

## R E V I E W

**By Assoc. Prof. Dr. Irena Lyubomirova Philipova,**  
Institute of Organic Chemistry with Centre of Phytochemistry, BAS

**Subject:** PhD thesis for the award of the educational and scientific degree "doctor", field of higher education 4.4 Earth Sciences, PhD program "Mineralogy and Crystallography"

**Author:** Rusi Ivanov Rusew

**Subject:** Synthesis, structural characteristics and antimicrobial activity of quaternary ammonium compounds

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

### **General description of the presented materials**

According to order № 115 RD-09-180/27.04.2021 of the Director of IMC – BAS, I have been approved as a member of the academic board for awarding the educational and academic degree "Doctor" of assistant Rusi Ivanov Rusew, PhD student in IMC – BAS in professional field of higher education 4.4 Earth Sciences, PhD program "Mineralogy and Crystallography". The title of the thesis is: "Synthesis, structural characteristics and antimicrobial activity of quaternary ammonium compounds". At the first meeting of the academic board I was chosen as a reviewer of the PhD thesis.

The submitted set of documents and materials meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations on the terms and conditions for awarding scientific and academic degrees in IMC – BAS and includes the following documents: application form; autobiography; a copy of the diploma for completed master's degree; protocols from successfully passed exams according to an individual training plan; PhD thesis; abstract; list and copies of scientific publications on the topic of the thesis; list of participations in scientific events. The documents are well organized.

### **Brief biographical data about the PhD student**

Rusi Rusew graduated from the University of Chemical Technology and Metallurgy - Sofia in 2017 as a chemical engineer with a master's degree in fine organic synthesis. In 2014 he started working at IMC - BAS successively as a chemist-specialist, PhD student and assistant.

Rusi Rusew is a co-author of 10 scientific publications, and in three of them he is the first author. He has participated in 4 research projects.

### **Relevance of the topic and expediency of the set goals and objectives**

The PhD thesis of assistant Rusi Rusew is in an intensively developing and perspective scientific-applied field. The versatility of quaternary ammonium salts (QAS), determined by

their unique structural specificity contributes to a wide range of applications, namely as cationic surfactants, phase transfer catalysts, ionic liquids, fabric softeners, fluorescent biological markers and many others. They have proven to be antibacterial, anticancer and antimalarial agents. Their use as antibacterial agents is of great interest. In this regard, the topic is definitely relevant and the results achieved are in a promising scientific field.

The main goal of the present thesis is the synthesis of a series of quaternary ammonium compounds derived from 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine by applying a facile one step synthetic procedure and evaluation for antimicrobial activity. The specific tasks to achieve this goal are in a logical sequence: synthesis, physicochemical and spectral characterization of the new compounds, and evaluation of the synthesized substances for antibacterial activity against five bacterial strains.

### **Knowledge of the problem**

The literature review of the thesis covers 178 literature sources in renowned journals, which shows that the PhD student is well acquainted with the state of the problem. It is structured in two main sections. The first part provides an overview of the synthetic methods known in the literature for the preparation of QAS. The putative mechanism of Menshutkin quaternization is described. The second part of the literature review focuses on the main applications of QAS. They are systematized in two sections: application in organic synthesis and industry, and application in medicine and biological sciences. The use of QAS as antibacterial, antimalarial, antifungal and antitumor agents, as antagonists of various receptors and fluorescent contrast agents is considered in detail. The literary review leaves a good impression with the concise and competent exposition of the cited works.

### **Research methodology**

Within the PhD thesis a significant experimental work is demonstrated. A series of 26 new quaternary ammonium compounds derived from 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine are synthesized applying a modified Menshutkin reaction. Reaction conditions are optimized by varying the solvent and reaction temperature. The products obtained are isolated in pure form by washing with a solvent or recrystallization.

The newly synthesized compounds were characterized by appropriate physicochemical and spectral methods. The purity and molecular structure of the obtained compounds were proved by NMR spectroscopy ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, HMBC, HSQC, COSY, NOESY). Differential scanning calorimetry was used as an additional assessment for the purity of the QAS. Powder X-ray Diffraction analyses were used to determine the phase composition of the resulting quaternary ammonium salts. The crystal structures of the synthesized quaternary ammonium compounds were characterized by single crystal X-ray diffraction analysis. Twenty-five new crystal structures of 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine QAS were reported, mainly in the form of bromine salts.

The synthesized compounds were also investigated for their absorption and fluorescence properties by UV/VIS and fluorescence spectroscopy. The antibacterial effect of the obtained QAS was evaluated against two Gram strains - positive and three Gram strains - negative

microorganisms by Kirby-Bauer disk-diffusion method. Minimal (MIC) and non-inhibitory (NIC) concentrations of active compounds were determined by the method of Broth micro dilution. This is to achieve the goal by performing the main tasks of the thesis by using appropriate synthetic procedures, applying isolation and purification techniques, as well as instrumental methods for characterization and structural elucidation of quaternary ammonium compounds.

