Appendix 12a

REVIEW

of dissertation for the acquisition of:

educational and scientific degree " **doctor** "	**X**
scientific degree **"Doctor of Science"**	
	the true is indicated by the sign "X"

Author of the dissertation:

Assist.		**Dilyana**	**Vasileva**	**Dimitrova**	**UCTM - Sofia**
academic position	scientific degree	name	middle name	last name	workplace

Topic of the dissertation:

Synthesis and biological activity of temporin analogues

Scientific area:

5	Technical Sciences
code	name

Professional area:

5.11	Biotechnology
code	name

Scientific specialty:

Technology of biologically active substances

The review was written by:

Professor	**PhD**	**Petar**	**Todorov**	**Todorov**	**UCTM - Sofia**
academic position	scientific degree	name	middle name	last name	workplace

1. Completion of the provided documents:

A) The dissertation and the competition documents are in full compliance with the Regulations.	4 points	**X**
B) The documents are complete but do not fully comply with the requirements of the Regulations.	2 points	
C) The documents are not completed in accordance with the requirements of the Regulations.	0 points	
		one of the answers given is marked with the

		sign "X"

Missing documents and violated standards must be described if response C is marked.
The submitted documents and materials of the competition fully comply with the Regulations.

2. Meeting the minimum requirements under the Regulations:

A) The candidate meets the minimum requirements	20 points	**X**
B) The candidate doesn't meet the minimum requirements	0 points	
		one of the answers given is marked with the sign "X"

It must be filled in if answer B is marked. The publication activity of the candidate is analyzed. The response of the results achieved (quoted) is analyzed.
The presented materials and results fully comply with the requirements of the Act for the development of the academic staff in the Republic of Bulgaria and the Regulations for its implementation, as well as those of UCTM-Sofia. The scientific results have been published in a total of three scientific articles, two of which are in the journal *Pharmaceutics* with an impact factor IF = 5.5 and a quartile Q1, carrying a total of 13.33 points, as well as one publication in the *Journal of Chemical Technology & Metallurgy*, which does not have an impact factor, but is indexed with SJR and carries 20 points. The PhD-student is the first author in all three publications, which is categorical evidence of her significant contribution to the conduct of scientific research, the analysis of the results and the preparation of the publications. PhD-student Dilyana Dimitrova has also actively participated in 23 scientific forums, at which she presented poster messages and reports related to the topic of the dissertation work. This shows high scientific activity, consistency and commitment to the popularization of the obtained scientific results to the academic community. The doctoral student's total point asset amounts to 33.33 points, which meets the required minimum threshold of 30 points.

3. The relevance of the topic of the dissertation:

A) The topic is relevant and new (there are no known results on the topic by other authors)	8 points	
B) The topic is relevant and results from other authors are known	6 points	**X**
C) The topic is not relevant, but results from other authors are known	2 points	
D) The topic is not relevant and no results from other authors are known	1 point	
E) The topic does not correspond to the level of dissertation	0 points	
		one of the answers given is marked with the sign "X"

The evaluation of the relevance of the dissertation must be substantiated
The PhD thesis is devoted to the synthesis, characterization and study of the biological activity of new structural analogues of the antimicrobial peptide temporin A. The topic is extremely topical and

significant, since antimicrobial resistance is a serious global problem with a direct impact on human health in all regions of the world. The increasing resistance of pathogenic microorganisms to existing antibiotics necessitates the development of new and more effective therapeutic approaches. In this regard, antimicrobial peptides (AMPs) are considered one of the most promising alternatives to conventional antibiotics and have been the subject of increased scientific interest in recent years. These compounds have the potential to be effective against a wide range of infections caused by both bacteria and other pathogens, including strains resistant to available drugs. An additional advantage of antimicrobial peptides is their relatively low molecular weight, which facilitates their preparation by chemical and biotechnological methods. Based on natural antimicrobial peptides, in recent years new modified analogues have been increasingly developed with the aim of improving their pharmacokinetic and pharmacodynamic characteristics, as well as preserving or enhancing their biological activity. Therefore, the development and study of structural analogues of temporin A represents a promising direction with the potential for creating new antimicrobial agents.

