Habilitation overview of the scientific contributions

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My application in the contest for "professor" position includes **39** scientific papers, excluding the publications for my PhD thesis (**3,4,7-3-13**) and promotion as "'assoc. prof." (**1,2,5,6,7,14-41**). The scientific papers for the current contest (**39**) are published in scientific journals indexed in *Scopus* and *Web of Science* as follows: in **Q1** journals **(9 papers)**; in **Q2** journals **(4 papers)**, in **Q3** journals **(5 papers)**; in **Q4** journals **(9 papers)**; in journals with **SJR without IF (1 paper)**; other **non-indexed journals** (**4 papers)**, in **conference proceedings (2 papers)**, **book chapter (1 paper)**. Until July 2019 **152** citations in total were noticed in journals indexed in *Scopus* and *Web of Science*, citing **35** of my publications: **64** of the works are citing publications included in my PhD thesis and promotion as "'assoc. prof."; while **88** of the works are citing publications presented in the current contest.

All of the scientific publications presented in the current contest are in the field of organic chemistry, and particularly – the synthesis of new organic compounds for medicinal applications, study on the structure, biological activities, mechanisms of action, relevant anion and radical anion intermediates, elucidation of protein secondary structure upon modification with ionic liquids and bioactive molecules, identification and characterization of organic and inorganic materials in art objects and archaeological artefacts.

1. Introduction

The human body is constantly exposed to free radical attack the sources of which might be exogenous as well as endogenous, formed during the normal cellular metabolism. The excessive concentration of reactive oxygen species (ROS) could disrupt the existing homeostatic balance and hinder the antioxidant capacity of the organism to inhibit the free radical mediated processes. All this inevitably leads to oxidative stress. Such processes include damage of proteins and lipids and are related to serious pathological conditions such as cancer, neurodegenerative disorders, diabetes, liver, pulmonary and cardio-vascular diseases [1-6].

The prolonged exposure to oxidative stress is a key factor in the cancer development because it damages cellular structures and impairs cellular functions related to DNA mutations, gene instability and cellular proliferation [7-9]. It is known that ROS act as a secondary precursor in the intracellular signaling pathway that induce and maintain the oncogenic phenotype of the cancer cells and at the same time ROS are able to induce aging and apoptosis which contributes to their anti-tumorogenic effect. The increased generation of ROS is characteristic for cancer cells where changes in the signal transduction are observed. The oxidative stress induces cellular redox imbalance that can be observed in different types of cancer cells compared to normal cells. The redox imbalance is related to oncogenic stimulation. The DNA mutations have a key role in cancerogenesis prove of which are the increased levels of oxidative DNA changes (8-OH-G). These are the reasons why antioxidants are a useful tool in the fight against cancer.

The high sensitivity of neuronal cells to oxidative stress is determined by different factors including stronger dependence on oxidative phosphorylation for energy in comparison to other cells; exposure to high oxygen concentration; metal ions accumulating in the brain with aging that can be a potent catalyst for oxidative species formation and also they are rich in polyunsaturated fatty acids that are prone to oxidation. At the same time, they contain relatively poor concentrations of antioxidants. During the progression of neurodegenerative conditions, the capacity of cells to maintain the redox balance decreases, leading to the accumulation of free radicals, mitochondrial dysfunction, and neuronal injury [10-12].

The liver also contains high amount of polyunsaturated fatty acids and is prone to lipid peroxidation, the reaction products of which demonstrate mutagenic and carcinogenic properties.

It is considered that MDA can react with some nucleosides and form adducts with desoxyadenosine and desoxyguanosine, as well as pyrimido-purinone – pyrimido[1,2-a]purine-10(3H)-on (M1G). This type of condensation is the reason why M1G might act as mutagen [13]. When the DNA repair systems are absent, the MDA-DNA adducts might cause mutations, chain breaking [13,14], cell cycle block and induced apoptosis [15].

Therefore, the administration of antioxidants is a leading strategy in the prevention and treatment of health disorders resulting from the decreased antioxidant capacity [1-3,7,12,16-20]. Във връзка с това са възникнали разнообразни подходи, основаващи се на прилагането на природни антиоксиданти – достъпни на човека чрез храната, получаването на техни синтетични производни или други органични съединения с антиоксидантни свойства, активиране на естествените антиоксидантни системи на организма и др.

