Abstracts of main results and scientific contributions of Assoc. Prof. Dr. Eng. Petar Todorov Todorov

Summary of scientific papers:

Total number of scientific papers submitted for participation in the competition	**26**
Total impact factor	**52,879**
Individual impact factor	**10,668**
1. Publications in journals referenced in *Scopus* and *Web of Science* data bases with Impact Factor or Scientific Journal Ranking	
A. *with Impact Factor*	**20**
B. *with Scientific Journal Ranking*	**6**
2. Participation in other scientific forums (reports / posters)	**41**
3. Total number of citations	>**240**
4. Participation in research projects for the period 2013-2021, funded by:	
A. Bulgarian National Scientific Fund	**4**
B. NIS-UCTM	**8**
C. Others	**7**

All applications submitted for participation in the competition are in the field of Organic Chemistry, in particular the design, synthesis and characterization of biologically active substances, as well as structure -activity relationship of biologically active molecules, in particular endogenous peptides and hydantoin derivatives.

The main scientific contributions can be summarized in two areas:

I. Synthesis, spectral characterization and study of the biological properties of new endogenous peptides.

II. Synthesis and characterization of new hydantoin derivatives.

I. Synthesis, spectral characterization and study of the biological properties of new endogenous peptides.

I.1. Synthesis, characterization and study of the biological activity of new hemorphin analogues.

Biologically active peptides are important starting structures in the search for and development of new potential anticonvulsant and antinociceptive agents. They bind to various receptors (opioid, non-opioid or both) and are involved in the control of various physiological processes. The synthesis and research of this type of compounds is a current scientific topic, directly related to the study and future development of new therapies used in medicine. The

development of drugs with low toxicity and the absence of side effects is a major problem, the solution of which is a challenge for scientists around the world.

Endogenous opioid peptides function simultaneously as hormones and neuromodulators. They are peptide molecules that are produced in the central nervous system as well as in various glands of the body. Hemorphins, or also called hemoglobin-active peptides, are natural peptides with affinity for opioid receptors (μ-, δ- and k-) and morphinomimetic properties. 10 hemorphins derived from one section of the β-globin chain of hemoglobin have been identified. They are found in various tissues and body fluids, such as brain, plasma, cerebrospinal fluid, spinal cord, erythrocytes and others. Under normal conditions, hemorphins are found in tissues as families of structurally related peptides that differ in their N- and C-terminal degradation. Some hemorphins have an established mechanism of action associated with the inhibition of the enzyme aminopeptidase-N and are functional antagonists of the biologically active angiotensin peptide angiotensin IV. Therefore, the synthesis of new N- and C-modified analogues of blood protein peptides, such as hemorphins containing non-proteinogenic and / or natural amino acids, aminophosphonic residue etc. with neurobiological activity is of interest both synthetic chemists and pharmacologists. Most of our studies are in the field of targeted synthesis and design of new peptide analogues of bioactive hemorphins, their characterization and determination of their biological activity, and are presented in publications № ***A11, A12, A13, A14, A15, A16, A19, A20, A22, A23*** and ***A26.*** The potential of this group of compounds as biologically active substances and the prospects of the work on the creation of new derivatives of hemorphins with neuropharmacological activity were the main motive in our scientific developments. The papers published in the last 2-3 years related to the synthesis and characterization of the new hemorphin analogues have been cited several times in the World Scientific Literature. This shows the importance of the investigations.

Based on the extensive experience we have in both solid-phase peptide synthesis and the preparation of α-aminophosphonic acids, we synthesized the new hemorphin analogues using solid-phase peptide synthesis (Fmoc-strategy (Shepard's synthesis)) and Rink-Amide-MBHA resin as a solid phase carrier. According to the conditions of the method, the construction of the peptide chain is carried out on a solid support - specially treated polyvinyl insoluble resin, which under certain conditions binds to the C-terminus of the first amino acid and also under certain conditions, the completed peptide chain is cleaved from the resin at the end of the synthesis.

The main contributions related to the new hemorphin analogues are:

- Article № ***A11*** presents the synthesis and antinociceptive activity of new analogues of VV-hemorphin-5, modified in position 1 and 7 with non-proteinogenic and / or natural amino acids following the structure: Xxx-Val-Val-Tyr-Pro-Trp- Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-Yyy-NH_2, where Xxx is Ile or Aib and Yyy is Lys / Orn / Dap / Dab. All tested peptides showed a short-term initial antinociceptive effect, with the exception of peptide H2, containing 2,3-diaminopropanoic acid (Dap) at position 7 and characterized by a prolonged and strong antinociceptive effect, while other peptide analogues showed more variable effects on visceral nociception depending on the dose and time after injection.
- Scientific work № ***A12*** presents the synthesis, characterization and study of the biological properties of a new peptide analogue of VV-hemorphin-5, containing

azobenzene residue with attitude to its E → Z photophysical properties. The compound was synthesized by modified solid phase peptide synthesis according to the Fmoc-dimerization strategy. To study the effect of the synthesized azopeptide on the electrical properties of cell membranes, the specific capacity of the lipid layers was measured. The results showed a decrease in membrane capacity in the presence of the azopeptide, as well as evidence of possible changes in the dielectric constant of the bilayer lipid layer. The peptide (Val-Val-Tyr-Pro-Trp-Thr-Gln)$_2$Azo was also tested in vivo for pre-anticonvulsant activity by a 6-Hz assay and an intravenous pentylenetetrazole seizure assay (PTZ) in mice. The Z-isomer has been found to reduce the possibility of PTZ-induced seizures and the peptide deserves further evaluation in other screening tests for anticonvulsant activity. In Article № ***A2***, chromatographic analysis of two-component charged lipid bilayers in aqueous solutions with controlled ionic strength at different pH of the medium was performed. Observations by phase contrast and fluorescence microscopy showed the coexistence of two structural phases in stearoyl oleoyl phosphatidylcholine (SOPC) membranes containing more than 10 mol% of the charged lipid dioleoyl phosphatidylserine.

- VV-Hemorphin-5, also known as Valorphin, is an endogenous opioid peptide from the hemorphin family with affinity for opioid receptors. The peptide molecules obtained by solid-phase peptide synthesis according to the Fmoc strategy are C-terminal amides. Following the structural model of VV-hemorphin-5, modifications were made in positions 1 and 7 with unnatural and proteinogenic amino acids (*Aaa*-Val-Val-Tyr-Pro-Trp-Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-*Aaa*-NH_2). The peptide derivatives were evaluated for their anticonvulsant activity in three acute seizure tests in male ICR mice, the maximal electroshock (MES), the 6 Hz psychomotor seizure test, and the timed intravenous pentylenetetrazole (ivPTZ) infusion test. Motor coordination was not affected by newly developed analogues of VV-hemorphin-5. Among the tested peptide analogues, V4 showed anticonvulsant activity in the three seizure tests that was comparable to the VV-Hemorphin-5 (V1) used as a positive control. While V5, V6, and V7 peptide derivatives exhibited anticonvulsant activity in the MES and 6 Hz test, they were inactive (V7) or showed pro-convulsant effect (V5 and V6) in the i.v. PTZ test. Docking study results suggest that kappa opioid receptor binding could be the mechanism of action of peptide derivatives with anticonvulsant activity. The results suggest that incorporation of nonproteinogenic and/or natural amino acids at position 1 and 7 of the VV-Hemorphin-5 scaffold deserve further evaluation in models of epilepsy and derivatization (papers № ***A14*** and ***A15***).
- For the first time an aminophosphonic residue was introduced in peptides of this type (articles № A16 and A23). A new series of N-modified analogues of VV-hemorphin-5 containing an aminophosphonic residue were synthesized, characterized, and tested for anticonvulsant activity. The new peptide molecules were synthesized by solid phase peptide synthesis according to the Fmoc strategy. The biological activity of the compounds was evaluated by performing an intravenous pentylenetetrazole seizure test (ivPTZ) and a 6-Hz psychomotor test in

experimental models in mice. Among the five tested peptide analogues, V3p was the most active against the ivPTZ test with effect comparable to that of the VV-hemorphin-5 (V1) used as a positive control. The peptide analogues V2p–V5p were able to suppress dose-dependent psychomotor seizures in the 6-Hz test. In contrast, the V6p peptide showed either a pro-convulsant effect in the iv PTZ test or was inactive in the 6-Hz test. No changes in motor coordination were noted with the novel peptides. Docking study results suggest that kappa opioid receptor binding could be the mechanism of action of peptide derivatives with anticonvulsant activity. The results suggest that incorporation of aminophosphonate moiety at position 1 of the VV-hemorphin-5 scaffold deserve further evaluation in models of epilepsy and derivatization.

