Appendix 12b

REPORT

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:

Assistant	**Eng.**	**Dilyana**	**Vasileva**	**Dimitrov**	**UCTM**
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 report was written by:

Professor	**PhD**	**Emilia**	**Dimitrova**	**Naydenova**	**UCTM**
academic position	scientific degree	name	middle name	last name	workplace

1. 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 is mandatory to fill in if answer B is marked. The publication activity of the candidate is analyzed. The response of the results achieved (quoted) is analyzed.

The doctoral student has presented a list of 3 scientific publications on the results obtained during the development of the dissertation work. The publications are published in scientific journals which are referenced and indexed in world-renowned databases of scientific information with IF or SJR and falling into quartiles Q1 and Q3 according to the grouping of scientific journals. 2 of the publications are published in the journal *Pharmaceutics* with Q1 and 1 review article in the *Journal of Chemical Technology and Metallurgy*, (Q3). The points that these publications carry are **33.33**, which satisfies the minimum requirements.

The doctoral student's active participation in national and international scientific forums makes an excellent impression. She participated in 11 scientific poster sessions and conferences in the country and 12 abroad.

2. 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 topic of the dissertation is appropriately selected, and meets the requirements for a dissertation, and the research conducted is current, promising and with an element of practical orientation for application in medicine

After the introduction of antibiotics in the 1950s for the treatment of microbial infections, the development of resistance by many bacteria to antibiotics used in the clinic was considered a negative effect. This leads to the need to search for new drugs to combat pathogenic microorganisms. Antimicrobial peptides (AMPs) have received special attention as an alternative approach to combat infections caused by antibiotic-resistant bacterial strains. AMPs are positively charged, amphipathic structures with a great diversity and small molecular length. They exhibit a wide spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, viruses and parasites in a wide range of pH and temperatures. In nature, they represent an important part of the innate immune defense of animals and insects.

3. Type of research:

A) Theoretical	4 points	
B) Applied	4 points	
C) Theoretical with application elements	4 points	**X**
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 dissertation and the presented publications show that the research is rather theoretical with elements of applications, as the potential for their use in medicine is sought, as new therapeutic agents to combat infections caused by antibiotic-resistant bacterial strains.

4. 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.
Based on the literature data, the goal of the dissertation is formulated precisely and clearly. It is of scientific and applied interest and is related to molecular design and synthesis of new structural analogues of the antimicrobial peptide temporin A with potential antibacterial activity and study of their biological properties. The tasks for its implementation are selected appropriately. The main tasks are: • Preparation of peptide analogues of temporin A by solid-phase peptide synthesis; • Characterization and study of their biological properties, such as antibacterial activity, cytotoxicity, phototoxicity and antiproliferative activity; • Determination of the hydrolytic stability of the newly synthesized analogues. • Establishing some relationships between structure and biological activity based on the results obtained.

5. 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 doctoral student has performed sufficient experimental work and has mastered various methods and techniques for synthesis, purification and characterization of new compounds. The conclusions correctly reflect the results achieved from the research carried out. The main contributions of the present dissertation are in the synthesis and determination of the biological activity of new analogues of the antimicrobial peptide temporin A with potential biological activity. ✓ Temporin A and 13 new analogues were synthesized and characterized by introducing various proteinogenic and non-proteinogenic amino acids in key positions for biological activity. ✓ The obtained and studied new analogues are grouped into 3 series. In the first series, Arg^{7} was replaced with Lys, Orn, Cit, Dab and Dap. Based on the biological studies performed, the structure-biological activity relationship was established. • It has been shown that the introduction of Dab at position ^{7} leads to an increase in antibacterial activity, while the introduction of Cit at the same position leads to a loss of antibacterial activity, reduced stability at alkaline pH, but increased selectivity towards the luminal type of breast cancer; ✓ In the synthesized second series of analogues, Phe^{1} was replaced with Tyr and fluorinated phenylalanine, and Ser^{10} was replaced with Tyr and Thr. • It has been found that the introduction of Tyr at position 10 leads to low cytotoxicity towards normal cells, high antiproliferative activity and selectivity towards the luminal type of breast cancer. ✓ A third series of analogues was synthesized in which in the analogues with the best indicators from previous studies, Phe^{1} was replaced with Phe(4-F), Phe(2-F) and Phe(4-Cl). • It has been found that the introduction of Phe(4-F) at position 1 leads to significantly higher antibacterial activity, as well as high antiproliferative activity and selectivity against luminal type breast cancer, but with reduced stability at alkaline pH.

6. Conclusion

A) The evaluation of the dissertation is **POSITIVE**	This evaluation is assigned to a total number of at least 40 points	**X** total 58 points
B) The evaluation of the dissertation is **NEGATIVE**	This evaluation is assigned to a total number below 40 points	
		one of the answers given is marked with the sign "X"

To be filled in at the request of the member of the scientific jury
Conclusion The dissertation is dedicated to a problem that is relevant to practice. In completing the assigned tasks, doctoral student Dilyana Dimitrova demonstrates very good knowledge of the literature on the topic, experimental setups and methods, as well as opportunities for independent research work and interpretation of the results.

In conclusion, I think that the presented dissertation fully meets the requirements for dissertations work and the UCTM Regulations for the award of scientific degrees in terms of volume, scientific and applied contributions and publications in the scientific literature. Based on the presented dissertation work and the achievements in it, I confidently vote **positively** and I suggest to the members of the Scientific Jury to render a positive decision for awarding the educational and scientific degree "Doctor" to **Dilyana Vasileva Dimitrova** in the Scientific Field: 5. Technical Sciences, Professional Direction 5.11. Biotechnologies, Scientific Specialty - Technology of Biologically Active Substances.

28.05.2026	The report was written by:	
date	Prof. Dr Ing. Emilia Dimitrova Naydenova	signature