BULGARIAN ACADEMY OF SCIENCES

Institute of Organic Chemistry with Centre of Phytochemistry

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PHTHALOCYANINE PHOTOSENSITIZERS FOR PHOTODYNAMIC METHOD TOWARDS DRUG RESISTANCE

ABSTRACT OF A DISSERTATION

Associating to a scientific degree „Doctor of Sciences“, in the specialty „Bioorganic Chemistry, Chemistry of the Natural and the Physiologically-Active Substances”

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The thesis is presented in **203** pages and it includes **63** Figures, **25** Schemes and **14** Tables, and **367** References. The results are part of **20** research articles and a book Chapter, all published during the years 2015-2020. The publications are citated **128** times till the end of April. 2021.

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Acknowledgements

The thesis presents scientific achievements in a very actual and under intensive development research field called Photodynamic Therapy (PDT). The topic has been considered as interesting for investigation in the Institute of Organic Chemistry with Centre of Phytochemistry since early 80^{ty} (XX cent.) starting by Profs. M. Shopova and N. Genov.

Nowadays, PDT as a curative method is well accepted not only in tumor therapy but more likely for preventions and control of the pathogen's circulation and for a safety environment. During the last years with the emergency of drug and multi-drug resistance because of the fast alteration of pathogens as well as the appearance of the new harmful pathogenic species, PDT is under consideration as an alternative therapy with fast outcome. ***Thanks to the scientists who started this exhausting and long-lasting topic with high impact on human health!***

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In honor of my grandmother Ivanka Christova Ivanchova.

ABBRIVIATIONS

Ac	acetyl
ADMA	Antracene-9,10-diyl-bis-methylmalonate
Ar	aryl
Boc	tert-butyloxycarbonyl (protecting group)
BSA	Bovine serum albumin (fraction V)
BQ	1,4-Benzoquinone (p-quinone)
CEL	Chremophor PEG 40
CHCA	α-Cyano-4-hydroxycinnamic acid
CLSM	Confocal Laser Scanning Microscopy
Conc.	Concentrated (stock solution)
DABCO	1,4-Diazabicyclooctane (triethylenediamine)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DHB	2,3-Dihydroxybenzoic acid
DIPEA	N, N-Diisopropylethylamine
DIT	Dithranol (matrix for MALDI)
DMEM	Dulbecco's Modified Eagle Medium
DMF	N, N-Dymethylformamide
DMS	Dimethyl sulfate or sulphate {$(CH_3)_2SO_4$}
DMSO	Dimethylsulfoxide
DMTMM	4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
DPBF	1,3-Diphenylisobenzofuran
EDTA	Ethylenediaminetetraacetic acid
Et	ethyl
HSA	Human serum albumin
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight ("Soft" ionization)
Me	methyl
MPc(s)	metallophthalocyanine(s) or metal phthalocyanine complex(es)
MTT assay	test for measuring of cellular metabolic activity (viability, cytotoxicity)
NMM	N-methylmorpholine

NIR	near infrared spectrum
n. d.	not determined (or not detected)
PBS	Phosphate Buffered Saline
PET	Photoinduced electron transfer
Ph	phenyl
PDT	Photodynamic Therapy
Pc(s)	phthalocyanine(s)
ROS	reactive oxygen species
RT	room temperature
S.D.	Standard (average) deviation
SDS	Sodium dodecyl sulfate (or sodium lauryl sulfate)
TCSPC	Time-correlated Single-Photon Counting
TFA	Trifluoroacetic acid
TLC	thin-layer chromatography
Tol	toluene
Ts	tosyl
UV	Ultraviolet spectrum (100-400 nm)
VIS	Visible spectrum (400-750 nm)
	(red visible spectrum: 620-750 nm)
v/v	ratio of volumes
WHO	World Health Organization

CONTENTS

I. INTRODUCTION

The research and development of new therapeutics, methods, and technologies with mechanism which is different from the actions of the well-known drugs such as antibiotics and chemotherapeutics have been acquired of high importance because of the fast development of drug resistance. Every year, the population takes more than 2500 tones of antibiotics sometimes without needs for treatment of bacterial infections. The problem appears because of the lowering of the efficiency of the current drugs year by year as a result of the natural mutations of pathogens but mostly because of the humans' overuse with abuse to these drugs. The health institutions such as WHO announced the drug resistance as a real threat to humanity with a near future of the "post-antibiotic era".

Photodynamic therapy (PDT) is an approved curative method with long lasting history of development which is still actual because of the non-specific local action, with a fast efficiency after application without development of resistance because of the mechanism of photodynamic action. Currently, the clinical PDT together with the approved photosensitizers has been applied for local treatment of diseases without another options. The nature of PDT method involves the action of three unharmful constituents, namely the non-toxic in the dark light-sensitive compound (photosensitizer), the light from visible to near infrared spectrum (630-850 nm), which is applied in nondamaging doses and surrounding of oxygen atmosphere. The photosensitizer must be localized in pathogens so that after irradiation the absorbed photon energy can initiate the electron transitions in the molecules. The molecules in their singlet exited state can undergo an energy loss by the lower energy triplet excited state which is a preferable relaxation mechanism for a photosensitizer. The molecule in its triplet excited state is a long-lived reactive form, which participates in the photochemical reactions through two possible mechanisms of photosensitization. The reaction with an electron or proton transfer from a molecule in the excited triplet state to the biomolecules as part of the cells is so called type I mechanism. The second mechanism involves an energy transfer from the triplet state of an excited molecule to the oxygen in the ground triplet state which leads to generation of singlet oxygen excited state (type II mechanism). The efficiency of the mechanisms of photosensitization are crucial part of the PDT procedure. The development of PDT as a method with different biomedical applications is possible because of the progress in chemistry of heterocyclic highly conjugated compounds with porphyrins` origins and the new more efficient light sources with spectrum of exposure in the so-called optical therapeutic window (630-850 nm).

There are several incidental and aimful scientific discoveries in the chemistry of the natural and synthetic porphyrins and the part of this group are phthalocyanines. The developments in the chemistry of complexes, the synthesis of monomers for cyclotetramerization reaction with formation of the macromolecule cycle, and the knowledges in chemical sciences let to further efforts in the development of new more effective photosensitizers overcoming the limitations of the present clinically approved drugs for PDT. The research efforts in the scientific achievements aim the optimization of the structure of the well-knowns photosensitizers to obtain close to the optimum values of the main physicochemical properties as well as the photobiological properties which will have advanced functionality to the photodynamic action.

Nowadays so called third-generation photosensitizers for PDT is under intensive development because of the fast development of drug resistance of pathogenic species, which seems to have a solution in this alternative method for inactivation. Nevertheless, that the existing chemo-drugs are under modification all the time, they have been lowering their efficiency. This focuses the attention of the scientific community to look for the new treatments with different mechanisms of action. In rare cases there are noticed also the resistance in tumor cells towards traditional chemotherapeutical drugs.

