5th Mini Symposium of Section of Medicinal and Pharmaceutical Chemistry

TUESDAY, NOVEMBER 30TH, 2021, 10 A.M.

on-line via ZOOM

Sponsor:

Moderators: Maja Beus, PhD & Đani Škalamera, PhD	
Morning Session	
10:00	***Opening Ceremony*** ***Vesna Gabelica Marković, PhD, Head of Section of Medicinal and Pharmaceutical Chemistry*** ***Adrijana Vinter, PhD, Managing Director and President of the Management Board, Fidelta***
10:10	**Manuela Panić**, Novel, Environmentally-friendly and Tunable Solvents for Application in the Pharmaceutical Industry, *Faculty of Food Technology and Biotechnology*, *University of Zagreb*
10:30	**Tomislav Stolar**, Discovery and Control of Vitamin C and B3 Cocrystal Polymorphism on Different Scales by Mechanochemistry, *Ruđer Bošković Institute, Zagreb*
10:50	**Silvio Jakopec**, Heterobimetallic Transition Metal Complexes of 1,2,3-Triazole Ligands, *Faculty of Chemical Engineering and Technology, University of Zagreb*
11:10	**Silvija Šafranko**, The Preparation and Modification of the Biomass-derived Carbon Quantum Dots – Investigating their Potential in Biomedical and Pharmacological Application, *Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek*
11:30	**Nikola Topolovčan**, Catalytic Approaches to Diverse Biologically Active Compounds, *Ruđer Bosković Institute, Zagreb*
11:50	**Gabrijel Zubčić**, C-H Amination Reactions via Radical Pathway; Repurposing Hofmann-Löfller-Freytag Reaction, *Faculty of Pharmacy and Biochemistry, University of Zagreb*
12:10	***End of Morning Session***
Afternoon Session	
14:00	**Hrvoje Rimac**, The Complementarity Principle in Docking Procedures: Evaluation of the Correctness of Binding Poses, *Faculty of Pharmacy and Biochemistry, University of Zagreb*
14:20	**Tana Tandarić**, Irreversible Inhibition of the Monoamine Oxidase B enzyme. A Computational Insight, *Ruđer Bošković Institute, Zagreb*
14:40	**Alojzije Brkić**, A New Insight into Mupirocin Resistance of Bacterial Isoleucyl-tRNA Synthetases, *Faculty of Science, University of Zagreb*
15:00	**Doris Crnčević**, Synthesis and Antibacterial Activities of New Amidoquinuclidines and Their Quaternary Salts, *Faculty of Science, University of Split*
15:20	**Martina Mušković**, First *In Vivo* Positron Emission Tomography Biodistribution Study of [^{68}Ga]Gallium Radiolabelled Amphiphilic Cationic Porphyrins with Potential Applications in Photodynamic Therapy, *Department of Biotechnology, University of Rijeka*
15 :40	***Break***
15:50	**Kristina Pavić**, Harmirins, Novel Harmine–Coumarin Hybrids as Potential Anticancer Agents, *Faculty of Pharmacy and Biochemistry, University of Zagreb*
16:10	**Ana Ratković**, Photochemical Synthesis and Functionalization of Benzobicyclo [3.2.1]octadienes as Potential Cholinesterase Inhibitors, *Fidelta Ltd., Zagreb*
16:30	**Mateja Toma**, Cytotoxic Activity of Ferrocene-substituted Purines Against Several Cancer Cell Lines, *Faculty of Pharmacy and Biochemistry, University of Zagreb*
16 :50	**Iva Zonjić**, Recognition of DNA:RNA Hybrid and Triplex Structures by a Series of Benzothiazole Ligands, *Ruđer Bošković Institute, Zagreb*
17:10	***Closing remarks***

Novel, Environmentally-friendly and Tunable Solvents for Application in the Pharmaceutical Industry

Manuela Panić*, Martina Bagović, Mia Radović, Marina Cvjetko Bubalo, Kristina Radošević, Ivana Radojčić Redovniković

University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia

*e-mail: mpanic@pbf.hr

Over the past decade, deep eutectic solvents (DES) have become promising solvents from both environmental and technological perspectives. The properties that have gained them the environmentally friendly label are nonvolatility (reduced air pollution), nonflammability (process safety), and excellent stability (potential for recycling and reuse). The number of structural combinations encompassed by DES is tremendous, thus it is possible to design DES with unique physicochemical properties for a particular purpose such as the design of solvents for efficient extraction of biologically active compounds or biocatalytic process. In the biomedical field, it has been reported that DES as novel solvents could improve the solubility, permeability, and absorption of active pharmaceutical ingredients (API). Furthermore, incorporation of API, as one of the components of a eutectic mixture, in so-called therapeutic deep eutectic solvent (THEDES) is also a promising approach for designing new drug formulations with better pharmacological properties. Over the last several years it was shown that pharmaceutical agents (anti-inflammatory, anti-fungal, and antiseptic APIs) can be formulated into liquids as THEDES which retain the activity of the API itself and present novel, more effective drug delivery systems. Furthermore, the preparation of the THEDES systems yields a 100% pure product, with no losses during production and no need for subsequent purification steps. Also, this approach is, due to its simplicity, relatively straightforward when it comes to the scale-up of the process. Although current literature search on that topic indicates that DES formulations have the potential to increase the solubility, permeation, and absorption of the APIs, which is of outstanding importance for the pharmaceutical industry, there is still plenty of work and research to be done, such as finding the proper DES to obtain a solubility-enhancing formulation of API, what is crucial for achieving better bioavailability of poorly soluble drugs.

