6th Mini Symposium of Section of Medicinal and Pharmaceutical Chemistry

TUESDAY, NOVEMBER 22ND, 2022, 1:00 P.M.

Selvita d.o.o.
Prilaz baruna Filipovića 29
10 000 Zagreb

Sponsor:

Selvita

Moderators: Maja Beus, PhD; Hrvoje Rimac, PhD & Đani Škalamera, PhD	
1st section	
13:00	***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, Selvita***
13:10	**Antonio Zandona**, Oximes Developed as Antidotes for Organophosphates Present a Scaffold for New Research(Es) Candidates
13:30	**Ana Matošević**, Design and Synthesis of Selective Butyrylcholinesterase Inhibitors as Multi-Target Directed Ligands in the Treatment of Alzheimer's Disease
13:50	**Goran Poje**, Design, Synthesis and Biological Activity of Harmicens
14:10	**Antonio Sabljić**, Synthesis and Antibacterial Activity of 3-Aminoquinuclidine Quaternary Ammonium Salts
14:30	**Marta Jurković**, Theranostic Agents for Tumor Cell and Virus-Targeted Photodynamic Therapy
14:50	***Coffee break***
2nd section	
15:10	**Andrea Radeljak**, Automation of Sample Preparation - Final Step in Application of Reference Method (LC-MS / MS) for Vitamin D Determination into Routine Workflow
15:30	**Mia Kapun**, Novel Disulfide Re-Bridging Strategy for The Synthesis of Antibody-Drug Conjugates (ADCs)
15:50	**Arben Beriša**, Enantioselective Organocatalytic Construction of a Congested Tetrasubstituted Stereogenic Center on Pyrrole *β*-(C3)-Position
16:10	**Tomislav Stolar**, Sustainable Solid-Form Screening Of 2,6-Diaminopurine By Mechanochemistry
16:30	***Coffee break***
3rd section	
16:50	**Ida Boček Pavlinac**, Imidazo[4,5-*b*]pyridine Derived Tubulin Polymerization Inhibitors
17:10	**Ivona Čipor**, Stokes Shifted Styryl Dyes – Interactions with DNA/RNA, Cytotoxicity and Application as Fluorescent Probes
17:30	**Iva Zonjić**, What Does a Chlorine Do?
17:50	**Tana Tandarić**, Aminoacylated tRNA Orthoester Intermediate – Myth or Reality?
18:10	***Closing remarks***

Oximes Developed as Antidotes for Organophosphates Present a Scaffold for New Research(es) Candidates

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Scaffolds for development of the antidotes for organophosphates, have one or more nucleophilic oxime group(s) along with the presence of aromatic rings, alcohol groups, nitrogen and chlorine atoms. Their main purpose is to reactivate activity of acetylcholinesterase in synapses upon covalent inhibition by highly toxic organophosphorus compounds (nerve agents, pesticides). However, a lot of designed and synthetized oximes do not reach the expected efficiency level in *in vitro* experiments as antidotes, and they usually get discarded from further evaluations. To prevent such compounds from being forgotten and unused for other research objective, we evaluated them thorough the cell-based assays and determined their new potential targets. We focused our research on a set of quinuclidinium oximes (Qox). Our results indicate that compounds having a longer alkyl chain in the structure disrupt cell and mitochondria membrane fluidity by mimicking fatty acids. Causing changes in membranes, they activate caspase 8 mediated by the death receptor. However, due to the simultaneous influence on the integrity of the membrane, we did not observe the activation of apoptosis but only immediate necrosis. Additionally, computational pharmacophore modelling revealed pharmacological potential of Qox as inhibitors for the enzyme ALDH1. Moreover, changes in ALDH1 lead to cancer progression and therapy resistance, which makes our findings interesting and worth further investigation.

This work was supported by the Croatian Science Foundation (HrZZ-UIP-2017-05-7260).

Design and Synthesis of Selective Butyrylcholinesterase Inhibitors as Multi-Target Directed Ligands in the Treatment of Alzheimer's Disease

