WYDZIAŁ CHEMII

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**Review of the doctoral dissertation of MSc. Agnieszka Natalia Staśkiewicz "Constrained secondary structures to develop bioactive peptides and peptidomimetics"**

The doctoral dissertation written by MSc Agnieszka Staśkiewicz was carried out under the supervision of Prof. dr. hab. Rafał Latajka (Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology) and Prof. dr. Anna Maria Papini (Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff", University of Florence). This fruitful collaboration between the Polish and Italian supervisors resulted in a chemically fascinating idea to design and understand the correlation between the structure and function of carefully designed oxytocin and Myelin Basic Protein peptidomimetics. MSc. Staśkiewicz brought this idea into life and described the most interesting findings of this project in the scope of the discussed thesis.

The dissertation has been prepared in a traditional form; it contains several appropriate sections and includes all elements required by relevant regulations. The work begins with an abstract in English, Polish and Italian, followed by a brief and informative introduction and a thorough literature review on oxytocin structure and function, oxytocin antagonists and on its receptor and receptor agonists. The reader also learns about the consequences of replacing the disulphide bond, about the application of click chemistry and about the possible modifications via triazolyl moieties.

The PhD candidate then guides the reader through the molecular causes of multiple sclerosis, focusing on Myelin Basic Protein (MBP), its chosen structural modifications and cross-reactivity between viral antigens and MBP epitopes. Considerable attention is

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also given to the ways in which we can mimic specific structural features in proteins and peptides.

In one of the most interesting chapters, "Aim and objectives of doctoral dissertation", the author clarifies the objectives of her work. She focuses on understanding the correlation between the precisely introduced structural modifications and the biological mode of action of two classes of important peptide-based families of compounds, oxytocin and Myelin Basic Protein.

Oxytocin, a cyclic nonapeptide, has limited biomedical use due to its short half-life and low receptor selectivity. However, designing oxytocin analogues could be valuable for imaging and targeting diseases. The study specifically aimed to replace the disulfide bridge in oxytocin with a 1H-[1,2,3]triazol-1-yl bridge to create metabolically stable oxytocin analogues with improved pharmacological properties. A series of C alpha 1-to-C alpha 6 side chain-to-side chain triazolyl-bridged oxytocin analogues were designed, varying in ring size, location, and orientation of the triazolyl groups; the number of -$CH_2$ groups in the surrounding of the triazolyl bridge also varied. The PhD candidate has also studied the sequence and structure of synthetic Myelin Basic Protein peptides used to identify specific antibodies in the sera of Multiple Sclerosis patients and discussed the relationship between the structure and bioactivity of these MBP peptides (Myelin Basic Protein peptides play a key role in recognizing IgM antibodies in Multiple Sclerosis, a demyelinating autoimmune disease affecting the central nervous system).

MSc. Staśkiewicz analyzed an impressive number of 20 peptides was althogether; she optimized the structure of oxytocin analogues using molecular modelling, synthesized, purified and analyzed the peptides and peptidomimetics, carried out their conformational studies and tested their biological activity. The experimental section gives the reader precise details about the used methods of molecular modelling, peptide synthesis, purification and analysis (among others *via* immunoassays, ELISA, CD, NMR).

The most interesting part of the thesis is described in the "Results and discussion" section. MSc. Satśkiewicz enhanced oxytocin's stability by replacing its disulfide bridge (between Cys1 and Cys6) with a more rigid 1H-[1,2,3]triazol-1-yl moiety. To achieve

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this, she used the Cu(I)-catalyzed azide-alkyne 1,3-cycloaddition (CuAAC) macrocyclization to stabilize β-turn secondary structures. The study presents the design, synthesis, conformational analysis, and pharmacological activity of several oxytocin analogues, varying in bridge length, orientation, and location of linking moieties. These findings offer valuable insights into the structure-conformation-function relationship.

In the second part of the thesis, the PhD candidate explored Myelin Basic Protein peptides, crucial for recognizing IgM antibodies in Multiple Sclerosis. MBP is an intrinsically disordered protein with an interesting a-helix motif that may act as a conformational epitope. The work shows newly designed sequences and structures of synthetic MBP peptides to identify specific antibodies in Multiple Sclerosis patient sera, focusing on the relationship between their secondary structure and bioactivity.

The discussed thesis triggers numerous questions and encourages the reader to make the author hypothesize about the meaning of the obtained results:

1. In the case of oxytocin peptiomimetics, analogues IV and IVR seems to have a tendency to form beta-turn conformation, just like the native oxytocin. These peptidomimetics possessed the same amount of -CH2 groups in the surroundings of the triazolyl bridge, although they differed in the orientation of the triazoles. I am curious about two issues: first, which feature correlates most with the partially beta-turn structure, and second, what seems to be more important for the biological role of these peptidomimetics in general – is it the number of -CH2 groups, or the orientation of the triazolyl bridge?

2. It is really exciting to see that oxytocin analogues IV and IVR were more stable in serum of pregnant women than the native oxytocin. Again, can you speculate on (i) why it is so – which structural feature could be responsible for the enhanced stability in serum and (ii) what could be the biological benefit of such a compound with enhanced lifetime, given to a pregnant patient (instead of native oxytocin)?