The thesis aims to summarize the achieved knowledge in the field of PDT topic which includes the research and development of the new generation photosensitizers based on the phthalocyanine molecule. The new compounds are synthesized by the modified already known and new original synthetic procedures. The proposed derivatives are obtained as complexes of different ions with advances to the physicochemical properties of the photosensitizers as well as with the substitution groups of the biologically active substances with different origin such as amino acids, carbohydrates, steroid, and inhibitors of microbial species, all in aspect to contribute to the biological functionality and the photoinactivation potential of the new phthalocyanine derivatives. The routine chemical characterizations and the light-associated studies of the main physicochemical properties are presented. The phthalocyanine derivatives showed potential as photosensitizers towards pathogenic bacterial species and a fungus with drug and multi-drug resistance. In addition, the photoinactivation of viruses and the aggressive tumor cell lines versus the normal cells are suggested the ability of the new phthalocyanine derivatives for selective photodynamic therapy.

The new phthalocyanine derivatives are obtained as complexes of the traditional metals such as **zinc**, **silicon, and palladium** as well as some unusual metals which are not so well investigated in PDT application such as **lutetium, tin, and nickel**. In previous our studies the cationic phthalocyanine complexes of Ga(III), In(III), Ge(IV) and Al(III) were evaluated with high efficiency towards harmful resistant pathogenic species. The related new phthalocyanine photosensitizers are synthesized with substitution groups by the chemical linkage in the peripheral and non-peripheral positions to the phthalocyanine ring or in two axial position of the coordinated silicon ion. The starting dinitriles which are the monomers for the cyclotetramerization reaction are obtained as new or as well-developed compounds by following the known and the new synthetic pathways. The proposed new schemes can be applicable for other new compounds beyond phthalocyanine chemistry. The structures of the obtained compounds were proven with the known chemical analyses. The properties of the ground and excited states of the phthalocyanine compounds as complexes and bioconjugates were investigated by a comparative method. All these properties were examined with an actual methodology which is updated based on the spectral characteristics of the studied phthalocyanines. The pharmacokinetic behaviors of uptake and localization were studied for pathogens in suspension and as biofilm cultured. The aggressive tumor cell lines were tested for localization of new compounds by using the typical red fluorescence of phthalocyanine which is not overlapping the spectra of the native cellular chromophores. The photodynamic efficiency was evaluated for several resistant pathogens as well as for tumor cell lines towards normal cells suggesting the selectivity potential of the selected phthalocyanines. The photodynamic efficiency was evaluated towards viruses such as enveloped forms and more difficult to treat non-envelop viruses, which were inactivated with high efficiency with PDT. This can be an effective treatment procedure also for the new challenges such as coronavirus as well. An example of the useful PDT application is the photodynamic opening of blood-brain barrier as a provider of specific medications. The structure-function relationship is discussed in relation to optical physicochemical properties, the uptake and localization, and *in vitro* PDT efficiency. The dependance on the nature, the position of the substitution groups and the coordinated metal or non-metal ions in phthalocyanine molecule on the PDT effectiveness are briefly remarked.

II. GOALS AND TASKS

The aim of the present work is to summarize the knowledge in the research and development of biologically active phthalocyanine complexes as photosensitizers for the method known as photodynamic therapy (PDT). PDT is an alternative curative procedure against pathogenic microorganisms especially for the resistant species which cannot be treated with the traditional medicine. The new phthalocyanine complexes are obtained as complexes with metal ions so that to possess the advanced photo- physicochemical properties which influenced on the photodynamic process. The proposed phthalocyanine complexes are bioconjugates chemically linked as substituents to the ring of specific substitution groups: 1) chromophore groups, 2) bioorganic molecules of biologically active compounds and 3) inhibitors with antibacterial properties.

The new photosensitizers are synthesized by following the well-known synthetic schemes but with some originality in the reaction conditions and additionally the new original synthetic schemes are developed. The selected functional groups are chemically linked by other groups or directly through chemical bond to the phthalocyanine ring in peripheral or non-peripheral positions, or axially to the coordinated silicon ion. The obtained compounds were characterized by the routine chemical analyses and by some specific physicochemical characteristic approaches for light-sensitive compounds. In addition to the photobiological properties such as the cell uptake, the localization and selectivity, the photodynamic efficacy are discussed in respect to the structural features of the proposed photosensitizers for PDT usage towards resistant microbial species.

The relationship between the chemical structure and the photodynamic activity is under consideration in respect to the nature of the substituents, the linkage atoms or groups to the ring, the position of the substituents , the nature of the coordinated ions and the atomic number of this ion, which can influence the molecular planarity and steric hindrance of the molecule and how the specific structural modifications can alter the properties which are influenced the photodynamic inactivation of the pathogenic resistant microorganisms.

III. RESULTS AND DISCUSSION

1. CATIONIC PHTHALOCYANINES WITH SUBSTITUENTS

1.1. COMPLEXES WITH PERIPHERAL AND NON-PERIPHERAL GROUPS

The coordinated metal ion is known to influence the physicochemical properties of the photosensitizers. The properties of the triplet state are of importance to the efficacy of the photosensitization which for phthalocyanines goes with generation of the singlet oxygen as a highly reactive and cytotoxic oxygen molecule [1]. It is known that phthalocyanine complexes possess *d* electron structure which is in further contribution in the photochemical properties with an energy relaxation through the triplet state of the excited molecule [2].

1.1.1. Synthesis and chemical properties

The phthalocyanine molecule has bad reputation because of its low solubility in almost all organic solvents. The initial efforts in the phthalocyanine chemistry aim to work out on the solubility problem. The quite easy attempt looks the linkage of the suitable substitution groups which aims to increase the solubility of the basic phthalocyanine molecule. In case of the identical substitution groups as for tetra substituted compounds, an increment of the solubility is usual, then the case of low symmetry molecular substitutions. This behavior is a result of regioisomers typical for tetra-substituted phthalocyanines. The octa- substituted derivatives which are not isomers are evaluated with lower solubility. On the other hand, the coordinated metal ion can limit the unfavorable formation of the photoinactive molecular associates in solutions as is the case with the metals with high atom number. Most of the phthalocyanines which are appropriate for different PDT applications are prepared as complexes with suitable metals or semimetals.

The phthalocyanine complexes with substituents of different functional groups were prepared from the substituted dinitriles by reflux with or without catalyst (DBU). The peripheral 2(3),9(10),16(17),23(24)-tetra substituted phthalocyanines were prepared from 4-substituted phthalonitriles, while the non-peripheral from 3-substituted analogues to give 1(4),8(11),15(18),22(25)-tetra substituted phthalocyanines (Scheme **1.1.1**). The obtained products of tetra- substituted phthalocyanines are a mixture of four isomers, which are differing in molecular symmetry (C_{4h}, C_{2v}, C_s, and D_{2h}).

