2 receptor ligand due to its non-classical binding mode to this target. Performed radioligand binding assays and SAR analysis indicated structural modifications of D2AAK2 that are allowed to maintain its activity. These findings were further rationalized using molecular modeling. In cAMP signaling assays three active derivatives were identified as antagonists of the D_2 receptor, and the selected most active compound was subjected to X-ray studies, which revealed its crystal packing in the solid state. Finally, its antipsychotic activity *in vivo* was determined in behavioral tests [2].

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P81

Effect of cyclooxygenase 2 inhibition on regulation of apoptosis on UVB and cannabinoids treated keratinocytes

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Apoptosis is a process of physiological cell death controlled by numerous factors. Among these factors, cannabinoids are of particular interest, as these compounds have a pro-apoptotic effect on some cells and an anti-apoptotic effect on others. Given that cyclooxygenase-2 (COX2) can metabolize cannabinoids, their activity may depend on the activity of this enzyme and factors regulating it, such as UVB radiation [1-2].

The aim of this study was to determine the effect of the interaction between COX2 and cannabinoids on the apoptosis of keratinocytes, whether exposed or not exposed to UVB radiation, in vitro. To achieve this, percentage of cells undergoing apoptosis (fluorescent microscopy), expression of caspases (qRT-PCR) and levels of prostaglandins in HaCaT keratinocytes were examined. The keratinocytes were divided into 2 groups: with/without a COX2 inhibitor, and further divided into subgroups: control, UVB, cannabidiol (CBD), anandamide (AEA), combination of UV+CBD, and UV+AEA.

In cells without a COX2 inhibitor, CBD inhibited the expression of caspases 2 and 8,9 without altering the percentage of cells undergoing apoptosis. AEA increased the expression of caspases 2 and 9, resulting in an increase in the percentage of cells undergoing apoptosis which may be attributed to the fact that AEA is metabolized by COX-2 to AEA-EA.. Following UVB irradiation, there was an increase in the expression of caspases 2, 8, and 9, as well as an increase in the percentage of cells undergoing apoptosis, which was inhibited by CBD and enhanced by AEA. In the case of cells with COX2 activity blocked by the inhibitor, both CBD and AEA reduced the expression of the tested caspases and the percentage of cells undergoing apoptosis, both in UVB-treated and non-irradiated cells. Radiation alone had a similar effect to cells with normal COX2 activity, although less severe. This may be due to the fact that UVB enhances the production of pro-apoptotic prostaglandins D and E, which is inhibited by celecoxib.

The results indicate that COX2 enhances UVB-mediated keratinocyte apoptosis. CBD has a protective effect on cells, and its action is not modulated by COX2. However, the action of AEA is strongly dependent on COX2, as its inhibition completely changes the effect of AEA, suggesting that the metabolites of this compound formed under the influence of COX2, such as AEA-EA, are responsible for the pro-apoptotic effect of AEA.

The research was performed under OPUS grant from National Science Center (NCN Poland).

Grant number: UMO-2021/43/B/NZ7/01732

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P82

Anticancer activity of the novel potential tubulin inhibitors 4-thiazolidinone derivatives against breast cancer (MCF-7) cell line

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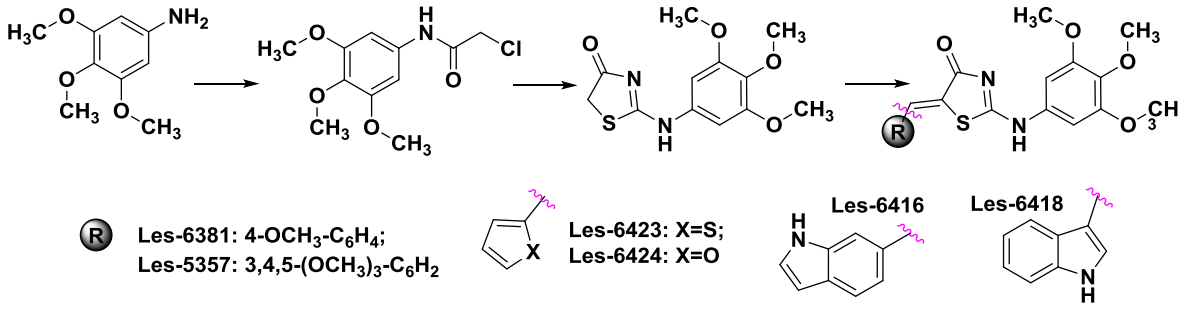
We present 6 novel 5-arylidene-2-(3,4,5-trimethoxyphenyl)aminothiazol-4(5*H*)-ones with anticancer properties as potential tubulin inhibitors [1]. Mentioned derivatives were synthesized according to the following scheme:



4-Thiazolidinone moiety has a confirmed anticancer activity against various types of cancer such as leukaemia, CNS cancer, non-small cell lung cancer, renal cancer, colon cancer and breast cancer [2].

