

## **S T A T E M E N T**

on PhD thesis for obtaining an educational and scientific degree "Doctor"  
in the scientific field **4.4. Earth Sciences – PhD program - Mineralogy and Crystallography**

Author of the PhD thesis: **RUSI IVANOV RUSEV** from the Institute of Mineralogy and  
Crystallography "Acad. Ivan Kostov "

Dissertation title: ***SYNTHESIS, STRUCTURAL CHARACTERISTICS AND ANTIMICROBIAL  
ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS***

Scientific adviser: **Prof. Dr. Boris Shivachev**

Reviewer: **Prof. Dr. Svetla Trifonova Danova, DSc**

### **1. CONCERNING THE PROCEDURE:**

I was elected as a member of the Scientific Jury, based on a Decision of the Scientific Council of IMC-BAS (protocol № 13 / 20.04.2021) and an Order (№115 RD-09 / 27.04.2020) of the Director, in accordance with Art. 4 of ZSRBS and Art. 30 of the Regulations for its implementation and art. 16 (4) of the Regulations for the conditions and the order for acquiring scientific degrees in IMC-BAS.

I declare that I have no common publications, projects or conflicts of interest of another nature within the meaning of paragraphs 1 items 3 and 5 of ZSRBS with the evaluated candidate for acquisition of ONS "Doctor".

### **2. EVALUATION OF THE DISSERTATION WORK**

#### **2.1. Relevance of the problem developed in the PhD thesis**

Presented for review PhD thesis sets important scientific and applied tasks related to solving significant social and scientific challenges. Namely, the antimicrobial resistance and the search for new means to limit it. It is no coincidence that the WHO has set a prize fund of \$ 7 billion to deal with this problem. Today, the emergence of multi- and pan-drug-resistant bacteria, or "superbugs," is seen as a far greater risk to human health than Covid-19, which threatens to return modern medicine "back to the dark ages" thus, the pre-antibiotic era. The global implications for public health of such emergency with drug-resistant bacteria are enormous. Therefore, additional search to prevent is needed and this determine the aim of presented thesis. Special attention is paid to the anti-microbial evaluation of quaternary ammonium compounds and the pathways for their synthesis. Although the author focuses on formulas known for their activity, he initially seeks for a methodical approach to their cost-effective production. All this gives me the reason to evaluate the

work as relevant, significant All this gives me reason to evaluate the work as relevant and significant. This is a modern scientific and scientific-applied research, meeting the scientific challenges of the day.

## **2.2. Literary awareness and theoretical qualification of the candidate**

The literary review is concise and precisely focused on the topic of the dissertation. The quaternary ammonium compounds (QAC) and the methods for their preparation are considered in a logical sequence. The literature review of the biological activity of various QAC outlines the scientific and applied significance of these compounds and justifies the prospects of new research. The work has an interdisciplinary character and requires serious theoretical and experimental preparation, which is evident both from the implementation of the 6 experimental tasks and in the way of presenting the results. The number of cited references is impressive - 255, with a predominance of those from the last 10-15 years.

The whole work is written in a concise scientific style, grammatically sound and very well technically shaped on 170 standard pages. It is richly illustrated with 52 color figures and 41 schemes. I would like to note the excellent way of schematically presenting some of the protocols and stages of the research, which allows to outline the original contributions in the work of the PhD student.

The author handles the various scientific terminology and concepts in the field of chemistry, mineralogy and crystallography, as well as microbiology. The presented summary of all specific schemes, figures and tables make a very good impression, which makes it easier to review and evaluate this voluminous work.

## **2.3. Analysis of the methodological approaches for achieving the goal of the dissertation**

Rusi Rusev has used a rich set of different modern methods, with proven reproducibility and relevance of the obtained data. Here I want to mention some of them, such as single crystal structural analysis, nuclear magnetic spectral analysis and others. The set of modern equipment and the combination of analyzes to achieve the goal of the dissertation is impressive. This gives me reason to believe that the PhD student has built very solid skills for the production / synthesis of new chemical formulas/crystals and for complex / targeted "mineralogical" and crystallographic analyzes of these new structures. The methodological approaches are described in two sections - "Synthetic Protocols" and "Methods of Analysis" and are presented in detail.

The selected biological approaches for evaluation of the obtained QAC fully correspond to the set task for determination of antimicrobial activity and are methodically performed correctly. I would recommend to the doctoral student the details of the tests for antibacterial activity (test cultures, algorithm of work, etc.) to be described in the section "Materials and methods".

## 2.4. Evaluation of the achieved results and contributions of the dissertation work

The goal is ambitious and in line with the latest research on such compounds. The goal and tasks are clearly and correctly formulated, but are presented in the section "Results and discussion". A separation in the part "Aim and Tasks" section of the dissertation would be more appropriate. For Task 6, I would recommend formulating a new task or subtask instead of "attempting to determine the structure-activity relationship" to be "Finding or determining the existing structure-activity relationship". Moreover, such a study may be sufficient as a work and volume, even for a dissertation. I.e. this remark of mine does not belittle the results achieved, but proves how large-scale and ambitious the goal of the work is.

The results are presented in detail on 89 pages, following the logical consistency of the experimental work and are very well illustrated with 45 color figures and 5 schemes. Already at the level of QAC synthesis the doctoral student has made a summary and conclusions, relevant for the subsequent stages of analyzes of the structures and their flexibility and stability.

Rusi Rusev manages to synthesize new in its structure and activity QAC. An original author's protocol, has been designed, which in addition is relatively easy and accessible. I evaluate the work as complex and complete because not only are new structures synthesized, but they are also complexly characterized (crystallographic, spectroscopic and microbiologically evaluated).

I highly appreciate the work of the PhD student's indisputable contributions to the development. They are both scientific and applied, and I think they are even modestly noted. Undoubtedly, we have original scientific contributions with the synthesis of 29 new QAC, not described in the literature. This gives me the right to summarize that as a goal, volume and results the dissertation many times exceeds the requirements for obtaining the ONS "Doctor". The material is dissertable and it is clear that it is the personal work of the candidate, probably under the targeted guidance of the supervisor. This is proved by both publications (with IF and impact rank), in which Russi Rusev is the first author.

*I would like to ask the PhD student which of the new compounds obtained would be the most promising for further development?*

## CONCLUSION

Based on the above, I can confidently say that the peer-reviewed dissertation is an original scientific work, with theoretical and applied significance. It meets all the conditions of the Law for development of the academic staff in the Republic of Bulgaria, the Regulations for its application and the Regulations of the Institute of Mineralogy and Crystallography "Acad. Ivan Kostov" - BAS.

All this gives me a reason for an overall high evaluation of the dissertation, on the basis of which I propose to the respected scientific jury to award **Rusi Ivanov Rusev the educational and**

**scientific degree "DOCTOR" in the scientific specialty 4.4. Earth Sciences - doctoral program - Mineralogy and crystallography .**

09.06.2021

Заличено  
съгласно чл. 2 от  
ЗЗЛД

/Prof. S. Danova/