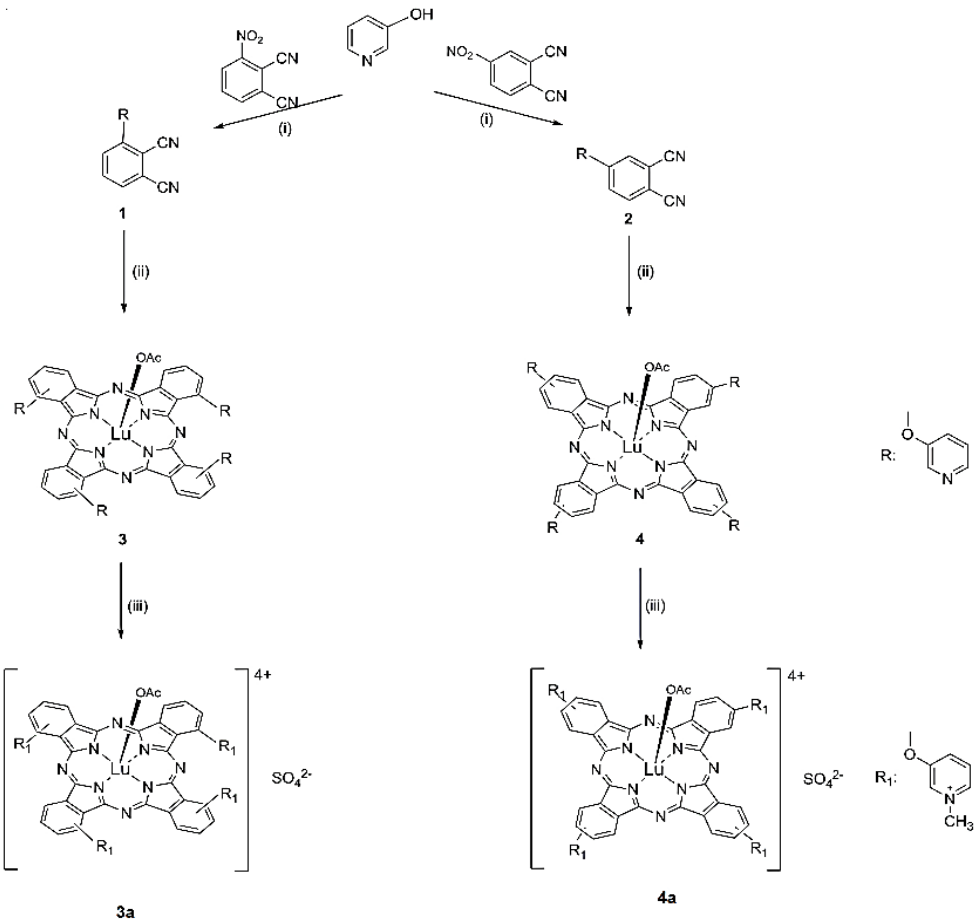


Схема 1.1.1. Synthesis of methylpyridyloxy substituted Lu(III)-phthalocyanines. (i) DMF, K_2CO_3, RT, 48h, Ar; ~ 60 %. (ii) $Lu(OAc)_3$, DBU, 1-pentanol, reflux, Ar, ~ 30 %; (iii) DMS, DMF, 120 °C, 3h, Ar, 79-93%.

The synthesis of phthalocyanine zinc complexes with pyridyloxy group which after quaternization turns to water-soluble derivative is a helpful approach to obtain cationic water-soluble derivatives, which was invented in MPcs chemistry by Wöhrle and co-workers in early 90^{ty} [3a-c]. The new achievement is the coordination of the metal ions with high atomic number, which is not possible to embedded in the ring. The intention is to prevent the undesirable formation of molecular associates.

Lutetium ion (Lu^{3+}) is of high interest among the metals suitable for coordination of phthalocyanines because of the unique photophysical properties of the monomolecular lutetium complexes (LuPcs) with respect to the photochemical properties and the efficiency of the photodynamic action. Two structurally different Lu(III)-phthalocyanines were synthesized to have four pyridyloxy substituents on four peripheral and non-peripheral

positions which after reaction with DMS turned to the water-soluble cationic derivatives (Scheme 1.1.1). The applied synthetic routine follows a known procedure for quaternization of different phthalocyanine complexes with pyridyl group [3a]. The critical part of the procedure is the insertion of lutetium which is a high energy reaction for the formation of the macrocycle of phthalocyanine. Two steps procedure which includes formation of the ring molecule as a ligand firstly and the next reaction to insert the metal ion. Another possible one step reaction requires a high energy to close the macrocycle (180-220 °C). The products of peripheral or non-peripheral tetra- methylpyridyloxy substituted Lu(III)-phthalocyanines were obtained as a mixture of regioisomers. The attempt to separate them was not successful. The studies with separated isomers showed no differences in their photophysical and photochemical properties with similarity in the singlet oxygen generation [4, 5]. It is believed that the phthalocyanines with four substituents of methylpyridyloxy groups showed better solubility than the octa-substituted analogues because of the existence as regioisomers [6, 7]. Following the similar scheme, the complexes of tin, palladium and nickel were obtained as in the previous our work for the complexes of gallium, indium, silicon and germanium [8, 9].

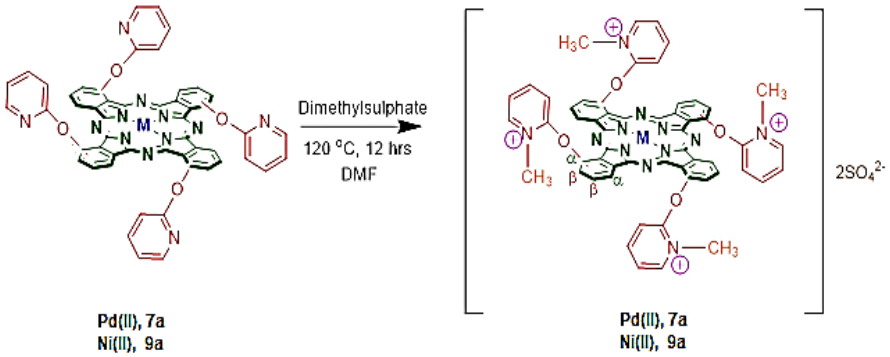
The reaction pathways with ions such as lutetium and tin involve two steps procedure without characterization of the intermediate product of lithium phthalocyanine. In the beginning the lithium pieces are dissolved and then the dinitrile compound is added. The reaction is carried out by reflux in n-pentanol at temperature ~ 140 °C, without catalyst. The reaction time is between 3-5 hours till the starting dinitrile is finished. In case of addition of a salt lutetium acetate, the formation of the complex is distinguishable by the precipitation of the water-soluble product. Two possible non-peripheral (LuPc, **3**) and peripheral (LuPc, **4**) pyridyloxy substituted Lu(III) phthalocyanines are prepared. Three possible products are isolated from the reaction mixture, namely metal-free or Li_2Pc, Lu(III)-phthalocyanine as monomolecular compound and the double-decker structure with one coordinated lutetium ion. At the applied reaction conditions, LuPcs (**3** and **4**) were in highest amount as compared to the other two products including the by-products. The new compounds are chemically characterized by the mass spectrometry (MALDI-TOF) with a matrix DHB. The spectra showed a molecular peak at (m/z): 1214.398 [M-OAc + DHB] for LuPc **3** and at 1214.718 [M-OAc + DHB] for LuPc **4**. The IR spectra of the both LuPcs with pyridyloxy groups are observed as intensive band at 1237 cm^{-1}, which is based on Ar-O-Ar between the macrocycle molecule and the substitution groups. The obtained ^{1}HNMR spectra showed a characteristic shift of the position of the obtained signals which as a result of the position of the substituents.

The well-known lutetium complex for antitumor PDT is a complex of texaphyrin with commercial name Lutrin® which presently is approved for clinical examination for applications in USA [10, 11].