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disease characterized by memory loss and personality changes. AD affects more than 50 million people worldwide with numbers continuously growing as a result of a globally aging population. The multifactorial nature of AD points to the existence of a number of possible targets, but the existing treatment of AD is manly symptomatic and based on increasing the concentration of the neurotransmitter acetylcholine (ACh) by inhibiting the action of the enzymes responsible for its hydrolysis, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).
As during disease progression, BChE activity progressively increases by about 40–90% of its normal activity, BChE is important in the regulation of brain ACh levels in late AD. Also, selective inhibition of BChE, as shown with respect to AChE in rodents, has a beneficial effect on the cognitive abilities of rodents with AD and reduces accumulations of amyloid plaques in their brains. Consequently, the selective inhibition of BChE has evolved into a promising new approach in the treatment of middle and advanced AD. In our study, the carbamate group was chosen as a pharmacophore, because the carbamates currently or previously in use for the treatment of AD displayed significant positive effects on cognitive symptoms. With the aim to propose new drug candidate/s for the treatment of AD as selective inhibitors for BChE, we synthesized 25 biscarbamates with different substituents at the carbamoyl and hydroxyaminoethyl chain on the benzene ring and evaluated their inhibition potency toward AChE and BChE. All biscarbamates were proven to be very potent inhibitors of AChE and BChE with inhibition rate constants up to 10^6 M^{-1} min^{-1}, with generally higher preference to BChE. Twenty-one biscarbamates were neither hepatotoxic, nephrotoxic nor neurotoxic. Ability to chelate at least one of biometals (Zn, Fe and Cu) was pointed out as additional beneficial property of these compounds thus they should be able to reduce the neurotoxic effects of biometal imbalances in AD brains. For three biscarbamates were determined to be able to pass the BBB by passive transport, while for nine biscarbamates this ability was slightly limited. According to our results, we could point to three carbamates as a promising compound for the development of more effective drugs for the treatment of middle and late stages of AD [1].

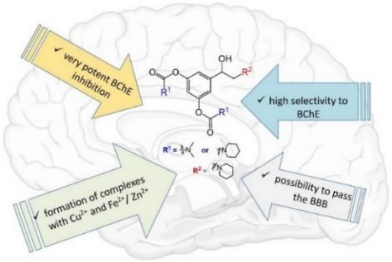

Figure 1. Schematic representation of the main results.

This work was supported by the Croatian Science Foundation Grants IP-2020-02-9343 (to A. Bosak) and IP-2018-01-7683 (to Z. Kovarik).

References

[1] A. Matošević *et al.*, *Pharmaceuticals* **2022**, *15*, 1220.

Design, Synthesis and Biological Activity of Harmicens

Goran Poje[a,*], Marina Marinović[a], Kristina Pavić[a], Marija Mioč[b], Marijeta Kralj[b], Lais Pessanha de Carvalho[c], Jana Held[c], Ivana Perković[a], Zrinka Rajić[a]

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Cancer and malaria are serious public health problems worldwide. The current treatment of these diseases faces many challenges, including drug resistance and high toxicity [1]. Therefore, the discovery of novel anticancer and antimalarial agents is of paramount importance. Herein, we present the design, synthesis, and evaluation of the biological activity of harmicens, hybrids comprising scaffolds with pronounced anticancer and antimalarial properties, namely harmine and ferrocene [2]. Triazole-type harmicens were prepared using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC), while the synthesis of amide-type harmicens was carried out by applying coupling reaction. The novel compounds were characterized by standard methods (^{1}H and ^{13}C NMR, IR, MS). The antimalarial activity of the prepared compounds was evaluated *in vitro* against the erythrocytic and hepatic stages of the *Plasmodium* life cycle, as well as the antiproliferative activity against a panel of human tumour cell lines (MCF-7, HepG2, HCT116, SW620, Hek293T). The results showed that the harmicens exerted moderate antiplasmodial activity (IC_{50} in submicromolar and low micromolar range) and significant and selective antiproliferative activity against the MCF-7 and HCT116 cell lines (IC_{50} in the single-digit micromolar range). Cell localization experiments showed clearly that HCT116-selective harmicene **1** had penetrated the nucleus. It induced G1 cell cycle arrest after 24 h, followed by G2/M arrest, which is in accordance with its localizations within the cell. The effect of nonselective compound **2** on the cell cycle was less pronounced.

Keywords: harmine, ferrocene, antiproliferative activity, intracellular localization, cell cycle

This research was funded by the Croatian Science Foundation under grant number UIP-2017-05-5160.

References

[1] A. V. Nordor *et al.*, *Open Biol.* **2018**, *8*, 180127.
[2] G. Poje *et al.*, *Int. J. Mol. Sci.* **2022**, *23*, 9315.

Synthesis and Antibacterial Activity of 3-Aminoquinuclidine Quaternary Ammonium Salts

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Bacterial resistance to available antibiotics is a growing problem in the combat against pathogens that cause various diseases in humans. Since quaternary ammonium compounds (QACs) have a membranolytic effect, they are known for their antibacterial properties. Literature data show that introducing an amide functional group into their structure results in *soft* QACs with improved antimicrobial properties [1]. By introducing an amide functional group into the structure of QACs provides the possibility of controlled regulation of their biological activity. The aim of this investigation is the *de novo* synthesis of QACs based on the 3-aminoquinuclidine scaffold with long alkyl chains with amide functionalization connected to the quaternary center by a methylene bridge (Figure 1).