The newly synthesized compounds were also investigated for their potential antinociceptive activities in formalin-induced model of acute and inflammatory pain in mice. The experiments were carried out on adult male ICR mice (publication № ***A23***).

Publication № ***A20*** shows a simple and rapid electrochemical method for the selective copper determination in water samples using non-toxic and easy degradable peptide molecules such as VV-hemorphin-5 analogues. The peptide sensor provided electrochemical and sensing excellent response with a low limit of detection (0.188 ng ml^{-1}).

- Paper № ***A19*** presents the synthesis, characterization and anticonvulsant activity of new analogues of VV-hemorphin-7, modified in position 4 and 7 with unnatural amino acids, following the structure Val-Val-Tyr-*Xxx*-Trp-Thr-*Yyy*-Arg-Phe-NH_2, where Xxx is Ac5c (1-aminocyclopentanecarboxylic acid) or Ac6c (1-aminocyclohexanecarboxylic acid), and Yyy is Dap (diaminopropanoic acid) or Dab (diaminobutanoic acid). The new synthetic peptide analogues were prepared by solid-phase peptide synthesis—Fmoc chemistry. A single intracerebroventricular (i.c.v.) injection at doses of 5, 10, and 20 μg/10 μl, respectively, was given before evaluation with timed intravenous pentylenetetrazole (ivPTZ) infusion test and 6-Hz psychomotor seizure test in mice. To explain the structure-active properties of the modified peptides, some physicochemical characteristic was obtained. The FT-IR spectra and their second derivatives of the amide I, II, and III bands of the peptides show ß-sheet structure conformation. Analogues 4 and 5 of VV-hemorphin-7 were the most active against the ivPTZ test, with the effect comparable to that of peptide 1 used as a positive control. Except compound 8, all other tested peptide analogues were ineffective to raise the threshold for the clonic seizures. The novel peptides did not show neurotoxicity in the rotarod test.
- Synthesized, characterized and tested for anticonvulsant activity against three tests for acute seizures in experimental models of ICR mice were a series of new analogues of hemorphin-4, modified with unnatural conformational inhibited amino acids, following the structure Aaa–Tyr–*Xxx*–Trp–Thr–NH_2 where Aaa is the low molecular weight lipophilic adamantyl residue and Xxx is Ac5c (1-aminocyclopentanecarboxylic acid) or Ac6c (1-aminocyclohexanecarboxylic acid).

The new synthetic neuropeptides were obtained by solid-phase peptide synthesis according to the Fmoc strategy. From the six synthesized peptide analogues, the P4-5 was the most active at doses of 1 and 3 μg in the three seizure tests. The order of potency of other peptides was as follows: P4 > P4-3 = P4-4 > P4-2 > Ang IV in MES, P4-4 ≥ P4-1 > P4-3 > P4-2 > P4 > Ang IV in 6-Hz test and P4-4 = P4-3 > P4-2 = P4 > Ang IV in ivPTZ test. None of the peptides displayed neurotoxicity in the rota-rod test. Docking study results suggest that direct H-bonding and ionic interactions between our synthetic ligands and residues, responsible for coordination of Zn^{2+} along with hydrophobic interactions between our ligands and IRAP active site are the most important for the ligand binding. The results propose that incorporation of adamantane and cycloalkane building blocks in the peptide chain of the hemorphin-4 scaffold is important for the potential high biological activity (paper № ***A22***).

- In this paper **№*A26*** a new analogue of hemophin-4 containing azobenzene has been synthesized and investigated for assessment of spectral, electrochemical, and biological effects. The synthesis was achieved by a modified solid-phase peptide synthesis (SPPS) by Fmoc-strategy. This compound represents a newly synthesized and unstudied peptide-based chemosensor bearing azobenzene side-chain with different spectral and electrochemical properties in the two trans-/cis-states depending on the solvent polarity. Their fluorescence intensity, as well as voltammetric behavior, was found to depend on both the polarity of the solvents and the type of isomers of the azopeptide compound. The novel biopeptide Azo-Tyr-Pro-Trp-Thr-NH_2 was explored in vivo for potential anticonvulsant activity by 6-Hz seizure test and maximal electroshock test (MES) in ICR mice. The cis-Az-H4 isomer showed stronger potency than the trans-Az-H4 against both the 6 Hz-induced psychomotor seizures and tonic-clonic seizures in the maximal electroshock test with 100% protection demonstrated at the lowest dose of 1 μg administered intracerebroventricularly in mice. None of the tested isomers displayed neurotoxicity in the rotarod test. Our results suggest that modified biopeptide in the N-terminus of hemorphin-4 with azobenzene deserve further exploration as a promising candidate with both anticonvulsant activity and as a chemosensor for pH determination.

I.2. Synthesis, characterization and study of the biological activity of new opioid peptides, nociceptin and endomorphin analogues.

Nociceptin is an opioid neuropeptide that is involved in the modulation of many processes controlled by the central nervous system. It affects the sensations of pain and fear, motor activity, training and others. In the peripheral nervous system, nociceptin affects the functions of the cardiovascular, digestive, excretory and respiratory systems. Opiate analgesics are widely used as painkillers. The study of endogenous opioids is aimed at detecting an analgesic with fewer side effects. The more significant contributions in this direction can be summarized as follows:

- The effects of two new N/OFQ(1-13)NH_2 derivates in which the N-terminal Phe was replaced with 1-[(methoxyphosphono) methylamino]cycloalkanecarboxylic

acids containing seven AFC7-N/OFQ(1-13)NH_2, (NC7) or eight-membered cycloalkane rings AFC8-N/OFQ(1-13)NH_2, (NC8) on the fast oscillations of the interpulse interval (IPI) as well as on the sympathovagal balance in conscious Wistar rats were investigated. Our results indicated that NC7 led to displacement of sympathovagal balance as a result of decrease of sympathetic determined fast oscillations, whereas NC8 involved a powerful mechanism responsible for long-lasting regulation of heart rate (paper ***№ A5***).

- Aiming to develop more potent analgesic substances a new series of hexapeptides containing b2-tryptophan analogues was synthesized. The Trp in position 4 and 5, respectively in Ac-Arg-Phe-Met-Trp-Met-Lys-NH_2 (opioid receptor antagonist) and Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2 (highly potent and selective NOP-receptor agonist) was substituted by the (S)-2-(1-methyl-1H-indol-3-yl)propionic residue or the (S)-2-(5-methoxy-1H-indol-3-yl)propionic residue. The analgesic effect of the four newly synthesized compounds has been evaluated in male Wistar rats. Replacement of Trp with b2-tryptophan analogues in 4th position (Ac-Arg-Phe-Met-Trp-Met-Lys-NH_2) led to increased and longer lasting analgesic effect. The results obtained permit us to assume that both opioid and NOP receptors take part in the newly synthesized compounds analgesic effects. (papers ***№ A1*** and ***A3***).
- Endomorphins are small endogenous neuropeptides that are produced by the body and act to reduce pain. They are tetrapeptides with the highest known affinity and selectivity for the μ-opioid receptor. This report ***№ A7*** refers to the synthesis and characterization of novel endomorphin analogues containing phosphonate moiety. The new endomorphins with N-terminal phosphonate were prepared using solid phase peptide synthesis by Fmoc chemistry. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction.