On the other hand, the chemical transformation of the drugs in the human body can lead to the formation of hazardous metabolites. For instance, drugs containing a nitroaromatic moiety such as nitrofurantoin, nimesulide, nilutamide, flutamide etc., have been associated with hepatotoxicity due to bioreduction of the nitro group [21-25]. The bioreduction of the nitroaromatic compounds undergoes a multistep conversion to nitro radical anions, nitroso intermediates, N-hydroxy derivatives and finally to the respective amines. The process is catalyzed by specific enzymatic systems including primarily cytochrome P450 (CYP) reductase as well as xanthine oxidase, aldehyde oxidase and quinone reductase [25]. The cytotoxicity of these compounds is due to the emerging reactive intermediates in the process of bioreduction, such as nitroanion radicals, which are capable of binding covalently to nucleophilic centres of proteins and nucleic acids. As a result of the redox cycling the oxidative stress increases, which also contributes to the overall toxicity. Hepatocytes, which were exposed to nitroaromatic drugs like flutamide, demonstrated decrease in the GSH/GSSG ratio [26] and also nitrofurantoin definitely caused intracellular oxidative stress [27]. Therefore, in the study of new drug candidates it is important to characterize the potential reductive products of nitroaromatic compounds. The first step in the mechanism of action of nitroheterocyclic drugs as cytotoxic agents for hypoxic cells is the reduction of the nitro group of the drug to the corresponding nitro radical anion [27].

2. Overview of scientific contributions

2.1. Combined DFT and IR study on the mechanisms and possible intermediates in the antioxidant action of natural antioxidants

We have studied a series of naturally occurring antioxidants: vanillin, its ketone analog – apocynin [28], and syringaldehyde [29], in order to clarify the mechanism of their antioxidant action at molecular level in polar and nonpolar media.

Vanillin, one of the most popularly used flavoring components has exhibit multifunctional effects such as antimutagenic, antiangiogenetic, anti-colitis, anti-sickling, and antianalgesic effects. However, the study of its antioxidant activity continues to attract much attention in order to clarify some inconsistencies in the experimental results. Apocynin (4-hydroxy-3-methoxyacetophenone, acetovanillone) is the biologically active substance in the roots of hymalayian Picrorhiza kurroa, and due to its inhibitory activity to nicotinamide adenine dinucleotide phosphate oxidase (NADPH), apocynin exhibit potent antiinflammatory effect.

The oxidation processes in biological systems involve various free radicals characterized by different reactivity: the most reactive hydroxyl radicals $^{\bullet}$OH that show little selectivity towards the possible sites of attack; the hydroxyperoxyl radicals, $^{\bullet}$OOH, which are less reactive, but they can diffuse into remote cellular locations and initiate the lipid peroxidation; the lipid alkoxyl radicals, $^{\bullet}$OR, formed from the reduction of peroxides, and the lipid peroxyl radicals $^{\bullet}$OOR. Those different reactivity of free radicals is connected with different scavenging capacity of the antioxidants, therefore in our study the reactivity of apocynin and vanillin against several free radicals was evaluated according different antioxidant mechanisms: hydrogen atom transfer (HAT), single-electron transfer (SET), sequential proton loss electron transfer (SPLET) in polar and nonpolar solvents [28].

The calculated reaction enthalpies showed that in nonpolar phase apocynin and vanillin would react exothermically with $^{\bullet}$OH and alkoxyl ($^{\bullet}$OCH3 and $^{\bullet}$OR) radicals via HAT mechanism with resulting negative ΔH(BDE) value, but not peroxyl ($^{\bullet}$OOH, $^{\bullet}$OOCH3 and $^{\bullet}$OOR) and alkyl ($^{\bullet}$R) radicals. It was also found that apocynin would readily scavenge $^{\bullet}$OH and alkoxyl ($^{\bullet}$OCH3 and $^{\bullet}$OL) radicals via SPLET mechanism in water. Scavenging of $^{\bullet}$OOH by apocynin

would only be possible via SET mechanism in water according to the calculated ΔH(IP). The comparable activity for apocynin and vanillin, provided by the theoretical estimated BDE values, is in good accordance with the experimental data from crocin bleaching inhibition [30] and oxygen radical absorbance assay (ORAC) [31]. In a cell-based antioxidant assay - oxidative hemolysis inhibition assay (OxHLIA), where oxidation of erythrocyte membranes is induced by AAPH-derived hydroperoxyl radical, apocynin showed superior activity compared to vanillin, but those result was attributed to superior lipophilicity of apocynin and higher resulting access to the lipophilic biomembrane of erythrocytes in OxHLIA [31].