Therefore, the cytotoxicity of the new structures has been researched for their activity on breast cancer cell line - MCF-7 in concentrations ranging from 0.1μM to 50μM using the MTT assay method. The most cytotoxic compounds were Les-6416, Les-5357 and Les-6381 with IC-50 values of 3.18μM, 4.27μM and 5.70μM respectively. Next we determined apoptosis by applying flow cytometry assessment of annexin V binding. In concentration of 5μM compounds Les-6416, Les-6418 and Les-6381 showed the best results with 60.3%, 52.2% and 50.3% apoptosis induction accordingly. In addition, in silico methods have been applied to investigate their ADME properties and possible sites of metabolism. In conclusion, we determined that those structures have promising anticancer properties and further tests have to be conducted.

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P83

Determination of the antimicrobial and antioxidant activity of *Verbascum macrosepalum* Boiss. & Kotschy ex Murb. and *Verbascum insulare* Boiss. & Heldr. (Scrophulariaceae)

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Verbascum L. genus is one of the important genera in the Schrophulariaceae family, distributed mainly in the Mediterranean, Iran-Turan and European-Siberian phytogeographic regions [1]. It is represented by 360 species worldwide, with 256 species and 132 hybrids in Turkey, and 201 endemic species [2]. Turkey is one of the important gene sources for *Verbascum* genus. In Turkey, the species *V. macrosepalum* and *V. insulare* known as “Dadaş Sığırkuyruğu”, “Ada Sığırkuyruğu” respectively. These species are endemic to the province of Mus (Turkey) [3].

Methanol extracts of plants and roots of *Verbascum macrosepalum* and *Verbascum insulare* species were used to determine their antimicrobial and antioxidant effects. It was determined that the species showed strong antioxidant effects according to total phenolic substance determination, DPPH and ABTS techniques. According to the results of microbroth dilution method and minimum bactericidal concentration techniques, *Verbascum macrosepalum* and *Verbascum insulare* species were found to have a certain antimicrobial effect.

[1] Davis, PH, RR Mill, K Tan. 1988. Flora of Turkey and the East Aegean Islands. Vol. 10. (suppl. 1). Edinburgh: *Edinburgh University Press*.

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P84

Determination of the cytotoxic effect of *Verbascum cheiranthifolium* Boiss. var. *cheiranthifolium* Boiss.

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Verbascum L. genus is one of the important genera in the Schrophulariaceae family, distributed mainly in the Mediterranean, Iran-Turan and European-Siberian phytogeographic regions[1]. It is represented by 360 species worldwide, with 256 species and 132 hybrids in Turkey, and 201 endemic species [2]. Turkey is one of the important gene sources for *Verbascum* genus. In Turkey, the species *V. cheiranthifolium* var. *cheiranthifolium* known as “Bozkulak” [3].

The MTT assay was used to determine the cytotoxic effects of methanol extracts of the herb and roots *V. cheiranthifolium* var. *cheiranthifolium* in the ovarian cancer (skov-3) cell line. For this purpose, cells were treated with *V. cheiranthifolium* var. *cheiranthifolium* skov-3 cells between 0.025-2 mg/ml. According to the results of the MTT assay, *V. cheiranthifolium* var. *cheiranthifolium* showed cytotoxic effect on skov-3 cells.

[1] Davis, PH, RR Mill, K Tan. 1988. Flora of Turkey and the East Aegean Islands. Vol. 10. (suppl. 1). Edinburgh: *Edinburgh University Press*.

[2]Teofilovski A. (2023): *Verbascum matevskii* (Scrophulariaceae), a new species from North Macedonia. – *Thaiszia – J. Bot.* 33 (1): 063-073.

[3] Karavelioğulları FA, Verbascum L. In: Güner A, Aslan S, Ekim T, Vural M, Babaç MT. (eds.). Türkiye Bitkileri Listesi (Damarlı Bitkiler). *Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını*, İstanbul, pp 850-70, 2012.

P85

Modified aminoflavones with potential applications in medicine and pharmacy

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Cancer has been and remains a significant threat that negatively affects the quality of life of human beings (1,2). To cure this fatal disease, researchers tend to develop diverse bioactive agents. Among others, pyrrole-based compounds expose eminent capabilities against tyrosine kinases, microtubule polymerization, histone deacetylase, cytochrome p450, and bcl-2 proteins (3). Another class of proven anticancer drugs encompasses flavonoids, which were noticed to cause the activation of apoptotic proteins, ROS production, and inception of DNA damage (4).

