REVIEW

of Ph.D. thesis for scientific and educational degree **„doctor of philosophy“**

prepared by Prof. Dancho Lyubenov Danalev, Ph.D.

in the field of higher education *4. Natural sciences, mathematics and informatics*

professional field *4.2. Chemical sciences*

doctoral program *Bioorganic chemistry, chemistry of natural and physiologically active substances*

Author: Boryana Krasimirova Yakimova

Topic: Design and synthesis of biologically active peptides as potential inhibitors of angiotensin converting enzyme (ACE I)

Head of Ph.D. thesis: Prof. D.Sc. Ivanka Borisova Stoyneva, IOCCPh of BAS

1. General description of the submitted materials

The materials presented for review on the Ph.D. thesis of Boryana Krasimirova Yakimova with supervisor Prof. D.Sc. Ivanka Stoyneva include a manuscript of Ph.D. thesis, an abstract in Bulgarian and English, the candidate's CV, the diploma of the doctoral student for higher education Master's degree, evidence both for the training of the doctoral student in the primary unit, in which she is enrolled as a doctoral student in self-study (Order №НО-05-06-23 / 11.12.2015), and for her scientific achievements, which include:

- list and copies of 4 publications on the topic of the Ph.D. thesis, including an official letter of acceptance of publication in the Pharmacia Journal;

- list and certificates for participation in scientific conferences with elaborations on the Ph.D. thesis;

- list of participations in projects on the topic of the Ph.D. thesis and training projects for acquisition of additional and upgrading of already acquired knowledge and skills;

- list of noticed citations of the articles on the Ph.D. thesis topic.

Here I would like to mention that the documents are extremely carefully arranged, which immediately makes a good impression on the responsible attitude of the doctoral student to the overall procedure for obtaining the degree for which she is a candidate.

A complete set of all materials is presented, according to the requirements of the Academic Staff Development Act of the Republic of Bulgaria, the Regulations for its implementation and the Regulations for development of the academic staff of BAS, which show that the candidate fully meets the criteria of these regulations for the acquisition of scientific and educational degree **„doctor of philosophy“**.

2. Brief biographical data about the doctoral student

According to the documents submitted by the candidate Boryana Krasimirova Yakimova, she graduated in 2001 with a high school degree in biology in one of the most elite schools in Veliko Tarnovo, Vasil Drumev Secondary School, then a Bachelor's degree and a Master's degree at Sofia University "St. Kliment Ohridski " in biotechnology, respectively in 2005 and 2007. The overall education of Assistant Professor Yakimova fully corresponds to her chosen profile for professional development. Immediately after her bachelor's degree, she was appointed to the IOCCPh of BAS as a specialist biologist, and after obtaining a master's degree, as an assistant professor in the laboratory "Chemistry and Biophysics of Proteins and Enzymes", then headed by the highly respected Prof. D.Sc. Bozhidar Chorbanov, at the same Institute. There she continued her professional development to this day in the field of her Ph.D. thesis, namely biocatalysis, bioorganic synthesis of peptides and glycopeptides, isolation of bioactive products from natural sources for biomedicine and ecology.

3. Relevance of the topic and expediency of the set goals and objectives

Peptides play various functions in the human body. They can be neurotransmitters, neuromodulators, hormones, etc. In the last decade, peptide structures have found application in medical practice as potent biologically active substances with diverse activity, vectors of biologically active molecules and diagnostic markers, as well as in the composition of polypharmacophores with potential application in pharmaceutical practice in diseases with multicomponent symptoms, for examples neurodegenerative diseases, treatment of tumors, treatment of symptomatic pain of various origins and many others. The widespread use of peptide preparations is based on the fact that they contain natural amino acids that are normally present in the human body as part of its metabolic functions, which does not imply the presence of secondary, side effects of their use due to the existence of natural pathways for their elimination.

The Ph.D. thesis presented on my attention for review targets angiotensin converting enzyme (ACE I), whose main function in the human body is related to the control of blood pressure and other related cardiovascular diseases. It is well known that due to the extremely dynamic and stressful lifestyle in the modern world, high blood pressure and hemostatic disorders, for which data are presented on pages 18 and 19 in this dissertation, occupy a worldwide almost equal place in distribution with tumors, and in some countries come first. From this point of view, the topic chosen by the doctoral student and her supervisor is extremely relevant and directly related to the needs of pharmacy and medical practice for prevention and control of morbidity and mortality from such diseases, widespread in modern society.