Cationic phthalocyanines which are complexes of different metals are prepared by reaction with iodomethane at 40 °C or dimethyl sulphate at 120 °C in DMF and argon atmosphere. Independently on the used reagent the quaternization reaction goes with high yields and purity of the products **3a** и **4a** (79-93 %). The successful attempts are made for quaternization of pyridyloxy group with reagents with longer chains by using propyl-, hexyl- and dodecyl iodide. The newly prepared compounds are with limited solubility as well as with not improvement of the optical physicochemical properties. The most suitable for the biomedical applications appears the methylpyridyloxy- group for substitution.

The new phthalocyanine complexes of tin such as **Sn(IV**)Pcs (**5** and **6**) are prepared by following the above described synthetic procedure. The choice of this metal ion is due to the use of tin metal in a clinical photosensitizer tin ethiopurpurin (SnET2) [11a-c]. Two oxidation states of tin determine the possibility to obtain two kind of complexes as Sn^{2+} or Sn^{4+}. The oxidation state of the tin complex depends on the ration between a ligand to tin salt, added in the reaction mixture [12]. The tin ion cannot be inserted in the vicinity of the Pc ring which can limit the aggregation as well. On the other hand, there are possible the steric hindrance effect due to the substitution groups to Sn^{4+}, which in case of two new Sn(IV)Pcs are two hydroxyl groups with contribution to the solubility of the complex. It happens to obtain in addition to the desired compounds also small amounts of the double – decker structure with one tin ion for coordination of two macrocyclic molecules. The synthesis of the both kinds of complexes Sn(VI/II) was carried out by a reaction based on the reflux in n-pentanol (137 °C) with a catalyst DBU or by addition of lithium cuttings. The reaction was carried out also in high boiling point solvent such as chinolin (quinoline) at temperature 220 °C with a tin salt ($SnCl_2$). After the reaction, the crude product was precipitated in ether and the product was purified by column chromatography with a gradient of the solvent mixture (DCM: EtOH, 10: 0.1-1.0). The conventional reactions of cyclotetramerization from the phthalonitrile derivatives, metal salt and catalyst by reflux was applied for preparation of new tetra-substituted phthalocyanine complexes. Both products turned to the water-soluble cationic derivatives (**5a** and **6a**) after reaction with DMS.

The phthalocyanine complexes of palladium **Pd(II)**Pcs are among the most effective photosensitizers because of the properties of the metal which refer to the main phthalocyanine complex to reach the high quantum yields of the triplet state with contribution to the photochemistry [13]. Two new palladium phthalocyanines with non-peripheral and peripheral pyridyloxy groups Pd(II)-Pcs (**7** и **8**) are prepared following the same reaction pathway as in Scheme 1.1.1. The procedure starts with nucleophilic substitution according to Wöhrle et al, 1990 [3a]. The cyclotetramerization involves the dinitriles **1** or **2**, palladium salt ($PdCl_2$), a catalyst DBU and reflux in 1-pentanol in argon flow. The crude products of non-peripheral (**7**) or peripheral (**8**) substituted with pyridyloxy groups Pd(II) phthalocyanines are purified on column chromatography (SiO_2, DCM: EtOH with a gradient). Similarly to the other cationic complexes, the new *Lu(III)*, *Sn(IV)* and *Pd(II)* phthalocyanines are obtained as a mixture of four regioisomers. Next step of the synthetic pathway includes the quaternization with DMS which turn to cationic derivatives **7a** and **8a**. In case of iodomethane the reaction temperature is 40 °C but the time for complete conversion is between 24-72 hours, while with DMS the reaction time is shorter at 120 °C. Both palladium complexes (**7** and **8**) have low solubility in the most organic solvents (DCM, THF, $CHCl_3$) but with a high solubility in DMF and DMSO. The non-peripheral derivative **7** is characterized with higher solubility than the peripheral analogue compound **8** in many organic solvents. Both cationic water-soluble Pd(II)-phthalocyanine complexes (**7a** и **8a**) are suitable for biomedical applications from saline. Following the similar synthetical procedure, the new phthalocyanine complexes of nickel (**9** and **10**) with the same substitution groups are synthesized (Scheme 1.1.2).



Scheme 1.1.2. Synthesis of non-peripheral Pd(II)- and Ni(II)-phthalocyanines. (Yield: 65-73 %).

The obtained palladium complexes are evaluated by the means of the known methods: FT-IR, UV-Vis, MALDI-TOF and ^{1}H NMR. The collected analytical data approved the predicted structures. The IR spectra show a band typical for the nitrile group ~2284 cm^{-1} which is not appeared after the formation of the ring molecule. The phthalocyanines have the

characteristic bands of the symmetric ether group (C-O-C) at ~1045 cm^{-1}, for the aromatic CH group the bands are between 3088–3092 cm^{-1}; the aromatic group C=C of the molecule ring has vibration at 1655 cm^{-1} which are shown in the spectra of the complexes **7** and **8**. After quaternization the compounds **7a** and **8a** are characterized with IR spectra with several bands between 1110-1182 cm^{-1} for S=O group. ^{1}H NMR spectra of both palladium complexes (**7** and **8**) are characterized with the number of protons that agree to the molecules which showed a shift due to different positions of the substituents to the ring, namely peripheral or non-peripheral substitution. Tetra- substituted compounds have ^{1}HNMR spectra which are often difficult to describe because of the isomerism. The cationic water-soluble phthalocyanines **7a** and **8a** are characterized with broad overlapping signals of multiplet of the pyridyloxy groups (28 протона). The protons of CH_3 are observed as singlets in a range between 3.7 ppm and 4.0 ppm for a solvent DMSO-d_6.

In conclusion can be said that new Lu(III), Sn(IV), Pd(II) and Ni(II) phthalocyanine complexes with peripheral pyridyloxy groups are synthesized, which after quaternization turned to cationic, water-soluble derivatives which is expected to be in contribution to the binding ability, uptakes and efficiency of photodynamic method towards pathogens.

1.1.2. Physicochemical properties

The water-soluble phthalocyanine complexes of lutetium (**3a** and **4a**) show the typical intensive Q band of absorbance with λ_{max} at 685 nm for **3a** and at 675 nm for **4a**, as the low intensity bands at 610 nm. Both complexes show the typical spectra of aggregated molecules in water solutions. The bands are distinguished with a low intensity and with a splitting in the spectral region 675-695 nm (**3a** and **4a**). Our previous work with the complexes of Ga(III), In(III), Si(IV) and Ge(IV) showed the lower aggregation behavior in water [8, 9]. The reducing of the photoinactive molecular associates was achieved by addition of the anionic detergent such as sodium dodecyl sulphate (SDS) or by incorporation in the vehicle molecules such as liposomes, albumins, and others.

Fluorescent properties of Lu(III) phthalocyanine complexes **3a** and **4a** are studied in solutions which are appropriate for cell studies (PBS and DMSO). The fluorescent spectra show the significant bathochromic shift of the emission band at 704 nm and 721 nm as

compared to the absorbances with λ_{max} (Q-band): 675 nm and 685 nm. The absorption and the excitation spectra of the studied compounds show identical bands.

