R E V I E W

by Snezhanka Metodieva Bakalova, PhD
Assoc. Prof. in the Institute of organic chemistry with Centre of phytochemistry, BAS

On the Doctoral Thesis for acquiring the scientific degree “Doctor of Sciences”
in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics";
professional area: 4.2. "Chemical Sciences";
scientific specialty "Bioorganic chemistry and chemistry of natural and physiologically active compounds”,
Presented by: Assoc. Prof. Vanya Nikolova Mantareva, PhD
- Institute of organic chemistry with Centre of phytochemistry, BAS
Topic: Phtalocyanine Photosensitizers for Photodynamic Method towards Drug Resistance

1. Subject of review

By order RD-09-114 of May 17, 2021 of the Director of IOCCP – BAS and in relation to the procedure of public defense of a Dissertation for acquisition of the scientific degree of Doctor of Sciences by Assoc. Prof. Dr. Eng. Vanya Nikolova Mantareva I was appointed a member of the scientific jury in the professional area: 4.2. "Chemical Sciences", scientific specialty "Bioorganic chemistry and chemistry of natural and physiologically active compounds”. At the first meeting of the scientific jury, held on 27.05.2021, it was decided that I would prepare a review.

The set of materials delivered by Assoc. Prof. Dr. Vanya Mantareva on paper and/or by electronic media is in accordance with the Law of development of the academic staff in the Republic of Bulgaria, the Regulations for its implementation, the set of rules of development of the academic staff of IOCCP – BAS, and of the criteria of IOCCP – BAS for acquiring the scientific degree "Doctor of Sciences". The candidate has attached 21 publications to support the claim. Some of the results of scientific research have been presented at 12 scientific meetings in Bulgaria and abroad.

2. Brief biographic data

Vanya Nikolova Mantareva was born on Nov. 21, 1966 in Sofia, Bulgaria. She graduated the Higher Chemical Technology Institute (now University of Chemical Technology and Metallurgy) and obtained master degree in chemical engineering in 1990. Since 1991 she is affiliated to the IOCCP in the field of bioorganic chemistry. In 1998 she defended

successfully a PhD thesis entitled “Zn(II)-2,3-naphthalocyanine complexes as photosensitizers in the photodynamic therapy of tumors”, under the supervision of Prof. DSc. Maria Shopova and Prof. Dr. Dieter Wörle. In 1999, the Higher Attestation Commission awarded her the educational and scientific degree of Doctor. In the period 1999 – 2003 years she carried out grant research work in the Institute of Macromolecular chemistry of the Bremen University, Germany, the Louisville University, KY, USA, and the Autonomous University of Madrid, Spain. These grants contributed to the expansion of her scientific horizons and experience. Since 2005 she has been conducting independent research at IOCCP - BAS. In 2014 she was awarded the Associate Professorship. She was the supervisor of master diploma works, as well as of one successfully defended doctoral student (Meliha Aliosman, 2019). Currently she participates in the teams of three running projects for basic research financed by the Bulgarian Research Fund, BRF and is the coordinator of one of them. A BRF project with her participation is already concluded. She was the leader of a bilateral cooperation project between BAS and TUBITAK, Turkey. All mentioned projects are related to the present dissertation. She has experience in working with colleagues from other scientific specialties. She has a good command of English.

3. Timeliness of the scientific topic and adequacy of followed goals and tasks

The main goals and results of the dissertation are in an important and prospective scientific area and are a continuation of the traditional scientific direction of the group of photodynamic therapy, PDT, in the IOCCP – BAS which was initially developed in the early 1980-ies by the renown scientists Maria Shopova and Nikolai Genov. Vanya Mantareva has managed to expand and develop the area in accordance with the new modern trends. The area of PDT is still very up-to-date and is currently used in diagnostics and treatment of tumors, as well as in environment control and protection of air, waste water, surface disinfection, etc. There are data on the use of antimicrobial PDT in COVID-19 control. Specific tasks set in the present dissertation are related to design and derivatization of phthalocyanines, synthesis and studies of their novel complexes suitable for photodynamic applications for tumor diagnosis and treatment, as well as for antimicrobial applications related to wastewater treatment. The scientific topic is complex and requires the participation of scientists from different specialties - chemistry, photophysics, biophysics, biology and others. There is the favorable impression that Dr. Mantareva collaborates well with Bulgarian scientists, as well as foreign specialists from *e.g.* the Gebze Technical university (Turkey), the Saratov State University and the Saratov State Medical university (Russia), the Institutes of Macromolecular Compounds and of Bioorganic Chemistry of the Russian academy of sciences, etc. The results of these collaborations are promising.