4. Knowledge of the problems, subject of research in the dissertation:

A) The doctoral student knows in detail the achievements of other authors on the topic of the dissertation	8 points	**X**
B) The doctoral student is partially familiar with the achieved results on the topic of the dissertation	4 points	
C) The doctoral student has no prior knowledge of the status of the problems in the dissertation	0 points	
		one of the answers given is marked with the sign "X"

The evaluation must be substantiated if answer C is marked.
From the overall presentation of the dissertation work, it is clearly seen that the PhD-student Dilyana Dimitrova has conducted a thorough research and analysis of the literary sources on the topic under consideration and is fluent in the available scientific information. This testifies to a very good theoretical preparation, a critical approach to scientific information and the ability to correctly interpret and apply it in the context of the research conducted.

5. Type of research:

A) Theoretical	4 points	
B) Applied	4 points	**X**
C) Theoretical with application elements	4 points	
D) It does not correspond to the level of dissertation	0 points	
		one of the answers given is marked with the sign "X"

The level of research must be substantiated if answer D is marked.
The PhD thesis covers the synthesis and study of the biological activity of new analogues of the antimicrobial peptide temporin A. The conducted research is aimed at the development of new peptide molecules with increased selectivity, better tolerability and potentially higher efficiency in the fight against antimicrobial resistance. The results obtained are of an applied nature, as they could contribute to the creation of new therapeutic agents against resistant pathogenic microorganisms. In

this aspect, the dissertation represents a contribution to research in the field of biologically active substances, biotechnology and the development of innovative antimicrobial agents.

6. Objectives of the research:

A) Realistic and of scientific and / or applied interest	8 points	**X**
B) Realistic, but not of scientific and / or applied interest	3 points	
C) Unattainable (unrealistic)	0 points	
		one of the answers given is marked with the sign "X"

Objectives must be specified. The type of the set objectives must be justified.
From the literature review it is clear that PhD-student Dilyana Dimitrova has become very familiar with the literature on the topic and is fluent in using it. Based on this, the goal of the dissertation work has been precisely and clearly formulated and the corresponding 6 tasks for implementation have been determined. The goals set for the conducted scientific research are realistic, and the results achieved on their basis have significant scientific and applied interest.

7.Methods of research:

A) Adequate to research and set objectives	8 points	**X**
B) Partially appropriate, enabling part of the scientific objectives and / or applications to be achieved	4 points	
C) Inappropriate methods	0 points	
		one of the answers given is marked with the sign "X"

Methods must be specified. The type of methods used is justified.
During the development of her PhD thesis, the PhD-student used the method of solid-phase peptide synthesis to obtain antimicrobial peptide analogues of temporin A. This method has become widely used in recent years due to a number of its advantages, related to efficiency, high selectivity and the possibility of obtaining peptides with a high degree of purity. High-performance liquid chromatography and mass spectrometry (HPLC/MS) were used to prove the structure and purity of the synthesized peptide analogues. The optical activity of the newly synthesized peptides was studied by determining the angle of optical rotation of the compounds, and the circular dichroism (CD) method was applied to analyze their secondary structure. In order to study the hydrolytic stability of the peptides, three pH systems were modeled, corresponding to the conditions in the stomach, blood plasma and small intestine of the human organism. This approach allows to assess the stability of the peptides in different physiological environments. All synthesized compounds were tested for potential biological activity: antibacterial, antifungal, antiproliferative, cytotoxicity and phototoxicity. The experiments conducted allow for a comprehensive assessment of the biological potential of the newly synthesized analogues. The results obtained clearly show that Dilyana Dimitrova has mastered and successfully applied a variety of modern methods and techniques for the synthesis and analysis of peptide molecules. This testifies to excellent experimental preparation and skills for independent research work.