The fluorescence lifetime as is well-known is a direct method for determination of the time a molecule stays a photoactive specie and can participate in the photochemical reaction with the formation of singlet oxygen. The curves of quenching of the fluorescence signals are registered at excitation wavelength 670 nm (Fig. 1.1.1). The obtained mono-exponential curves show the high purity of the compounds and in monomeric state in solutions with lifetimes of 2.24 ns for the non-peripherally substituted LuPc, **3a** and 3.27 ns for the peripherally substituted LuPc, **4a**. The fluorescence lifetime of both lutetium complexes was measured with values lower than for the unsubstituted complex **ZnPc** (3.99 ns). It was observed a fast quenching of fluorescence for **3a** (2.24 ns) as a result of the close distance of the substituents to the main ring while the peripherally substituted LuPc **4a** was evaluated with fluorescent life-time of 3.27 ns.

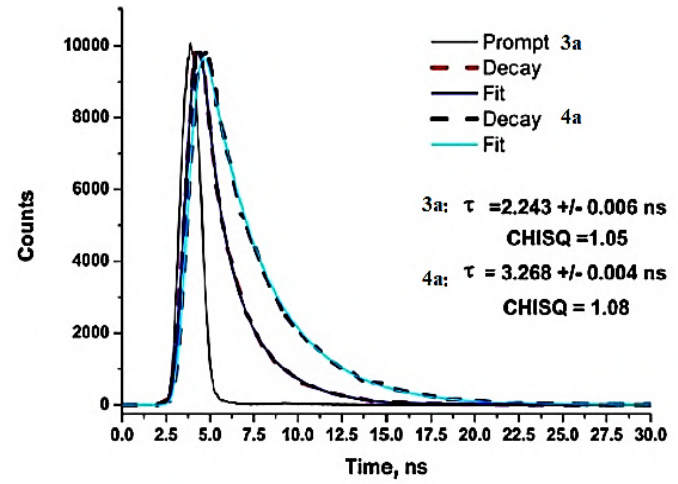


Figure 1.1.1. Exponential curves of the cationic Lu(III)-phthalocyanines (**3a** and **4a**) obtained at excitation spectra 670 nm (inserted: the values of the fluorescent life-times).

The absorption and fluorescent spectra of both Sn(IV)Pcs with non-peripheral (**5a**) and peripheral (**6a**) methylpyridyloxy groups are measured with λ_{max}: 681 nm for the absorption Q-band and 707 nm for the fluorescent band of **6a** and at 697 nm for the absorbance and 719 nm for fluorescence of **5a** in DMSO. As for the lutetium complexes, Sn(IV)Pcs show the significant shifts of the fluorescence maxima to the far red region. At excitation with a spectrum of 365 nm, 635 nm and 660, the identical bands of the excitation spectra as for the absorption spectra are observed. The obtained values for the fluorescent life-time (τ_F) of Sn(IV)Pcs are as followed: 1.65 ns (**5a**) and 1.86 nm (**6a**). The fluorescent quantum yields of the complexes of the metals with high atom numbers such as Lu^{3+} и Sn^{4+} are characterized with relatively low values as is shown for the other complexes with these metals [14-16]. In case of LuPcs the fluorescence

quantum yields are 0.012 (**3a**) and 0.018 (**4a**), which is approx. 10 times lower than the used as a standard ZnPc (ϕ_{Fl} = 0.20, [17]).

The absorption spectra of Pd(II)Pcs (**7**, **8**, **7a** and **8a**) showed the characteristic Q- band in the spectral region 670-680 nm and B-band between 320-334 nm with low intensity which is typical for the monomeric state of MPcs. The bands in the red spectrum are with λ_{max}: 680 nm and 683 nm for the non-peripheral PdPcs (**7** and **7a**) and shifted to the long wavelength as compared to the peripheral PdPcs (**8** and **8a**). The absorption Q band has a significant bathochromic shift to the near infrared region for the non-peripheral substitution to the phthalocyanine ring [18]. The quaternization improves the solubility of the complexes **7a** and **8a**, nevertheless the position of the substituents of the methylpyridyloxy- groups. The fluorescence spectra were obtained with λ_{max}: 682 nm (**8**) and 684 nm (**8a**) as for the non-peripheral complexes at 689 nm (**7**) and 691 nm (**7a**) in DMSO. The differences of the position of the excitation band and the respected absorption spectrum are not determined as was observed for other phthalocyanine complexes.

The ability of the quaternized phthalocyanine complexes to participate in the reactions with generation of singlet oxygen was studied with DPBF as a reductor with an absorption maximum at 417 nm in DMSO. The studies in water was not possible because of the aggregation ability. The quantum yields of the singlet oxygen was calculated by comparative method (ZnPc, Φ_{Δ}=0.56 in DMF). The obtained values show similarity: **0.35** for **3a** and **0.32** for **4a**. The quantum yields are in agreements with the values obtained for the other phthalocyanine complexes with diamagnetic ions [15]. The cationic Sn(IV)Pcs are evaluated with singlet oxygen quantum yields of **0.38** (**5a**) and **0.43** (**6a**). The replacement with palladium ion leads to highest values of the singlet oxygen quantum yields: Φ_{Δ} **0.65** (**7a**) and **0.68** (**8a**) in DMSO.

The general conclusion of this study is that the replacement of zinc with another high atom number ion such as lutetium and tin influences on the properties of the triplet excited state showing the low fluorescence quantum yield as well as the lifetime. However, there are not great differences in physicochemical parameters as observed by chosen metal ion, but on the other hand the insertion of the high atom numbers metal ion of Lu(III) and Sn(IV) or Pd(II) facilitate the triplet state properties which are responsible for singlet oxygen generation and PDT efficiency.

In addition to the metal ion, the linkage to the substitution groups tends to decrease the fluorescence lifetime in the order: ZnPc > PdPc > LuPc > SnPc. The theory said that the quantum yields of triplet state follow the opposite order namely the order is increasing expecting the higher efficiency of the generated singlet oxygen. The complex of zinc possesses an electron configuration $3d^{10}$ (closed-shell) which is in advance to the more effective transition to the excited triplet state because of the more effective spin-orbital interaction. The complexes of palladium PdPcs, (**7a** and **8a**) with configuration $4d^8$ (open-shell) with diamagnetic properties and low fluorescent quantum yield of the d–π interactions, as well as with relatively high quantum yield of the triplet state of the molecule which results in high quantum yield of the singlet oxygen generation (Φ_Δ). As known, the values of the quantum yield of singlet oxygen are in dependance of several inner and outer factors such as: 1) the triplet state's quantum yield, lifetime and energy E_T ; 2) the nature, size and positions of the substituents with effect on the excited states of the molecule and 3) the efficiency of the photosensitization by an energy or electron transfer to the biomolecules or/and to the molecular oxygen. The obtained values are also in dependance on the substitution groups and can be consider as optimal for phthalocyanine complexes.

