

## Evaluation of PhD Thesis

### ***„Constrained secondary structures to develop bioactive peptides and peptidomimetics”***

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The doctoral thesis of Agnieszka Natalia Staśkiewicz concerns two different topics. The topics are not tightly bonded with each other from a biological point of view but connected with the rationale approach of peptide design and structure-activity relationship.

The first topic is related to Oxytocin. Oxytocin (OT) is a peptide hormone and neurotransmitter involved in various biological functions through interacting with its cognate G protein-coupled receptors. Oxytocin is a cyclic nonapeptide with a disulphide bond between Cys1 and Cys6. The biomedical use of oxytocin is limited by its short half-life *in vivo* and by the low receptor selectivity. The studies presented in this doctoral thesis aim to increase the stability of oxytocin by replacing the disulphide bridge with the stable and more rigid 1*H*-[1,2,3]triazol-1-yl moiety. The Cu(I)-catalysed side chain-to-side chain azide-alkyne 1,3-cycloaddition (CuAAC) macrocyclisation strategy was employed to stabilise  $\beta$ -turns secondary structures. This doctoral dissertation reports the design, synthesis, conformational analysis, and *in vitro* pharmacological activity of a series of Ca1-to-Ca6 side chain-to-side chain 1*H*-[1,2,3]triazol-1-yl-containing oxytocin analogues differing in the length of the bridge and the orientation and location of the linking moieties. By developing this macrocyclisation method, the production of a series of compounds that provided attractive insight into the structure-conformation function relationship is provided.

The second topic concerns Myelin Basic Protein (MBP) peptides. These peptides play a significant role in effectively recognising IgM antibodies in Multiple Sclerosis (MuSc). MuSc is a demyelinating, neuroinflammatory, autoimmune disease that attacks the central nervous system. The MBP is an intrinsically disordered protein demonstrating an interesting  $\alpha$ -helix motif, which can be considered a conformational epitope. This doctoral thesis investigates the role of the sequences and structures of synthetic MBP peptides that have been used to identify specific antibodies in Multiple Sclerosis patient sera. Therefore, the studies of the relationship between the secondary structure and the bioactivity of the series of MBP peptides are discussed.

**The structure of the thesis** agrees with the classic recommendations. Following the list of abbreviations chapters 1 and 2 report, respectively, the introduction and the literature review. The second chapter reports a comprehensive and detailed background for future studies, with more than 260 cited articles. The chapter is well presented and comprehensive and reports a

very ordered and complete background for both topics, even if the tentative to take together the two topics of the thesis not always succeed.

**The experimental part** of the thesis is presented in Chapter 4. This chapter describes the synthetic methodologies to obtain the linear and the cyclized peptides. All the experiments are clearly described, and peptide synthesis is performed according to up-to-date techniques. The obtained peptides, for both topics of the research are fully characterized, also for their structure in solution, with appropriate analytical methods, in particular by Circular dichroism and NMR studies. Also, the pharmacological experiments on Oxytocin and its new synthesized analogues, are clearly described. For major clarity I suggest moving the point concerning the immunoassay studies of MBP peptides (4.2.5) after the NMR characterization and before the Pharmacological studies.

**Results and discussion:** Chapter 5 presents all results obtained, and a deep discussion on the various aspects of synthetic procedures, both for the obtainment of linear and cyclized peptides, of the structural behavior in solution of obtained peptides and of their biological and pharmacological behavior.

During the thesis a total of 14 linear precursors of Oxytocin analogues peptides have been synthesized and the corresponding 14 cyclized peptides in which the disulphide bridge present in the natural Oxytocin is replaced by 1*H*-[1,2,3]triazolyl-containing bridge. The 14 analogues differ for the length of the spacers in the bridge as well for the orientation of the 1*H*-[1,2,3]triazolyl moiety. Moreover, is reported the synthesis of 6 longer peptides for the MBP topic. For all peptides the synthetic procedures and their complete characterization are well documented and deeply discussed. I agree with the conclusions reported after the evaluation of CD data and NMR studies, as concerns with the conformation and structure in solution of all synthesized peptides.

**Questions to be discussed with the Defendant** are as follows:

The candidate reports a deep analysis of the structure and conformation by CD and NMR of the 14 OT analog peptidomimetics; she also reports the pharmacological activity of these compounds by displacement studies with the radioligand. The results obtained are very interesting, but I would like a deeper comparison between the pharmacological behavior of the synthesized compounds with all the other OT agonist and antagonists reported in the literature.

Moreover, a comparison between the two series of the OT peptidomimetics in terms of yield and purity should be reported and discussed.

**Conclusion:** The thesis is original in its concept, multifaceted, presents a comprehensive approach to the research problems and provided very interesting results which give new insight to demonstrate how constrained secondary structures allow to develop bioactive peptides and peptidomimetics. Several compounds for both topics were synthesized, purified, characterized, and evaluated towards their bioactivity. The work contains comprehensive discussion of the methods and results. The way of preparing the thesis, the presentation and interpretation of the results, as well as raising new research questions and opportunities

proves that the PhD candidate has not only extensive knowledge concerning the subject of research but also synthetic and analytical skills as well, she is able precisely evaluate results of own work.

In conclusion, the thesis meets the conditions specified in Article 187, sections 1-2, of the Act of July 20, 2018, Law on Higher Education and Science.

Date: 13/10/202

Signature:  
Giancarlo Morelli

A handwritten signature in black ink, appearing to read 'Giancarlo Morelli', written in a cursive style.