II. Synthesis and characterization of new hydantoin derivatives.

Hydantoins and their derivatives are widely used in various fields of science, industry and everyday life. Especially valuable is the use of some of them as medicines. A number of hydantoin derivatives are used as antiepileptic drugs, arrhythmia, antitumor agents, in the treatment of asthma, as aldose reductase inhibitors and others. Hydantoins are also of interest as starting materials for the synthesis of non-proteinogenic amino acids and peptides with their participation. Hydantoins, which are substituted for C-5, are known to be important drugs. The discovery of the anticonvulsant effects of 5-ethyl-5-phenylhydantoin and its use as an antiepileptic agent provoked the synthesis and study of a large number of 5,5'-disubstituted hydantoins, which have found various applications in medicine. The hydantoin derivative that is most important as a medicinal product is 5,5'-diphenylhydantoin (Phenytoin), also known as Dilantin, and Epanutin. The following major contributions have been made in this direction:

- For the purposes of the studies, it was necessary to synthesize preparative amounts of the ligands 3-amino-5,5'-dimethylhydantoin and 3-amino-5,5'-diphenylhydantoin. They were prepared by the reaction of the corresponding C-5 substituted hydantoin and hydrazine hydrate by a method modified by us. The resulting starting ligands were purified and characterized using modern techniques

and methods. Hydantoin derivatives, as well as their complexes with transition metal ions, have been intensively studied in recent years due to their wide application in various fields of medical practice. The structural characteristics of hydantoins and their metal complexes are interesting from the point of view of the development of both new drugs and for a better understanding of the relationship between structure and activity (publications № ***A4, A9, A10, A18*** и ***A25***). Publication № ***A4*** presents the synthesis, spectral characterization, and in vitro antitumor activity of two new complexes of 3-amino-5,5'-dimethylhydantoin with Cu (II) and Co (II) ions. The test compounds showed different antiproliferative activity against HT29, MDA-MB-231, HepG2 and HeLa cell lines after 24 hours of treatment. HepG2 cells are most sensitive at different concentrations of test compounds, followed by HT29. Article № ***A9*** presents the synthesis and characterization of new complexes of 3-amino-5,5'-diphenylhydantoin with Cu (II). Analyzes showed that 3-amino-5,5'-diphenylhydantoin acts as a bidentate ligand, including the carbonyl oxygen and the nitrogen atom of the amino group in the coordination of the metal ion. The obtained results gave us a reason to expand and deepen our research by synthesizing and analyzing new complexes of 3-amino-5,5'-dimethylhydantoin with Ni (II) and Zn (II) ions, which would be useful for future development of methods for the determination of the specified metals in different media (publication № ***A10***). In order to find practical application, as well as due to its favorable electrical and optical properties, some of the synthesized ligands and complex compounds, such as Cu (II) 3-amino-5,5'-dimethylhydantoin (CLP) and Ni (II) 3-amino-5,5'-dimethylhydantoin (NLP) with different concentrations were applied in composite films of the azopolymer PAZO (publication № ***A18*** and ***A25***).

- Four cycloalkanespirohydantoins with 5-, 6-, 7- and 8-membered rings have been synthesized, purified and characterized: 1,3-diazaspiro[4.4]nonane-2,4-dione (CSH (5)); 1,3-diazaspiro[4.5]decane-2,4-dione (CSH (6)); 1,3-diazaspiro[4.6] undecane-2,4-dione (CSH (7)); and 1,3-diazaspiro[4.7]dodecane-2,4-dione (CSH (8)) in order to further study the mechanism and electrochemical behavior of these biologically active compounds. They were obtained by the Bucherer-Bergs reaction from the corresponding cyclic ketones under the action of KCN and $(NH_4)_2CO_3$. The proposed mechanisms of the studied cycloalkanespiro-5-hydantoins could be essential in terms of revealing the main pathways by which such compounds exert their biochemical action (publication № ***A8***).
- Publications № ***A13, A17, A21*** and ***A24*** present the synthesis, structural, photophysical and electrochemical properties of a series of new Schiff bases containing a hydantoin ring. The compounds were synthesized by a condensation reaction between 3-amino-5,5'-dimethylhydantoin / 3-amino-5,5'-diphenyl-hydantoin and various aromatic aldehydes. All compounds were characterized by different spectral and electrochemical methods, as well as by X-ray diffraction analysis of a single crystal. Also, the photochromic and molecular switching behavior of the azomethine -CH = N- groups in Schiff bases was studied. These compounds are newly synthesized and unstudied photoswitches with different spectral properties in both trans- / cis states, depending on the polarity of the

solvents. The results showed that by varying the medium from the solvent, direct control of the two-way switching behavior from one type to another can be achieved, and can be referred to as photochromic switches.

An indication of the relevance and importance of the research conducted are the numerous citations in the World Literature on hydantoins and their derivatives observed in the last year.

Abstracts of main results

A1. Rositsa Zamfirova, Nikola Pavlov, **Petar Todorov**, Polina Mateeva, Jean Martinez, Monique Calmès, Emilia Naydenova. Synthesis and changes in affinity for NOP- and opioid receptors of novel hexapeptides containing β^2-tryptophan analogues. *Bioorganic & Medicinal Chemistry Letters, Volume 23, Issue 14*, *15 July 2013*, *Pages 4052-4055,* (*IF=2.338*).

We report the synthesis and the biological activity of new analogues of Ac-RFMWMK-NH_2 and Ac-RYYRWK-NH_2, modified in position 4 and 5, respectively, with incorporation of newly synthesized ß^2-tryptophan analogues. Trp was substituted by the (S)-2-(1-methyl-1H-indol-3-yl) propionic residue or by (S)-2-(5-methoxy-1H-indol-3-yl)propionic residue. The biological activity (pEC_{50} and E_{max}) of these compounds was tested on electrically stimulated preparations of rat vas deferens. The 5-methoxy ß-tryptophan group reverses the affinity of the compounds.

A2. Denitsa Mitkova, Angelina Stoyanova-Ivanova, Stela Georgieva, **Petar Todorov**, Nikolay Kozarev, Yury A. Ermakov, Victoria Vitkova. Charged lipid bilayers in aqueous surroundings with low pH. *Book Series: Advances in Planar Lipid Bilayers and Liposomes, Volume 18, 2013, pages 1-20. ISSN 1554-4516*, http://dx.doi.org/10.1016/B978-0-12-411515-6.00001-1.

The present chapter discusses our experimental results on morphology, stability, and the mechanical properties of two-component charged lipid bilayers in aqueous solutions with controlled ionic strength at low pH. Observations by phase-contrast and fluorescent microscopy revealed coexistence of two structural phases in membranes from stearoyl oleoyl phosphatidylcholine (SOPC), containing more than 10 mol% of the charged lipid dioleoyl phosphatidylserine. At temperatures lower than 29 ^oC, the existence of nonfluctuating “hard” domains was registered in fluid membranes at pH 5.0. The method of shape-fluctuation analysis of quasi-spherical vesicles was applied for the determination of the bending modulus of homogeneous liquid bilayers. The value obtained for this material constant of bilayers with 0.2 molar fraction of charged lipid is around 30% higher than the bending modulus of uncharged (SOPC) membranes in the same surrounding solution. The experimental findings, presented and discussed in the chapter, qualitatively agree with our previous results for the curvature rigidity of charged bilayers as measured by vesicle micromanipulation.

