

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:

		Sirine	Sleiman	Jaber	UCTM - Sofia
academic position	scientific degree	name	middle name	last name	workplace

Topic of the dissertation:

Synthesis and biological activity of analogs of (KLAKLAK) ₂
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Scientific area:

4	Natural Sciences, mathematics, and informatics
code	name

Professional area:

4.2.	Chemical Sciences
code	name

Scientific specialty:

Bioorganic chemistry, chemistry of natural and physiologically active substances
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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"
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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 presented results were published in 2 scientific publications in the journal Molecules with a high impact factor (IF = 4.412) and Q1 /Q1 = 25 points/, and one citation was noted. PhD-student Sirine Jaber has taken an active part in 10 scientific forums with poster presentations and reports on the topic of her dissertation.

Indicator G (publications in journals referenced in Scopus or Web of Science) is completed with 50 points /2 scientific publications in Q1/, with a minimum of 30 points required.

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 presents a topical issue for bioorganic chemistry and chemistry of natural and physiologically active substances, namely the creation of new and improvement of already known bioactive peptide molecules, as well as the study of their biological activity, in particular the discovery of new antimicrobial peptides with antitumor activity. The topic of antimicrobial peptides with antitumor

activity is especially relevant today, as it has a direct bearing on the so-called. targeted therapy, which is one of the most reliable therapies for cancer.

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 whole exposition, it is clear that the PhD-student Sirin Jaber has gotten acquainted in detail with the scientific literature on the topic and is fluent in it.

5. 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 PhD-thesis covers the design, synthesis, and characterization of new analogs of (KLAKLAK)₂ as potential biologically active compounds. Important dependencies on the structure-biological activity relationship have also been derived. Therefore, the type of research can be categorized as theoretical with application elements.

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 Sirine Jaber is very well acquainted with the scientific literature on the topic and is fluent in it. Based on this, the purpose of the dissertation is formulated precisely and clearly and the respective 7 tasks for implementation are defined. The set goals of the conducted research are realistic, and the results achieved on their basis have a 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 his dissertation, the PhD-student used one of the modern methods for the synthesis of biologically active peptides, namely solid-phase peptide synthesis. From the obtained results it is clear that Sirine Jaber has mastered this method of synthesis, as well as the appropriate characterization of the newly synthesized compounds /hydrolytic stability; determining the angle of optical rotation; determination of melting points, etc./. Reverse phase high-performance liquid chromatography (RP-HPLC) has been used to determine the purity of the peptides, and electrospray ionization mass spectrometry (ESI-MS) has been used to prove the structures of the compounds. In vitro cytotoxicity studies (3T3 NRU test) were also performed, and the antiproliferative and antimicrobial activities of peptide analogs of (KLAKLAK)₂ were determined.

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. Design, synthesis, and characterization of analogs of the single and double sequence of the antimicrobial peptide with additional antitumor activity (KLAKLAK)₂-NH₂ containing unnatural amino acids Nle and β-Ala into its primary structure;

2. Design, synthesis, and characterization of bioconjugates of the truncated and duplicated analogs of the single-sequence antimicrobial peptide (KLAKLAK)₂-NH₂ with a second pharmacophore with proven antitumor activity 1,8-naphthalic anhydride and caffeic acid;

3. Antibacterial properties, antiproliferative effect, and cytotoxicity of all newly synthesized analogs and conjugates of (KLAKLAK)₂-NH₂ have been studied and important structure-biological activity relationships were derived;

4. The hydrolytic stability of the newly synthesized analogs of the single and double sequence (KLAKLAK)₂-NH₂ for 72 hours at three different pH values simulating the conditions in the human body has been studied and it was found that all newly synthesized compounds have complete stability under the tested conditions.

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 completely dissertable, with an original contribution to science in a very interesting and promising field, such as peptide chemistry. The obtained results are up-to-date and have a significant scientific contribution. During the development of the dissertation, Sirine Jaber has mastered varied methods and techniques for synthesis, analysis, and research, which are very well interpreted with understanding and in good scientific language. The results are presented in 2 scientific publications in Molecules with high IF (IF = 4.412) and Q1 and one citation were noted. It should be noted that the PhD-student is in first place in both publications. Sirine Jaber has participated in 10 scientific forums with poster presentations and reports on the topic of her dissertation.

11. Critical notes:

A) Lack of critical notes	8 points	
B) Critical notes of a technical nature	7 points	X
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 dissertation is written understandably with a logical sequence in the exposition and summarization of the results. I have the following questions and remarks for the PhD-student:

- it would be good to present the chromatograms and mass spectra of all newly synthesized compounds;
- the yield of the obtained compounds is not indicated;
- how is the theoretical yield of a peptide obtained by the solid phase method calculated?
- the purity (%) of most of the final peptides is given to be close to 100%. How exactly is this purity calculated?
- What are the yields of peptide compounds containing caffeic acid, and how would you explain their high purity, given that you used unprotected caffeic acid in condensation reactions?

12. Conclusion

A) The evaluation of the dissertation is POSITIVE	This evaluation is assigned to a total number of at least 65 points	X (97 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 Sirine Jaber and recommend the esteemed members of the scientific jury to support her award of the educational and scientific degree "Doctor" in the scientific area 4.2. Chemical Sciences (Bioorganic chemistry, chemistry of natural and physiologically active substances).

18.05.2022	The review was written by:	
date	Professor PhD Petar Todorov	signature