REVIEW

for obtaining the scientific degree "Doctor of Sciences" on the topic

PHTHALOCYANINE PHOTOSENSITILIZERS FOR PHOTODYNAMIC METHOD IN DRUG RESISTANCE”

by professional field 4.2. Chemical Sciences (Bioorganic Chemitry, Chemisty of Natural and the Physiologically-Active Substances)

Dissertation: Assoc. Prof. Dr. Vanya Nikolova Mantareva in the SOA Lab, IOCH CP-BAS

Reviewer: Professor Tsonko Mitev Kolev, University of Plovdiv "P Hilendarski" and IMB "Roumen Tsanev" - BAS

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The doctoral student has attached all the necessary documents for the defense.

Brief biographical data about the doctoral student.

From 20.03.2014. - She currently holds the academic position of Associate Professor. Works at the Institute of Organic Chemistry with the Center for Phytochemistry, Bulgarian Academy of Sciences. Str. "Acad. Georgi Bonchev”, Bl. 9, 1113 Sofia. Conducts research, supervising a doctoral student, project management, publishing scientific results, reports at international forums. Research in the field of natural sciences - organic synthesis, electron spectroscopy, etc.

I. General description of the submitted materials

1. Data on the submitted documents

The candidate. Assoc. Prof. Dr. Vanya Mantareva presented a dissertation and abstract, as well as the mandatory tables for IOCH CP from the Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions in IOHCF - BAS. Other documents (in the form of official notes and certificates from the employer, project manager, financing organization or project assignor, references and feedback, awards and other relevant evidence) supporting the applicant's achievements are also presented.

Notes and comment on the documents.

The documents submitted for the defense by the candidate comply with the requirements of the Law on the Protection of Scientific and Technological Information and Practice and the Regulations on the Terms and Conditions for Acquisition of Scientific Degrees and Occupation of Academic Positions in the IOCH CP.

2. Details of the candidate

Professional and biographical data about the candidate.

Vanya Nikolova Mantareva was born on October 11, 1966, in Sofia, Bulgaria.

Assoc. Prof. Dr. Vanya Nikolova Mantareva is a member of the Structural Organic Analysis Section

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Brief biographical data

Education:

In October, 1998

Defense of PhD Dissertation on the topic of phthalocyanine chemistry and spectroscopy by the Higher Attestation Commission and the scientific and educational degree "Doctor" was awarded. Scientific supervisors: .Senior Res. Fellow. Ds M. Shopova and Prof. Dr. D. Vorle

June, 2014 Habilitation

The dissertation is presented on 203 pages and contains 63 figures, 25 diagrams and 14 tables and a total of 367 references. Some of the results were published in 20 articles and a chapter of a book, all of them from the period after the habilitation (March 2014), with 128 citations as of April. 2021

The dissertation was discussed and directed for defense at a meeting of the Colloquium "Functional Materials, Computer Modeling and Technology", Institute of Organic Chemistry with Center for Phytochemistry - BAS (Minutes № 29 / April 2021).

The dissertation is in a very relevant scientific field, summarized by the name of the method "photodynamic therapy" (PDT), which began to develop in Bulgaria at the Institute of Organic Chemistry with the Center for Phytochemistry, BAS, in the early 80's (XX century) by scientists M. Shopova and N. Genov. Nowadays, the PDT method, as a photobiotechnology, is not limited to the application of antitumor therapy, but increasingly for prevention, control for a safe environment, and in recent years with exacerbation of drug resistance caused by rapidly changing and emerging are pathogenic microorganisms, and as an alternative therapy to deal with the problem. For phthalocyanines as photosensitizers for many different applications, the dissertation was studied in the laboratories of Prof. Dieter Wöhrle, Bremen University and later by Prof. Tomas Torres, Autonomous University of Madrid. to cover in its entirety is the expertise of colleagues with different scientific interests such as Assoc. Prof. Ivan Angelov, physics; Assoc. Prof. Veselin Kasovski, microbiology; Prof. Lachezar Avramov and Assoc. Prof. Ekaterina Borisova, biophysics; Assoc. Prof. Ivan Iliev and Assoc. Prof. Anton Kril, cell cultures and colleagues from the analytical laboratories at IOHCF. The dissertation describes the results obtained with funding from the Research Fund, MES, of four projects after 2014 in competitions for basic research.