A3. Adriana Bocheva, Hristina Nocheva, Nikola Pavlov, **Petar Todorov**, Monique Calmès, Jean Martinez, Emilia Naydenova. Synthesis and analgesic effects of novel β^2-

tryptophan hexapeptide analogs. *Amino Acids*, *2013, October 2013, Volume 45, Issue 4, pp 983-988,* (*IF*=3.653).

Aiming to develop more potent analgesic substances a new series of hexapeptides containing ß^2-tryptophan analogues was synthesized. The Trp in position 4 and 5, respectively in Ac-Arg-Phe-Met-Trp-Met-Lys-NH_2 (opioid receptor antagonist) and Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2 (highly potent and selective NOP-receptor agonist) was substituted by the (S)-2-(1-methyl-1H-indol-3-yl)propionic residue or the (S)-2-(5-methoxy-1H-indol-3-yl)propionic residue. The analgesic effect of the four newly synthesized compounds has been evaluated in male Wistar rats by PP and HP tests and compared to the native templates. Further estimation of the mechanisms of action of each compound was achieved using specific antagonists—naloxone for opioid and JTC801 for the NOP receptor. Replacement of Trp with ß^2-tryptophan analogues in 4th position (Ac-Arg-Phe-Met-Trp-Met-Lys-NH_2) led to increased and longer lasting analgesic effect. The results obtained permit us to assume that both opioid and NOP receptors take part in the newly synthesized compounds analgesic effects.

A4. Stela Georgieva, **Petar Todorov**, Diana Wesselinova. Synthesis, Characterization and Cytotoxic activity of Novel Cu(II) and Co(II) Complexes with 3-amino-5,5-dimethylhydantoin. *Comptes Rendus Chimie* 2014, *Volume 17, Issue 12*, *December 2014*, *Pages 1212-1220*, (*IF=1.713*).

Novel mixed complexes of copper (II) and cobalt (II) with 3-amino-5,5-dimethylhydantoin were synthesized and their in vitro anticancer activity was investigated. The structures of the compounds were confirmed by IR, UV–Vis spectrometry, voltammetry and elemental analysis. The cytotoxic effects of novel complexes of copper (II) and cobalt (II) with 3- amino-5,5-dimethylhydantoin were tested against a panel of human tumor cell lines. All of the compounds investigated exhibited different concentration-dependent antiproli- ferative effects against the HT29, MDA-MB-231, HepG2 and HeLa cell lines after 24 h of treatment. The most sensitive cells were the HepG2 cells at various concentrations of both tested compounds followed by HT29.

A5. R. A. Girchev, P. P. Markova, **P.T. Todorov**, E. D. Naydenova. Sympathovagal balance after application of N-modified nociceptin analogues. *Bulgarian Chemical Communications, Volume 47, Number 1 (pp. 45 – 49) 2015,* (*IF=0.32*).

The effects of two new N/OFQ(1-13)NH_2 derivates in which the N-terminal Phe was replaced with 1-[(methoxyphosphono) methylamino]cycloalkanecarboxylic acids containing seven AFC7-N/OFQ(1-13)NH_2, (NC7) or eight-membered cycloalkane rings AFC8-N/OFQ(1-13)NH_2, (NC8) on the fast oscillations of the interpulse interval (IPI) as well as on the sympathovagal balance in conscious Wistar rats were investigated. The NC7 or NC8, was applied by i.v. bolus injection in dose 100 nmol/kg b.w. and its effects were studied for nine consecutive 10 minute long intervals. The spectrograms for IPI were derived by using Fast Fourier Transform algorithm. The spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band typical for rats (20-195; 195-605; 605-3000 mHz, respectively) was studied. The sympathovagal balance was determined by the relation of power of mid to high frequency band PMF/PHF. Application of NC7 led to decrease of PMF/PHF from 0.52±0.04 to 0.31±0.08 ($p<0.05$); 0.24±0.06 ($p<0.01$) and to 0.23±0.07, ($p<0.01$) in the first three 10 min long intervals

as a result of decrease of mainly sympathetic mediated oscillations of IPI (PMF). Depended by parasympathetic branch of autonomic nervous system fast oscillations of IPI (PHF) were not influenced. Application of NC8 did not affect fast oscillation of IPI as well as sympathovagal balance, but provoked a sustained decrease of heart rate in the course of 70 min, ($p<0.05$). Our results indicated that NC7 led to displacement of sympathovagal balance as a result of decrease of sympathetic determined fast oscillations, whereas NC8 involved a powerful mechanism responsible for long-lasting regulation of heart rate.

A6. S. Georgieva, **P. Todorov,** Z. Genova, P. Peneva. Interdisciplinary Project for Enhancing Students' Interest in Chemistry. *Chemistry: Bulgarian Journal of Science Education. Khimiya. 25, 122-136 (2016). SJR (2014) = 0.210.*

A student research project for sampling and analysis of drinking water from different Bulgarian regions has been parallel incorporated into analytical and organic courses at the second level of high engineering education in Bulgaria. The degree of quality of drinking water has been evaluated in fi ve cities from different Bulgarian regions: Stara Zagora, Gorna Oryahovitsa, Parvomai, Dupnica and Sofi. The students analyzed water samples for pH, hardness, organic compounds, sodium, potassium and heavy metals ions like cadmium, copper, iron, lead and manganese using titrimetric methods, atomic absorption and atomic emission spectrometry, potentiometry and voltamperometry. The article also describe pre-lab activities related to the "water quality project" in order to increase student motivation, independence, and critical thinking skills. Students have been motivated to perform the seminar and practical work and get used to an approach often used in the chemical engineering' practice. Students have been explored parameters concerning water quality from different sources and had an opportunity to evaluate the data critically and to answer the pre-lab questions. Based on student results the "water project" became overwhelmingly popular with students while challenging them to think critically and work independently on problem solving tasks. Thanks to the "water quality project" we found that the student's willingness to carry out a scientifi c work is increased. The students become more motivated and committed to the learning process.

A7. **Petar T. Todorov,** Petia N. Peneva, Zlatina N. Genova, Emilia D. Naydenova. Synthesis and characterization of new endomorphin analogs with N-terminal phosphonate. *Bulgarian Chemical Communications, Special Issue E, (pp. 31–34) 2017. (IF=0.32)*

Endomorphins are small endogenous neuropeptides that are produced by the body and act to reduce pain. They are tetrapeptides with the highest known affinity and selectivity for the μ-opioid receptor. This report refers to the synthesis and characterization of novel endomorphin analogues containing phosphonate moiety. The new endomorphins with N-terminal phosphonate were prepared using solid phase peptide synthesis by Fmoc chemistry. The phosphonate moiety was incorporated by modification of Kabachnik-Fields reaction. The crude neuropeptides were purified on a reversed-phase high-performance liquid chromatography and the molecular weights were determined, using electrospray ionization mass-spectrometry, and also determining of the specific angles of optical rotation.