Therefore, the aim of this work was to prepare 3 different THEDESs containing APIs - ciprofloxacin, acetyl-ciprofloxacin or ciprofloxacin-2-methylpentanoyl by (i) DES design for better solubility of API, and to conduct (ii) stability, permeability and bioavailability studies of prepared THEDES.

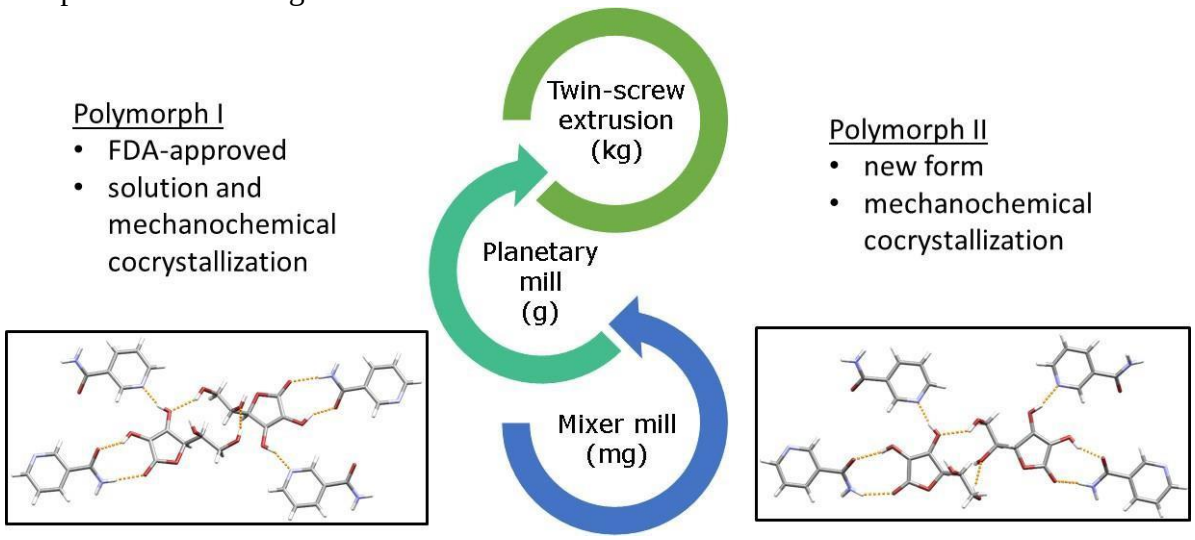
Discovery and Control of Vitamin C and B3 Cocrystal Polymorphism on Different Scales by Mechanochemistry

Tomislav Stolar[a,*], Stipe Lukin[a], Bahar Karadeniz[a], Gordana Matijašić[b], Zvonimir Katančić[b], Igor Dejanović[b], Krunoslav Užarević[a]

[a] Ruđer Bošković Institute, Zagreb, Croatia
[b] University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia

*e-mail: tomislav.stolar@irb.hr

We demonstrate a controllable mechanochemical synthesis of cocrystal polymorphs of L-ascorbic acid (vitamin C) and nicotinamide (vitamin B3) on different scales and without using bulk solvents [1]. Next to the previously described polymorph of the 1:1 cocrystal, which is one of the first cocrystals approved for human consumption, we report here a new, thermodynamically more stable polymorph detected during in situ synchrotron powder X-ray diffraction monitoring of ball milling reactions. The new polymorph is currently available exclusively by mechanochemical synthesis, and its crystal structure was determined from synchrotron powder X-ray diffraction data. Laboratory *in situ* monitoring by Raman spectroscopy provided direct insight into the cocrystal polymorphs formation and was further used to optimize the manufacturing procedure. Sub gram synthesis using a laboratory mixer mill was transferred to the 10 g scale on a planetary ball mill and continuous manufacturing using a twin-screw extruder. Cocrystal polymorphs perform excellently in tabletting, thus alleviating the notoriously poor compactible properties of vitamin C, while maintaining its antioxidant properties. This study shows the utility of mechanochemical techniques that follow the principles of green chemistry, for the research and development of active pharmaceutical ingredient solid forms.