According to the calculations, the SPLET mechanism is preferred in ionization supporting solvents such as water, hence the corresponding oxyanion of apocynin was generated and characterized in more details by IR methods and DFT computations.

Another subject of our studies were the antioxidant mechanisms and reaction intermediate of syringaldehyde, a naturally occurring phenolic antioxidant with antimicrobial, antifungal, antiparasite, antidiabetic and antioxidant properties [29]. Its ability to scavenge free radicals by HAT, SET and SPLET mechanisms was elucidated by computing bond dissociation enthalpy, ionization potentials and proton affinities at DFT B3LYP/6-311++G** level. Gas phase, benzene, water and DMSO calculations were carried out in order to account for different environment (nonpolar lipid membranes and polar physiological liquids) where the antioxidant action in the living organism could take place and various experimental *in vitro* conditions.

The calculations in gas phase and benzene, indicated the HAT mechanism as the most favorable one in nonpolar medium. Comparison of the BDE values of syringaldehyde to those of vanillin outlined the syringaldehyde as a better radical scavenger than vanillin via HAT. It was also shown that syringaldehyde should effectively prevent the lipid peroxidation as its BDE value is lower than the one of methanol used as model lipid radical in the study. The relative stability of vanillin and syringaldehyde radicals was estimated by spin density distribution as measure of the odd electron delocalization through the conjugated system. In benzene, representing the nonpolar conditions, the calculations revealed smaller spin density at the phenoxyl oxygen in the syringaldehyde than in the vanillin radical, thus supporting the higher stability of the syringaldehyde radical and its experimentally observed higher radical scavenging capacity via HAT.

Further it was shown that syringaldehyde is characterized by lower calculated IPs than the vanillin in accordance with the experimentally determined higher ability of syringaldehyde to donate an electron [30]. Comparison to the IP of phenol was suggested by Wright et al. [32] as effective approach to estimate the probability certain compounds to react via SET mechanism: when IPs of the studied antioxidants drop to ca. 167 kJ mol^{-1} below phenol, the SET mechanism would gain importance in solution [32]. However, the IPs of vanillin and syringaldehyde in water are higher than the IP of phenol in water calculated at the same theoretical level, hence the SET mechanism should be excluded as possible mechanism of action for both compounds.

On the other hand, the PA values showed lower energy requirements for the SPLET mechanism than HAT in polar medium. Beside the small difference in the PAs of syringaldehyde and vanillin, according to the calculated ETE values, the phenoxy anion of the former is more prone to donate its electron to the free radical (in the second step of the SPLET mechanism). This, combined with the better radical stability of the resulting radical, lead to the conclusion that syringaldehyde should be regarded as more potent radical scavenger via SPLET as well. The same relative activity of vanillin and syringaldehyde and their dendrimers was shown in DPPH assay where the experiment is conducted in polar methanol medium and the ionization of the studied compounds through SPLET contribute to their radical scavenging activity [30,33,34]. Having in mind the importance of the anionic form in the SPLET mechanism, we studied the ability of syringaldehyde to form an anion in DMSO-d_6 solution [29]. The IR spectral analysis showed that the conversion into oxyanion causes a strong decrease in the stretching C=O frequency and a shift of the stretching Ph-O coordinate to higher frequency, accompanied by intensity increase of the formyl stretching C-H band and aromatic skeletal bands in the region 1600-1400 cm^{-1}. Based on these spectral data and the calculated structural parameters, it was established that the structure of the oxyanion shows quasiquinonoidal geometry of the para-phenylene ring.