Base catalyzed synthesis of the convenient amide with alkyl chains of different lengths (12, 14 and 16 C-atoms) was carried out in dry dichloromethane from 5 °C to room temperature. 3-aminoquinuclidine quaternization reactions were carried out in dry acetonitrile with the appropriate amount of synthesized amide at reflux conditions. For these QACs cLog*P* values, i.e., lipophilicity coefficients were calculated using the online tool SwissADME. Obtained values indicate that compounds can easily penetrate the bacterial membrane and cause the leakage of cytoplasmic contents and cell lysis. The microbiological activity of the newly synthesized compounds was tested using the microdilution method against a panel of Gram-positive and Gram-negative bacteria. However, the obtained values of minimum inhibitory concentrations (MIC) were higher than expected (≥100 μM). Future steps, currently underway in our laboratory, involve investigation of their spontaneous hydrolysis in basic or acidic conditions which will yield inactive degradation products that do not threaten human health and the environment.

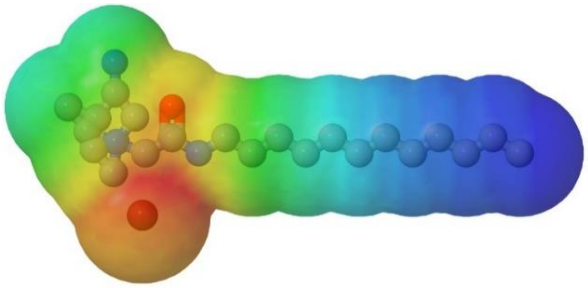

Figure 1. Model of synthesized 3-aminoquinuclidine QACs.

References

[1] A. A. Ryan *et al., Bioorg. & Med. Chem. Lett.* **2017**, *27*, 2107–2112.

Theranostic Agents for Tumor Cell and Virus-Targeted Photodynamic Therapy

Marta Jurković[a*], Ksenija Božinović[b], Davor Nestić[b], Matthias Ferger[c], Evripidis Michail[d], Ivo Piantanida[a], Dragomira Majhen[b], Todd B. Marder[c], Christoph Lambert[d]

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In the recently published paper, three tetracationic bistriarylboranes with bis(2,6-dimethylphenyl-4-ethynyl)arene linkers showed high binding affinities towards DNA and RNA, reporting the binding simultaneously by Raman(SERS) and fluorescence changes [1]. In here presented study [2], mentioned dyes (Figure 1) exhibited very promising photo-induced biological activity on human cell lines and adenovirus type 5 (HAdV5), thus acting as theranostic agents for photodynamic therapy (PDT). PDT is non-invasive antitumor therapy which has several advantages over the traditional treatment modalities [3]. All compounds efficiently entered living cells, showing negligible antiproliferative activity. In addition, bis-thiophene- and anthracene-analogues bind non-covalently to HAdV5 virus with high affinity. The anthracene-analogue itself causes a moderate antiviral effect, i.e. decreases the ability of the virus to infect human cells. Most intriguingly, irradiation of bis-thiophene- and anthracene- analogues with visible light (400-700 nm) caused a very rapid (within 1 minute) and strong increase in cytotoxicity, as well as an order of magnitude increased antiviral activity. Additionally, both analogues induced the formation of reactive oxygen species (ROS) in human cells. Photochemical studies of the compounds revealed that they produce singlet oxygen upon irradiation, which correlates with the observed light-induced bioactivity.

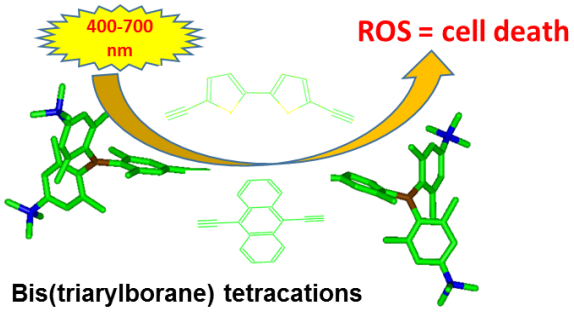

Figure 1. Formation of ROS due to irradiation of compounds with visible light, resulting in cell death.

Keywords: borane, singlet oxygen, photodynamic therapy, fluorescence, theranostic

This work was financially supported by Croatian Science Foundation within the project „BioMultiChromoProbes" (HrZZ IP-2018-01-5475).

References

[1] M. Ferger *et al.*, *Chem. Eur. J.* **2021**, *27*, 5142-5159.
[2] K. Bozinovic *et al.*, *J Photochem. Photobiol. B* **2022**, *234*, ARTN 112523.
[3] G. M. F. Calixto & J. Bernegossi, *Molecules* **2016**, *21*, 342.