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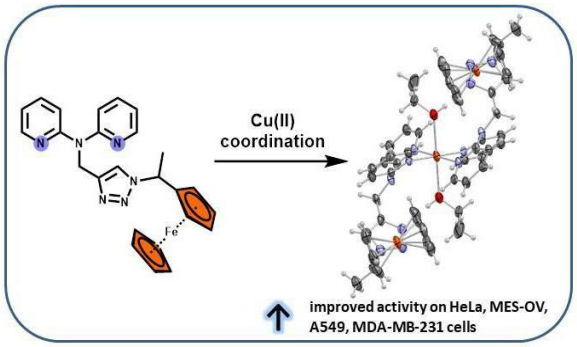
Heterobimetallic Transition Metal Complexes of 1,2,3-Triazole Ligands

Silvio Jakopec[a,*], Natalija Pantalon Juraj[b], Anamaria Brozovic[b], Gilles Gasser[c], Berislav Perić[b], Srećko I. Kirin[b], Silvana Raić-Malić[a]

[a] University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia
[b] Ruđer Bošković Institute, Zagreb, Croatia
[c] Institute of Chemistry for Life and Health Sciences, Chimie ParisTech, PSL University, Paris, France

*e-mail: sjakopec@fkit.hr

Organometallic and metal-based complexes have been widely used in drug discovery and some of them have entered clinical trials for the treatment of cancer. Design of new chemotherapy agents based on metal complexes is a fast-growing research area in which the biological activity of antitumor agents is based on metal-specific modes of action [1]. The ferrocene pharmacophore is often introduced into the structure of drug-like molecules and bioactive hybrids with the aim of improvement of physicochemical properties and potency. In continuation of our research on coordination of mono- and bis-1,2,3-triazole derivatives[2], we have synthesized and characterised ferrocene ligands as well as their Zn(II) and Cu(II) complexes by NMR, IR and UV-Vis spectroscopy and SC-XRD. Cytotoxic activity of *N*-heterocyclic ferrocene derivatives and their Zn(II) and Cu(II) complexes was evaluated.



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The Preparation and Modification of the Biomass-derived Carbon Quantum Dots – Investigating their Potential in Biomedical and Pharmacological Application

Silvija Šafranko*, Stela Jokić

Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

*e-mail: silvija.safranko@ptfos.hr

In recent years, agro-industrial waste valorization has gained significant interest in their reuse, both from an economical and environmental aspect. The research on biowaste valorization for sustainability is mainly focused on adequate waste managing/disposal and conversion of wastes into useful materials. Carbon quantum dots, an emerging class of zero-dimensional carbonaceous nanomaterials have gained considerable attention owing to their unique physicochemical and optical properties, biocompatibility, exhibiting also good fluorescent properties. Due to their good chemical and optical stability, solubility in water, stability in a wide range of pH and high ionic strength media, carbon dots are extensively studied in a broad range of applications, especially in pharmaceutical and biomedical purposes. A biomass waste, as a green, cheap, abundant, easily available and more importantly rich source of carbon, has been considered as a potential and promising precursor for carbon quantum dots obtaining, applicable for bioimaging, biosensing, and environmental monitoring purposes.

The aim of this study is to present a novel approach to waste utilization and valorization toward obtaining biocompatible carbon quantum dots with full potential in metal ion sensing, cellular imaging, and as fluorescent ink. New approaches in the preparation and material modification toward properties enhancement will be also discussed. A special emphasis will be given on the particle size control, surface modification, and chemical composition which in general defines their pharmacological and biological activity (specific antitumor effect and antioxidative activity). Finally, some of the challenges and future outlooks in carbon quantum dots research will be briefly outlined.

Acknowledgements: This work has been supported by the Croatian Science Foundation under the project “Application of innovative techniques of the extraction of bioactive compounds from by-products of plant origin” (UIP-2017-05-9909).

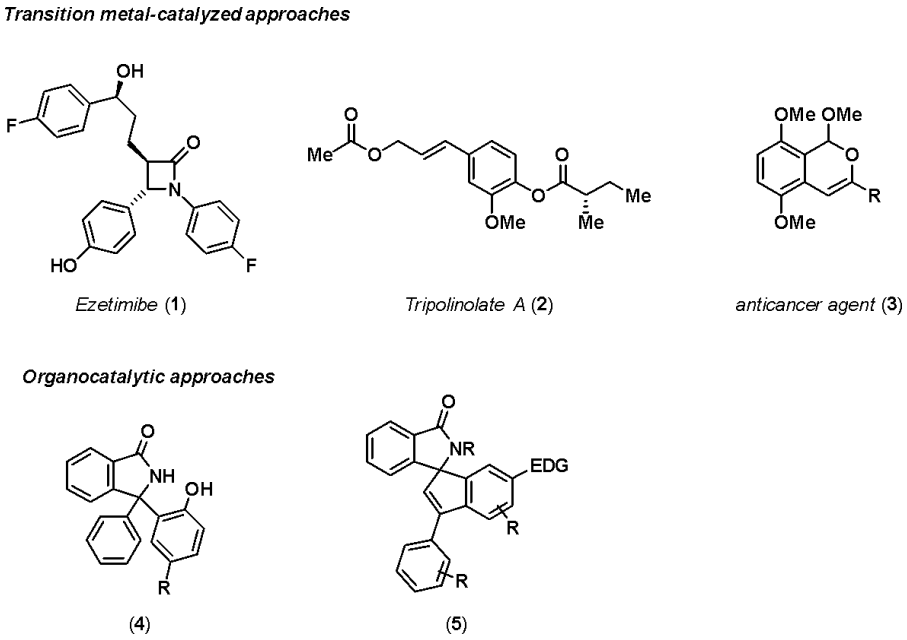
Catalytic Approaches to Diverse Biologically Active Compounds

Nikola Topolovčan*

Ruđer Bosković Institute, Zagreb, Croatia

*e-mail: ntopolov@irb.hr

The development of practical and straightforward routes for the production of medicinally important substances have been a long-standing challenge for synthetic chemists. Endeavours to shorten the synthesis in an atom-economic and cost-effective manner resulted in implementation of catalytic strategies that circumvent the neccessity for sometiomes tedious functional group interconversions. In this line, protocols based on transition metal-catalysis for the synthesis of strong cholesterol absorption inhibitor ezetimibe (**1**), inhibitor of proliferation of glioma and colorectal cancer cells, tripolinolate A (**2**), and isochromene (**3**) exhibiting antitumor activity against breast cancer will be discussed. Parallel to these efforts the recent progress in organocatalytic synthesis of α-triphenylmethylamines (**4**) and spiroisoindolinone indenes (**5**), which are highly valued structural motifs in biologically active compounds and chemical sensors will be reported as well.