Addressing the issue related to the dearth of efficient antitumor drugs, we resorted to the hybrid pharmacophore approach, which afforded new promising compounds. The synthetic strategy entailed the application of the Paal-Knorr pyrrole synthesis, originating from aminoflavones through their one-step condensation with various 1,4-diketones. As a result, we synthesized 6- and 7-(1-pyrrolyl) flavones that eventually showed great promise as potential anticancer agents. The isolated products underwent characterization using NMR spectroscopy, UV-vis spectroscopy, mass spectrometry, and Microtox® analysis. For all derivatives monocrystals were obtained and subjected to X-ray analysis. What is more, the preliminary biological study performed on the achieved compounds through the MTT assay with 5637 cells (human bladder grade II carcinoma) showed a distinct drop in cancer cell viability, especially in the case of derivatives possessing 2-methyl-5-phenylpyrrolyl moiety. The obtained data suggest persuading results, encouraging further exploration of the obtained molecules along with the discovery of new ones.

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P86

Drug-likeness analysis of purinergic $P2Y_{14}$ receptor antagonists

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Purinergic $P2Y_{14}$ receptor ($P2Y_{14}R$) is characterized by predominant expression on immune cells. This expression profile suggests that the $P2Y_{14}R$ and its ligands UDP-sugars play a pivotal role in immune or inflammatory responses and creates the potential opportunity for therapeutic applications of $P2Y_{14}R$ antagonists in the inflammation-associated diseases. In fact, the use of $P2Y_{14}R$ antagonists showed the efficacy in animal models of asthma, viral infections, neuropathic pain and acute kidney injury [1-3].

The prototype and most studied group of $P2Y_{14}$ receptor antagonists arise from zwitterionic structure of piperidinylphenyl-2-naphthalene derivative - 4-((Piperidin-4-yl)-Phenyl)-7-(4-(Trifluoromethyl)-Phenyl)-2-Naphthoic Acid (PPTN). The greatest limitation of those groups of compounds is a high lipophilicity resulting in poor bioavailability. Only a few alternative classes of antagonists were developed so far to overcome those drawbacks, including the substitution of naphthalene moiety in PPTN with alternative bioisosteres, like phenyl-triazole ring system or replacement of naphthalene with 3-amide-benzoic acid fragment [4-7]. The modifications of original PPTN structure helped to reduce lipophilicity, but on some occasions introduced other liabilities, like interaction with hERG channel or inhibition of cytochrome P450 isoforms.

In current study an in-depth characterization of selected drug-like properties was conducted for the series of twenty-nine $P2Y_{14}R$ antagonists to enable a broader structure-activity relationship analysis for most critical ADMET parameters in parallel with evaluation of $P2Y_{14}R$ affinity. The following tests were performed in the study: parallel artificial membrane permeability, inhibition of CYP3A4 and CYP2D6 isoforms and hERG channel inhibition assays.

Obtained data allowed for identification of the key structural elements responsible for undesired characteristics of $P2Y_{14}R$ antagonists, which will be used for rational planning of further modifications and will help to select the structures with optimized ADMET parameters that could provide improved efficacies *in vivo*.

The work was supported by the National Science Centre, Poland, under research project No. UMO-2021/43/D/NZ7/02190

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P87

The interactions of monomeric acridines and unsymmetrical bisacridines (UAs) with DNA duplexes

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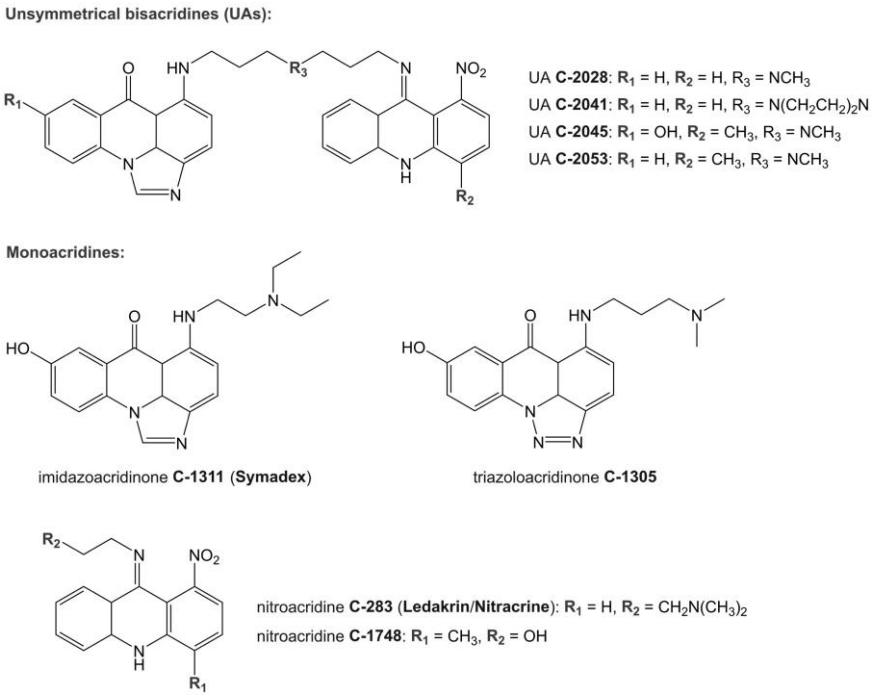

