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Evaluation of PhD Thesis

„Constrained secondary structures to develop bioactive peptides and peptidomimetics"
Presented by: Agnieszka Natalia Staśkiewicz

MSc Agnieszka Natalia Staśkiewicz completed her doctoral thesis entitled "Constrained secondary structures to develop bioactive peptides and peptidomimetics" at the Faculty of Chemistry, Wrocław University of Science and Technology and the Department of Chemistry "Ugo Schiff" University of Florence. The thesis was supervised by Prof. Rafał Latajka from the Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology and Prof. Anna Maria Papini from the Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff" University of Florence.

The research topic taken up in the dissertation concerns the design and synthesis of peptides/peptidometics with a rigid secondary structure, which significantly improves the functional parameters of peptides. The search for methods of synthesis of peptides with a stable spatial structure is one of the research trends that allow for a wider use of peptides as drugs. Due to their specialized structure and the ability to precisely interact with various cell receptors, peptides are increasingly used in medicine as drugs.

Peptides as potential drugs are increasingly used due to: high specificity: peptides can be designed to interact with very specific molecular targets, which minimizes side effects; high efficacy: due to precise interaction with the target, peptides can demonstrate high therapeutic efficacy; biocompatibility: peptides are natural compounds, which means they are well tolerated by the body; broad spectrum of action: peptides can be used to treat many different conditions, from metabolic diseases to cancer.

Peptides are used in the treatment of many diseases, including: diabetes: insulin, a peptide, is one of the most important drugs used in the treatment of diabetes; cancer: peptides can inhibit the growth of cancer cells, induce their death and strengthen the immune system; circulatory system diseases: peptides can lower blood pressure, prevent the formation of blood clots and improve the functioning of the heart; neurodegenerative diseases: peptides can slow the progression of diseases such as Alzheimer's and Parkinson's; infections: Peptides can inactivate bacteria and viruses. Despite their many advantages, peptides as drugs also have certain limitations: low resistance to digestive enzymes: peptides administered orally are often broken down in the digestive system, which limits their bioavailability; short half-life: many peptides are quickly removed from the body, which requires frequent administration of the

drug. Despite these challenges, peptides are considered one of the most promising classes of drugs. The development of peptide synthesis methods and a better understanding of the mechanisms of their action mean that we can expect that in the future peptides will play an increasingly important role in the treatment of many diseases.

The undertaken research topic fits into the contemporary research areas concerning medicinal chemistry and peptide chemistry, which is an interdisciplinary branch of science dealing with the design and creation of structures of new drugs, including peptide drugs. Therefore, the doctoral thesis of MSc Agnieszka Staśkiewicz, fits into the intensively developing branch of science and is up to date.

The dissertation has a multi-level structure, consistently adapted to achieve the overarching research goal. The work contains two main goals:

1) to replace the disulphide bridge in native oxytocin with 1*H*-[1,2,3]triazol-1-yl-containing bridge to attend to the requirement for metabolically stable OT-derived drug candidates and chemical probes with desired pharmacological activities. It was designed a series of Cα1-to-Cα6 side chain-to-side chain disubstituted-1,4- or 4,1-(1*H*-[1,2,3]triazol-1-yl)-bridged OT analogues presenting a systematic permutation of the ring size, location, and orientation of the triazolyl moieties

2) studies of the relationship between the structures and the bioactivity of the series of synthetic MBP peptides that have been used to identify specific antibodies in MuSc patient sera.

Such an ambitious and complex goal could be achieved by implementing many specific objectives, including: structure optimisation of analogues of OT and native OT by molecular modelling; synthesis, purification, and analysis of peptides and peptidomimetics; conformational studies of peptides and peptidomimetics; biological activity assays of peptides and peptidomimetics.

The reviewed dissertation has 154 pages. The layout of the dissertation is classic and includes an abstract, list of abbreviations, introduction, literature review, aim of the work, experimental part, results and discussions, conclusions, references, list of figures, list of tables and scientific achievements.

The literature review includes subchapters regarding: Characterisation of oxytocin, Replacement of the disulphide bridge, "Click chemistry", Application of the molecules possessing the triazolyl moieties, Peptides antigens as biological targets, Multiple Sclerosis, Myelin Basic Protein, Experimental autoimmune encephalomyelitis in Multiple Sclerosis, Cross-reactivity between viral antigens and MBP epitopes, Structures of peptides and proteins. This chapter is 33 pages long and contains 261 references. Despite the great diversity of topics of individual fragments of the literature review, coherence is maintained through careful selection of referenced publications and consistent subordination of all components to the overarching goal of the work. In general, this allows for a high assessment of this part of the dissertation. Unfortunately, the PhD student was not able to avoid errors in this part of the thesis. For example: on page 29 the PhD student writes about methods of obtaining triazoles. Path I described by the PhD student starts with β-amino-α,β-unsaturated

esters or ketones, is the correct formula in Figure 7? There is also an error in Figure 9. Path B is incorrectly presented. Please discuss both issues correctly during the defense. The subchapter Structures of peptides and proteins was presented very briefly and superficially, I would ask the PhD student to present this issue in more detail, because it is directly related to the use of peptides as potential drugs.

The next main chapter presents the aim of the research, which was prepared precisely, the presentation of peptides/peptidomimetics planned to be obtained makes it easier to follow the text.

The next main chapter, "Experimental Part", contains descriptions of research methods. This part is described in detail, which allows for reproducing the obtained results. . All fragments of Experimental part are wholly informative and confirm the proficiency and experience in laboratory experiments. The methods are clearly defined and their use is justified. Compounds described in the Thesis were obtained with up-to-date techniques. Appropriate analytical methods: HPLC, LC/MS, NMR, confirmed their purity and structure. The biological activity of synthesized compounds was carefully evaluated by *in vitro* assays. The conformation, spatial structure was studied by CD, molecular modeling and docking and biological activity of synthesized compounds determined by *in vitro* assays. The results are reliable and of high scientific relevance, which was already confirmed by the publication of them in international peer-reviewed journals characterized by high for this discipline Impact Factors.