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C-H Amination Reactions via Radical Pathway; Repurposing Hofmann-Löfller-Freytag Reaction

Gabrijel Zubčić*, Marijan Marijan, Davor Šakić

University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

*e-mail: gzubcic@pharma.hr

Modern synthetic approaches try to incorporate already known chemical entities and then modify them using late-stage functionalization (LSF) procedures. Hofmann–Löffler–Freytag (HLF) reaction was originally discovered by Hofmann [1–3] in synthetic studies of unconventional *N*-halo amines. This reaction has the ability to functionalize inactivated C-H bonds and thus belongs in the LSF toolbox. Recently, Del Castillo and Muñiz [4] have modified the HLF reaction for stereoselective nicotine synthesis. Their modification implies an experimental trial and error approach. On the other hand, the quantum chemical approach provides the means for the theoretical determination of parameters that guide the intramolecular HAT step which constitutes the essence of the HLF reaction as well as evaluating the role of the substituents. Therefore, emphasis is set on these parameters, which include the stability of *N*- and *C*-centered radicals and the activation barrier. In pursuit after them, N-H bond dissociation energies and related stability values of *N*-centered radicals, as well as stability values of *C*-centred radical and corresponding C-H bond dissociation energies are calculated, and with the implementation of the Bell-Evans-Pollany principle, the corresponding activation barriers for the crucial HAT step is obtained. Finally, particular attention is given to prevalent organic solvents that may have an effect on the stability of radicals besides themselves participating in rearrangement steps. Establishing a clear picture of the underlying parameters and the role that substituents have on the crucial step may streamline HLF reaction in accordance with green chemistry principles.

References

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The Complementarity Principle in Docking Procedures: Evaluation of the Correctness of Binding Poses

Hrvoje Rimac[a,*], Maria Grishina[b], Vladimir Potemkin[b]

[a] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[b] South Ural State University, Higher Medical and Biological School, Chelyabinsk, Russian Federation

*e-mail: hrimac@pharma.hr

Even though the first docking procedures were developed almost 40 years ago, they are still under intense development, alongside with their validation methods. We are proposing the use of the quantum free-orbital AlteQ method in evaluating the correctness of ligand binding poses and their ranking. The AlteQ method calculates the electron density in the interspace between the ligand and the receptor and, in this way, it evaluates the quality of contacts between the ligand and the receptor, bypasses the drawbacks of using ligand RMSD as a measure of docking quality, and can be considered as an improvement of the "fraction of recovered ligand−receptor contacts" method.

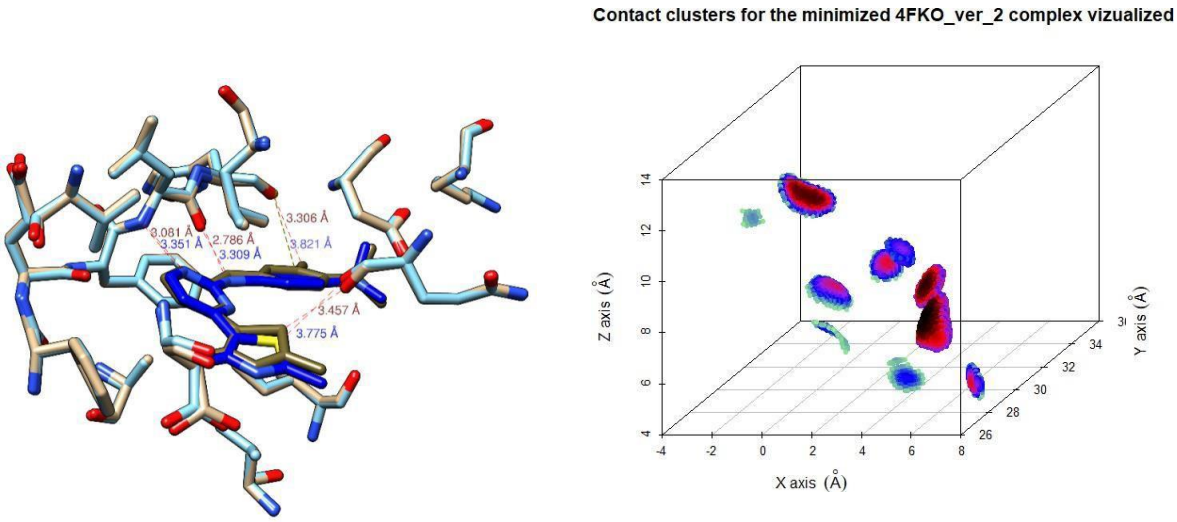


Figure 1. Left: Superimposition of the minimized (brown and tan) and best docked (dark and light blue) 4FKO complexes. Right: Contact clusters for the minimized 4FKO complex visualized in 3D space.