A novel class of anticancer compounds, exhibiting high antitumor activity, i.e., the unsymmetrical bisacridines (UAs), consist of two heteroaromatic ring systems. One of the ring systems is an imidazoacridinone moiety, with the skeleton identical to the structural base of Symadex (a.k.a. C-1311). The second one is a 1-nitroacridine moiety, hence it may be regarded as Nitracrine's (a.k.a. Ledakrin, C-283) structural basis. Ledakrin was the first Polish anticancer drug, introduced to clinical treatment in 1970's, whereas Symadex has successfully passed Phase I clinical trials and was recommended to Phase II several years ago. While constituting the unsymmetrical bisacridines, these monoacridine units are connected by an aminoalkyl linker, which vary in structure. In theory, these unsymmetrical dimers should act as double-stranded DNA (dsDNA) bis-intercalators, since the monomeric units (C-1311, C-283) were previously reported to exhibit an intercalating mode of binding into dsDNA. On the contrary, our earlier, preliminary studies have suggested that specific and/or structurally well-defined binding of UAs into DNA duplexes might not be the case. Therefore, we have revisited and carefully examined the dsDNA-binding properties of monoacridines C-1305, C-1311 (Symadex), C-283 (Ledakrin/Nitracrine) and C-1748, as well as bisacridines C-2028, C-2041, C-2045 and C-2053 using advanced NMR techniques, aided by molecular modelling calculations and the analysis of UV–VIS spectra, decomposed by chemometric techniques. These studies have contradicted some well-established paradigms regarding DNA-binding mode of nitroacridines and acridinones, hence allowed us to explain why the properties of UAs are not a simple sum of the features exhibited by the acridine monomers[1,2].

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This study was supported by Polish National Science Centre under grant No 2019/33/B/NZ7/02534.

P88

2-(Cyclopropanecarboxamido)pyridin-4-yl substituted benzenesulfonamides as new dual GSK3β and IKK2 kinase inhibitors for Alzheimer's disease therapy

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The lack of effective therapy for Alzheimer's disease forces scientists to challenge the problem. The multifactorial nature of AD opens the possibility for the search of multifunctional ligands, targeting key nodes of the disease pathomechanism. Among the interesting targets, we selected two enzymes: glycogen synthase kinase 3β (GSK3β) and IKK2 kinase. Under AD conditions, GSK3β is associated with pathological hyperphosphorylation of tau protein, resulting in the formation of toxic neurofibrillary tangles (NFTs), one of the causes of neuronal death [1]. IKK2 activation stimulates inflammatory gene expression in glial cells, thus being closely related to neuroinflammatory processes in the brain, and actively contributing to neuronal loss [2]. Based on the structures of known GSK3β and IKK2 inhibitors, we designed and synthesized novel dual inhibitors, derivatives of 4-substituted benzenesulfonamides with the *N*-(pyridin-2-yl)cyclopropanecarboxamide scaffold (Figure 1). We then assessed the inhibitory activity of compounds towards GSK3β and IKK2 using the Kinase-Glo^{TM} bioluminescent assay, revealing the nanomolar range of IC_{50} values for the most potent derivatives. To investigate the anti-neuroinflammatory potential of new compounds, we evaluated levels of selected markers (nitric oxide, TNF-α, and IL-6) in a standard model of neuroinflammation – lipopolysaccharide-stimulated BV-2 microglial cells [4]. Our new multifunctional ligands constitute a perfect starting point for further optimization and development of innovative anti-neuroinflammatory agents with the potential for testing in AD models.

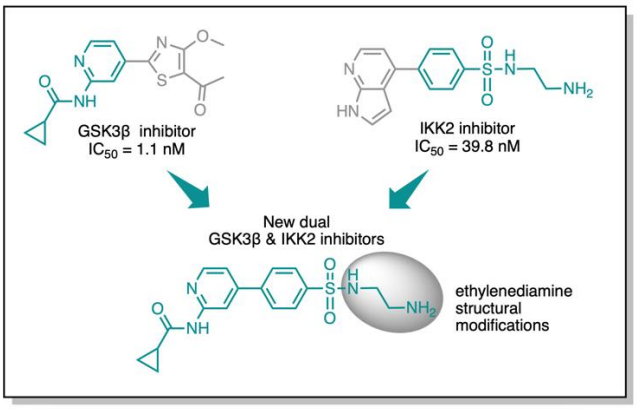

Figure 1. Series of new dual inhibitors of GSK3β and IKK2 kinases.