Contributing to this work is a project with Prof. Mahmut Durmush, Technical University, Gebze, Turkey in agreement with TUBITAK.

The gratitude of the dissertation expressed to everyone makes a very impressive impression

Colleagues who took part in these interdisciplinary research

Assoc. Prof. Dr. Mantareva has structured her dissertation in the generally accepted way.

I. INTRODUCTION is set out on 4 pages

II. GOALS AND TASKS are formulated clearly, informatively and understandably.

The dissertation aims to summarize the achievements as new scientific knowledge in the development of photosensitizers containing phthalocyanine molecule, which includes synthetic schemes and procedures for obtaining and further study of new derivatives with chemical analysis, physicochemical methods and photodynamic activity. All phthalocyanines obtained are complexes of non-metallic and metallic ions that contribute to their photosensitizing properties. They are also obtained as bioconjugates with biologically active compounds such as amino acids, carbohydrates and sterol, and with chromophore groups, and with inhibitors as substitutes, with properties suitable for the application photodynamic method

III. RESULTS AND DISCUSSION In this section a division of the synthesized and characterized classes of compounds is made as follows:

1. CATIONIC PHTHALOCYANIN COMPLEXES in this subsection the dissertation has made the following important conclusions:

Replacing Zn (II) with another diamagnetic ion with a significantly higher atomic number has a significant effect on the properties of the triplet excited state, contributing to the basic physicochemical characteristics that are optimal for derivatives of phthalocyanine complexes. In the new Lu (III) and with the exception of Pd (II), there is no significant difference in the quantum yields of singlet oxygen, compared with those obtained for phthalocyanine complexes of Ga (III) and In (III), with the possibility of physical quenching from the position of the ion in the complex. All complexes obtained follow the main order of increasing the quantum yield of singlet oxygen (() with increasing atomic number of the coordinated ion, as well as increasing ΦΔ for the complexes depending on the substituent groups, with the contribution of derivatives in non-peripheral positions. the solubility of phthalocyanines - a key problem when using them in solution.

1.1. COMPLEXES WITH NON-PERIPHERAL AND PERIPHERAL GROUPS

The coordinated metal ion, as well studied, affects the properties of phthalocyanine complexes by changing their basic physicochemical characteristics with a contribution to the generation of singlet oxygen. It is well known that phthalocyanine complexes of diamagnetic ions with d electronic structure have advantages in the photochemical properties of the triplet excited state of the molecule, which is important for the efficiency of the photodynamic process. In this subsection an assessment of

The phthalocyanine macrocycle lacks low solubility in most organic solvents. The first functionalized phthalocyanines were intended to improve solubility so that phthalocyanines could be tested for solutions from solutions. The most common approach is by chemically coupling appropriate substituent groups that increase the solubility of the phthalocyanine macrocycle.

1.2. SILICON COMPLEXES WITH AXIAL GROUPS. The study of these complexes is related to the fact that they are the first allowed in the United States for clinical use.

The new phthalocyanine complexes of silicon have been proven by known analytical methods: FT-IR, 1H NMR, MALDI-TOF. FT-IR spectra showed characteristic bands at 1078 cm-1 and 1080. cm-1 for the Si-O-C connection, other vibration bands are 1520 - 1519. cm-1 for -C = C- oscillation, as in 3062 and 3035. cm-1. cm-1cm-1 for aromatic -CH, respectively aliphatic -CH oscillations occur at 2922–2853 cm-1 and 2919–2849 cm-1

2. Zn (II) PHTHALOCYANINS WITH BIOLOGICALLY ACTIVE GROUPS. My assessment of this section is very high due to the introduction of chiral carbon atoms, which leads to the formation of substances that crystallize in non-centrosymmetric spatial groups. Undoubtedly, this will be the next step in the research of Assoc. Prof. Mantareva - the use of the only absolute structural method, namely single-crystal X-ray diffraction.