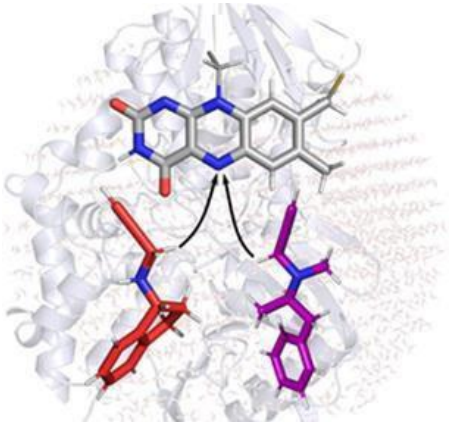
Irreversible Inhibition of the Monoamine Oxidase B enzyme. A Computational Insight

Tana Tandarić*, Robert Vianello

Ruđer Bošković Institute, Zagreb, Croatia

*e-mail: tana.tandaric@irb.hr

Monoamine oxidase B (MAO B) is a flavoenzyme [1] responsible for the regulation of levels exogenic and endogenic amine in the human body, including amine neurotransmitters in the brain, which is why it represents a crucial pharmacological target for treating Parkinson's and Alzheimer's diseases. This computational investigation elucidates the mechanism of the irreversible MAO B inhibition with clinical propargylamine inhibitors, rasagiline and selegiline [2], and their derivatives desmethyl-selegiline and methyl-rasagiline. The quantum-chemical analysis within the cluster model showed that this reaction proceeds in three steps, with the rate-limiting abstraction of the inhibitor's α-methylene H^- anion by FAD in the first step [3].



The obtained results are in excellent agreement with experimental observations. The proposed mechanism is further characterized by the Empirical Valence Bond approach on the entire enzyme structure, whereas molecular dynamics simulations identified residues crucial for the binding. The offered insight provides important guidelines for the development of new and more effective MAO B inhibitors [4].

Acknowledgements**:** We thank the University of Zagreb Computing Centre (SRCE) for granting computational resources on the ISABELLAcluster.

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A New Insight into Mupirocin Resistance of Bacterial Isoleucyl-tRNA Synthetases

Alojzije Brkić[a,*], Marc Leibundgut[b], Nenad Ban[b], Ita Gruić Sovulj[a]

[a] University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia
[b] Institute of Molecular Biology and Biophysics, ETH Zurich, Zurich, Switzerland

*e-mail: alojzije.brkic@chem.pmf.hr

Ribosomal protein biosynthesis is a key cellular process because proteins are the main cellular building blocks on all scales of life. Ensuring substrates for protein biosynthesis, evolutionarily conserved aminoacyl-tRNA synthetases (aaRSs) covalently couple amino acids to cognate tRNAs. This makes aaRSs important targets for natural and man-made antibiotics. A good example are isoleucyl-tRNA-synthetases (IleRS) which are susceptible to inhibition with mupirocin, a naturally produced antibiotic from the bacterium *Pseudomonas fluorescens*. Bacterial IleRSs cluster into two clades differing in susceptibility to mupirocin inhibition. IleRS1, present in most pathogenic bacteria, are up to 9 orders of magnitude more susceptible to mupirocin inhibition than bacterial IleRS2, which share similarities with eucaryotic IleRS, thus making IleRS1 favorable pharmaceutical targets. To investigate the origin of mupirocin insensitivity in IleRS2, crystal structures of IleRS2, and IleRS1 in a complex with mupirocin and a nonhydrolyzable analog of the reaction intermediate, Ile-AMS, were solved. *Bacillus megaterium* was chosen because it has genomic copies of both IleRS1 and IleRS2. By analyzing the solved structures we have shown that mupirocin binding to the two IleRS types, although similar, differs in the number of established mupirocin interactions and steric rearrangements which coincide with mupirocin binding. In IleRS2, mupirocin lacks two active site interactions compared to IleRS1 and the binding coincides with steric rearrangements of key active site parts. In contrast, the IleRS1 active site seems preformed for high-affinity mupirocin binding, with only a little steric rearrangement taking place upon the mupirocin binding. Thus, we propose a mechanism wherein the differences between mupirocin resistance of IleRS1 and IleRS2 are not only correlated to the differences in the interactions mupirocin establishes in the active site, but also to the steric rearrangements (or lack thereof) of the IleRS active site that accompany mupirocin binding.