Acknowledgements: This research was funded by National Science Center, Poland grant No. 2019/34/E/NZ7/00090, and from Jagiellonian University Medical College subsidy No. N42/DBS/000308.

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P89

Non-steroidal CYP17A1 inhibitors with dual CYP/AKR activity against prostate cancer

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This research focuses on the synthesis and assessment of compounds that substitute the nitrogen-containing heterocyclic ring in the chemical structure of cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1) inhibitors [1] with a phenyl group containing a sulfur-based substituent. Initial screening identified compounds that inhibit CYP17A1 activity. Subsequently, the compounds' selectivity was tested against cytochrome P450 21-hydroxylase. Additionally, the compounds displayed mild inhibitory effects on aldo-keto reductase 1C3 (AKR1C3). The compounds' influence on steroid hormone levels was also evaluated, revealing synergistic modulatory effects. This research lays the foundation for the development of more potent dual inhibitors that specifically target both CYP17A1 and AKR1C3.

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P90

Isoxazole derivatives as new potential antimicrobial agents for non-healing wound treatment

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Staphylococcus aureus, *Pseudomonas aeruginosa* and *Candida albicans* are a leading cause of hospital-acquired infections. Non-healing wounds left untreated and improperly managed, can result in significant medical issues, including infection, sepsis even death of the patient. Chronic wounds are often co-colonised by *S. aureus* and *P. aeruginosa*, and once contaminated with *P. aeruginosa* the infected wound becomes highly resistant to treatment. Biofilm is present in 90% of chronic wounds and plays a key role in chronic wound infections [1]. Although, the use of topically administrated agents is substantial part of treatment, the microbial tolerance/resistance against clinically-used antiseptics is observed. Taking into account, there is still a need to search for new efficient agents. Among the wide range of pharmacologically important heterocycles, isoxazoles play a significant role in the field of medicinal chemistry. Emerging research interest on the isoxazole moiety results from fact that this moiety is a common synthetic building block in searching for new compounds exhibiting antimicrobial and antifungal activities [2]. Thus, the synthesis of new isoxazole derivatives is a very attractive aspect in the research and development field for both medicinal and organic chemistry. Due to these facts as well as to its relatively easy synthesis, isoxazole rings have become an object of our interest. Inspired by these facts we synthesized a series of isoxazole derivatives using different methods of functionalization such as Passerini multicomponent reaction and Michael addition. Then, compounds were examined against leading biofilm wound pathogens *S. aureus* and *P. aeruginosa*, and against *C. albicans* fungus. Obtained results showed that all isoxazole derivatives displayed antimicrobial activity and low cytotoxic effect, but antimicrobial activity of **PUB9** (2-(cyclohexylamino)-1-(5-nitrothiophen-2-yl)-2-oxoethyl 5-amino-3-methyl-1,2-oxazole-4- carboxylate) and **PUB10** (2-(benzylamino)-1-(5-nitrothiophen-2-yl)-2-oxoethyl 5-amino-3-methyl-1,2- oxazole-4-carboxylate), was noticeably higher compared to the other compounds. The **PUB9** and **PUB10** derivatives were able to reduce more than 90% of biofilm-forming cells, regardless of the species, displaying at the same time none or moderate cytotoxicity against fibroblasts and cytocompatibility against these wound cells. Taking into consideration the clinical demand for new antiseptic agents for non-healing wound treatment, the compounds **PUBX9** and **PUBX10** seem to be promising candidates to be further tested in more advanced animal models and later, if the satisfactory results are obtained, in the clinical setting[3].

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P91

The strategy for antimicrobials' search in the series of thieno[2,3-d]pyrimidine-6- and -4- carboxamides

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One of the promising strategies of antimicrobial drug development, with some problems and limitations [1] is the search for inhibitors for the bacterial protein targets, which in case of their whole cell high activity could even form a new class of antibiotic or antibacterials. One of these attractive targets is bacterial TrmD. Blockage of this enzyme leads to +1 frameshifting at the ribosomal translation stage of protein synthesis, which leads to misfunctioning of the outer membrane of Gram-negative bacterial cells.

In our research, we tried combining the molecular docking strategy with antimicrobial activity screening studies using the recently disclosed information about the structure of *Pseudomonas aeruginosa* TrmD [2]. Previously the authors [2] found the lead compounds in the series of thieno[2,3-d]pyrimidine-5-carboxami]des with benzylamide linker. We decided to carry out the docking studies and *in vitro* antimicrobial activity tests for the series of thieno[2,3-*d*]pyrimidine-6-carboxamides and also the series of thieno[2,3-d]pyrimidine-4-carboxamides. The last were made readily available due to the development of the highly effective procedure for synthesis of thieno[2,3-d]pyrimidine-4-carboxylic acids [3].