4. Knowledge of the problem

The literature survey in the dissertation, as well as the discussion give me reason to believe that the author has a very good knowledge of the majority of the enormous volume of studies in the field of PDT and has skillfully selected which of them to take into account as the most relevant to her research and the results described and discussed in the dissertation.

5. Research methodology

The main research of Assoc. Prof. Dr. V. Mantareva is in an interdisciplinary field, joining organic and bioorganic chemistry with photochemistry, photophysics, biophysics, biology and others. The performed comparative analysis of the properties of the newly synthesized compounds with the properties of photosensitizers known from the literature was used as a basis in the design of new derivatives.

In my opinion, the chosen research methodology, including the measurement of a large volume of various characteristics, allows the achievement of the set tasks and adequate analysis of the obtained results.

6. Characterization and evaluation of the thesis

The Thesis written on 202 pages, contains 63 figures, 25 diagrams and 14 tables, and consists of six chapters, (*i*) Introduction (3 pages), (*ii*) Literature survey (33 pages), (*iii*) Objectives and tasks (1 page), (*iv*) Results and discussion (108 pages), (*v*) Experimental part (24 pages), and (*vi*) Contrbutions (2 pages). Finally, there is a section of appendices, including a list of publications, information on noticed citations, a list of projects, participations in scientific meetings. Cited literature is separated into three sections, related to the literature survey, to results and discussion, and to the experimental part, which to some extent facilitates reading.

The introduction describes briefly photodynamic therapy, PDT, areas of its application and established photosensitizer mechanisms of action. The main goals of the dissertation are given briefly: a summary of basic achievements in the development of novel phthalocyanine-based photosensitizers, namely design, synthesis and characterization of novel derivatives and studies of their photodynamic activity and their basic pharmacological characteristics.

The literature survey is well structured, using 157 references. The timeliness of PDT, underlying principles of the method, requirements for a good photosensitizer, mechanisms

of photodynamic activity and strategies for optimization of the photodynamic process are considered. Special attention is paid to phthalocyanines as photosensitizers for PDT, their advantages and shortcomings, as well as to the main ways of their structure modification to obtain derivatives with improved properties: better solubility and/or stability, smaller propensity for aggregation, etc. The main synthetic schemes for the synthesis of phthalocyanines are also considered, and their advantages and/or shortcomings are commented. Described and interpreted are the main characteristics of their electronic spectra, as well as the most frequently observed photochemical processes.

Goals and tasks of the dissertation are defined briefly, but clearly: (*i*) synthesis of novel phthalocyanine derivatives - metal ion complexes with either chromophore substituents, or biologically active groups, or inhibitors; (*ii*) study of their main physicochemical, photophysical and photochemical properties; (*iii*) discussion of structure to photobiological and photodynamic activity relationships.

Results and discussion:

Syntheses of novel phtalocyanine complexes, unknown to the literature are described:

- Lu(III), Sn(IV), Ni(II) and Pd(II) with four peripheral or non-periperal susbtituents. Subsequent quaternization reaction produces the corresponding water-soluble cationic complexes.
- Si(IV) with a pair of bulky axial substituents.
- Derivatives of Zn(II) phthalocyanine with four or eight aminoacid residues (tyrosine, phenylalanine, lysine, arginine) in peripheral positions.
- Derivatives of Zn(II) phthalocyanine with galactopyranose (4), obtained by two reaction schemes – by glycosylation or click-reaction, producing an ether bond or triazole bridge, resp.
- Zn(II) phthalocyanine with sterols (mestranol and ethynylestradiol) by click-reaction and subsequent quaternization to cationic derivatives
- Si(IV) with two parabene (methyl, ethyl, propyl or buthyl) axial substituents
- Hybrid structure of Zn(II) phthalocyanine with four peripheral dodecylpyridyloxy groups, absorbed in the crystal lattice of TiO_2
- Physical conjugates of Zn(II) phthalocyanine with two polymer brushes useful in photodynamic inactivation in water

In the latter two cases phthalocyanine is in its photoactive monomeric state.

This part of the Thesis refers to 205 literature entries.

Descriptions of all complexes include (*i*) synthetic procedures and their modifications against literature examples; (*ii*) chemical characteristics – elemental analyses, IR, proton

NMR and mass-spectra; (*iii*) photophysical properties – electronic spectra, fluorescence quantum yields and lifetimes; (iv) photochemical properties – singlet oxygen generation and photostability. Discussed are monomeric against aggregate complexes in solution. For the majority of complexes is studied also their toxicity against cell strains. There are comparisons of properties of novel compounds with known phthalocyanine complexes and corresponding conclusions.