1.2. Si(IV) PHTHALOCYANINES WITH AXIAL SUBSTITUENTS

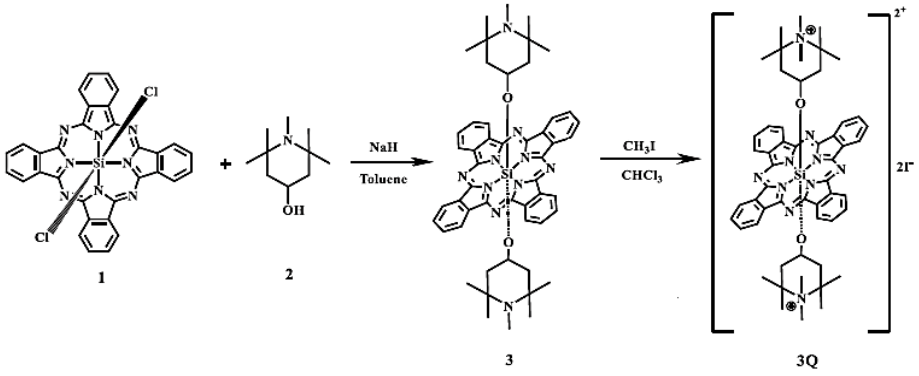
The aggregation behaviour of phthalocyanines in solutions are part of the synthesis, purifications, and the chemical analyses [19-21]. The effective control on the process can be achieved with structural modifications by using the bulky groups for substitutions to the coordinated metal ion. A typical example of this approach is a silicon which allows axial substitutions and limit the formation of aggregates. The silicon complexes are among the most studied phthalocyanines after zinc complexes, and there is a clinically approved photosensitizer **Si4** for application in antitumor therapy in USA [22-24].

1.2.1. Synthesis and chemical properties

The silicon phthalocyanine derivatives with axial substituents are synthesized from the complex $SiCl_2Pc$ (**1**), which is a commercial product, but the synthesis is possible in the laboratory conditions following the literature procedure [22]. Si(IV) phthalocyanines with two kinds of substitutions on axial positions are synthesized (**3** и **4**) and after quaternization the cationic water-soluble derivatives **3Q** and **4Q** are obtained (Scheme 1.2.1). The synthesis was

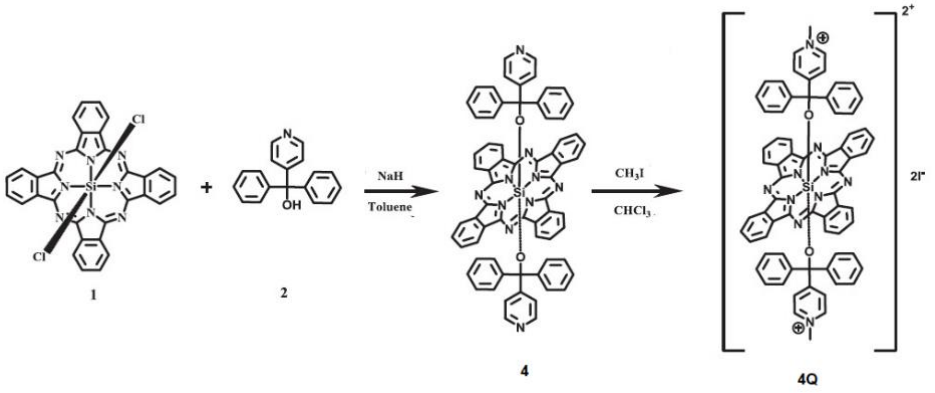
carried out by a nucleophilic substitution of 1,2,2,6,6-penthamethyl-4-piperidinol in dry toluene, with addition of a strong base such as sodium hydride (NaH) and by reflux for 24 hours under argon. The purification was done on column chromatography (Al_2O_3) with a mixture of solvents (DCM: EtOH; 10: 0.5).

A.



Scheme 1.2.1. Synthesis of axially substituted cationic water-soluble Si(IV)-phthalocyanines (A and B); 12-14 %.

B.



The quaternized complex **3Q** was obtained with methyl iodide in chloroform at RT. During the reaction, a sediment is formed which suggests the water-soluble product **3Q.** The next step involved the filtration and washing by several solvents (DCM, EtAc, Ac). Both SiPcs (**3** and **3Q**) were characterized by the means of FT-IR, ^{1}H NMR, MALDI-TOF spectrometry. The FT-IR spectra of **3** and **3Q** showed characteristic vibration with bands 1078 cm^{-1} and 1080 cm^{-1} for Si-O-C band, the vibration bands are recorded between 1520 - 1519 cm^{-1} for -C=C- group and at 3062 cm^{-1} and 3035 cm^{-1} for the aromatic -CH, as well as for aliphatic -CH groups which appear at 2922–2853 cm^{-1} and 2919–2849 cm^{-1}. The substituents after quaternization showed no difference in FT-IR spectra of **3Q** which is like the spectrum of **3.** ^{1}HNMR spectrum of **3** shows the aromatic protons at 9.69 and 8.50 ppm, and for **3Q** at 9.75 and 8.60 ppm. The aliphatic proton of CH as for the group Si-O-CH show signals in the negative range of the spectra (-2.77 ppm), which is a result of the magnetic anisotropy. The mass spectra were obtained by MALDI-TOF spectrometer and a matrix of

DHB for **3** and of CHCA for **3Q**. The spectrum of **3** showed the mass of the fragments: m/z = 851.02 $[M-2CH_3]^+$, 798.07 $[M-6CH_3]^+$, 695.90 $[M-C_{11}H_{23}NO]^+$, and for **3Q**: m/z = 730.939 $[M-(2I)-12CH_3]^+$ and 559.058 ([M-(2I)+ CHCA+ H_2O] + 2) /2.

Di-(α,α-diphenyl-4-pyridylmethoxy) Si(IV) -phthalocyanine (**4**) and the cationic derivative (**4Q**) were obtained (Scheme 1.2.1, B). The newly prepared complexes are chemically analyzed by FT-IR, UV–Vis, ^{1}H NMR, MALDI-TOF. FT-IR spectra of the compounds showed similarity. The vibrations are found at 1080 cm^{-1} (Si-O-C), between 1524 cm^{-1} and 1529 cm^{-1} (-C=C-), as well as in between 3082 cm^{-1} and 3054 cm^{-1} (arom. -CH). The complex **4Q** has characteristic vibration between 2919 cm^{-1} and 2849 cm^{-1} of the aliphatic -CH group. The obtained ^{1}HNMR spectra of compound **4** shows signals in the region 9.63 ppm, 9.55-8.47 ppm and 8.08-5.75 ppm. The spectra of **4Q** showed signals of the protons of the ring at 9.72 ppm, 8.57 ppm, 6.90-6.86 ppm, 6.34-6.31 ppm and 5.76 ppm. The protons of the methyl group appear as singlet signal at 1.23 ppm in spectrum of **4Q**. The mass spectra of compounds **4** and **4Q** were identified by the mass spectrometer MALDI-TOF with a matrix DHB for **4** and without the matrix for **4Q**. The signals appear in the spectrum of **4** at 1084.056 $[M+Na]^+$ and 1233.811 $[M+DHB+H_2O]^+$ and with the signals at 588.032 $[M-(2I)+Na+H]^{2+}$ and 1115.778 $[M-(2I)+Na+H]^+$ for derivative **4Q**.