8. Contributions of the dissertation:

A) With lasting scientific and / or applied response, they form the basis for new research and applications	20 points	**X**
B) They are of significant scientific and / or applied interest, complete and / or summarize previous research	16 points	
C) They are of scientific and / or applied interest	12 points	
D) Lack of significant contributions	8 points	
E) Lack of contributions	0 points	
		one of the answers given is marked with the sign "X"

Contributions must be specified. The type of results achieved must be justified.
The contributions to the dissertation are precisely and clearly formulated, and can be summarized as follows: 1. Nine analogues of the antimicrobial peptide temporin A have been synthesized and characterized for the first time, containing the non-proteinogenic amino acids Dab, Dap, Cit, Orn, Phe(4-F), Phe(2-F) and Phe(4-Cl). 2. Three new analogues of the antimicrobial peptide temporin A have been designed, synthesized and characterized, in which the natural amino acids Thr and Tyr have been introduced for the first time at positions 1 and 10, respectively. 3. Important relationships between the structure and biological activity of all newly synthesized analogues of temporin A have been established with respect to their antimicrobial properties, antiproliferative effect, cytotoxicity and hydrolytic stability. 4. As a result of the conducted studies, the following important dependencies were established: ✓ introduction of Dab at position 7 leads to an increase in antibacterial activity, while substitution with Cit at the same position causes a loss of antibacterial activity, reduced stability at alkaline pH and at the same time increased selectivity towards the luminal type of breast cancer; ✓ introduction of Tyr at position 10 leads to low cytotoxicity towards healthy tissue models, high antiproliferative activity and good selectivity towards the luminal type of breast cancer, which defines this analogue as a promising potential therapeutic agent; ✓ introduction of Phe(4-F) at position 1 leads to a significant increase in antibacterial activity, as well as high antiproliferative activity and selectivity towards the luminal type of breast cancer, albeit with reduced stability in an alkaline environment. The above contributions have both scientific and applied nature and represent a significant contribution to the development of research on antimicrobial peptides and the possibilities for developing new biologically active compounds with potential therapeutic application.

9. Evaluation of the compliance of the dissertation summary with the dissertation:

A) Full compliance	4 points	**X**
B) Compliance of the main parts	2 points	
C) Lack of compliance of the main parts	0 points	
		one of the answers given is marked with the sign "X"

The evaluation must be substantiated if answer C is marked.

The dissertation summary meets the requirements of the Regulations of UCTM-Sofia and fully reflects the results of the research.

10. Participation of the doctoral student in the achievement of the results of the dissertation:

A) The doctoral student has at least an equal participation	8 points	**X**
B) The doctoral student has secondary participation	5 points	
C) The participation of the doctoral student is unnoticeable	0 points	
		one of the answers given is marked with the sign "X"

Critical notes must be provided if one of the items B or C is marked.
The presented dissertation is fully dissertable, with a real contribution to science and practice. The scientific results presented by the doctoral student have been published in three scientific articles, and she is the first author in all three publications. This is categorical evidence of her active and substantial participation in the planning and conduct of the research, the analysis and interpretation of the results, as well as in the preparation of the scientific publications.

11. Critical notes:

A) Lack of critical notes	8 points	**X**
B) Critical notes of a technical nature	7 points	
C) Critical notes that would partially improve the results achieved	4 points	
D) Significant critical notes	0 points	
		one of the answers given is marked with the sign "X"

Critical notes must be provided if one of the answers C or D is marked.
The PhD thesis is written in a comprehensible manner, with a logical order to the exposition and summary of the results. There are minor stylistic and technical errors, but they do not detract from the enormous amount of results obtained.

12. Conclusion

A) The evaluation of the dissertation is **POSITIVE**	This evaluation is assigned to a total number of at least 65 points	**X** **(98 points)**
B) The evaluation of the dissertation is **NEGATIVE**	This evaluation is assigned to a total number below 65 points	
		one of the answers given is marked with the sign "X"

To be filled in at the request of the reviewer
Based on the above arguments in my review, I confidently vote "**positive**" assessment of the dissertation of Asst. Dilyana Vasileva Dimitrova and recommend the esteemed members of the scientific jury to support her award of the educational and scientific degree "Doctor" in the field of higher education 5. "Technical Sciences", professional direction 5.11 "Biotechnologies", scientific specialty "Technology of Biologically Active Substances".

28.05.2026	The review was written by:	
date	**Professor PhD Petar Todorov**	signature