2.1. FROM AMINO ACIDS Chemical conjugates of phthalocyanines with amino acids were first published by the group of Lukyanetz et al. In these first steps, the goal is to create phthalocyanine and amino acid-based structures that contribute to the solubility of the hydrophobic molecule, as well as to optimize the basic photophysical properties of absorption and fluorescence. For applications in biomedicine, the requirements for cell specificity, for interaction with receptors, for membrane permeability and as a final result for high selectivity and photocytotoxic effect of the compounds are added to the listed properties. Selected amino acids belong to the essential amino acids, with important physiological functions for the body, as supportive, with a healing effect and are used as drugs of the type "prodrugs". In addition to their biological function, these amino acids have a specific fluorescence such as tyrosine and phenylalanine, and lysine and arginine are cationic in a physiological environment and are characterized by their ability to cross cell membranes. I see a good continuation of these studies due to the possibility of using the other 16 amino acids and some more accessible dipeptides.)

2.2. OF CARBOHYDRATES Carbohydrate molecules chemically linked as functional groups to photosensitizers are among the most studied biologically active molecular groups, contributing to photodynamic action. The initial idea for this type of structure was to obtain phthalocyanine compounds of amphiphilic and even hydrophilic nature. Since the first publication by Maillard et al., 1989, scientific interest in phthalocyanines as carbohydrate conjugates has increased due to the biological activity of the resulting new structures for antitumor photodynamic therapy. Carbohydrates are suitable as functional groups for phthalocyanine, on the one hand because of their contribution to increasing solubility and on the other hand because of the effect on biological processes at the cellular level through receptor groups for carbohydrate molecules present in large amounts in tumor cell membranes, for receptor-directed transport with optimal accumulation and localization of the new structure.

Photochemical studies of the bio conjugate with non-peripheral n-GalZnPc substituents have shown a quantum yield of singlet oxygen with a higher value with comparable photo stability of the peripherally substituted p-GalZnPc. In galactose groups to the phthalocyanine ring and application of an irradiation spectrum (635 nm) with appropriate dose and energy, selectivity of the photo cytotoxic response was observed in studies on tumor versus normal cell lines.

2.3. FROM STEROLS The structural features of photosensitizers for the photodynamic method are considered critical for their photophysical and photochemical properties, as well as for their interaction with the membrane structures of pathogenic cells, which is decisive for the phototoxic effect. Accumulated knowledge of the optimal structure for a photosensitizer points to suitable compounds with a positive charge and amphiphilic nature, as well as substituents in a spatial arrangement around the plane of the ring molecule, as well as with biological functionality.

In the composition of cell membranes, natural sterol molecules have the physical function of membrane "anchors", in the sense of molecules that simultaneously give strength, flexibility and protective function to the membranes. Sterols participate as building blocks with the role of "foundation" in cell growth. For photosensitizers, phospholipids with functions to transfer and stimulate the binding of liposomally incorporated photoactive compounds to cell membranes by receptor recognition of pathogenic cells have been well studied. The dissertation shows that sterols

evolved with the birth of the Earth's atmosphere, with fungi containing egrosterol along with cholesterol. Cholesterol as part of the membrane structures of cells is a well-studied target molecule for oxidative reactions in pathogenic processes. A serious plus of the work is that a small number are studies of photosensitizers linked to sterols for photodynamic applications.

2.4. FROM PARABENS one of the first approved complexes for PDT of tumors is Si (IV) - phthalocyanine with axial asymmetric groups as substitutes - Pc4. For Europe, various metal-containing phthalocyanines, including silicon complexes, have recently been granted a patent for photodynamic inactivation applications. To create an effective structure, a successful approach is to link a photoactive compound such as SiCl2Pc with a p-benzoic acid ester as an inhibitor of pathogens. Parabens are one of the most common preservatives in cosmetics and pharmaceuticals, with important applications in consumables and materials for medicine, as well as in the food industry.