Automation of Sample Preparation - Final Step in Application of Reference Method (LC-MS / MS) for Vitamin D Determination Into Routine Workflow

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a reference method for determination of vitamin D concentration, whose application to routine work in clinical laboratory is burdened by the high cost of equipment, complexity of analysis and demanding pretreatment of samples. In order to reduce the staf workload and respond to the progressive increase of requests for vitamin D determination during the COVID-19 pandemic, we launched the automation of sample preparation. In accordance with the requirements of the HR EN ISO 15189 prior to introduction into routine work we have done verification of this protocol and compared patient results obtained automated vs. manually preparation. Commercial control samples and reagent kit (RECIPE ClinMass®) were used to verify precision on CLAM-2030 coupled to LC-MS/MS (UPLC NEXERA X2-LCMS-8050 (Shimadzu) according to CLSI EP15-A3. Bias assessment was performed analyzing EQA samples (N=4), provided by Referenzinstitut für Bioanalytik and achieved average deviation of 3.9% from the median of the group (≤30%).

Recipe ClinChek controls	25-OH D3 (nmol/L)	Repeatability	Intermediate precision	Within-laboratory precision
Level 1	38.8	5.6 %	3.4 %	5.7 %
Level 2	104.2	5.3 %	3.8 %	6.0 %

Table 1. Results of precision verification of automated sample pretreatment meet the set criteria for LC-MS/MS methods according to CLSI C62-A (CV≤15%).

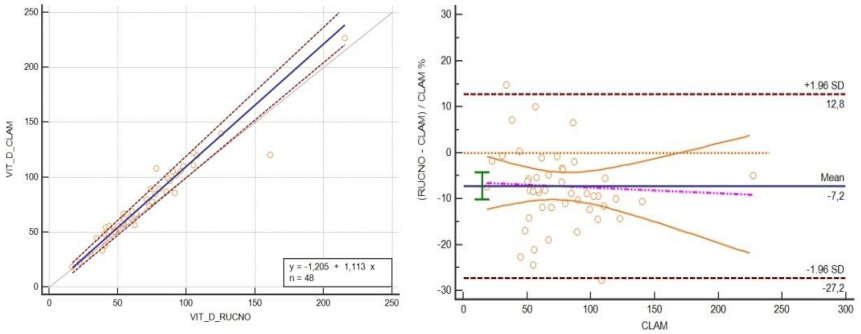

Figure 1. and 2. Comparison of vitamin D concentration in patients samples (N=48), using different sample preparation methods (automated vs. manually) assessed using Passing-Bablok linear regression, meet the desired criteria in accordance with biological variability.

Obtained results allowed us rapid introduction of automated sample pretreatment into routine work and full automation of processes required for determination vitamin D concentrations by LC-MS/MS method.

Keywords: vitamin D, mass spectrometry, automation, sample preparation

Novel Disulfide Re-Bridging Strategy for the Synthesis of Antibody-Drug Conjugates (ADCs)

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Antibody-drug conjugates (ADCs) are a cutting-edge class of cancer-targeting therapeutic agents that can achieve a wide therapeutic window by combining the selectivity of monoclonal antibodies (mAb) with the antitumor activity of a cytotoxic payload. Current FDA-approved ADCs are prepared from IgG1 or IgG4 antibodies via stochastic modification of surface exposed lysine residues or site-selective cysteine modifications. However, current modification technologies fail to successfully modulate the drug-antibody ratio (DAR) to afford homogeneous ADCs without the use of protein engineering. Such highly heterogeneous ADCs, with varying conjugation sites and a wide distribution of antibody-drug loading, have been shown to negatively impact the safety and efficacy of ADCs. Herein, we present a method that enables precise modulation of DAR in integer increments (DAR = 1,2,3 and 4) via click chemistry upon disulfide reduction and re-bridging using diviniylpyrimidine (DVP) strategy (Figure 1). Analysis of these bioconjugates in HER2-positive cell lines indicates improved efficacy compared to the native antibody.

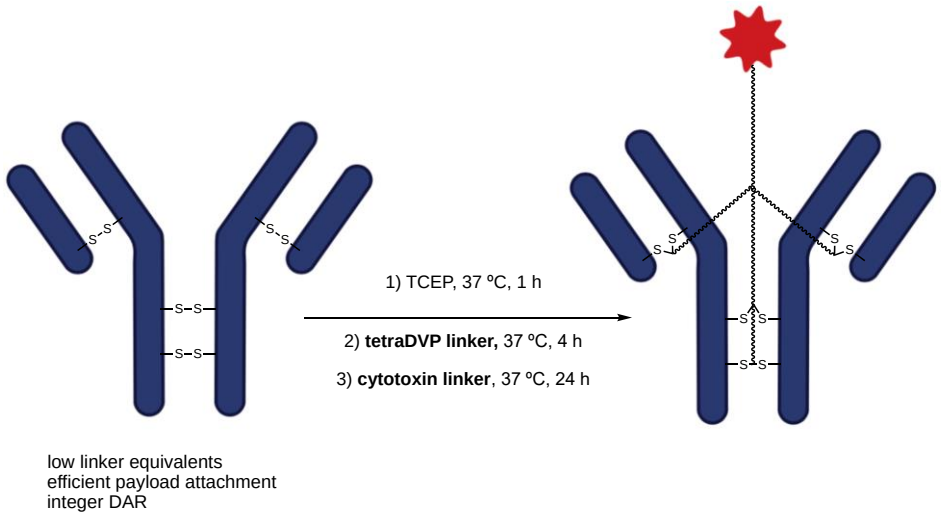

Figure 1. Antibody modification with tetraDVP.