The most extensive part of the dissertation is the chapter "Results and Discussion". The dissertation includes two groups of peptides/peptidometics. One of them concerns oxytocin. The PhD student tried to find the answer to the question how the replacement of the native $-CH_2-S-S-CH_2-$ bridge in oxytocin by -(CH_2)m-1,4-/4,1-1*H*-([1,2,3]triazol-1-yl)-(CH_2)n- (analogues of oxytocin) will affect receptor selectivity, binding affinity, the potency of signalling, metabolic and conformation stability.

The PhD student designed and obtained fourteen cyclopeptides (I-VII and IR-VIIR) being derivatives of oxytocin. The oxytocin analogues were obtained by replacing Cys1 and Cys6 at positions *i* and *i*+5 with *N*α-Fmoc-ω-azido-α-amino acids and *N*α-Fmoc-ω-ynoic-α-amino acids with (CH2)n/m (n/m = 1-4 or 2-3 or 1 -1). The intramolecular cyclisation of the linear precursors was carried out by solution-phase and on-resin microwave strategy. The [1,2,3]-triazolyl-bridging approach was applied to stabilise the β-turn conformation of oxytocin analogues. Within this framework, circular spectroscopy measurements were performed. According to the CD spectra performed in water. The PhD student found that peptidomimetics IV, V, IVR, and VR showed a tendency to form β-turn conformation. The NMR studies of oxytocin and its analogues confirmed that oxytocin preferentially adopts a type I β-turn conformation in water.

The PhD student found a similar relationship for the derivatives IR, II, IIR, IVR, V-VII, and VR-VIIR. Based on biological studies, it was found that peptidomimetics IV and IVR possess the most interesting pharmacological properties. Analogue IV presents characteristic features of a weak partial agonist, while peptidomimetic IVR is a weak competitive antagonist. What is important, the only structural difference between analogues

IV and IVR is the reversed orientation of the 1 *H*-[1,2,3]triazol-1-yl moieties in the bridges. In addition, both analogues, IV and IVR, demonstrate a meaningful increase in metabolic stability relative to OT when incubated in serum taken from pregnant women at the 40th week.

The second part of the doctoral student's research concerned obtaining series of MBP peptides possessing a significant role in efficient IgM antibody recognition in Multiple Sclerosis. MBP peptides 1-6 were evaluated in SP-ELISA. In the research, the PhD student found two peptides MBP(81-106) (1) and MBP(76-116) (2) with the best activity. Based on NMR conformational analysis studies, the PhD student showed that the peptides MBP(81-106) (1) and MBP (76-116) (2) are characterized by a stable helical conformation along residues 87-96, while the remaining regions display unordered conformation. The results of the study were also confirmed by CD analyses, where the presence of α-helix structures was found for MBP (81-106) (1) and MBP (76-116) (2).

The Conclusions chapter, divided into two subchapters, is prepared correctly. The graphic design of the doctoral dissertation also deserves appreciation. The collection of cited literature references is also impressive, comprising 305 items.

In the dissertation I lacked a short subchapter discussing future research plans concerning both oxytocin analogues and MBP peptides, which should be undertaken in order to bring the results obtained by the PhD student closer to studies on animal models. I ask for a discussion on this topic during the defense of the doctoral dissertation.

I highly value the choice of an ambitious research topic, fully consistent with contemporary directions of basic work and application potential. I highly value the use of diverse and modern research methods, as well as the difficult and interdisciplinary nature of the experimental work performed. The proficiency in using various synthetic methods and the efficiency in using complex, modern analytical and biological techniques, which the PhD student was able to implement in the research, deserve special recognition. These skills provide the best evidence of the PhD student's thorough knowledge and scientific maturity.

MSc Agnieszka Natalia Staśkiewicz is a co-author of 7 publications, including 4 from the JCR list. Three publications are directly related to the doctoral dissertation. Additionally, the PhD student is also a co-author of 15 communications presented at national and international conferences.

Conclusion: The thesis as a whole is an original in its concept, multifaceted, presents a comprehensive approach to the research problems. The way of preparing the thesis, the presentation and interpretation of the results, as well as rising new research questions and opportunities proves that the PhD candidate has not only extensive knowledge concerning the subject of research but also analytical skill and ability to precisely evaluate results of own work. All planned compounds were synthesized, purified, characterized, and their biological activity was investigated.

In my opinion, the reviewed doctoral dissertation meets the conditions specified in art. 187 paragraphs 1-2 of the Act of 20 July 2018 - The Law on Higher Education and Science (consolidated text: Journal of Laws of 2023, item 742, as amended). In view of the above, I hereby apply to the High Council of the Discipline Chemical Sciences, Wrocław University of Sciences and Technology (Politechnika Wrocławska) to admit MSc Agnieszka Natalia Staśkiewicz to further stages of the doctoral procedure.

W mojej opinii przedstawiona do recenzji rozprawa doktorska spełnia warunki określone w art. 187 ust. 1-2 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (t.j. Dz. U. z 2023 r. poz. 742 z późn. zm.).
W związku z powyższym, Radzie Dyscypliny Naukowej Nauki Chemiczne Politechniki wniosek o dopuszczenie mgr Agnieszki Natalii Staśkiewicz do dalszych etapów przewodu doktorskiego.

Prof. dr hab. inż. Beata Kolesińska

Łódź, 29. 10. 2024