4. Knowledge of the problem

The Ph.D. thesis is based on a review of 124 literature sources, of which 44 deal with the issue in the years before 2000, 73 in the period 2000-2010 and 7 literature sources are from the period after 2010. The doctoral student has examined in great detail and depth the works of other scientific groups on the topic of the dissertation, and in some places made an analysis and comparisons with their own results obtained during the development of the dissertation. In addition, different groups of ACE I inhibitors have been considered (pp. 21-26 of the dissertation), which has helped to select a direction for the design and synthesis of new ACE I

inhibitor molecules to be synthesized as part of the present dissertation. In the context of realization of the tasks from page 26 to page 34 of the dissertation the two main methods for synthesis of peptides, namely synthesis in solution and synthesis on solid phase polymer carrier are considered in sufficient detail and in depth. Attention is paid to some of their features, advantages and disadvantages. A brief place is given on page 35 of microwave-assisted peptide bond synthesis, but in light of the area in which the PhD student is developing, brief information on the use of enzymes in peptide bond synthesis could be added.

Based on an in-depth analysis of the data from the literature, the goals and tasks of the dissertation are defined very precisely, clearly and concretely on page 10. This allows to easily tracing their implementation in the course of the dissertation development. However, I believe that the place of the section Aim and tasks of the Ph.D. thesis is after the section Literary review, as the experiment set in the dissertation is the result of the analysis of current data in the literature.

5. Methodology of study

There are several modern methods for the synthesis of biomolecules, and the correct selection of the method of obtaining the target molecules and the design of appropriate schemes for their synthesis is the result of clearly defining the purpose of the study and the capabilities of the research team. In this context, the doctoral student has chosen and mastered both the main methods, namely synthesis in solution and synthesis on a solid-phase polymer carrier, together with their features and challenges. In addition, the PhD student studied the reaction of transesterification of aglycone amino acids with carbohydrate substrates. In both cases the synthesis of peptide and carbohydrate potential inhibitors of ACE I she worked with multifunctional target molecules, which is an extremely complex task in the experiment, requiring in-depth knowledge of the nature, the reactivity of the functional groups and the peculiarities, including the conformational freedom of the target substrates. This gives me reason to say that during her work on the Ph.D. thesis the doctoral student has acquired key and in-depth knowledge and competencies in the field of peptide and carbohydrate chemistry, as well as the purification and analysis of biomolecules. The doctoral student has applied modern HPLC technique for purification and realization of some of the tasks in the dissertation. The structures were detected by NMR and MS analysis. IR spectroscopy was also used to determine the conformation of some of the target molecules. This shows that during her work the doctoral student has worked and mastered the most modern methods existing in practice for purification and analysis of biomolecules with potential biological activity. All obtained data are accurately and correctly interpreted and described in the dissertation. Docking simulations of some of the active compounds were performed to show how the inhibitor binds in the active site of the enzyme, which allowed the PhD student to acquire knowledge in the field of mathematical modeling of biological objects. The conducted biological researches *in vitro* and *ex vivo* give full completion and fulfillment of the set tasks in the dissertation work.

6. Characterization and evaluation of Ph.D. thesis

The Ph.D. thesis is based on 7 main sections - Introduction, Goals and Tasks, Literary Review, Experimental Part, Results and Discussion, Conclusions and Contributions. The sections

Literary Review, Experimental Part as well as Results and Discussion, are in-depth with a large number of subsections, covering and discussing the necessary prerequisites for directing development in a certain direction or presenting the used methodologies and results and discussion.

The literature review covers the subsections Description of the Renin-angiotensin system (RAS), Hypertension - current situation in our country and worldwide, Classifications of ACE inhibitors and mechanism of action and Synthesis of peptides. Each of them examines in sufficient depth the developed problem of good scientific style, which helps a clear and precise definition of the goals and objectives for development in the dissertation.

The synthetic methods used, as well as the methods for performing kinetic studies, mathematical modeling and biological tests are described in the Experimental Part in sufficient detail, which would undoubtedly allow their future use in the development of similar problems.

The Results and Discussion section presents in depth and well systematized as tables and figures all the target biomolecules synthesized by the doctoral student, the *in vitro* inhibitory activity investigations, mathematical modeling and observed *ex vivo* biological activity. The discussion on individual moments of the work is in-depth enough and undoubtedly points to the conclusions and contributions made at the end of the dissertation.

7. Contributions and significance of development for science and practice

I would formulate the main contributions from the Ph.D. thesis in three directions:

- Synthesis, isolation and characterization of new ACE I inhibitors based on peptides and carbohydrates, showing good ACE I inhibitory activity in the μM range

- Mathematical modeling of docking of newly synthesized inhibitors in the active site of the target enzyme ACE I

- Determined *in vivo* inhibitory activity and *ex vivo* biological activity of newly synthesized ACE I inhibitors

The dissertation uses modern methods for synthesis and innovative approaches using activated cyanomethyl esters of amino acids to achieve the goals and objectives. On the one hand, the results achieved have a scientific contribution, as they provide some key structure-activity relationships by substituting different amino acids in the target peptide inhibitors of ACE I and reveal some structurally key binding fragments in the active site of the target enzyme. In addition, the role of the conformational freedom of the peptides on the effective binding in the active site of ACE I was studied. The set goals and objectives were achieved by using the most modern approaches, methods for characterization and analysis of biomolecules. On the other hand, the obtained new biomolecules have the possibility in the future to be applied in practice as food additives, as some of them show good activity in the μM range.