A8. S. Georgieva, **P. Todorov,** E. Naydenova. Investigation of redox mechanisms of biologically active hydantoin derivatives by different voltammetric methods. *Analytical & Bioanalytical Electrochemistry. 9 (2), 214-231, 2017. (IF=0.64)*

The electrochemical behavior of four biologically active cycloalkane spirohydantoins with 5-, 6-, 7-, and 8-membered rings: 1,3-diazaspiro[4.4] nonane-2,4-dione (CSH(5)); 1,3-diazaspiro[4.5]decane-2,4-dione (CSH(6)); 1,3-diazaspiro[4.6]undecane-2,4-dione (CSH(7)); and 1,3-diazaspiro[4.7]dodecane-2,4-dione (CSH(8)) has been investigated at the pH range 1.81-12 by modern electrochemical techniques as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). The electrochemical studies were carried out on a hanging mercury drop electrode (HMDE) at room temperature. CSH(5) and CSH(6) at pH 5.50 and 7.63 and CSH(7) at pH 5.50 showed a single quasireversible peaks. For CSH(7) at pH 7.63 and CSH(8) at two pH values the irreversible nature of the electrode process was proved. The effect of scan rate, pH and concentration on peak current and peak potential was investigated. Physical parameters like diffusion coefficient and heterogeneous electron transfer rate constant were determined from scan rate and concentration effects. The number of proton transfer in the electrochemical reaction was determined from the peak potential shift as a function of pH and a mechanism of cycloalkanespiro-5-hydantoins derivatives on the basis of CV, SWV and DPV results was proposed. The proposed mechanisms of the cited cycloalkane spiro-5-hydantoins could be essential in regard to the hidden pathways by which such compounds exert their biochemical actions.

A9. S. Georgieva, **P. Todorov**. Spectroscopic and Voltamperometric studies of Cu(II) complex with 3-amino-5,5-diphenylhydantoin. *Journal of Chemical Technology and Metallurgy. 53 (3), 465-472, 2018.*

New copper(II)-aminohydantoin complexes ($[CuAPh_2Cl_2(OH_2)_2]$; $[CuAPh_2(NH_3)_2(OH_2)_2]$, where APh is 3-amino-5,5-diphenylhydantoin), were synthesized and analyzed by means of elemental analysis, atomic absorption spectroscopy, UV-Vis spectroscopy and voltamperometric method. The complex forming processes in the Cu(II)-aminophenytoin (APh) system are studied by differential pulse polarography (DPP). The experiments are carried out at temperature of 20±1^{o}C in ammonia buffer solution (pH = 8.2±0.1) at ionic strength I = 0.1. The reversibility of the reduction of the peak is proved and the equations of De Ford and Hume and these of Leden to DPP data are applied. The existence of two complexes (n =1 and 2) is proved in the presence of high ligand concentration (CAPh = 2.10^{-4} - 3.10^{-3} mol l-1) and the values of its total stability constants are found. A satisfactory good coincidence between the results obtained by the procedure proposed and the literature data is observed. The atomic absorption spectroscopy and elemental analysis confirm the compounds stoichiometry. The IR and UV/Vis spectra show that APh act as bidentate ligands with the coordination involving the carbonyl oxygen and the nitrogen atom of amino group.

A10. Stela Georgieva, **Petar Todorov**, Artem Bezfamilnii, Anton Georgiev. Coordination behavior of 3-amino-5,5-dimethylhydantoin towards Ni(II) and Zn(II) ions: Synthesis, spectral characterization and DFT calculations. *Journal of Molecular Structure*, *Volume 1166, 15 August 2018, Pages 377-387* (*IF=2.12*)

The interaction of 3-amino-5,5'-dimethylhydantoin with nickel and zinc ions was investigated at different conditions by means of UV/Vis, FTIR spectroscopy and electrochemical methods as well as DFT quantum chemical calculations. Through calculated DFT frequencies and experimental FTIR spectra of the complexes the correlation analysis has been made. We have shown that complexes with 1:2 stoichiometry were formed at pH closed to physiological. The stability constant was determined calculating the value of total stabitlity constant of the two complexes. The results of the study are useful for incorporation with chemical equilibrium models for evaluation of the speciation and the reactions of metals with hydantoin derivatives in order to develop methods for determination of these metals in different milieu.

A11. **Petar Todorov,** Petia Peneva, Daniela Pechlivanova, Stela Georgieva, Elena Dzhambazova. Synthesis, characterization and nociceptive screening of new VV-hemorphin-5 analogues. *Bioorganic & Medicinal Chemistry Letters*, *Volume 28, Issue 18, 1 October 2018, Pages 3073-3079. https://doi.org/10.1016/j.bmcl.2018.07.040,* (*IF=2.448*).

In the present study, some new analogues of VV-hemorphin-5, modified at position 1 and 7 by the non-proteinogenic and/or natural amino acids followed the structures Xxx-Val-Val-Tyr-Pro-Trp-Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-Yyy-NH_2, where Xxx is Ile or Aib and Yyy is Lys/Orn/Dap/Dab were synthesized to investigate their potential antinociceptive activities. We report also the redox potentials and the acid/base properties as pKa values of these peptide analogues which were compared toward electrochemical behaviour of tryptophan containing peptides. All analogues showed a short lasting initial antinociceptive effect, however H2 hemorphin analogue is characterized with prolong and strong antinociceptive effect, while the other peptide analogues exerted more variable effects on the visceral nociception depending on the dose or time after the intracerebral injection.

A12. **Petar T. Todorov,** Petia N. Peneva, Stela I. Georgieva, Jana Tchekalarova, Victoria Vitkova, Krassimira Antonova, Anton Georgiev. Synthesis, characterization and anticonvulsant activity of new azobenzene-containing VV-hemorphin-5 bio photoswitch. *Amino Acids (2019) 51: 549-563, https://doi.org/10.1007/s00726-018-02691-1. (IF=3.063).*

A novel analog of VV-hemorphin-5 containing azobenzene moiety has been synthesized and investigated for anticonvulsant activity in relation to its $E \rightarrow Z$ photophysical properties activated by long wavelength light at 365 nm. The synthesis was achieved by a modified SPPS by Fmoc-dimerization strategy. The electrochemical behavior before and after UV illumination was investigated using different voltammetric modes. The number of electrons transferred, heterogenic rate constant and diffusion coefficient for *E*- and *Z*-isomers were also evaluated. Revealing the governing principles involved in signaling and nerve pulse propagation requires the detailed characterization of the electrical properties of cell membranes. For probing the effect of synthesized azo-peptide on the membrane electrical properties, we measured the specific capacitance of lipid bilayers, representing a basic physical model of biomembranes with their simple reproducibility in laboratory conditions at controlled membrane composition and physicochemical parameters of the surrounding aqueous medium.

Our results have shown reduced membrane capacitance in the presence of the azo-peptide, thus providing evidences for possible alterations in the dielectric permittivity of the bilayer. The (Val-Val-Tyr-Pro-Trp-Thr-Gln)$_2$Azo peptide was explored also in vivo for preliminary anticonvulsant activity by using the 6-Hz seizure test and pentylenetetrazol (PTZ) seizure test in mice. The *Z*-isomer has exhibited higher potency compared to *E*-isomer most pronouncedly in the 6 Hz test for psychomotor seizures where the compound had activity at all three tested doses. It was found that the *Z*-isomer decrease the latency for onset of clonic seizures induced by PTZ. These results demonstrate that the *Z*-isomer deserves further evaluation in other screening tests for anticonvulsant activity.