1.2.2. Photophysical and photochemical properties

The absorption spectra of the axially di-substituted Si(IV) phthalocyanines **3** and **4** have the characteristic Q band in the far red region with maxima at 680 nm and 676 nm in DMSO. The spectrum of the quaternized complex **3Q** obtained in PBS buffer shows λ_{max} at 688 nm. The bands in the UVA region are registered around 360 nm for both derivatives (**3** and **3Q)**. The monomeric state of the molecule was proven for a range of concentrations in DMSO (**3** and **3 Q**) and in PBS buffer for quaternized complex (Fig. 1.2.1). The absorption spectrum of **3** shows the lack of aggregation as for derivative **3Q** in DMSO and PBS buffer at the studied concentration region (2. 10^{-6} – 1. 10^{-5} M). The complexes **4** and **4Q** were evaluated with absorption spectra with Q-band at 676 nm and 677 nm, and with low intensity B-bands around 357 nm, which are shifted as compared to a silicon complex $SiCl_2Pc$. On the other hand, both derivatives exist as monomeric molecules in solutions for a wide concentration range. The other pair of compounds **4** and **4Q** show similar spectra of absorbance and fluorescence as for **3** and **3Q.**

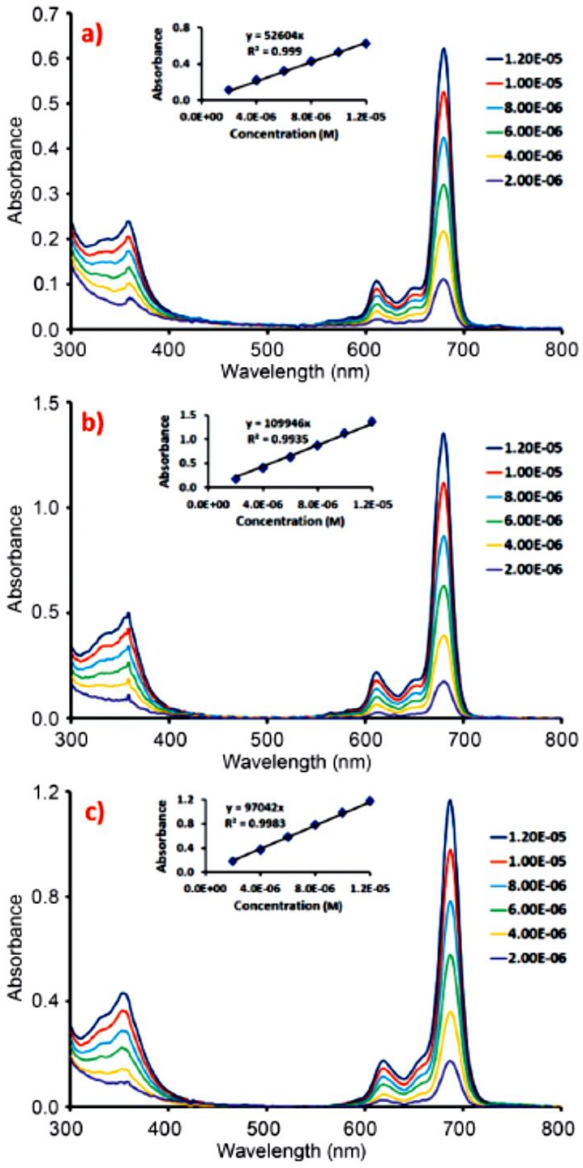


Figure 1.2.1. Absorption spectra for a range of concentrations for phthalocyanines **3** (a) and **3Q** (b) in DMSO as well as for **3Q** in PBS buffer (c).

The tendency for aggregation remains low as showed the spectra obtained for solutions of DMSO and PBS. The fluorescence spectra are obtained at the spectrum of excitation 610 nm and 645 nm and the position of the fluorescence bands have λ_{max}: 680 nm (**4**) and 684 nm (DMSO) and 691nm (**4Q**) in PBS. The spectra of absorbance and fluorescence, and the spectrum of excitation suggested the homogeneity and a single structure of the studied compound in solutions. The fluorescence band of the studied silicon complexes shows the typical bathochromic shift to the far-red region which for phthalocyanines is as short as overlapping shortly the absorption maximum. The fluorescence peak is recorded at 684 nm for complexes **3** and **3Q** in DMSO. The cationic complex **3Q** was studied in PBS with fluorescent maximum shifted to 693 nm. In Table 1.2.1. are summarized the data for fluorescent properties of **3** and **4** which are relatively high for the complexes of silicon. The cationic complexes **3Q** and **4Q** are measured with relatively high values of fluorescent lifetimes (5.27 ns and 4.94 ns) in DMSO as well as in PBS. The fluorescent quantum yields of both **4** and **4Q** are evaluated with values below 0.2, which is in agreement with the properties of an effective photosensitizer. In DMSO, the complex **4** has an yield of 0.17 and for the quaternized derivative **4Q** it is much lower (0.08).

Table 1.2.1. Fluorescent properties and the singlet oxygen quantum yield of SiPcs.

MPcs	Solvent	Φ_{fl}	τ_{fl}, (ns)	$\phi\Delta$
3	DMSO	0.31	5.44	0.31
3Q	DMSO (PBS)	0.26 (0.25)	5.27 (4.94)	0.18 (0.15)
4	DMSO	0.17	5.36	0.33
4Q	DMSO	0.08	5.27	0.09
$SiCl_2Pc$*	DMSO	0.44	5.37	0.15
ZnPc**	DMSO	0.20	3.99	0.67

*[22]; **[15]

The fluorescence lifetime (τ_{fl}) was measured by a direct method of a single photon counting. The curves are following a monoexponential dependances which suggest that the studied solutions contain structurally identical molecules. The obtained values of 5.36 ns (**4**) and 5.27 ns (**4Q**) are relatively high and close to the value obtain of the starting compound $SiCl_2Pc$ (5.37 ns) without bulky substituents.

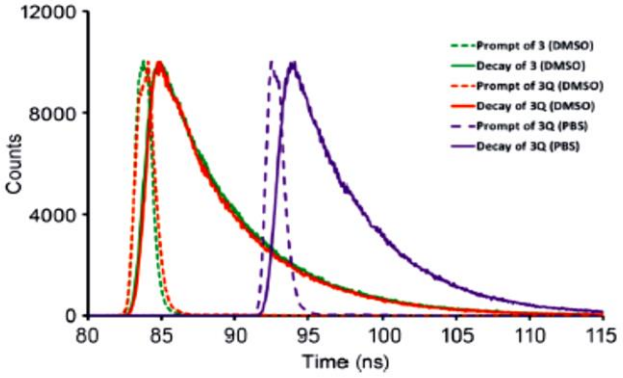


Figure 1.2.2. Exponential curves of the fluorescence lifetimes of Si(IV) phthalocyanines: **3** in DMSO and **3Q** in DMSO and in PBS at exc: 610 nm and 645 nm.

The singlet oxygen is a main oxidative specie which is generated during the irradiation of the phthalocyanine dyes [25, 26]. The singlet oxygen quantum yield ($\phi\Delta$) was determined by indirect photochemical reaction with a reductor compound. The used in our experimental work compounds have an absorption maximum at 417 nm (DPBF in DMSO) and for measurements in water at 380 nm (ADMA in PBS).

The singlet oxygen quantum yield of complex **3** in DMSO has relatively high value of 0.31 which is appox. twice higher than that of the complex **3Q** in PBS (0.15). The singlet oxygen generation of complex **4** is twice higher than of a standard $SiCl_2Pc$ (0.15).

The new SiPcs showed high chemical stability without structural changes due to the generated singlet oxygen. Phthalocyanines are described with high thermal and light stability in solid state [27-30]. While in solutions (< 10 μM), the light plus oxygen can destroy the molecule which is easily visualized by the changes in coloration of solutions. The studies on the photostability were carried out by absorbance. The obtained spectra showed the molar absorptivity (λ_{max}) with slight decrease, which suggest relatively high photostability.