Synthesis and Antibacterial Activities of New Amidoquinuclidines and Their Quaternary Salts

Doris Crnčević*, Antonio Sabljić, Renata Odžak, Matilda Šprung

University of Split, Faculty of Science, Department of Chemistry, Split, Croatia

*e-mail: dcrncevic@pmfst.hr

In recent years, the demand for development of new and more effective antiseptics and disinfectants has risen. One reason for this is the COVID-19 pandemic and the widespread use of these formulations provoking higher incidence of bacterial resistance in future years [1].
Quaternary ammonium compounds (QACs) have been shown to be effective antimicrobial candidates that tend to disrupt the bacterial membrane, leading to leakage of cytoplasm and subsequent cell lysis [2]. We and others have shown that natural products could be used as chemical scaffolds for quaternization to yield QACs of potent antimicrobial activities [3,4]. Here we report the new series of such natural scaffold-guided structures containing amide bond as a potential substrate for protease degradation. The introduction of amide bond might result in biodegradable QACs, hence variants that have much shorter lifetime in the environment. The 3-aminoquinuclidine was substituted with alkyl chains of different length (10, 12 and 14 C-atoms) to yield precursors for quaternization (Figure 1). The quaternization was performed with appropriate alkyl halide reagents to obtain amidoquinuclidine QACs in good yields (Figure 2). The minimum inhibitory concentrations (MICs) were determined for both, amidoquinuclidines and their QACs, against a panel of Gram-positive and Gram-negative bacteria. As expected, the activity of the QACs was significantly better than for corresponding amidoquinuclidines which is in good correlation with calculated lipophilicity coefficients (cLogP). Our results suggest that amidoquinuclidine QACs could be good antibacterial candidates. The future experiments will elucidate weather these candidates are substrates for protease degradation.

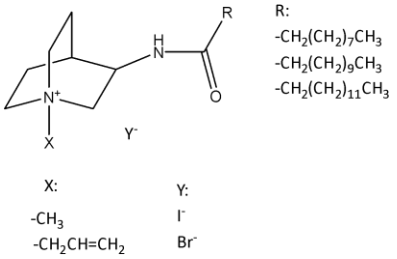

Figure 1. Structures of synthesized amidoquinuclidines

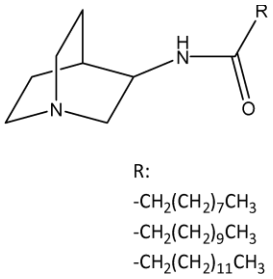

Figure 2. Structures of synthesized amidoquinuclidine QACs

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First *In Vivo* Positron Emission Tomography Biodistribution Study of [^{68}Ga]Gallium Radiolabelled Amphiphilic Cationic Porphyrins with Potential Applications in Photodynamic Therapy

Martina Mušković[a], Juozas Domarkas[b], John D Wright[b], Nela Malatesti[a,*], Ross W Boyle[c], Steve J Archibald[b]

[a] University of Rijeka Department of Biotechnology, Rijeka, Croatia
[b] University of Hull, Positron Emission Tomography Reseach Centre, Hull, United Kingdom
[c] University of Hull Department of Chemistry, Hull, United Kingdom

*e-mail: nela.malatesti@biotech.uniri.hr

Amphiphilic porphyrins, quaternised to increase water solubility and conjugated with alkyl chains to facilitate cellular uptake, are being intensively investigated as photosensitisers for photodynamic therapy (PDT) [1]. However, pharmacokinetic profiles and *in vivo* biodistribution of this type of porphyrin is not well studied.

Known as good metal ion chelators, porphyrins can be easily labelled with radiometals allowing real time monitoring of their biodistribution using positron emission tomography (PET) [2]. PET is a nuclear imaging technique utilising biologically active molecules labelled with positron emitting radionuclides, such as [^{18}F]fluorine or [^{68}Ga]gallium. The emitted positron undergoes rapid annihilation with an electron, generating two coincident 511 keV gamma rays at ca. 180º, which can be detected by a PET scanner [3]. [^{68}Ga]Gallium has a short nuclear decay half-life of 68 min, compatible with a biological half-life of fast biodistributing small molecules, and is conveniently available from a ^{68}Ge/^{68}Ga generator [4].

In this work, we designed, synthesised and labelled with [^{68}Ga] or [^{nat}Ga]gallium a group of four tricationic porphyrins carrying variable length alkyl chains (C1, C10, C14, C18), and investigated their properties, such as lipophilicity (logD), metal chelate stability, serum stability and formulation properties (with bovine serum albumin (BSA) and low density lipoprotein (LDL)). *In vivo* biodistribution was studied by PET imaging in naive mice. Studies in a tumour bearing mouse model are on-going.

References:

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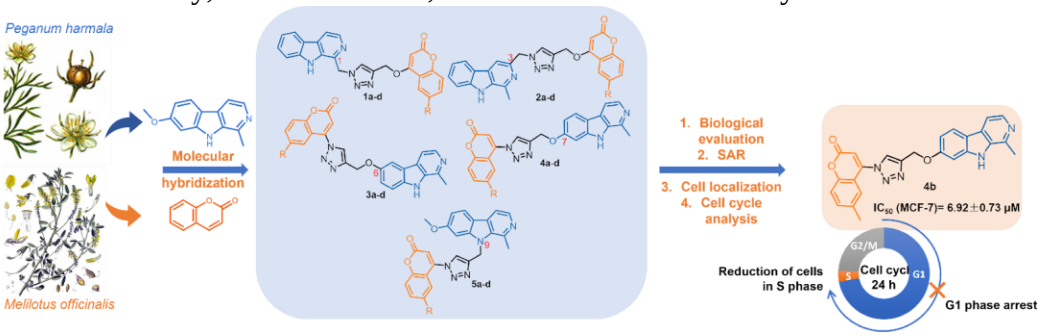
Harmirins, Novel Harmine–Coumarin Hybrids as Potential Anticancer Agents