Keywords: antibody drug conjugation

References

[1] F. M. Dannheim *et al.*, *Chem. Sci.* **2022**, *13*, 8781–8790.

Enantioselective Organocatalytic Construction of a Congested Tetrasubstituted Stereogenic Center on Pyrrole *β*-(C3)-Position

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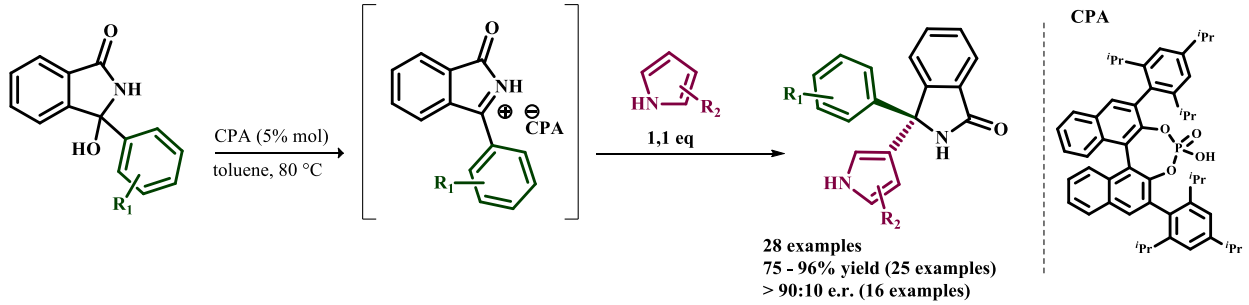
Stereoselective functionalization of pyrroles is a challenging task. Because of their inherent small molecular size, the coordination of pyrroles with chiral catalysts results in weak steric interactions, which leads to difficulties in controlling the enantioselectivity. Reactions on pyrroles predominantly occur at its more nucleophilic *α*-(C2)-position. On the other hand, stereoselective functionalization *β*-(C3)-position of pyrrole is usually more difficult to achieve. *β*-Functionalized pyrroles serve structural cores of many biologically active compounds such as Prodigiosin[1] and Rhazinilam.[2] Only two systematic studies are reported for the construction of a tetrasubstituted stereogenic center on pyrrole *β*-(C3) position, and both rely on usage of chiral transition-metal complexes.[3,4] However, in the contrast to these elegant examples, there are no reports in literature for the preparation of such compounds in an organocatalytic fashion. Herein, we report an enantioselective C-H functionalization of pyrroles for the construction of a tetrasubstituted stereogenic center on C3 position mediated by chiral phosphoric acid (CPA). Key to the success of this transformation is the *in situ* generation of the reactive ketiminium species from 3-hydroxyisoindolinones. The transformation proceeds rapidly with a broad range of ketimines and 2,5-disubstituted and 2-monosubstituted pyrroles to afford products in up to 96% yield, and up to 98,5:1,5 e.r. (Scheme 1). The mechanism of stereochemical induction is investigated, and the reaction is successfully conducted on a larger scale.



Scheme 1. Enantioselective functionalization of pyrrole on *β*-(C3)-position with tetrasubstituted stereogenic center.

Keywords: enantioselective synthesis, tetrasubstituted stereogenic center, pyrrole, organocatalysis

References

[1] A. Fürstner, *Angew. Chem. Int. Ed.*, **2003**, *42*, 3582.
[2] J. Gaunt *et al.*, *Angew. Chem. Int. Ed.*, **2012**, *51*, 9288.
[3] E. Meggers *et al.*, *Org. Chem. Front.*, **2016**, *3*, 1319.
[4] Z. Wang *et al.*, *Chinese J. Chem.*, **2022**, *40*, 195.

Sustainable Solid-Form Screening of 2,6-Diaminopurine by Mechanochemistry

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Mechanochemistry complies with the principles of green chemistry and is known for its efficiency in solid form and polymorph screening. 2,6-diaminopurine (DAP) is well known for treating genetic diseases and occurring as a nucleobase in viral DNA, yet its solid-state chemistry is unknown. In this work, with a total solvent consumption of 0.6 mL, we discovered five new DAP solid forms by mechanochemical and thermal routines (Figure 1) [1].