2.2. Studies on the mechanism of antioxidant action of synthetic antioxidants

Melatonin (N-acetyl-5-methoxytriptamine), a pineal gland hormone, is one of the primary choices as therapeutic agent to treat liver pathophysiological conditions due to its strong direct and indirect antioxidant properties. The structural similarity between the molecules of melatonin and benzimidazole is based on the imidazole and benzimidazole heterocycle resemblance and the

possibility to introduce substituents in the side chains. The strategy to design novel antioxidants based on the structural similarity between N-substituted benzimidazole derivatives and melatonin has demonstrated promising results in previous studies [35-38]. It was demonstrated that the introduction of hydrazone groups in the molecule of melatonin increases the antioxidant activity [37]. The effect could be due to extending the stability and electron delocalization of the indole ring which supports the radical-scavenging properties by forming a stable indole radical cation.

We have successfully used the strategy to develop benzimidazoles as structural analogues of melatonin by obtaining new N,N'-disubstituted benzimidazole-2-thiones through novel synthetic method of *aza*-Michael addition [38]. A pilot study was conducted on rat hepatocytes isolated by a method providing higher amount of live and metabolically active cells. The study identified the most perspective biologically active molecules showing high antioxidant and hepatoprotective activities *i.e.* the 5-substituted benzimidaozle-2-thiones. In order to estimate the influence of the structure on the biological properties, structural characterization of the studied compounds was performed by X-ray diffraction analysis and DFT methods. The most probable mechanisms of antioxidant action were suggested based on DFT calculations. For the ester derivatives of N,N'-disubstituted benzimidazole-2-thione, it was suggested that in nonpolar medium the hydrogen atom abstraction occurs from the activated alkyl groups in the side chains - next to the next to the carbonyl group. Taking into account the estimated lipid BDE values, it was concluded that the ester derivatives would be an efficient radical scavenger of lipid alkoxyl radicals but nor peroxyl radicals. Therefore, the protective effect of the ester benzimidazole-2-thiones against lipid peroxidation should be exerted by scavenging the highly reactive $HO^{\bullet}$, which initiates the degradation process, and the alkoxyl radicals $LO^{\bullet}$ formed from the reduction of lipid peroxides. For the hydrazide derivatives, two more sites for hydrogen atom abstraction were found for each N-alkyl chain – from the amide N-H bond and the amino N-H bonds. The DFT calculations showed that the BDE value for amide N-H bond is lower than that of the lipid peroxyl radicals, therefore the hydrazide derivatives would be able to trap lipid peroxyl, as well as alkoxyl radicals, and inhibit directly the lipid peroxidation process. Moreover, the studied benzimidazole-2-thiones are expected to easily transfer an electron to the lipid radicals and form radical cations in polar medium. As in case of melatonin, the electron transfer might be immediately followed by proton transfer and complimented by the formation of a cyclic intermediate of the benzimidazole-2-thiones. The latter is a neutral radical able to scavenge free

radicals (adduct formation) and inhibit the lipid peroxidation. On the other hand, due to the substantial spin density localized over the S-atom in the radical cation, it is suggested that another possible way is to bind a lipid radical at this site and form a cation adduct.

This research was the starting point to further development of similar compounds with potential application as oxidative stress inhibitors for liver disorders. We have synthesized a series of N,N'-disubstituted benzimidazole-2-thiones with extended side chains as melatonin analogues and studied their hepatoprotective and antioxidant properties in a model of *tert*-butyl hydroperoxide-induced oxidative stress in isolated rat hepatocytes [39]. In vitro chemical assays of Fe(II) induced oxidative damage demonstrated that the observed hepatoprotective effect could be attributed to the capability of the tested compounds to preserve biologically important molecules from oxidative damage. The established structure-activity relationship provided important insights for further structural optimization, namely unsubstituted benzimidazole-2-thione core proved to be more beneficial than 5-substituted core for cytoprotective activity as well as presence of phenyl hydrazine moiety with electron donating substituent (methoxy group) is more favorable than phenyl hydrazine moiety with electron withdrawing substituent (fluorine atom).