Kristina Pavić[a*], Maja Beus[a], Goran Poje[a], Lidija Uzelac[b], Marijeta Kralj[b], Zrinka Rajić[a]

[a] University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[b] Ruđer Bošković Institute, Zagreb, Croatia

*e-mail: kpavic@pharma.hr

Novel anticancer agents are constantly needed as cancer remains one of the greatest global health burdens. As a continuation of our work on harmine derivatives, we have prepared five series of harmirins – novel hybrid molecules of 1,2,3-triazole-type comprising harmine and coumarin pharmacophores (Figure). Harmine, a β-carboline representative, and coumarins are two important classes of natural products both possessing anticancer properties. Here, we report their synthesis, antiproliferative activity, cell localization, and influence on the cell cycle.



Harmirins were obtained by applying the standard Cu(I) catalyzed azide-alkyne cycloaddition. Their antiproliferative activity was evaluated *in vitro* against four human cancer cell lines (HepG2, SW620, HCT116, MCF-7) and one human non-cancer cell line (HEK293T). Harmirins **3** and **5** with the smallest substituents (H or F) on the coumarin ring showed the highest cytotoxicity against all tested cell lines. SAR analysis revealed that seven harmirins display activities in the single-digit micromolar range against MCF-7 and HCT116. Among them, harmirins **2b** and **4b**, substituted at *C*-3 and *O*-7 of the β-carboline core and bearing methyl substituent at the position 6 of the coumarin ring, were the most active compounds with the highest selectivity towards cancer cells, in comparison to HEK293T. Harmirin **4b** and MCF-7 cell line were chosen for further investigation. Cell localization experiments demonstrated that **4b** remains exclusively in the cytoplasm. Furthermore, cell cycle analysis has shown that the treatment of MCF-7 cells with harmirin **4b** induced a strong G1 arrest, accompanied by a drastic reduction in the percentage of cells in the S phase. These results might suggest that harmirin **4b** exerts its antiproliferative activity through inhibition of DNA synthesis, rather than DNA damage. Our future experiments will focus on the elucidation of molecular mechanisms involved in the anticancer activities of harmirins.

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Photochemical Synthesis and Functionalization of Benzobicyclo[3.2.1]octadienes as Potential Cholinesterase Inhibitors

Ana Ratković*

Fidelta Ltd., Zagreb, Croatia

*e-mail: ana.ratković@fidelta.eu

A continuous flow-photochemistry setup was evaluated with the aim to enable a more efficient protocol for the synthesis of benzobicyclo[3.2.l]octadiene skeleton by intramolecular [2+2]-photocycloaddition reaction. This was the first application of the flow-photochemistry on *β*-heteroaryl-*o*-divinylbenzenes and *o*-vinylphenyl substituted butadienes.

Numerous compounds with the bicyclo[3.2.l]-skeleton are proven as potent inhibitors of dopamine and serotonin transporters and also play a crucial role in the treatment of central nervous system and Alzheimer's disorders. Some of the previously prepared photoproducts showed good cholinesterase inhibition activity, but the main disdvantages were high lipophilicity values and low solubility. Further functionalization of photoproducts was explored in order to achieve better ADME properties.

New amines were synthesised using amination reaction, while the addition to the isolated double bond resulted in new epoxides, alcohols and ethers. The furan ring as part of benzobicyclo[3.2.1]-core was suitable substrate for the synthesis of new oximes and oxime ethers and acyl derivatives. A library of amine, oxime, ether, epoxy and acyl derivatives of the benzobicyclo[3.2.1]octene were synthesized and evaluated as inhibitors of both human acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The majority of the tested compounds exhibited higher selectivity for BChE.

a) b)

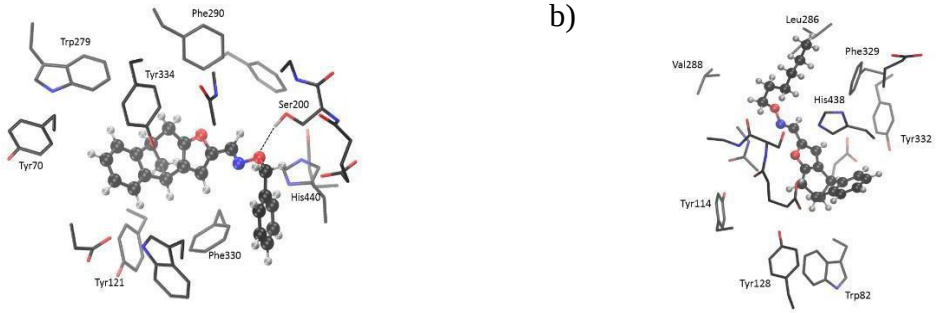


Figure 1. a) optimized structure of molecule **41** within AChE active site and
b) optimized structure of molecule **36** within BChE active site.

Structural adjustment for AChE seems to have been achieved by acylation, and the furan ring opening of furo- benzobicyclo[3.2.1]octadiene. From all obtained results, several cholinesterase inhibitors with a potential for further development as potential drugs for treatment of neurodegenerative diseases were identified.