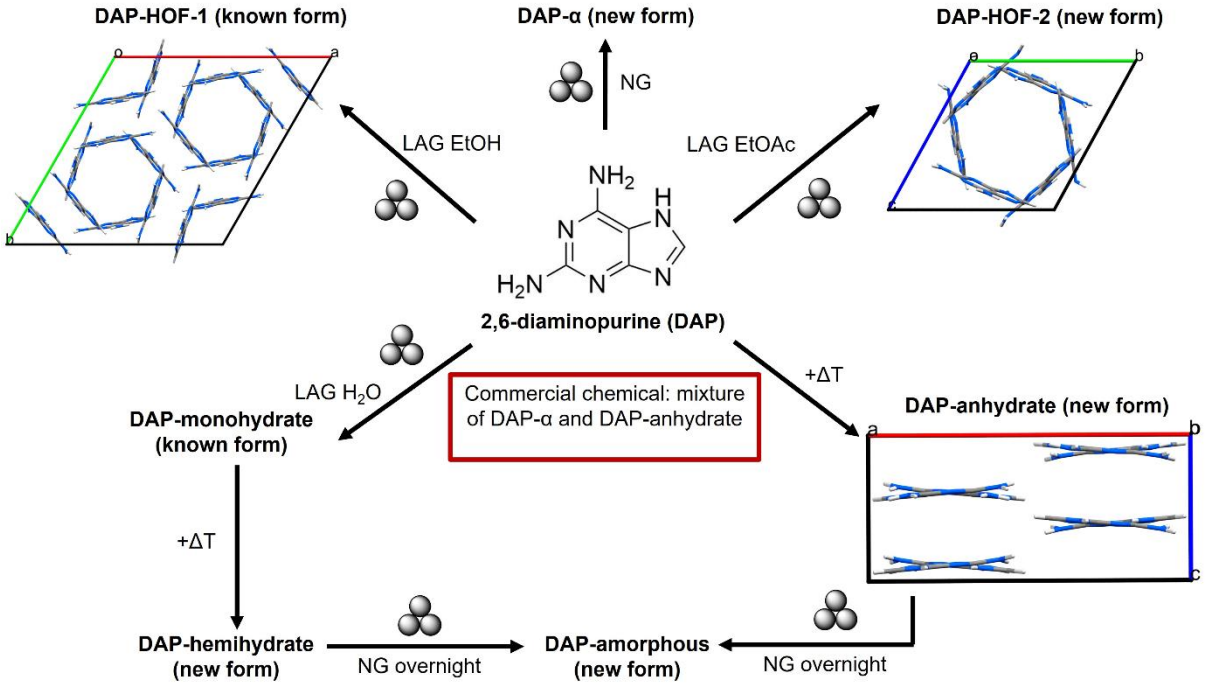

Figure 1. Overview of DAP solid forms obtained in this work.

Keywords: mechanochemistry, sustainability, solid form screening, active pharmaceutical ingredients

References

[1] T. Stolar *et al.*, *CrystEngComm*, **2022**, *24*, 6505–6511.

Imidazo[4,5-*b*]pyridine Derived Tubulin Polymerization Inhibitors

Ida Boček Pavlinac[a*], Lucija Hok[b], Leentje Persoons[c], Dirk Daelemans[c], Robert Vianello[b], Marijana Hranjec[a]

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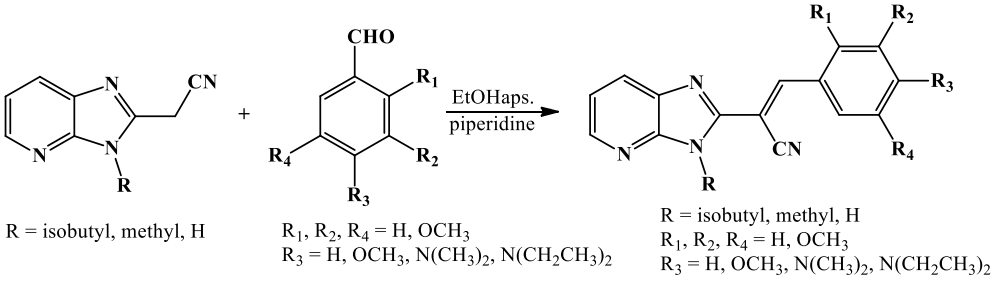
In the development of novel antitumor drugs considerable interest is focused on the development, design and biological activity of novel heteroaromatic systems as potential tubulin polymerization inhibitors. Microtubules, which are formed through the polymerization of heterodimers, have an important role in cellular shape organization, cell division, mitosis and intracellular movement and thus are a key dynamic structural components in cells. Since imidazo[4,5-*b*]pyridine derivatives, as one of the privileged medicinal scaffolds, have not been fully explored as tubulin polymerization inhibitors, we have designed novel imidazo[4,5-*b*]pyridine derived acrylonitriles, described their synthesis and structural characterization. (Scheme 1) Antiproliferative activity was tested *in vitro* against nine human cancer cells in order to study the influence of the substituents placed at the phenyl ring and at the N-atom of the imidazo[4,5-*b*]pyridine nuclei.[1] Both experimental and computational methods confirmed the same biological target. Combination of docking and molecular dynamic simulation further explained interaction with biological target. (Figure 1)



Scheme 1. Synthesis of acrylonitrile derivatives.