A13. **Petar T. Todorov,** Petia N. Peneva, Stela I. Georgieva, Rusi I. Rusew, Boris Shivachev, Anton Georgiev. Photochromic and molecular switching behaviour of new Schiff base containing hydantoin ring: Synthesis, characterization and crystal structure. *New Journal of Chemistry, 2019. http://dx.doi.org/10.1039/C8NJ05748F, 43, 2740-2751 (IF=3.288).*

The present study reports synthesis, structural, photophysical and electrochemical properties of five new Schiff bases containing hydantoin ring. The compounds were synthesized via condensation reaction between 3-amino-5,5'-dimethylhydantoin and five different aromatic aldehydes. All compounds were characterized using different spectroscopic and electrochemical tools as well as single crystal X-ray diffraction analysis. The photochromic and molecular switching behaviour of the switchable -CH=N- groups of Schiff bases were investigated by real time UV-Vis spectroscopy in DMF under long wavelength UV light at λ = 365 nm for 90 min (mostly *E*→*Z*) and dark relaxation at room temperature for 60 min (mostly *Z*→*E*) in the spectral region 250 – 600 nm at equal concentrations. The electrochemical properties of the compounds were investigated on a glassy platinum electrode (Pt-) in DMF using cyclic voltammetric (CV) technique before and after UV irradiation. The effect of functional groups on reduction potential of the Schiff bases was discussed. Electrochemical study has shown that the reduction of -CH=N groups are mostly quasi-reversible with the adsorption controlled process for (*E*)-3-((2-hydroxybenzylidene)amino)-5,5'-dimethylimidazolidine-2,4-dione (SB2) and (*E*)-3-((4-(dimethylamino)benzylidene) amino)-5,5'-dimethylimidazolidine-2,4-dione (SB4) and intermediate process for the rest compounds.

A14. **Petar Todorov,** Miroslav Rangelov, Petia Peneva, Nadezda Todorova, Jana Tchekalarova. Anticonvulsant evaluation and docking analysis of VV-Hemorphin-5 analogues. *Drug Development Research*, Volume 80, Issue 4, page 425-437, 2019. DOI:10.1002/ddr.21514 (*IF*=1.902).

VV-Hemorphin-5 is an endogenous opioid peptide of the Hemorphin family with affinity at opioid receptors. A series of C-amide analogues have been synthesized, based on the structure of VV-Hemorphin-5, modified at position 1 and 7 by the un/natural amino acids (*Aaa*-Val-Val-Tyr-Pro-Trp-Thr-Gln-NH_2 and Val-Val-Tyr-Pro-Trp-Thr-*Aa1*-NH_2) using SPPS, Fmoc-chemistry. The peptide derivatives were evaluated for their anticonvulsant activity in three acute seizure tests in male ICR mice, the maximal electroshock (MES), the 6 Hz psychomotor seizure test and the timed intravenous pentylenetetrazole (ivPTZ) infusion test. Their neurotoxicity was assessed in the rotarod test. Among the tested peptide analogues, V4

showed anticonvulsant activity in the three seizure tests that was comparable to the VV-Hemorphin-5 (V1) used as a positive control. While V5, V6 and V7 peptide derivatives exhibited anticonvulsant activity in the MES and 6 Hz test, they were inactive (V7) or showed pro-convulsant effect (V5 and V6) in the iv PTZ test. At a dose of 10 µg/mouse the peptide V2 was effective against clonic seizures induced by PTZ. Motor coordination was not affected by newly developed analogues of VV-Hemorphin-5. Docking study results suggest that kappa opioid receptor binding could be the mechanism of action of peptide derivatives with anticonvulsant activity. The results suggest that incorporation of non-proteinogenic and/or natural amino acids at position 1 and 7 of the VV-Hemorphin-5 scaffold deserve further evaluation in models of epilepsy and derivatization.

A15. Stela Georgieva, **Petar Todorov**, Petia Peneva. Investigation of electrochemical behavior of new hemorphin-5 analogue. *Journal of Chemical Technology and Metallurgy, 54, 5, 947-951, 2019.*

A biologically active VV-hemorphin-5 (Valorphin: Val-Val-Tyr-Pro-Trp-Thr-Gln) analogue was characterized electrochemically. In this regard the oxidation and adsorption reactions of the peptide on an electrode surface in a phosphate buffer solution have been studied and compared with those of valorphin and polarographic active tryptophan and tyrosin. For investigation of the interfacial behavior of the VV-hemorphin-5 derivative (H2) voltamperometry with platinum electrode as working electrode, with Ag/AgCl, (3 mol L^{-1}) KCl electrode as a reference electrode, and a carbon electrode as an auxiliary electrode, have been used. The redox potentials were determined under various mode of sweep - differential pulse (DP) and cyclic voltamperometry (CV). Analysis of the wave shape, pH dependence, and concentration dependence of the oxidation process leads to the conclusion that the valorphin derivative is adsorbed on the Pt-electrode. The detection of a low concentration ($2x10^{-5}$ mol L^{-1}) leads to the conclusion for possible application of the voltamperometry as a method for determination of low concentrations from this class peptides.

A16. **Petar Todorov,** Petia Peneva, Jana Tchekalarova, Miroslav Rangelov, Stela Georgieva, Nadezhda Todorova. Synthesis, characterization and anticonvulsant activity of new series of N-modified analogues of VV-Hemorphin-5 with aminophosphonate moiety. *Amino Acids (2019), https://doi.org/10.1007/s00726-019-02789-0. November 2019, Volume 51, Issue 10–12, pp 1527–1545. (IF=3.063)*

A new series of *N*-modified analogues of the VV-hemorphin-5 with aminophosphonate moiety have been synthesized, characterized and investigated for anticonvulsant activity. The novel peptide analogues were prepared by solid-phase peptide synthesis–Fmoc-strategy and were evaluated in the timed intravenous pentylenetetrazole infusion test (ivPTZ) and 6-Hz psychomotor seizure test in mice. The acute neurological toxicity was determined using the rotarod test. The redox potentials at glass carbonic electrode (GC) and the acid/base properties as pKa values of these peptide analogues were compared with the electrochemical behaviour of tyrosine- and tryptophan-containing peptides using different voltamperometric modes. Among the five tested peptide analogues, V3p was the most active against the ivPTZ test with effect comparable to that of the VV-hemorphin-5 (V1) used as a positive control. Dose-dependent elevation of the seizure threshold for myoclonic twitch and generalized clonic

seizures was observed after i.c.v. administration of V2p, V4p and V5p as well as for forelimbs tonus in V4p peptides. The peptide analogues V2p–V5p were able to suppress dose-dependent psychomotor seizures in the 6-Hz test. In contrast, the V6p peptide showed either a pro-convulsant effect in the iv PTZ test or was inactive in the 6-Hz test. No changes in motor coordination were noted with the novel peptides. Docking study results suggest that kappa opioid receptor binding could be the mechanism of action of peptide derivatives with anticonvulsant activity. The results suggest that incorporation of aminophosphonate moiety at position 1 of the VV-hemorphin-5 scaffold deserve further evaluation in models of epilepsy and derivatization.