Si (IV) -phthalocyanines with axial substituents of various parabens are soluble in most organic solvents (DCM, DMF, THF) and slightly soluble in DMSO. It is well known that the photoactivity of the compound decreases with the formation of molecular associates. The studies were performed in DMF solutions. The obtained spectra show that no associates are formed for the concentration range 1.2 x 10-5 - 2.0 x 10 -6 M. Similar dependences were obtained with compounds with the maximum in the red spectrum λmax = 682 nm.

3. PHTHALOCYANIN COMPOSITES The photocatalytic effect of titanium dioxide when irradiated with daylight is well studied and is used to purify toxic products from industrial wastewater and to maintain an environment free of infectious pathogens. Another broader application is the antimicrobial effect of using titanium and zinc or other oxides in materials for the prevention and control of pathogens transmitted through surfaces in the environment. Although it shows high activity in general, titanium dioxide is part of water purification technology, but as an antimicrobial agent, its effect is limited. The reason is considered to be a defect in the irradiation spectrum, which for natural light is only 3% of the spectrum of metal oxides, as various additives of metallic and non-metallic oxides in the composition slightly increase the photocatalytic activity. Foster et al. describe TiO2 in the inactivation of pathogenic microorganisms by a photocatalyst mechanism as suitable for controlling and preventing the spread of pathogens. The combination of the two photosensitizer and photocatalyst mechanisms is expected to increase the type and amount of toxic products generated, as well as the application of natural sunlight as a source.

3.1. With titanium dioxide

3.2. With polymer brushes. A phthalocyanine hybrid with polymer brushes based on hydrophobic interactions between molecules of different origins was obtained. The result is a water-soluble photosensitizer suitable for the purposes of the photodynamic inactivation of pathogens method. As is known, the release of the active ingredient in a medicinal product depends on the acidity, polarity, charge, enzymes in the medium, which can be used as an approach for good accumulation of active molecules in the target cells. For hydrophobic phthalocyanine, for example, the polymer can serve as a good solvent for its applications from solutions, including water. Zn (II) - phthalocyanine without substitutes (ZnPc) exists as a monomer in organic solvents, showing

photosensitizing properties, with good photoactivity, but due to its lipophilic nature its application for biological purposes is difficult. DMSO solutions were used for these studies.

4. PHOTODYNAMIC METHOD FOR DRUG RESISTANCE

The material of the dissertation contains a brief summary of the main results obtained in photo biological studies for accumulation, localization and photodynamic activity of the developed phthalocyanine derivatives for PDT applications in resistant pathogenic microorganisms. Despite their long history of development and clinical trials, the available phthalocyanines have drawbacks such as low selectivity and no specificity of accumulation, as well as dark toxicity to complexes without substitutes and, as a result, low photo activity. The photodynamic effect was observed depending on the accumulation and localization of the compounds in bacterial strains of Gram (+) Staphylococcus and Streptococcus, Gram (-) Pseudomonas aeruginosa, Aerumonas hydrophila and Salmonella enteritidis, which cause severe infections as well as bacteria. The most common fungal strain Candida albicans was also used for the research.

4.1. OF PATHOGENIC BACTERIA AND CANDIDA The tests for antimicrobial activity were performed with cationic Lu (III), Sn (IV) and Pd (II) phthalocyanine complexes with peripheral and non-peripheral substitutes (item The studies performed with Gram (-) bacterial strain aeruginosa and fungal C. albicans, as a suspension (~ 106 CFU. mL-1) and with biofilms of these pathogens, as in nature bacteria multiply and form biofilms, the effect on biofilm samples formed on a glass slide was studied The Lu (III) Pcs and Sn (IV) Pcs complexes were incubated at concentrations between 1 μM - 30 μM and activated with a spectrum of nm or 665 nm LEDs and a dose of 50 J. cm-2 (60 mW. Cm-2). With optimal photophysical properties of the complexes, the effect of complete photoinactivation is observed for relatively high concentrations between 20 and 30 μM With respect to the coordinated metal ion, for Lu (III) Pcs complexes the effect is higher > log 3 compared to complexes of Sn (IV) Pcs under analogous experimental conditions. The effect of higher than log 3 is considered to be an indicator of good photodynamic activity, as the complex with peripheral substituents (LuPc,) requires two higher concentrations for inactivation compared to a complex of zinc with the same groups as substituents in peripheral position. The main conclusion of these studies is that the replacement of a zinc ion with another with a higher atomic number, such as lutetia or tin, contributes to the physicochemical properties of the resulting complexes, but does not affect the effectiveness of inactivating pathogens.