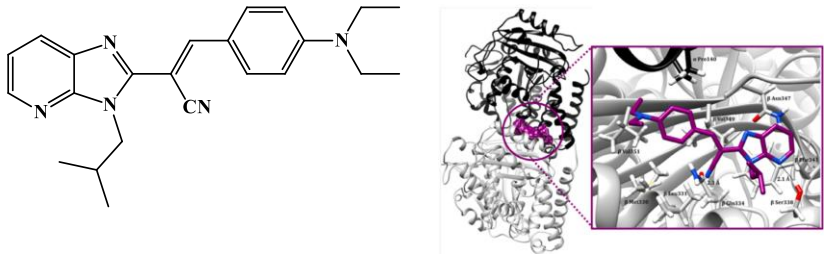


Figure 1. Structure of most active compound and binding to the active site.

Keywords: acrylonitriles, imidazo[4,5-*b*]pyridine, docking simulations, tubulin polymerization

References

[1] I. Boček *et al.*, *Bioorg. Chem.*, **2022**, *127*, 106032.

Stokes Shifted Styryl Dyes – Interactions with DNA/RNA, Cytotoxicity and Application as Fluorescent Probes

Ivona Čipor[a,*], Atanas Kurutos[b], Georgi M. Dobrikov[b], Fadhil S. Kamounah[c], Dragomira Majhen[a], Davor Nestić[a], Ivo Piantanida[a]

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[b] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[c] University of Copenhagen, Department of Chemistry, Copenhagen, Denmark
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The excellent optical properties of styryl dyes (photostability and ease of synthesis [1]) gave rise to applications, such as in optical devices or as biosensors and cellular or tissue staining agents [2]. In this work, nine novel styryl dyes were designed and prepared as the continuation of previous work [3], to further study the relation of the structure of styryl dye to photophysical properties and binding to biomolecules. Various N-quaternary heterocyclics, ranging from the smallest aromatic structure of pyridine (**P-PY, O-PY**) to the largest, benzo[*e*]indole (**BZ-IND**), were employed. The dyes fluorescence spectra showed remarkable Stokes' shifts dependent on the heterocyclic moiety. All the studied dyes showed a strong fluorescence increase upon binding to DNA/RNA with micromolar affinity. Joined results of several methods supported the binding of dyes into the DNA minor or RNA major groove, with no significant selectivity between various types of ds-DNA or ds-RNA. The most of dyes showed no cytotoxic effect on human cells, except for **BZ-IND** which was toxic even at 1μM concentrations. Intracellular localization studies showed that most dyes localize in the mitochondria, again except for **BZ-IND** which distributes equally between mitochondria and lysosome, and benzo[d]selenazole (**BZ-Se**) which accumulates preferably in the lysosome. Here presented results show excellent properties of these novel dyes for use as intracellular staining agents or, in the case of the bioactive **BZ-IND** derivative, possible application as a theranostic agent [4].

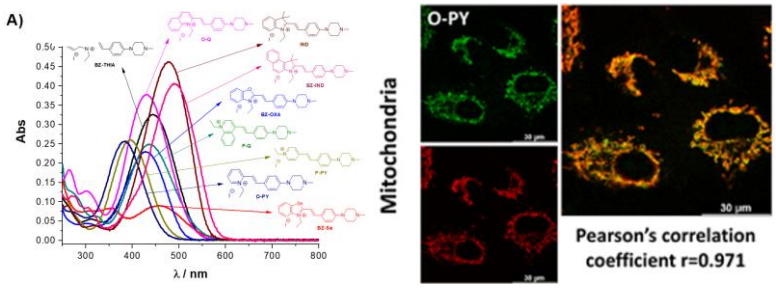

Figure 1. Structures and absorption spectra of studied styryl dyes, intracellular distribution [4].

This work was supported by Croatian Science Foundation, CSF, project IP-2018-01-5475.

Keywords: styryl dyes, Stokes shift, DNA/RNA binding, theranostic agent

References:

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[2] K. Supabowornsathit *et al.*, *Sci. Rep.*, **2022**, *12*, 1-17.
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What does a Chlorine do?