A17. Anton Georgiev, **Petar Todorov**, Deyan Dimov. Excited State Proton Transfer and E/Z photoswitching performance of 2-hydroxy-1-naphthalene and 1-naphthalene 5,5'-dimethyl- and 5,5'- diphenylhydantoin Schiff bases. *Journal of Photochemistry and Photobiology A: Chemistry. https://doi.org/10.1016/j.jphotochem.2019.112143. 2019, Volume 386, 1 January 2020, 112143, (IF=4.291).*

The paper presents the synthesis and photoinduced enol/keto tautomerization as well as E/Z photoswitching behavior of four 3-amino-5,5′-dimethyl- and -5,5′-diphenylhydantoin Schiff base derivatives containing 2-hydroxy-1-naphthyl and 1-naphthyl moieties. These compounds represent newly synthesized and unstudied photoswitches with different spectral properties in the two switched states depending on the polarity of solvents. Steady-state fluorescence measurements were performed in different solvents and the results show strong sensitivity of environmental polarity on the efficiency of conversion (ηT) of the excited state intramolecular proton transfer. It was observed a high degree of conversion in polar solvents such as AcCN and MeOH, while in nonpolar 1,4-DOX is low. The kinetics of enol/keto tautomerization and E/Z photoswitching to photostationary state (PSS) was studied by real time UV–vis spectroscopy in AcCN by long wavelength UV-light activation at 350 nm. The quantitative and qualitative performance of the switching behavior was evaluated by the degree of photoisomerization (R) and rate constant (k). It was found that 2-hydroxy substituted Schiff bases have lower R (9.6% and 19.7%), compared to the unsubstituted one (32.4% and 37.7%). The speed of back Z/E relaxation ($k=3.62\times10^{-4}$ s^{-1} and $k=5.49\times10^{-4}$ s^{-1}) is unusual slower compared to the very fast E/Z photoconversion ($k=4.07\times10^{-2}$ s^{-1} and $k=1.08\times10^{-1}$ s^{-1}). The behavior of the compounds was analyzed through optimization of the molecular geometry of enol and keto tautomers as well as E- and Z-isomers by DFT calculations B3LYP/6-31+G(d,p) level of theory using IEFPCM in AcCN. The ratio of the emissions of enol and keto tautomers of 2-hydroxy substituted bases was studied by real time fluorescence spectroscopy upon pump laser illumination at 355 nm.

A18. Georgi Mateev, Ani Stoilova, Dimana Nazarova, Lian Nedelchev, **Petar Todorov**, Stela Georgieva, Yordanka Trifonova, Vanya Lilova. Photoinduced birefringence in PAZO polymer nanocomposite films embedded with particles of biologically active metal complexes. *Journal of Chemical Technology and Metallurgy*, *54, 6, 1123-1127, 2019*.

The composite materials based on azo polymers doped with metal particles attract considerable attention due to their advantageous electrical and optical properties. The present

communication reports results from measuring the photoinduced birefringence in composite films of the azopolymer (poly[1-[4-(3-carboxy-4-hydroxyphenylazo) benzenesulfonamido]-1,2-ethanediyl, sodium salt]), shortly PAZO, doped with metal complexes of Cu(II) 3-amino-5,5'-dimethylhydantoin (CLP) and Ni(II) 3-amino-5,5'-dimethylhydantoin (NLP) of different concentrations. The thin film materials are prepared via spin coating. The thickness of the samples is in the range between 500 nm and 800 nm. The photoinduced birefringence is measured by a classical polarimetric setup, where a vertically polarized He-Cd laser emitting at $\lambda = 442$ nm is used as a pump laser, while a DPSS laser emitting at $\lambda = 635$ nm is used as a probe laser. The influence of the dopants concentration on the maximal induced birefringence and the time of response are discussed.

A19. **Petar Todorov,** Petia Peneva, Jana Tchekalarova, Stela Georgieva. Potential anticonvulsant activity of novel VV-hemorphin-7 analogues containing unnatural amino acids: synthesis and characterization. *Amino Acids (2020) 52: 567–585, DOI: 10.1007/s00726-020-02836-1, (IF=*3.52*).*

Herein, some new analogues of VV-hemorphin-7, modified at position 4 and 7 by the unnatural amino acids followed the structure Val-Val-Tyr-Xxx-Trp-Thr-Yyy-Arg-Phe-NH_2, where Xxx is Ac5c (1-aminocyclopentanecarboxylic acid) or Ac6c (1-aminocyclohexane carboxylic acid), and Yyy is Dap (diaminopropanoic acid), or Dab (diaminobutanoic acid) were synthesized, characterized and investigated for anticonvulsant activity. The new synthetic peptide analogues were prepared by standard solid-phase peptide synthesis - Fmoc chemistry. A single intracerebroventricular (i.c.v.) injection at doses of 5, 10, and 20 µg/10 µl, respectively, was given before evaluation with timed intravenous pentylenetetrazole (ivPTZ) infusion test and 6-Hz psychomotor seizure test in mice. The acute neurological toxicity was determined using the rotarod test. To explain the structure-active properties of the modified, peptides some physicochemical characteristic was obtained. The FT-IR spectra and their second derivatives of the amide I, II, and III bands of the peptides shows ß-sheet structure conformation. The calculation of isoelectric points by potentiometric determination of dissociated constants is in the range from 9.79 to 10.84. In this study for the first time are also reported the reduction-oxidative potentials of the guanidine at Arg-moiety on such kind of peptides containing arginine and tyrosine residues in different medium and electrode surface. The VV-hemorphin-7 analogues 4 and 5 were the most active against the ivPTZ test with effect comparable to that of peptide 1 used as a positive control. Except of compound 8 all other tested peptide analogues were ineffective to raise the threshold for the clonic seizures. The peptide analogue 5 showed 100 % protection in the 6-Hz test while the other seven VV-hemorphin-7 analogues have dose-dependent activity against psychomotor seizures comparable to 1. The novel peptides did not show neurotoxicity in the rotarod test.

A20. Stela Georgieva, **Petar Todorov**, Petia Peneva, Marian Varbanov, Kristina Gartsiyanova. VV-Hemorphin-5 analogue for trace copper determination in water samples. *Journal of the Iranian Chemical Society, 17, pages 2885–2894 (2020), (IF=2.019). DOI: 10.1007/s13738-020-01968-1*

Natural and anthropogenic sources produce mainly metal ions which can be accumulated in sediments that have a significant environmental impact on local areas,

especially for their river water quality. The environmental analysis applies chemical sensors having the inherent ability to detect in real-time analytes as cations, anions, or small molecules in water systems. In this regards, the aim of our work was the development of a simple and rapid electrochemical method for selectively copper determination in water samples using non-toxic and easy degradable peptide molecules such as a hemorphin analogue. Analytical characterizations as sensibility, linear dynamic range (LDR), the limit of detection (LOD), and limit of quantification (LOQ) have been also discussed. The results revealed that the peptide sensor provided electrochemical and sensing excellent response with a low limit of detection (0.188 ng ml^{-1}). The precision of the method proposal evaluated by the relative standard deviation is 0.7%.

A21. **Petar Todorov,** Stela Georgieva, Petia Peneva, Rusi Rusew, Boris Shivachev, Anton Georgiev. Experimental and theoretical study of bidirectional photoswitching behavior of the 5,5'-diphenylhydantoin Schiff bases: synthesis, crystal structure and kinetics approaches. *New Journal of Chemistry, https://doi.org/10.1039/D0NJ03301D 2020, 44, 15081-15099, (IF=3.591).*

Herein, the synthesis and characterization of four novel 5,5'-diphenylhydantoin Schiff bases containing different aromatic species have been presented. Their structure-property relationship was studied by the X-ray, optical and electrochemical methods as well as DFT calculations in terms of their *E/Z* photoisomerization and *enol/keto* phototaumerization. The big challenges in photoinduced motion are to achieve control and stability over the two isomers. Solvent-driven bidirectional photoswitching behavior was studied in nonpolar 1,4-dioxane and polar aprotic DMF. It was observed T-type photochromism in 1,4-DOX and opposite behavior in DMF as P-type switches (bistable system). The obtained results lead to a conclusion that by variation of solvent environment the direct control over the bidirectional switching behaviour from T-type to P-type can be achieved.