Structurally related 1,3-disubstituted benzimidazole-2-imines were synthesized using 5(6)-substituted-benzimidazolethiols as precursors and their antioxidant potential was evaluated by spectrophotometric quantification of the production of malondialdehyde (MDA) as biomarker for lipid peroxidation (LP), based on the formation of MDA-thiobarbituric acid complex [40]. The ester of the 5-benzoylbenzimidzolyl derivative, [3-(2-ethoxy-5-benzoyl-2,3-dihydro-1H-benzimidazole-1-yl] acetate, has shown the most potent lipid peroxidation inhibitory effect 74,04% (IC_{50} = 141.89 μg/mL). The study included 2-substituted thiazolobenzimidazolones as well: 4-fluorobenzylidene-7-(phenylcarbonyl)-[1,3]thiazolo[3,2-a]benzimidazole-3(2H)-one was the most potent compound in the group of 2-substituted thiazolobenzimidazolones with inhibition effect 90,76% (IC_{50} = 53.70 μg/mL). On the basis of calculated bond dissociation enthalpies, it was suggested that the iminobenzimidazoles might act as radical scavengers via hydrogen atom abstraction preferably in the a-C atom of the side chains attached to the iminobenzimidazole rings. Based on the higher BDE for the alkylidene group in benzimidazoles, the HAT was excluded as possible mechanism of action of these compounds. It was suggested that due to their electron-donating properties, the thiazolobenzimidazolones might undergo a

stepwise oxidation via SET mechanism and produce radical cations able to scavenge the lipid alkoxyl ($LO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$) or hydroxyl ($^{\bullet}OH$) radicals, then form an intermediate adduct and terminate the process by a proton transfer.

The structure and radical scavenging activity of another group of compounds, isoxazolo- and thiazolohydrazinylidenechroman-2,4-diones, was studied by experimental methods and DFT calculations [42]. The estimation of the scavenging capacities of the studied molecules towards nitric oxide ($NO^{\bullet}$), superoxide anion radical ($O2^{\bullet-}$) and DPPH• radicals revealed remarkably higher activity of the compounds possessing a thiazolidine ring in the test with nitric oxide ($NO^{\bullet}$) than the coumarins with isoxazolidine ring. Explanation of this result was found in the fact that according to the DFT calculated reaction enthalpies SET mechanism would be competitive to the HAT one for the compounds possessing a thiazolidine ring in water. In this way, the superior activity of this group of compounds most probably is due to their capacity to deactivate free radicals simultaneously by two mechanisms (HAT and SET) in polar medium.

2.3. Studies on the pro-oxidant action of nitroaromatic compounds

The 5-nitro benzimidazole derivatives have shown one of the highest hepatotoxicity on isolated rat hepatocytes within the series of N,N'-disubstituted benzimidazole-2-thiones monitored by the cell viability and changes in lactate dehydrogenase (LDH), glutathione (GSH) and malondialdehyde (MDA) levels [38,39]. Taking into consideration that reduction of the nitro group to a nitro radical anion might be the origin of the higher hepatotoxicity, we investigated the feasibility of a nitro radical anion formation from the ester 5-nitrobenzimidazole derivative through electrochemical generation and IR measurements in DMSO solution [41]. The IR data and DFT calculations demonstrated that the conversion into radical anion causes an increase in the frequency of the C-NO2 bond due to its shortening and increase of its bond order, and a decrease in the frequency of the N-O bonds as a result of their lengthening. It was possible to draw conclusions on several molecular characteristics related to the conversion: the neutral compound and the radical anion exhibit coplanar orientation of the nitro group towards the aromatic system which most likely contributes to the observed toxicity and extended electronic conjugation; the major spin density (c.a. 71 % of the odd electron) is localized over the nitro group in the radical anion; the radical anion shows extended electronic conjugation compared to

the neutral compound; and the presence of thione function and N-alkyl chains in the benzimidazole ring contributes favorably to reduce the propensity for nitro radical anions generation. By analyzing the calculated energies of the lowest unoccupied molecular orbital, energy differences between the lowest unoccupied and the highest occupied molecular orbital, adiabatic electron affinities, and energy difference between the radical anion and the molecule for the studied compound and other nitroaryl compounds, it was found that the ease of nitro reduction of studied nitrobenzimidazole in biological systems should be comparable to that of nitrobenzene and nimesulide and much lower than those of nitrofurantoin.