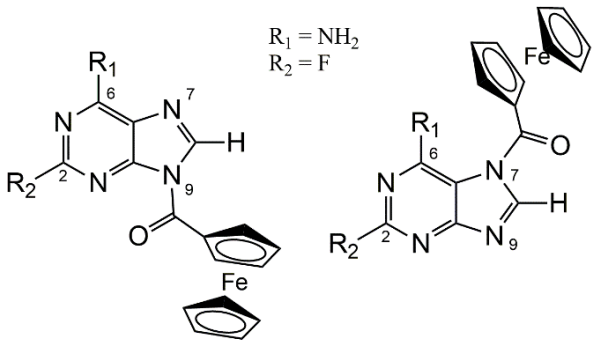
Cytotoxic Activity of Ferrocene-substituted Purines Against Several Cancer Cell Lines

Mateja Toma[a,*], Ana Marija Marjanović Čermak[b], Ivan Pavičić[b], Ivana Vinković Vrček[b], Valerije Vrček[a]

[a] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[b] Institute for Medical Research and Occupational Health, Zagreb, Croatia

*e-mail: mtoma@pharma.hr

A series of ferrocene-substituted purines with carbonyl group as linker between the organometallic and heterocyclic part were synthesized by regioselective acylation [1] and their cytotoxic activity against several cancer cell lines was investigated. Since ferrocenyl compounds are shown to exhibit diverse biological activity through redox mechanism by generating reactive oxygen species (ROS) [2], electrochemical and ROS generation studies were carried out. Cyclic voltammetry showed that all measured compounds follow a reversible one-electron oxidation in the range of 300-450 mV with the N7 isomers being better oxidants than the N9. While nucleobases coupled with ferrocene generate ROS in acellular media, ferrocene and nucleobases itself were not active. Cytotoxic effect against L929 representing normal cells, and Panc-1, A549 and MCF-7 cancer cell lines was determined based on IC_{50} values using MTT viability assay. N7 and N9 isomers containing fluorine atom at position 2 and amino group at position 6 of the purine ring, were the most active against all examined cell lines with IC_{50} value between 1 and 5 μM. Other compounds showed higher IC_{50} values (50-200 μM). The oxidative status of cells treated with compounds showing low IC_{50} values was assessed by cellular ROS generation with DCFH reagent and glutathione production with monochlorobimane. Collected data showed no correlation between cytotoxic effect and acellular ROS generation and/or the redox potential of ferrocene-substituted purines.



IC_{50} (N9, Panc-1) = 1,0 μM IC_{50} (N7, Panc-1) = 0,9 μM

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Recognition of DNA:RNA Hybrid and Triplex Structures by a Series of Benzothiazole Ligands

Iva Zonjić[a,*], Lidija Marija Tumir[a], Filip Šupljika[b], Ivo Crnolatac[a], Livio Racane[c], Marijana Radić Stojković[a]

[a] Ruđer Bošković Institute, Zagreb, Croatia
[b] University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[c] University of Zagreb, Faculty of Textile Technology, Zagreb, Croatia

*e.mail: izonjic@irb.hr

DNA:RNA hybrids and triplexes are formed as intermediate structures during many biologically important processes such as DNA replication, transcription, telomere replication and modulation of structure and/or function of specific genes (antigene strategy for gene regulation) [1,2,3,4,]. Interactions of an array of nucleic acid structures with a small series of benzothiazole ligands (**1-9**) were screened by competition dialysis assay. The main aim of this study was the detection of benzothiazole structure/s with preferential binding to DNA:RNA hybrids and ATT triplex in regard to regular (non-hybrid) DNA and RNA duplexes and single-stranded forms. Complexes of nucleic acids and benzothiazoles, screened by this method, were then characterised by UV/Vis, fluorescence and circular dichroism (CD) spectroscopy and isothermal titration calorimetry.
Compounds **1** and **6** showed the highest affinities toward 13 nucleic acid structures while **5**, despite lower affinities, yielded higher selectivity among studied compounds.
While both **6** and **1** exerted high binding affinities toward ATT, the latter showed stronger binding to rAdT hybrid whereas the former preferred dArU hybrid. Modes of binding to ATT triplex, dArU and rAdT hybrids were determined via CD spectroscopy.

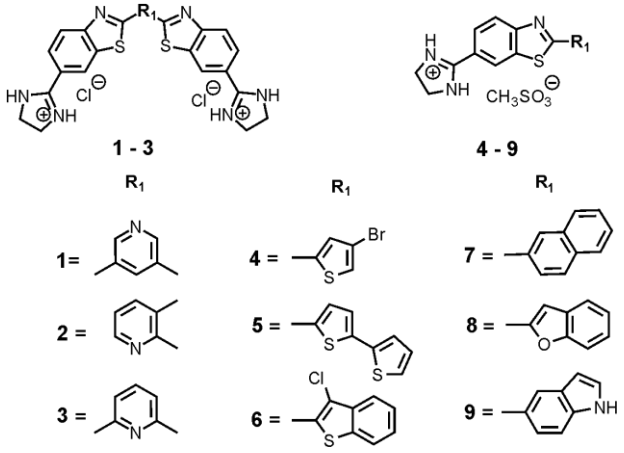

Figure 1. Structure of benzothiazole derivatives in the study.

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