The experimental part describes the synthesis of eight novel compounds, which are still unpublished. The chemicals and solvents used are described. The conditions of fluorescence and photochemical studies are described in detail, as well as of the studies of photostability and photodynamic activity. This part uses 10 literature references.

Contributions are summarized on two pages.

7. Contributions and importance of the results to science and practice

- Design and directed synthesis of 40 novel phthalocyanine complexes, unknown to the literature. The analysis of the results of conducted photophysical and photochemical studies (generation of singlet oxygen and photostability) shows their potential as sensitizers for photodynamic applications.
- Novel compounds are designed and synthesized on the basis of structural modifications of known photosensitizers in two main directions: (*i*) metal replacement in the heterocycle, and (*ii*) modification of type, number and positions of substituents.
- Synthesized are silicon complexes, where the substituents are coordinated directly to the metal ion in axial positions
- Two types of hybrid structures are obtained with hydrophobic phthalocyanines – with TiO_2 and two types of polymers. *In vivo* studies show the hybrid structures based on polymers as effective transport system for hydrophobic photosensitizers suitable for dynamic inactivation of pathogens
- A pharmacokinetic method is developed on the basis of extraction and fluorescence intensity for quantitative determination of the accumulation, retention and excretion of phthalocyanines. Cell accumulation and localization and photodynamic activity of synthesized novel compounds are studied for photodynamic applications against resistant pathogenic microorganisms. The effectiveness of complexes against viruses is estimated using the method of photodynamic inactivation.

7. Assessment of the papers related to the thesis

The dissertation summarizes 21 scientific papers (Q1 – 6; Q2 – 3; Q3 – 6; Q4 – 3; full text reports at international meetings with SJR – 2; a book chapter). Notably, all papers are published after 2015, that is, after the promotion of the candidate to Assoc. professorship. This indicates very high publication activity. In the period up to April 2021 128 citations of the above papers have been noticed (according to the candidate based on the information in sonix.bas.bg). The candidate is first author of 6 publications, second – of one, and corresponding author of 13 papers, of which 3 have two corresponding authors. In the years 2016 – 2020 the candidate has participated in 12 scientific meetings. 7 of the presentations/posters are given at 5 international meetngs. The candidate is the presenting author in all cases, and first author of 9 reports.

8. Personal participation of the author

The main contributions of the candidate are in the field of design, synthesis and chemical characterization of the novel compounds and conjugates. The candidate shows also in-depth knowledge of photophysiscs and photochemistry. Discussions of the results deftly exploit all used investigations and offer interpretations of the observed phenomena in comparison with known so far photosensitizers. The fact that the candidate is the author of correspondence in 62% of the scientific articles included in the thesis shows that she has a general view of all the research included in them.

10. Abstract

The abstract, both in Bulgarian and English is prepared according to the requirements of the respective regulations. It gives in concise form the basic results reported in the thesis and their analyses, skipping the literature survey and the experimental part.

11. Remarks and recommendations

At many places in the dissertation the position of the maxima of the electronic spectra is discussed and "significant" batochromic shifts of the fluorescence maxima of some of the newly synthesized compounds, as well as increased Stokes' shifts are reported. As can be seen from presented values, however, the differences are actually very small and in most cases do not exceed 600 - 700 cm^{-1}. Therefore, in my opinion, the energies of the longest wavelength absorption and of the fluorescence maximum should not be considered in the list of parameters for comparative analyses of the compounds in relation to their usefulness for photodynamic applications.

12. Personal impressions

I have personal impressions of Assoc. Prof. V. Mantareva, as she is a member of the team of an ongoing project, financed by he Bulgarian Science Fund and coordinated by me. She works quickly, always after discussion of the tasks and her results are of good quality. She participates actively in discussions of the team, having both critical remarks and ideas of her own.

C O N C L U S I O N

The thesis contains substantial and significant original fundamental and applied scientific contributions in the field of bioorganic chemistry, which meet all requirements of the Law of Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the implementation of the same Law and the specific requirements in view of the corresponding Regulations in the IOCCP - BAS.

The thesis shows that Assoc. Prof. Vanya Mantareva has in-depth knowledge and professional skills in the scientific specialty "Bioorganic chemistry and chemistry of natural and physiologically active compounds", which she has demonstrated in the conducted research and summarized in the form of original and significant scientific contributions, as well as in the present thesis.

On the basis of the above considerations, I strongly propose to the honorable members of the Scientific jury to award the scientific degree "Doctor of Sciences" to Associate Professor Vanya Nikolova Mantareva, PhD in the professional area 4.2. Chemical Sciences, scientific specialty "Bioorganic chemistry and chemistry of natural and physiologically active compounds".

August 5th 2021 Reviewer:

Assoc. Prof. Snezhanka Bakalova, PhD