4.2. OF VIRUSES Studies with various phthalocyanine derivatives were performed by the method of photodynamic inactivation to evaluate the effectiveness of the complexes in viruses. Research on the subject has been recognizable for the past thirty years, such as Al (III) complexes have shown efficacy in inactivating various viruses (Sindbis virus, VSV and HIV-1) in blood products. Structurally related cationic porphyrin derivatives Tri-P (4) have shown a good effect in inactivating viruses: bovine viral diarrhea virus (BVDV), VSV, HIV-1 and pseudorabies virus. Studies on the photodynamic activity of cationic Zn (II) -phthalocyanine (ZnPcMe) and anionic Zn (II) -phthalocyanine (ZnPcS) were performed on viruses originating from different taxonomic groups (BVDV, HSV-1 and VV). Characteristic of these viruses is that they have a "lipid envelope". At the start of research on viruses (after 2008), there was almost no information on the structure-antiviral activity relationship and on the charge dependence of the photodynamic effect

of viruses. The lipid envelope can be considered as a target of the photosensitizer, where it binds and after irradiation the generated singlet oxygen disrupts its structure by oxidation of the lipid. Given that uncoated viruses are relatively resistant to therapeutics, the results obtained for photo inactivation with cationic tetra- and octamethylpyridyloxy substituted Ga (III) -phthalocyanines, especially in uncoated viruses such as human adenovirus, confirm the efficacy of photodynamic inactivity. phthalocyanine derivatives as a reliable antiviral therapy, especially in cases of resistance. In connection with the viral epidemic that has spread around the world, these studies are becoming especially relevant.

REFERENCES A total of 367 literature sources are cited, which shows that Assoc. Prof. Mantareva is well acquainted with the state of research on the topic.

IV. CONTRIBUTIONS WITH SUMMARY

1. New phthalocyanine derivatives have been obtained, such as complexes with traditional ions for photosensitizers such as zinc and silicon, as well as complexes of lutetium, tin, palladium and nickel, which have not been studied for these purposes.

1.1. To the coordinated metal ion:

- the complexes of lutetium, tin, palladium and nickel with pyridyloxy groups located in peripheral and non-peripheral positions of the ring molecule and after quaternization methylpyridyloxy groups for the hydrophilic cationic derivatives, with a total of 16 new compounds.

1.2. Compared to the substituents, the obtained new compounds are 24 derivatives, which are complexes of zinc and silicon and with functional groups as follows:

- the amino acids tyrosine, phenylalanine, arginine and lysine;

- carbohydrates galactose (galactopyranose);

- with sterol mestranol (estradiol);

- with inhibitors such as methyl-, ethyl-, propyl- and butyl parabens.

1.3. Hybrid structures with titanium dioxide and polymers (2 types).

2. Original synthetic schemes based on well-studied chemical reactions have been developed, with proposed new reaction conditions for obtaining the target structures of the new complexes, as well as the bioconjugates of zinc phthalocyanine complexes with the following molecular groups as substituents:

1) with amino acids linked through an aminophenoxy group via an amide bond;

2) with carbohydrates and parabens, by direct connection with an ether bond;

3) with carbohydrates and sterols linked through the azidoethoxy group of the triazole ring.