The electrochemical conversion of two nitroaromatic drugs, associated with hepatotocixity – the nitrofurantoin) [43] and nimesulide (aulin) [44] was recently performed in DMSO-d_6 solution and the IR spectral changes arising from the conversion were monitored. In the case of nitrofurantoin, the observed frequency shifts accompanying the conversion into radical anion were larger than those found with the conversion of dinitrobenzenes [45] and cyanobenzonitriles [46]. The substantial odd electron localization in the nitro group indicated high reactivity of the formed nitrofurantoin radical anion and strong ability to initiate production of various ROS via electron donation. Concerning the nimesulide conversion, based on the comparison with theoretically predicted spectra of possible reduction products, i.e. (i) radical anion resulting from one-electron reduction and (ii) dianion radical resulting from one-electron reduction accompanied by deprotonation, it was possible to conclude that in these conditions the electrochemical reduction of nimesulide lead to generation of a radical dianion [44].

2.4. Synthesis, biological activity and structure-activity relationship studies on didepsipeptide, benzimidazole, thienopyrimidine and 2-amino-5-alkylidenethiazol-4-one derivatives

The scientific contributions in this research area comprise a wide range of synthetic, structural and pharmacological properties spanning from the synthesis of new organic compounds of pharmacological interest to the application of spectroscopic techniques such as IR and NMR methods for characterization of the new compounds, supported by detailed theoretical analysis of the geometry and electronic structure of the molecules, and characterization of

structural features influencing the biological action and the interactions with the different biological targets. Several types of biological assays such as antibacterial, anticancer, antiparasitic, inhibition activity towards xanthine oxidase, DNAse I, were carried out. The computational studies on structure and ligand-protein interactions provided useful conclusions on the structure-activity relationship.

The synthesis, biological activity and structural characterization of didepsipeptide derivatives are discussed in papers [47-51]. Papers [52,53] deal with the tautomerism and isomerism of different groupd of benzimidazoles, investigation of the inhibitory activity towards DNAse I and interaction modes with the enzyme. Papers [54-56] are focused on the antihelminthic, anticancer activity of thieno[2,3-d]pyrimidine-4-ones and inhibitory activity towards DNAse I, along with clarification of the structural features influencing the activity, possible mechanisms of action through interactions biological targets. Xanthine oxidase inhibitory properties and anti-inflammatory activity of 2-amino-5-alkylidenethiazol-4-ones are presented in paper [57].

The data gathered from the studies enabled selection of the most promising candidates of the newly synthesized compounds for potential pharmacological applications. Besides, the structure-activity relationship of the studied compounds can be used for future design novel derivatives with even more potent pharmacological properties and lower toxicity.

3. Future research plans (for the upcoming 5 years):

Based on my current research interests and expertise, I plan to focus my work (in cooperation with the colleague from our laboratory and researchers from other scientific institutions) in the following scientific areas in the next 5 years:

3.1. Design, synthesis, structural characterization and study on the mechanisms of biological action of new benzimidazole derivative

The potential of the benzimidazole architecture for the development of novel antineoplastic compounds in well knows. Many benzimidazoles have found application in the therapeutic practice such as Nocodazole, Bendamustine, Dovtinib, Hoechst 33342. 2-Aminobenzimidazole heterocycle can be seen as a structural fragment in different biologically active molecules and is of main importance as a precursor for the synthesis of novel benzimidazoles with antitumor activity. Considerable number of benzimidazole derivatives, some of which with hydrazine fragments, have been studied and have shown high activity against different cell lines. Having in mind the scientific data related to the connection between the oxidative stress and the processes of carcinogenesis, it is of interest to study compounds with antioxidant properties as a potential antineoplastic agents and also to study the most probable mechanism of antioxidant action. Considering the fact that every modification in the chemical structure may lead to a change in the activity, toxicity and selectivity of a particular group biologically active compounds, several groups of benzimidazole compounds are considered of interest for our team: novel benzimidazole derivatives containing aryl hydrazone fragment or thienopyrimidine substituent; 1,3,5-substituted benzimidazole-2-thione hydrazones; 1,3,5-substituted benzimidazole-2-imine hydrazones; 5(6)-substituted benzimidazole-2-hydrazones; 2-substituted thiazolobenzimidazolones etc. Further goals will be: screening of the antineoplastic activity of the synthesized compounds; screening of the antioxidant activity in biologically relevant systems and studying the mechanisms of antioxidant action; screening of the hepatotoxicity and possible hepatoprotective properties; computation of molecular descriptors and study on the probable mechanisms of antioxidant action; clarification of the receptor-ligand interactions by theoretical methods.