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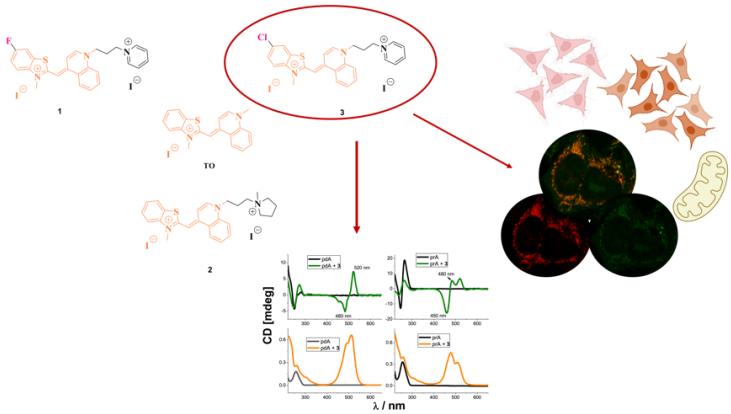
Cyanine dyes are compounds that find wide applications in many fields but initially were used in the development of photographic films, then as luminescent markers of biomacromolecules or sensitive probes for biomembrane fluidity [1] and potential. Substituents placed at different positions of the aromatic system can improve the photophysical properties of cyanine dyes, water solubility and interactions with DNA, but also affect the electronic properties of chromophores. [2] Our intent was to evaluate the influence of three cyanine dyes derivatives on recognition of various single-stranded, double-stranded, and triple-stranded DNA/RNA using several biophysical methods including thermal melting, fluorometry and circular dichroism. Confocal microscopy and MTT test were applied for *in vitro* study. All synthesized derivatives caused triplex formation of consecutive rA/dA-containing nucleic acid helices. In the case of derivative **3**, the most pronounced effect was the induction of H-aggregate formation, which depended on the presence of rA strand in all forms of nucleic acids (single, double, and triple-stranded). Compound **3** exerted strong antiproliferative effect on all tumor cell lines. It's also shown to be particularly good mitochondrial probe which, in addition to demonstrated cytotoxic activity, makes this derivative promising therapeutic lead.



Keywords: Cyanine dyes, DNA/RNA recognition, Triplex helix formation, H-aggregate, Mitochondrial dyes, Cell viability

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Aminoacylated tRNA Orthoester Intermediate – Myth or Reality?

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Translation of proteins is one of the key biochemical processes and as such has been the focus of scientific research for years. Despite the fact that translation has been extensively researched, there remain numerous open questions concerning the microscopic mechanisms involved. The central molecule in protein biosynthesis is certainly tRNA, an adapter molecule that links the genetic information transcribed on mRNA into the corresponding amino acid on the ribosome. Aminoacyl tRNA is synthesized by aminoacyl tRNA synthetase enzymes (aaRS) that catalyze the formation of amino acid and ribose esters at the 3' end of tRNA. As the terminal ribose has two free -OH groups, 2' and 3', the amino acid can be attached in two possible ways. AaRS are specific for a single amino acid and its tRNA. There are two types of aaRS, type I, which catalyzes the reaction with the amino acid and the 2'-OH group, and type II, which catalyzes the reaction with the 3'-OH group. After synthesis, transacylation occurs, so in equilibrium in the aqueous solution we find both isomers of tRNA, 2' and 3', in a ratio of 1:2 in favor of the 3' isomer [1]. The transcription factor, EF-Tu, stabilizes the both the free tRNA and the 3' isomer and brings the charged tRNA to the ribosome where it interacts with the mRNA template whereby the amino acid becomes part of the newly formed protein. Importantly, the 3' isomer is required for protein synthesis and successful hydrolysis of aminoacylated tRNA. In this work, we study the mechanism of transacylation of aminoacylated Phe-tRNA by quantum chemical (QM) methods (Figure 1.). An orthoester tRNA intermediate was located, the existence of which was controversial until now [2,3]. The dynamics of Phe-tRNA isomers, free and in complex with the EF-Tu protein, were studied using molecular dynamics simulations and PARENT software [4] for the calculation of configurational entropy. The analysis of simulations using standard tools of structural bioinformatics showed that there is a significant difference in the dynamics of different isomers of Phe-tRNA in the free form, as well as in the form bound to the stabilizing protein (EF-Tu). Entropy calculations also indicate significantly different dynamics, both of the entire system and of individual parts of the system (e.g. anticodon loop). The obtained results represent relevant new knowledge about the molecular system essential for life as it exists on Earth.

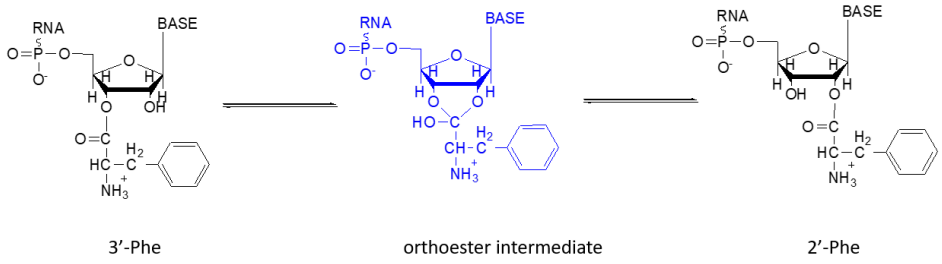

Figure 1. Proposed mechanism of aminoacyl-tRNA transacylation.

Keywords: molecular dynamics, tRNA, orthoester intermediate, quantum mechanics, allostery

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