Here I would like to note that it would make a good impression if at the end of the Ph.D. thesis the doctoral student's views on some future perspectives for work on the topic of the dissertation, as well as on its scientific development were noted.

8. Evaluation of the publications on the topic of Ph.D. thesis

All **4** presented publications on the topic of the dissertation are in journals with IF, which significantly exceeds the requirements of the Academic Staff Development Act of Republic of Bulgaria and the regulations to it for the acquisition of scientific and educational degree **„doctor of philosophy“**. Two of the publications cover the synthesis, characterization and study of the biological activity of newly synthesized ACE I inhibitors. One publication is related to the study of the conformational freedom of peptides at different pH and the fourth publication presents studies on the stability of target peptides.

The importance of the published results is proven by the mentioned 11 citations of two of the publications. This has contributed to the acquisition of h-factor 2, which is a very good certificate for recognizing the work of the doctoral student in the scientific community working in the same or related fields.

9. Personal involvement of the Ph.D. student

The Ph.D. student participated in a large number of National and International scientific forums with 14 presentations, which shows a very good promotion of the results obtained by her among the scientific community.

In two of the presented publications related to the synthesis and study of the biological activity of newly synthesized biomolecules the doctoral student is in first place, in one related to the study of conformational freedom of peptides at different pH is in second place and in one concerning the stability of target peptides she has fourth place between authors that undoubtedly proves her main participation in the development of the dissertation, the systematization and description of the obtained results and the achieved contributions.

10. Abstract of Ph.D. thesis

The abstract presented by the Ph.D. student student fully covers five of the main sections Introduction, Purposes and tasks, Results and discussion, Conclusions and Contributions from the main dissertation. It also contains scientific publications on the topic of the dissertation, noticed citations, participation in scientific forums with results of the dissertation, as well as participation in projects and educational activities as a scientific consultant of several diploma theses. The abstract is prepared in accordance with the requirements of the Academic Staff Development Act of the Republic of Bulgaria, the Regulations for its implementation and the Regulations for development of the academic staff of BAS, reflecting all the necessary results and contributions from the dissertation.

11. Critical notes and recommendations

I have some technical notes to the dissertation:

- the used literature is inconsistently cited in places;

- in some places foreign words such as coupling (condensation), leucine (leucine) are used, the amino acids are written with whole names in Latin, etc. which I recommend to be avoided in the future;

- in some places in the dissertation there is talk about concentration, indicating the value, the amount of active substance in mmol. I would like to draw the doctoral student's attention to the fact that the concentration is presented as the amount of a substance in a certain volume of solvent (mixture of solvents). The mmol units indicate the amount of substance, but they do not reflect the concentration in the absence of the amount of solvent in which they are present.

I recommend the doctoral student to be intimidated in the future and to present reports at National and International conferences, as I believe that the scientific results she has achieved are worthy of it.

12. Personal impression

I know Assistant Professor Boryana Yakimova from her participation in the Bulgarian Peptide Symposia and other scientific and social events organized by the Bulgarian Peptide Society. My personal impressions are that the works presented by her are in-depth, she has excellent scientific thought and skillfully, at a good scientific level is able to defend her scientific observations and achievements, which gives me a reason for the excellent overall impression and the final conclusion formulated below from reviewing her Ph.D. thesis.

13. Recommendations for future use of dissertation contributions and results

I recommend Assistant Professor Yakimova to expand her collaboration with other groups and scientists outside the institutes of BAS, including in other scientific and educational organizations at home and abroad, which I firmly believe will contribute to its future development in the chosen scientific field.

CONCLUSION

The Ph.D. thesis presented to my attention for review **contains** scientific and scientific-applied contributions, representing an original contribution to science in the specific and related scientific fields, to which the doctoral student applies for the acquisition of scientific and educational degree **"Doctor of philosophy"**. The developed dissertation **fully meets** and even **exceeds** the requirements of the Academic Staff Development Act of the Republic of Bulgaria, the Regulations for its implementation for the acquisition of scientific and educational degree **"Doctor of philosophy"**. The dissertation shows that the candidate has acquired significant theoretical and practical knowledge and skills in the claimed scientific specialty Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances, and undoubtedly has shown during her work qualities and skills for independent scientific work.

Taking into account all the above, I confidently give my **positive assessment** of the presented Ph.D. thesis and propose to the esteemed scientific jury to award scientific and educational degree "**Doctor of philosophy**" to the candidate Boryana Krasimirova Yakimova in the field of higher education 4. Natural sciences, mathematics and informatics, professional field

4.2. Chemical sciences, doctoral program Bioorganic chemistry, chemistry of natural and physiologically active substances.

10.05.2021 Reviewer: .....

Prof. Eng. Dancho Danalev, Ph.D.