3. In the case of analogues VII and VIIR, the orientation of the triazolyl ring did not impact the representation of their secondary structures (both were unordered). Do you think you could hypothesise about a specific rule – when does the orientation of

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the triazole matter, and when does it not matter for the overall secondary structure of the peptidomimetic?

4. MBP peptides 1-6 were random coils in water, while in a mixture of H2O:TFE (50:50, v:v), peptides 1-3 were at least partially alpha-helical. Taking into account that TFE is a well know alpha-helix inducer, can you speculate why some sequences can be made a-helical more easily than others? Do the same rules apply here that apply to the a-helical prediction of peptide structure without the presence of TFE?

5. On what basis was the reference dataset chosen in Dichoweb predictions?

6. Congratulations on the NMR structures of the peptidomimetics. Structures of peptides of this length are usually not easy to solve because of an extensive proton exchange that is going on in solution. Could you tell us what was the biggest challenge of this part of the work?

The comments included in the review arise mainly out of curiosity and do not affect the very high substantive level of the work, the way it is presented, or the conclusions drawn from it.

In terms of scientific quality, the output of the thesis is excellent, as it highly advances our understanding of the structure-function relationship of biologically significant peptidomimetics.

The main body of the dissertation is written on 154 pages, contains 40 figures and 11 tables, and cites 305 literature references. The 86-page supplement contains further 59 figures and 45 tables, and gathering the selected part of the work in supplementary materials makes the main body of the thesis very pleasant to read. In general, the dissertation is very well written, and the research conducted by Msc. Staśkiewicz and presented in the dissertation is of very high standard. I did not notice any serious errors or shortcomings in the work, apart from a very minor editorial ones, which are minor enough not to be mentioned in writing, and do not affect my very high assessment of the doctoral dissertation.

The PhD Candidate has spent around 16 months in Florence, where she acquired complementary skills in the synthesis od peptidomimetics. She also received an impressive number of scholarships and travel grants, and presented her work, very often in the form of oral presentations (7) or posters (also 7) at international

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conferences. Publication-wise, the outcome of the thesis is described in three excellent publications, strictly connected with the topic of the dissertation; the role of Msc. Staśkiewicz is clear in all of them. In total, the PhD candidate is the co-author of seven publications, which proves her ability to deal with a variety of scientific topics.

The dissertation is an original solution to an important scientific problem and in my opinion, the thesis not only meets, but significantly exceeds the requirements set for doctoral dissertations following the provisions of the Law on Higher Education and Science of July 20, 2018. The candidate has demonstrated great research independence and the ability to comprehensively analyze results, which deserves high praise and the distinction of the dissertation. The proposal to distinguish the Candidate's dissertation is based on the very positive assessment of the work, its high impact on the field, on its interdisciplinary nature, and on the robust amount of scientifically meaningful achievements gathered in the scope of this thesis, on its outstanding scientific quality, novelty, the huge amount of valuable data, the potential possibility of using them in therapy, and above all, on the fact that the conclusions of the work definitely enrich the chemistry of therapeutic peptides. Therefore, I recommend the Scientific Discipline Council of Chemical Sciences at the Wrocław University of Technology to admit MSc Agnieszka Staśkiewicz to the further stages of the procedure for the award of the doctoral degree in the discipline of chemical sciences and I am applying for a distinction of the work.

The Polish version of the last paragraph follows:

„Rozprawa stanowi oryginalne rozwiązanie istotnego problemu naukowego i, moim zdaniem, nie tylko spełnia, ale i znacznie przewyższa wymagania stawiane rozprawom doktorskim zgodnie z przepisami ustawy Prawo o szkolnictwie wyższym i nauce z dnia 20 lipca 2018 r. Kandydat wykazał się wysokim stopniem samodzielności badawczej oraz umiejętnością wszechstronnej analizy wyników, co zasługuje na szczególne uznanie i stanowi podstawę do wyróżnienia rozprawy.

Propozycja wyróżnienia opiera się na bardzo pozytywnej ocenie pracy, jej znaczącym wpływie na dziedzinę, interdyscyplinarnym charakterze oraz na licznych, naukowo istotnych osiągnięciach zawartych w tej rozprawie. Wyróżnia się ona wybitną jakością naukową, nowatorskim podejściem, dużą ilością cennych danych, a także

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potencjałem ich praktycznego wykorzystania w terapii. Przede wszystkim, wnioski płynące z pracy znacząco wzbogacają obszar chemii peptydów terapeutycznych.
W związku z powyższym rekomenduję Radzie Naukowej Dyscypliny Naukowej Nauki Chemiczne Politechniki Wrocławskiej dopuszczenie mgr Agnieszki Staśkiewicz do dalszych etapów postępowania o nadanie stopnia doktora w dyscyplinie nauki chemiczne oraz wnioskuję o wyróżnienie pracy."

With my best regards,

Signed by /
Podpisano przez:

Magdalena Alina
Rowińska-Żyrek
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Date / Data:
2024-10-29 23:03

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