Among the tested compounds many showed high antimicrobial activity against the standard strain of *Pseudomonas aeruginosa* ATCC 27853, which well correlated with good or sufficient results of the docking studies.

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P92

A multi-step mechanochemical synthesis of compound PZ-1190 with potential antipsychotic properties

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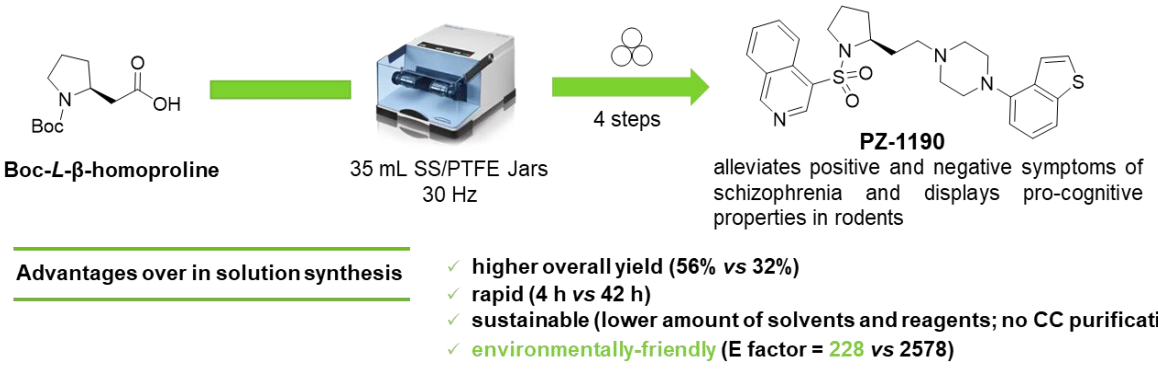
Mechanochemistry has been recently recognized as an efficient and green method for organic synthesis [1,2], providing an alternative to classical in-solution processes. Indeed, growing efforts from pharmaceutical industry and academia are focused on the development of sustainable procedures for the synthesis of pharmaceutically active ingredients (APIs) leading to coin the term medicinal mechanochemistry [3].
Extending the concept of medicinal mechanochemistry, we applied a solid-state approach for the synthesis of compound PZ-1190, a multi-target ligand for serotonin and dopamine receptors from a group of azinosulfonamide derivatives of arylpiperazines, with potential antipsychotic properties in rodents [4].
All mechanochemical reactions were performed using a vibratory ball-mill – Retsch Mixer Mill MM 400, operated at 30 Hz and 35 mL stainless steel jars equipped with one stainless steel ball. All of the reactions were carried out under air and ambient temperature.



The developed mechanochemical protocol offers several advantages over classical batch synthesis, including improvement of the overall yield (from 35% to 56%), reduction of reaction time (from 42 to 4 h), limitation of the use of toxic reagents and solvents, and the formation of by-products. All synthesized intermediates and final compound PZ-1190 were isolated with high purities by simple extraction, without the need for column chromatography purification. Of note, the unexplored mechanochemical reduction of carboxylic function of *N*-protected α and β-amino acids was successfully performed in high yields (84–91%). The advantages of the mechanochemical strategy over classic thermal methods were finally confirmed by the assessment of the green chemistry metrics Ecoscale (< 50 *vs* > 70) E factor (2578 *vs* 228).
To sum up, the obtained results confirm relevance of mechanochemistry as a sustainable and efficient method for the synthesis of biologically active compounds for preclinical development.

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P93

GPCR-target search for a new library of ligands

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Drug discovery and development process is an extremely costly and time-consuming area of pharmacy. The use of computer-aided drug design (CADD) makes it possible to significantly accelerate the work on a new drug and streamline research at an early stage. Nowadays, *in silico* research is at the core of any drug development process.

In this work, a library of structurally various ligands obtained by Prof. Dr. Vitalii Palchykov, was examined for their predicted biological activity on the G protein-coupled receptor (GPCR) family. Possible biological targets of the new ligands were predicted using a ligand-based method and the results of these predictions were validated using molecular docking workflow. Selected complexes were then subjected to molecular dynamics (MD) simulations. Biological affinity of the most promising ligands will be examined in further *in vitro* assays.

