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:

		Boryana	Biserova	Borisova	UCTM
academic position	scientific degree	name	middle name	last name	workplace

Topic of the dissertation:

Design and synthesis of phosphodiesterase inhibitors with dual activity

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	Doctor of Sciences	Lyubomir	Todorov	Vezenkov	pensioner
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.
Results included in the PhD Thesis are summarized in four prestigious scientific publications, which shows the good publication activity of Ass. Eng. Boryana Borisova. She is the first author in all publications, which shows its essential role in the development of the PhD Thesis The publications are in journals with quartile (for all) and impact factor for three of them and Scopus CiteScore for one of them: „Pharmaceuticals, 16 (8), 2023, 1183". ((IF = 4.8 and Q1), "Biomedicines, 9 (11), 2023, 3265". ((IF = 3.9 and Q1), "Current Research in Biotechnology, 10, 2025, 100332". (IF = 4.72 and Q2), „Journal of Chemical Technology & Metallurgy 2025" . (Scopus CiteScore = 1.4 and Q3). Part of the results are presented at 17 scientific forums: 12 posters and 5 presentations. Ass. Eng. Boryana Borisova fully meets the requirements according to the Regulations.

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	X
B) The topic is relevant and results from other authors are known	6 points	
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 currently available drugs are not effective enough for the treatment of inflammatory diseases, which determines the need to search for new drugs and approaches. It has been found that the phosphodiesterase enzyme isoform is considered a prospective therapeutic target for the treatment of inflammatory and/or lung diseases. In this regard, the research presented in this dissertation is extremely relevant in the field of drug discovery. They aim at the preparation and characterization of new phosphodiesterase inhibitors based on peptides, new isothiazolone and pyridazinone analogues having a - COOH group and bioconjugates between a pyrrole heterocycle and a peptide with potential analgesic activity.

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 objectives and experiments performed in the dissertation are aimed at the design and the synthesis of new peptides, isothiazolone and pyridazinone analogues possessing -COOH groups and their characterization. After performing biological tests for analgesic and anti-inflammatory activity of several of the newly synthesized peptides, important structure-biological activity relationships were made. . All this emphasizes the theoretical nature of the PhD research, but some of the more active compounds have potential for practical application.

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.
-A significant goal set in the dissertation work is to perform a large number of different modifications of the tetrapeptide Phe-Glu-Leu-Leu, such as introducing hydrophobic residues, amidation of the C-terminus, introducing an additional Leu residue into the C-terminus, replacing a Phe residue with L- or D-Tyr or with a 4-fluorophenylalanine residue. The conducted studies are of scientific interest and are realistic also due to the extensive experience of the supervisor from Bulgaria in solid-phase peptide synthesis. - Another important goal achieved is related to the design, synthesis and characterization of new isothiazolone analogues and their active structures, bearing in positions 4 and/or 5 (hetero)aryl substituents, possessing an acidic function, in order to ensure their direct binding to the N-terminus of previously prepared peptides. Two bioconjugates between peptides and isothiazolone analogues were synthesized. In this difficult research part, the PhD candidate was able to rely on the extensive experience of the supervisor from France. The research is realistic and interesting in scientific terms and with potential for future development of the topic. - New 2-substituted pyridazinone derivatives have been synthesized using various indole analogues. A linking segment containing an ester group has been successfully incorporated into the most active pyridazinone derivatives to allow further hydrolysis and subsequent conjugation with a peptide fragment. Such studies are of scientific interest.

-To determine the biological activity of the novel peptides, in vitro biological tests of 12 peptide analogs were conducted. This study highlighted that the most active analog is Phe-Glu-Leu-Leu -NH_2, Testing the novel peptides, one isothiazolone and one pyridazinone analog for anti-inflammatory activity using the Carrageenan-Induced Paw Edema test, found that the Tetrapeptide amide Phe-Glu-Leu-Leu-NH_2 significantly reduced paw edema at all time points. This research raises the scientific level of the dissertation work and increases the potential of peptides for practical application.

-An interesting goal is the synthesis of new bioconjugates by connecting pyrrole fragments with two tetrapeptides. The analgesic activity of the newly synthesized bioconjugates was studied and it was found that two of the newly synthesized hybrid conjugates exhibit a measurable analgesic effect. This part also effectively combines fundamental research with practical considerations.

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 of this dissertation are mainly of significant scientific interest. In general, they concern the synthesis of biologically active compounds with potential real-world applications.

Isothiazolone and pyridazinone analogues have a large number of activities, including anti-inflammatory activity. This allows the synthesized analogues containing a carboxyl function to be linked to peptide residues with different biological activities and thus open up new directions of research with potential applications and synergetic activities. More significant contributions of the dissertation are:

-Synthesis and characterization of 19 new analogues with the general formula Phe-Glu-X_3-X_4 .Based on the performed biological tests for analgesic and anti-inflammatory activity important structure- activity relationships were made.

-Another strong point in the thesis is the finding of new suitable conditions and/or improved reaction conditions for the preparation of some intermediates and for the preparation of the final new isothiazolone analogues containing a carboxyl function

-The synthesis and characterization by 1 H NMR, C13 NMR, MS, IR and melting point of 11 new isothiazolone analogues.

-Synthesis and characterization of 9 new (dihydro)pyridazinone derivatives bearing indole fragments at position 2 -Synthesis and characterization of 6 new bioconjugates involving pyrrole derivatives with peptides – 4 number and isothiazolone analogues with peptides – 2 number

6. Conclusion

A) The evaluation of the dissertation is **POSITIVE**	This evaluation is assigned to a total number of at least 40 points	X
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 PhD Thesis presented to me fully complies with the requirements for awarding the educational and scientific degree "Doctor" according to both the Law on the Development of the Academic Staff in the Republic of Bulgaria and the *Regulations* on the *terms* and *conditions* for acquiring *scientific degrees* and holding *academic positions* at UCTM, Sofia. I can also add my good personal impressions of Ass. Eng. Boryana Borisova, who in the course of her research has established herself as a good scientific researcher. On this ground, I recommend to the respected members of the Academic Board to award the Academic Degree „Doctor/" on Boryana Biserova Borisova in the **Scientific area** 5. Technical Sciences, **Professional area:** 5.11. Biotechnology, **Scientific Specialty**: Technology of biologically active substances.

28.11.2025	The report was written by:	
date		signature