A22. **Petar Todorov,** Petia Peneva, Jana Tchekalarova, Stela Georgieva, Miroslav Rangelov, Nadezhda Todorova. Structure–activity relationship study on new Hemorphin-4 analogues containing steric restricted amino acids moiety for evaluation of their anticonvulsant activity. *Amino Acids (2020) 52, pages 1375–1390, https://doi.org/10.1007/s00726-020-02898-1, (IF=*3.52*).*

In the present study, several new analogues of hemorphin-4, modified with unnatural conformationally restricted amino acids followed the structure Aaa-Tyr-Xxx-Trp-Thr-NH_2, where Aaa is the low molecular weight lipophilic adamantyl building block, and Xxx is Ac5c (1-aminocyclopentanecarboxylic acid) or Ac6c (1-aminocyclohexane carboxylic acid) were synthesized, characterized and investigated for anticonvulsant activity in three seizure tests, the maximal electroshock test (MES), 6-Hz psychomotor seizure test and timed intravenous pentylenetetrazole infusion (ivPTZ) test. The acute neurological toxicity was determined using the rota-rod test. The new synthetic neuropeptide analogues were prepared by solid-phase peptide synthesis - Fmoc chemistry and were evaluated in three doses of 1, 3 and 5 μg, respectively, administered intracerebroventricularly in male ICR mice. The physicochemical properties of these peptide analogues were evaluated as pKa and pI values were calculated

using potentiometry. The IR spectrum of the compounds were recorded and the characteristic lines of both adamantane moiety and the peptide backbone were registered in the wavelength range from 4000 to 400 cm^{-1}. The hexapeptide Ang IV was used as a positive control. From the six synthesized peptide analogues, the P4-5 was the most active at doses of 1 and 3 μg in the three seizure tests. The order of potency of other peptides was as follows: P4 > P4-3 = P4-4 > P4-2 > Ang IV in MES, P4-4 ≥ P4-1 > P4-3 > P4-2 > P4 > Ang IV in 6-Hz test and P4-4 = P4-3 > P4-2 = P4 > Ang IV in ivPTZ test. None of the peptides displayed neurotoxicity in the rota-rod test. Docking study results suggest that direct H-bonding and ionic interactions between our synthetic ligands and residues, responsible for coordination of Zn^{2+} along with hydrophobic interactions between our ligands and IRAP active site are the most important for the ligand binding. The results propose that incorporation of adamantane and cycloalkane building blocks in the peptide chain of the hemorphin-4 scaffold is important for the potential high biological activity.

A23. Borislav Assenov, Daniela Pechlivanova, Elena Dzhambazova, Petia Peneva, **Petar Todorov.** Antinociceptive Effects of VV-Hemorphin-5 Peptide Analogues Containing Aminophosphonate Moiety in Mouse Formalin Model of Pain. *Protein & Peptide Letters,* Volume 28, Issue 4, Page: [442 - 449], 2021, *doi: 10.2174/0929866527666200813200714, (IF=1.89).*

Hemorphins are endogenous hemoglobin-derived peptides that belong to the family of "atypical" opioid peptides with both affinities to opioid receptors and ability to release other endogenous opioid peptides. Objective: In the present study, peptide analogues of Valorphin (VV-hemorphin-5) containing amino phosphonate moiety synthesized by solid-phase peptide synthesis (Fmoc-strategy) were investigated for their potential antinociceptive activities and compared to the reference VV-H in formalin-induced model of acute and inflammatory pain in mice.

A24. S. Georgieva, **P. Todorov**, A. Bezfamilnyi. Development of voltammetric method for trace Cu(II) determination in the presence of Fe(III) in water samples using 5,5'-diphenylimidazolidine-2,4-dione derivative. Journal of Chemical Technology and Metallurgy, 56 (5), 999-1007, ***2021***.

A new cyclic voltammetric method has been described for the determination of Cu(II) in the presence of Fe(III) in tap and surface water samples using complexing properties of 5,5'-diphenylimidazolidine-2,4-dione derivative. In acetate buffer solution (0.1 mol/L; pH 5.25 ± 0.01) containing minimum ten-fold excess the imidazolidine derivative, copper metal ion was determined as complex compound following redox process into the working hanging mercury drop electrode (HMDE) versus Ag/AgCl, 3mol/L KCl using as a reference electrode. Limits of detection as low as 0.0415mg/L Cu(II) was achieved. The interfering effect of various cations as K(I), Na(I), Mg(II), Ca(II), Al(III), Zn(II), Fe(II), Fe(III) and anions: Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, most common in surface and tap water samples were also assessed.

A25. A.Stoilova, G.Mateev, D.Nazarova, L.Nedelchev, E.Stoykova, B.Blagoeva, N.Berberova, S.Georgieva**, P.Todorov.** Polarization holographic gratings in PAZO polymer films doped with particles of biometals. Journal of Photochemistry and

Photobiology A: Chemistry, Volume 411, 15 April 2021, 113196; https://doi.org/10.1016/j.jphotochem.2021.113196, (*IF=4.291).*

The paper presents a study of the diffraction efficiency of polarization holographic gratings recorded in thin films of the azopolymer PAZO (poly[1-[4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido]-1,2-ethanediyl, sodium salt]) doped with Cu(II) 3-amino-5,5′ -dimethylhydantoin (CLP) and Ni(II) 3-amino-5,5′ -dimethylhydantoin (NLP) at three different concentrations, namely 1, 2 and 5 wt.%. The influence of the dopants composition and concentration on the parameters of the polarization holographic gratings recorded in the thin composite films has been discussed. The gratings are recorded with a He-Cd gas laser with wavelength 442 nm. The polarization of the recording beams was left and right circular and the recording angle was 20°. Along with the anisotropic grating in the volume of the media, surface relief is also formed. The diffraction efficiency kinetics is probed at 635 nm and the height of the relief gratings is determined by AFM. Diffraction efficiency (η) higher than 30% was achieved for the hybrid samples, as well as 585 nm surface relief height.

A26. **Petar Todorov,** Stela Georgieva, Petia Peneva, Jana Tchekalarova. Spectral and electrochemical solvatochromic investigations of newly synthesized peptide-based chemosensor bearing azobenzene side chain bio photoswitch. Dyes and Pigments, Volume 191, July 2021, 109348. https://doi.org/10.1016/j.dyepig.2021.109348. *(IF=4.889).*

In the present study, a novel analogue of azobenzene-containing hemorphin-4 has been synthesized and investigated for assessment of spectral, electrochemical, and biological effects. The synthesis was achieved by a modified solid-phase peptide synthesis (SPPS) by Fmoc-strategy. This compound represents a newly synthesized and unstudied peptide-based chemosensor bearing azobenzene side-chain with different spectral and electrochemical properties in the two trans-/cis- states depending on the solvent polarity. Their fluorescence intensity, as well as voltammetric behavior, was found to depend on both the polarity of the solvents and the type of isomers of the azopeptide compound. Both isomer forms have shown pH dependence, as the fluorescence peak and intensity are significantly distinguished in acidic and neutral medium. The electrochemical behavior before and after 90 min UV illumination was investigated using a cyclic voltammetric mode at three-electrode system with HMDE electrode as the working electrode.

The novel biopeptide Azo-Tyr-Pro-Trp-Thr-NH_2 was explored *in vivo* for potential anticonvulsant activity by 6-Hz seizure test and maximal electroshock test (MES) in ICR mice. The cis-**Az-H4** isomer showed stronger potency than the trans-**Az-H4** against both the 6 Hz-induced psychomotor seizures and tonic-clonic seizures in the maximal electroshock test with 100 % protection demonstrated at the lowest dose of 1 μg administered intracerebroventricularly in mice. The effect of 1 and 5 μg cis-**Az-H4** and 5 μg trans-**Az-H4** was comparable to the positive control valproate in the 6-Hz test. None of the tested isomers displayed neurotoxicity in the rotarod test. Our results suggest that modified biopeptide in the N-terminus of hemorphin-4 with azobenzene deserve further exploration as a promising candidate with both anticonvulsant activity and as a chemosensor for pH determination.