P94

Structural evaluation and preliminary anticancer studies of newly synthesized 5-(fluorophenyl)-1,3,4-oxadiazol-2-amine derivatives

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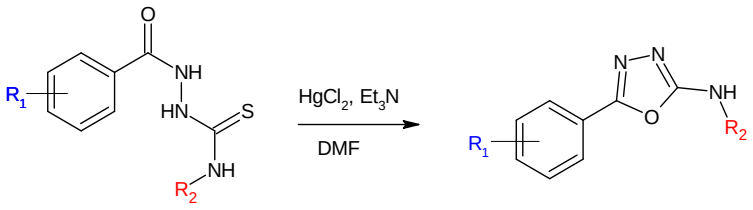
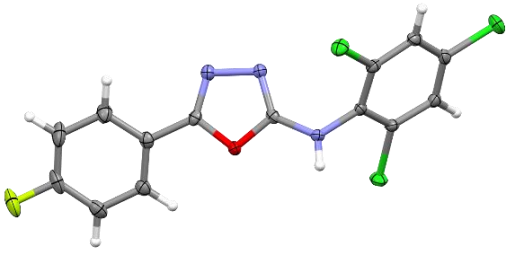
Core structures were chosen based on experience gained in working with similar heterocyclic derivatives in previous research [1-2] and scientific reports showing their cytotoxic potential. Final derivatives were synthesized via desulfurization-cyclization of suitable thiosemicarbazides (**Scheme 1**) [1,3]. Structures were investigated using NMR techniques, and X-Ray analysis.



Scheme 1. Synthesis of 5-(fluorophenyl)-1,3,4-oxadiazol-2-amine derivatives.

X-ray diffraction experiment was performed for 5-(4-fluorophenyl)-N-(2,4,6-trichlorophenyl)-1,3,4-oxadiazol-2-amine. The analyzed derivative (**Fig 1.**) crystallizes in the monoclinic system, space group $P2_1/n$, with four molecules in the unit cell.

Figure 1. Perspective view of 5-(4-fluorophenyl)-N-(2,4,6-trichlorophenyl)-1,3,4-oxadiazol-2-amine molecular structure.



A preliminary evaluation of cytotoxicity was done by the MTT method, showing the high activity of synthesized compounds on used cancer cell lines. Three of the 24 synthesized compounds are showing high activity ($IC_{50} \leqslant 10$ μM) on used cancer cell lines. Final results will be presented during the conference.

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Regioselective synthesis of new (2-imino-4-oxo-1,3-thiazolidin-5-yl)acetic acid derivatives. Identification of possible isomers and their biological activity.

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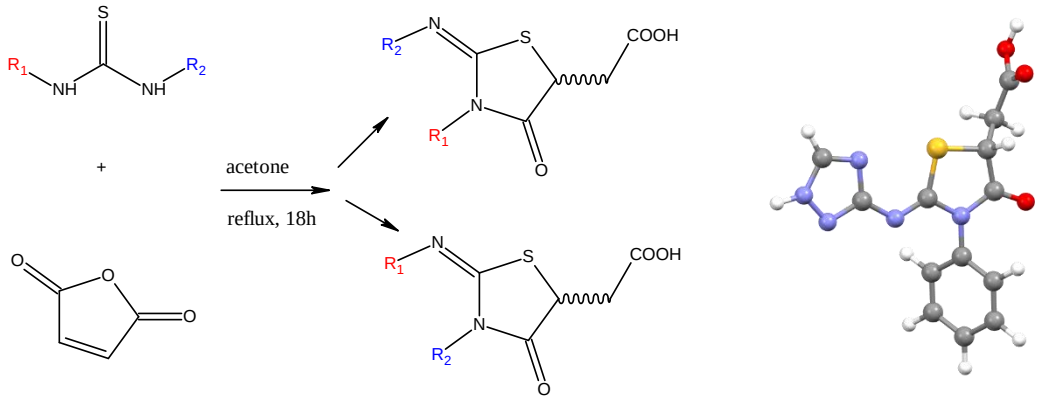
Recent studies confirmed that 1,3-thiazolidin-5-yl derivatives may possess broad cytotoxic effects [1-5]. The group of new compounds with a skeleton structure of 1,3-thiazolidin-5-yl has been obtained, using three different heterocyclic scaffolds. The chosen synthesis method is depicted in **Scheme 1**.



Scheme 1. Synthesis of novel compounds and example perspective view of {(2Z,5S)-4-oxo-3-phenyl-2-[(1H-1,2,4-triazol-3-yl)imino]-1,3-thiazolidin-5-yl}acetic acid.

Structures were confirmed by NMR. Afterward, preliminary anticancer tests were conducted with the usage of the MTT method to establish IC_{50} (μM) values [the concentration of the compound that corresponds to a 50% growth inhibition of the cell line (as compared to the control) after the cells were cultured for 72 h with the individual compound]. All 23 synthesized derivatives were screened for their *in vitro* cytotoxic properties towards a panel of cancer and normal cell lines. Two of examined compounds showed high activity against non-small-cell lung cancer line A549, human primary colon cancer SW60 and human breast cancer MDA-MB-231. The 1,3-thiazolidin-5-yl derivatives with the highest potency in the MTT assay will be transferred for further studies. Details related to research will be disclosed in the publication.

