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:

	Assist. Eng.	Dilyana	Vasileva	Dimitrova	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:

Prof.	DSc	Ivanka	Borisova	Stoineva	IOCCP(associate member)
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 Dilyana Dimitrova has presented a list of three scientific publications indexed in internationally recognized scientific databases. Two of the publications are published in journals ranked in the Q1 quartile and with a high impact factor, while one is published in the *Journal of Chemical Technology and Metallurgy* (Q3). The total number of points accumulated through the publication activity is 33.30, thereby meeting and exceeding the minimum national requirements for the acquisition of the educational and scientific degree "Doctor". The doctoral student's active participation in scientific forums is particularly noteworthy, with a total of 23 presentations at national and international conferences and scientific events. This demonstrates a high level of scientific engagement, dedication, and the strong quality of the research outputs achieved. In addition, the doctoral student has participated in the implementation of three research projects, one of which is international in scope, further confirming her ability to work effectively within scientific teams and her successful integration into research activities.

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
Antimicrobial resistance (AMR) represents one of the most serious global threats to public health. According to the World Health Organization (WHO), antibiotic resistance has been steadily increasing over the past two decades, leading to infections that are increasingly difficult or, in some cases, impossible to treat with existing antibacterial agents. In this context, research related to the design and synthesis of new drugs, and in particular novel antibiotics, is of growing importance, especially in light of recent pandemics and their long-term consequences. The main focus of this dissertation is the design and synthesis of synthetic analogues of the antimicrobial peptide temporin A with potential antibacterial activity. The conducted research is highly relevant, scientifically significant, and promising, as it opens new opportunities for the development of innovative therapeutic agents with potential applications in medical practice.

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 results obtained from the conducted research are partly theoretical in nature (deriving some SAR dependencies) and with elements of application in medicine as new therapeutic agents.

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.
The goals and objectives of the dissertation are clearly formulated and fully realistic in terms of their implementation, being grounded in the extensive scientific and research experience of the supervisors, as well as in the doctoral student's demonstrated interest in the research topic and the scientific challenges addressed. The main aim of the dissertation is the synthesis of novel structural analogues of the antimicrobial peptide temporin A, alongside an in-depth investigation of the biological properties of the newly synthesized compounds. The achievement of this objective has the potential to contribute to the expansion of current knowledge in the field of bioactive peptides and to support the development of new molecules with promising applications in medicine and pharmacy.

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.
In accomplishing the ambitious objectives and tasks of the dissertation, the doctoral student has successfully carried out the design, synthesis, and structural characterization of twelve novel analogues of the antimicrobial peptide temporin A using modern analytical techniques. A major contribution of the work is the successful implementation and application of contemporary solid-phase peptide synthesis methodology, which enabled the preparation of nine temporin A analogues containing non-proteinogenic amino acids (Dab, Dap, Cit, Orn, Phe(4-F), Phe(2-F), and Phe(4-Cl)) and three analogues incorporating the natural amino acids Thr and Tyr at positions 1 and 10, respectively. Another significant scientific contribution is the systematic investigation of the relationships between the structure and biological activity of the newly synthesized peptides. The biological evaluation included antimicrobial activity, antiproliferative effects, cytotoxicity, and hydrolytic stability. The obtained results revealed important structure–activity relationships (SARs). In particular, substitution with Dab at position 7 enhanced antibacterial activity, whereas incorporation

of Cit at the same position reduced antibacterial potency but improved selectivity toward luminal breast cancer cells. The analogue containing Tyr at position 10 exhibited low cytotoxicity toward healthy cells while maintaining high antitumor activity and selectivity. Furthermore, the introduction of Phe(4-F) at position 1 significantly improved both antibacterial and antiproliferative activities, as well as selectivity toward luminal breast cancer.
These findings provide a valuable basis for the development of rational strategies for structural modification and optimization of antimicrobial peptides and contribute to the design of novel peptide analogues with promising antibacterial and antitumor potential for biomedical applications.
The circular dichroism studies of temporin A and all newly synthesized analogues revealed no characteristic spectral features associated with well-defined secondary structures, suggesting the absence of pronounced structural organization under the experimental conditions employed.

6. Conclusion

A) The evaluation of the dissertation is **POSITIVE**	This evaluation is assigned to a total number of at least 40 points	58
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

The analysis of the submitted documents, the quality of the scientific publications, and the scientific contributions achieved during the doctoral studies identify Dilyana Vasileva Dimitrova as a promising young researcher with a strong interest in developing innovative solutions through the synthetic design of novel compounds with potential pharmacological significance. She has demonstrated solid theoretical knowledge, excellent experimental skills, and the ability to conduct independent scientific research.
Based on the presented dissertation, the published scientific results, and, above all, the original scientific contributions and achievements of the dissertation work, I give my unequivocally positive assessment of the thesis.
Therefore, I confidently vote in favor of awarding the educational and scientific degree “Doctor” to Dilyana Vasileva Dimitrova in Scientific Field 5. Technical Sciences, Professional Direction 5.11. Biotechnologies, Scientific Specialty “Technology of Biologically Active Substances”, and respectfully recommend that the members of the Scientific Jury make a positive decision regarding the conferment of this degree.

01.06.2026	Prof. DSC Ivanka Stoineva	
date	The report was written by:	signature