The synthesized benzimidazoles must possess a variety of structural features – amide, different substituents and a few possible sites for hydrogen bonding that could affect their physico-chemical properties and therefore their biological activity and interactions with biological targets. In this respect the first step in achieving these objectives is the design and synthesis of benzimidazole-2-thiones and benzimidazole-2-imines through variation of their structural characteristics. The changes in the structure of model compounds can enhance the interactions of the newly synthesized compounds with biological targets. The next step is the characterization of their structural features and the related to them physicochemical properties, using different analytical methods. The study of their properties and possibilities of forming metal complexes is also envisaged, since the interaction with catalytically active centers of the metalloenzymes and DNA bonding is an important characteristic.

Benzimidazoles derivatives, such as albendazole, fenbendazole, mebendazole, nocodazole, oxibendazole, parbendazole, and luxabendazole have been widely used for antinematodal or other therapeutic applications and can selectively bind with beta-tubulin in parasites, as well as mammalian beta-tubulin. As the key structural basis of microtubule, tubulin is considered as a highly attractive target for anticancer therapy. Having this in mind, the ability of the benzimidazole derivatives to interfer with tubulin polymerization will be studied in details.

In continuation of our ongoing research activities, considerable part of the work will be directed towards exploring the cytoprotective i.e. hepatoprotective and neuroprotective capacity of benzimidazole compounds.

3.2. IR studies on the protein secondary structure

In the recent years, I have broadly expanded my expertise in the IR studies of protein secondary structure studying the modification of several proteins in cooperation with colleagues from the Department of Chemistry and Biophysics of Proteins and Enzymes of our institute. This led to the successful publication of a series of papers covering diverse aspects of the modification, stability, secondary structure modulation and biological activity of lipase, insulin, and hemocynanin [58-64]. In the following years the studies on these macromolecule will be

continued and further developed by elucidation of the interactions of albumin with therapeutically important compounds, new drug candidates etc.

3.3. Spectroscopic studies on cultural heritage materials

Globally, the study of the composition of various materials of art and archeological artifacts is unquestionably up-to-date topic. The rich culture and art that have developed millennia on the territory of modern Bulgaria are prerequisite for the progress of national values and identity, and have an important reflection on the growth of a number of economic sectors. This poses great challenges in their study and preservation and it is an important responsibility for the country, cultural, educational and scientific institutions and society as a whole. Science and technology play a significant role in accomplishment of these activities.

Over the past few years in a laboratory “Structural organic analysis” in partnership with the Department of restoration in NAA and Leibniz-Institut für Polymerforschung Dresden e.V., began the systematic work in analyzing of inorganic and organic materials from cultural valuables. So far are analyzed and restored paintings from various galleries and museums and are constructed and protected a number of master's thesis. In the implementation of a project, funded by National Science Fund of Bulgaria based on vibration analysis combined with X-ray powder diffraction, elemental analysis (XRD, SEM-EDS), differential scanning calorimetry (DSC) etc., were studied works of fine and applied art from different periods such as: Tracian tombs with frescos decorate from 4-5 century BC; a church with frescos decorate dating from 14 century, a part of the ancient cultural and communication complex "Serdika"; murals of the Central church "The Nativity of the Virgin" of the Rila monastery, executed in different time periods by some of the prominent zographs of the Bulgarian Revival – Dimitar Zigrapf and Zahari Zograps; murals of Kurilo Monastery “St. Ivan Rilski”, Bulgaria, painted in 1596; polychrome iconstand from Kurilo monastery and many others. The work in this area resulted in several publications [65-68].

4. References:

(* the papers presented in the current contest for "professor" position are marked in bold)

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