### **Characteristics and evaluation of the thesis**

The thesis is well organized and structured and meets the established requirements. The exposition of the material in the thesis is formed on 157 pages and includes: Literary review, Materials and methods, Results and discussion and Conclusion, Contributions and Literature. It contains 52 figures, 41 diagrams, 8 tables. Quoted are 255 literature references. The results of the thesis are presented and discussed in seven sections. The thesis is clearly written in good scientific language. The detailed spectral characterization of the obtained compounds by applying a combination of modern NMR techniques makes a good impression.

### **Contributions and significance of development for science and practice**

The results obtained in the thesis are an enrichment of existing knowledge and experience in the field of chemistry of QAS. The main contributions to the thesis can be formulated as scientific and scientific-applied. The results of the conducted researches on the synthesis, characterization and application of QAS as antibacterial agents are presented. The main contributions and merits of the thesis can be summarized as follows:

- A facile one-pot synthesis of aromatic heterocyclic quaternary ammonium compounds has been developed. Twenty-six novel QAS derivatives of 4-pyrrolidino pyridine, quinoline and 4,4'-bipyridine have been obtained and isolated.
- The crystal structures of 25 new quaternary ammonium compounds have been successfully solved and part of the crystal structures has been deposited in the Cambridge Structural Database.
- For five of the synthesized compounds, similar or better inhibition activity against *Staphylococcus aureus* was found compared to the Kanamycin control.
- Based on the obtained results, an attempt was made to determine the relationship "Structure-antibacterial activity" of QAS. A basic model is given setting guidelines for future design of QAS with improved antibacterial activity.

### **Assessment of the publications and personal participation of the PhD student**

The scientific results of the thesis have been reflected in two articles in *Crystals* (IF: 2.404, Q2) and *Bulg. Chem. Comm.* (SJR 0.137). The PhD student is the first author in both articles, which confirms his personal participation in the elaboration and interpretation of the published results. The results of the thesis are presented at a scientific forum with poster presentations. I have no doubts about the personal participation of Rusi Rusew in the implementation of the tasks and the achievement of scientific results in the dissertation under the guidance of his supervisor.

## Abstract

The abstract of the thesis is 37 pages long. It is written in accordance with the established rules and accurately and correctly reflects the main results achieved in the dissertation in summary form. The conclusions and scientific contributions, publications and participation in scientific forums in connection with the thesis are noted. The list of publications related to the PhD thesis includes one that does not correspond to the topic of the thesis:

A. A. Petrova, R. I. Russev, I. A. Nikolchina, V. B. Kurteva, B. L. Shivachev, R. P. Nikolova, S. M. Angelova (2015). Novel 13-membered cyclic dioxatetraaza scaffolds – synthesis, solution and solid state characterization. *Bulg. Chem. Commun*, 47, 1, 208-220.

I recommend this article to be removed from the list provided.

## Critical remarks and recommendations

In general, the thesis is well-done and carefully written. However, some technical errors and inaccuracies are noted. I have a few remarks and recommendations. As a critical remark I would point out the short and insufficient description of the synthetic procedures in the section Materials and methods. There is no information on methods for isolation and purification of the individual products. This would make difficult to reproduce the procedures. Why are the names of the synthesized compounds written in English? I think that they should be in Bulgarian.

Linguistic inaccuracies have been admitted in several places. For example, 'reactivity', '*N*-heterocyclic compounds' and 'fluorescent properties' are not translated correctly. There is the use of some foreigners, such as "commercial".

I assume that the discrepancy in the reported number of newly synthesized compounds is a technical error. In the Conclusion section the given number is 26, and a little further down, in the Contributions section, they are already 29. The same mistake was made in the Abstract and the Author's Reference. I recommend that to be corrected.

The observed inaccuracies and technical errors are insignificant and do not affect in general my excellent impression of the research and the scientific level of the dissertation.

## Personal impressions

I have no personal impressions of the PhD student's work, but judging by the thesis, he undoubtedly has a very good theoretical and experimental training, based on which I can conclude that they were productive in the implementation of his research work.

## CONCLUSION

The PhD thesis contains **scientific and scientific-applied results, which represent an original contribution to science and meet all the requirements** of the Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for application of

LDASRB and the respective Regulations of BAS and IMC – BAS. The presented materials **fully** comply with the specific requirements of IMC – BAS.

The thesis unequivocally shows that the PhD student **Rusi Ivanov Rusew** has theoretical knowledge and professional skills for independent research.

Based on the above, I give my **positive assessment** of the PhD thesis **and propose to the esteemed academic board to award the educational and academic degree "Doctor"** of Rusi Ivanov Rusew in the field of higher education 4.4 Earth Sciences, PhD program "Mineralogy and Crystallography".

03.06.2021 г.

Sofia

Reviewer:..

Заличено  
съгласно чл. 2 от  
ЗЗД

/Assoc. Prof. Irena Philipova/