The synthetic production scheme includes the following approaches:

1) by coupling the biologically active molecule as a functional group to phthalonitrile at the 3- or 4-position and subsequent cyclotetramerization to phthalocyanine;

2) by connecting to the possible peripheral (four and eight) and non-peripheral only four positions (due to spatial obstruction) of the ring phthalocyanine molecule; 3) by nucleophilic substitution in the silicon ion of the Cl2Si (IV) complex - phthalocyanine with chromophore "volume" groups and others with proven antibacterial properties.

3. The basic optical physicochemical properties of the new phthalocyanine derivatives have been studied with an original experimental setup developed for experimental studies in the light spectrum of phthalocyanine compounds. The obtained values of the main photophysical (absorption and fluorescence) and photochemical (singlet oxygen generation and photostability) properties prove the potential of the developed new photosensitizers for biomedical applications with photodynamic method.

4. A method for pharmacokinetic studies based on chemical extraction and measurement of the fluorescence signal intensity with quantification of accumulation, retention and clearance for phthalocyanine compounds has been developed. The method is applicable due to the typical fluorescence (> 680 nm) for phthalocyanines, which does not overlap with that of cellular chromophores. Relatively high accumulation has been demonstrated in resistant and multidrug-resistant Gram (+) and Gram (-) pathogenic bacteria, and for galactosylated Zn (II) - phthalocyanines.

5. Protocols have been developed for (in vitro) photo biological studies with pathogenic microorganisms, as well as for tumor cell lines, which are based on a standard procedure and with a new stage of light irradiation. The permissible concentration range for the study of phthalocyanines (0.1 - 20 μM), the permissible for radiation doses (12 - 60 J.cm-2) and the optimal radiation energy (50 - 100 mW.cm-2) without thermal or another effect of light, the results obtained being reliable and reproducible. The developed methodology is also applicable for studies of the photodynamic efficacy of other photosensitizers and for other pathogenic microorganisms to determine the effectiveness of inactivation by photocytotoxic effect.

1. List of publications as part of the dissertation - 21 scientific papers in international, specialized journals with impact factor.

2. Information about the citations

According to the completed in "Sonix.bas.bg/bg": 128 citations of publications included in the dissertation as of April. 2021

Assoc. Prof. Mantareva is the leader of 4 projects with the Ministry of Education and is a participant in 1 project.

3. List of personal participation in scientific events - 12 scientific events. As in all, she is a leading author

4. Supervisor of a full-time PhD student: Assoc. Meliha Bahri Aliosman (Suleiman) - enrolled on 01.01.2014 with order № RD-09-12 / 30.01.2014; Defense within 19.07.2019 with order № RD-09-103 / 08.05.2019, with a diploma for ONS "Doctor".

When reviewing this dissertation I found no significant errors in terms of: staging; analyzes and generalizations; methodological level; accuracy and completeness of the results; literary awareness. I found only inaccuracies in the spelling of some forms in the Abstract, written in Bulgarian, for example, in which cases oxis (correct Bulgarian word) is written in other places oxide (rough foreign). All these remarks do not change my high opinion about the qualities of the dissertation.

Conclusion

After getting acquainted with the presented dissertation, abstract and other materials, and based on the analysis of their significance and contained in them scientific and scientific-applied contributions, I confirm that the scientific achievements meet the requirements of ZRASRB and the Regulations for its application and the relevant IOHCF Regulations for obtaining the scientific degree "Doctor of Physical Sciences". In particular, the candidate satisfies the minimum national requirements in the professional field and no plagiarism has been established in the dissertation, abstract and scientific papers submitted at the competition.

I give my positive assessment of the dissertation.

II. OVERALL CONCLUSION

Based on the above, I strongly recommend the scientific jury to awarded the scientific degree "Doctor of Sciences" in the professional field .4.2 Chemical Sciences, Chemitry, Chemisty of Natural and the Physiologically-Active Substances to Assoc. Prof. Dr. Vanya Nikolova Mantareva

.27.07.2021 Prepared the review: .....

Sofia (Prof. Tsonko Kolev)