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P96

Assessment of toxicity of indoximod - novel potential candidate against colon cancer

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Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide. Despite groundbreaking discovery in oncology that has been made in past years, the CRC effective treatment of CRC is still unavailable [1]. Recently it was postulated that tumor cells acquire the ability to avoid immune mechanisms which leads to their increased growth. One of these mechanisms is a modification of tryptophan (TRP) metabolism via the kynurenine pathway (KP). It has been reported that cancer cells secrete TRP catabolizing enzymes, such as indoleamine 2,3-dioxygenase (IDO1, IDO2) and tryptophan 2,3-dioxygenase (TDO2), which drive the formation of an immunosuppressive microenvironment [2]. Due to that fact inhibitors of this pathway – e.g. indoximod becomes a new agent that can be used in an antitumor treatment. Indoximod acts as an IDO1/TDO2 inhibitor and restores immune defense response [3]. The aim of the study was to evaluate toxicity of indoximod using the zebrafish model and cell viability assay. The FET (Fish Embryo Toxicity) was conducted with modifications [4], whereas cytotoxicity of indoximod was estimated by MTT assay. New fertilized zebrafish embryos (0-2) Hpf or 72 hpf larvae were transferred to 24-well plates filled with standard medium and series of concentrations of indoximod (100, 300, 1000 μM). Every 24 h indicators of lethality, early spontaneous movement, hatching rate, additional development alteration, and embryo malformations were observed. In case of embryos and larvae 24, 48,72 and 96 h after treatment the survival rate, heart rate, total body length and morphological deformities were examined. Survival rate was significantly lowered at concentration of 1000 μM in zebrafish embroys and larvae. Additionally, at concentration of 1000 μM the most prominent effects on the embryo and larvae phenotypic features was observed (spinal scoliosis, pericardial and yolk sack edema and tail curvature) (***$p<0.001$). Exposure to indoximod did not affect the embryo hatching rate, cardiac function, and total body length. Moreover, the effect of indoximod on the viability of colon adennocarcinoa cell line DLD-1 assessed by MTT assay showed no cytotoxic potency on cells. Our study suggests that indoximod has the potential to be considered as a safe and well-tolerated anticancer formulation, and seems to be a promising candidate for future treatment of patients with colon cancer.

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P97

Dialkyl disulfide derivatives of 6-mercaptopurine – synthesis, physicochemical characterization, and conjugation with nanoparticles

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Introduction: Currently, pharmaceutically active ingredients (APIs) within the group of purines constitute the essential drugs in the treatment of cancers, autoimmune diseases, viral and parasitic infections. One of the most important APIs in this regard is 6-mercaptopurine and its prodrug azathioprine. Besides critical therapeutic applications, these molecules also reveal some limitations, including short plasma half-time, low bioavailability, gastrointestinal, hepatic, and bone marrow toxicity, narrow therapeutic index, and a strong first-pass effect. The knowledge gathered about purine APIs is a driving force for further research. In recent years, the studies of new purine analogs with antiparasitic activity and the use of nanotechnology in overcoming the therapeutic limitations of these compounds have been undertaken [1], [2].
Aim: The main aim of the study was to synthesize and characterize new 6-mercaptopurine derivative of potential anti-leukemic and immunomodulatory properties.
Methods: 6-Mercaptopurine was methylated in the presence of methyl iodide and alkaline conditions. 2,2'-Disulfanediylbis(ethan-1-ol) was acetylated, purified by extraction, and subjected to the conjugation reaction with 6-methylmercaptopurine using a cross-dehydrogenative coupling approach [3]. The final product was purified by flash column chromatography. The chemical identity of all products was confirmed by NMR spectra. Functionalization of titanium dioxide nanoparticles with novel 6-mercaptopurine derivative was performed using the following steps: mixing, centrifuging, and drying. The characterization of modified titanium oxide material was performed using FT-IR spectra. The pharmacokinetic and physicochemical parameters of the obtained derivative and its possible metabolites were evaluated using the SwissADME tool [4].
Results: The synthesis and physicochemical characterization of 7-[2-acetoxy-1-(2-acetoxyethyldisulfanyl)ethyl]-6-methylsulfanylpurine with a yield of 33% was performed and was accompanied by the successful deposition of this molecule on titanium dioxide nanoparticles surface. A number of physicochemical and pharmacokinetic parameters were assessed for the new compound using the SwissADME tool.
Conclusions: The collected data will allow the continuation of the study on 7-[2-acetoxy-1-(2-acetoxyethyldisulfanyl)ethyl]-6-methylsulfanylpurine and the prospective synthesis of other 6-mercaptopurine disulfide derivatives. The assessment of the biological activity of novel molecule alone and in combination with nanoparticles is envisaged. Computational studies using SwissADME showed that the compound complies with Lipinski and Ghose's rule of five.

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