2. PHTHALOCYANINE COMPLEXES WITH BIOLOGICALLY ACTIVE SUBSTITUTION GROUPS

The symmetrical planar macroheterocycle molecule of phthalocyanine characterizes with total 16 possible positions for functionalization to the main ring molecule. The substitution with biologically active moieties is a suitable approach with several aims: an effective transport in the bloodstream; an increment of the selectivity towards pathogens or tumor cells; a strong interaction with cellular membranes [31]. In addition, to these aspects, the main biological functions of the linked to phthalocyanine biologically-active molecules can support the efficacy of the photosensitive process. Considering the cell's environment as a target of the photosensitizer, the peripheral positions of phthalocyanine molecule seem more likely to be useful for these substitutions because the resulted molecule is more exposed to the cells (Fig. 2.1.1).

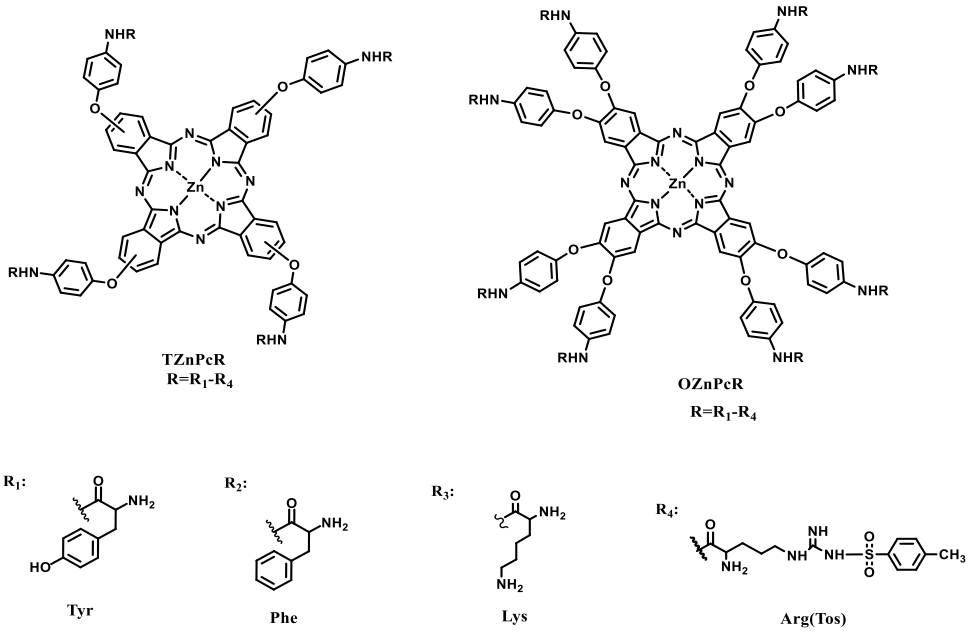


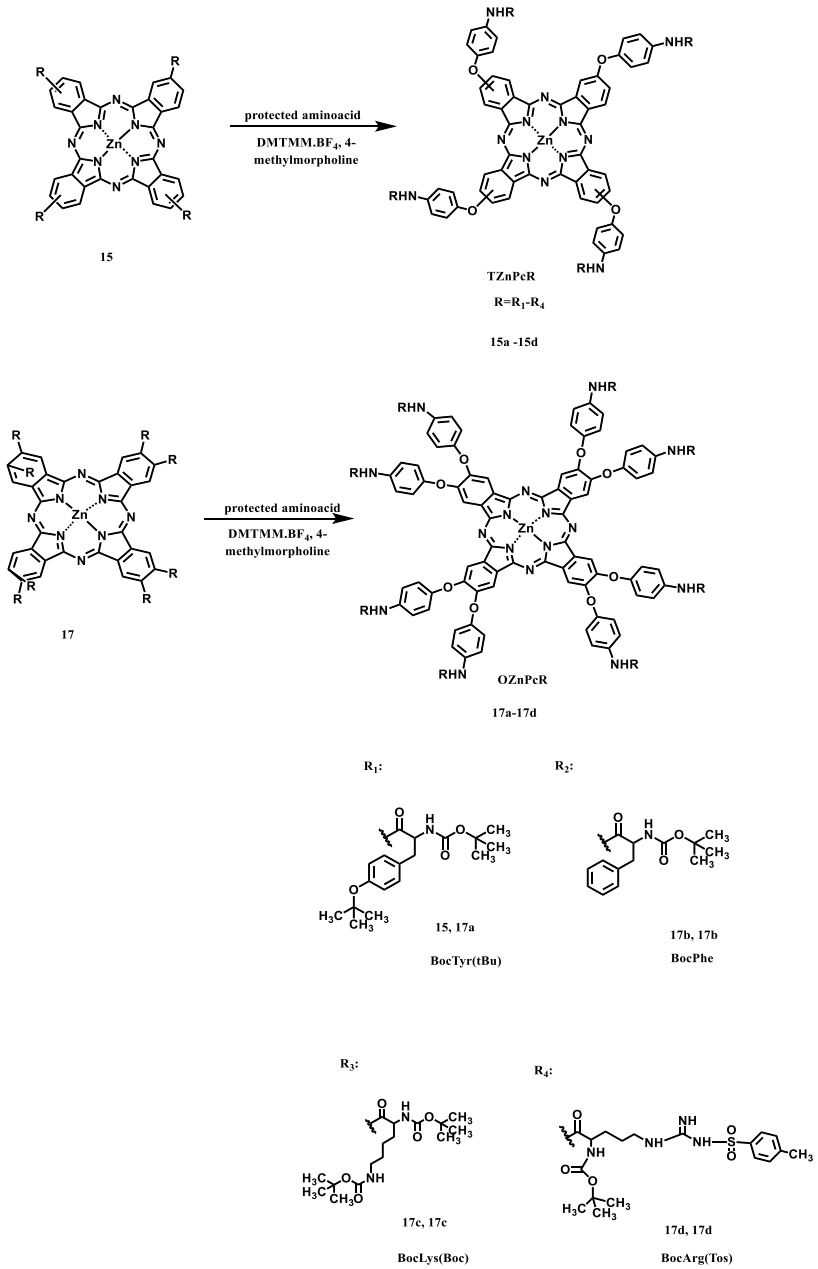
Figure 2.1.1. Bioconjugates of Zn(II)-phthalocyanines with four and eight amino acids such as tyrosine, phenylalanine, lysine and arginine in peripheral positions.

2.1. Bioconjugates of phthalocyanine with amino acids

The chemical linkage of phthalocyanine molecule to amino acids was firstly announced in the publications of Russia scientists from the group of Professor Lukyanetz [32, 33]. The main idea for the new structure was to create a phthalocyanine compound which is soluble and with advance to the main photophysical properties of absorption and fluorescence. The amino acids which are selected for chemical linkage to phthalocyanines are non-essential and have important physiological impact on humans' such as restorative and therapeutic acting as prodrugs [34]. In addition to the biological functionality, the chosen amino acids tyrosine and phenylalanine are among the rare amino acids which possess the fluorescence. The others are lysine and arginine, which are of high interest because of their cationic charge in physiological conditions as well as they are famous with the ability to cross the membranes easily and are preferable for improvement of the penetration into cells.

2.1.1. Synthesis and chemical properties

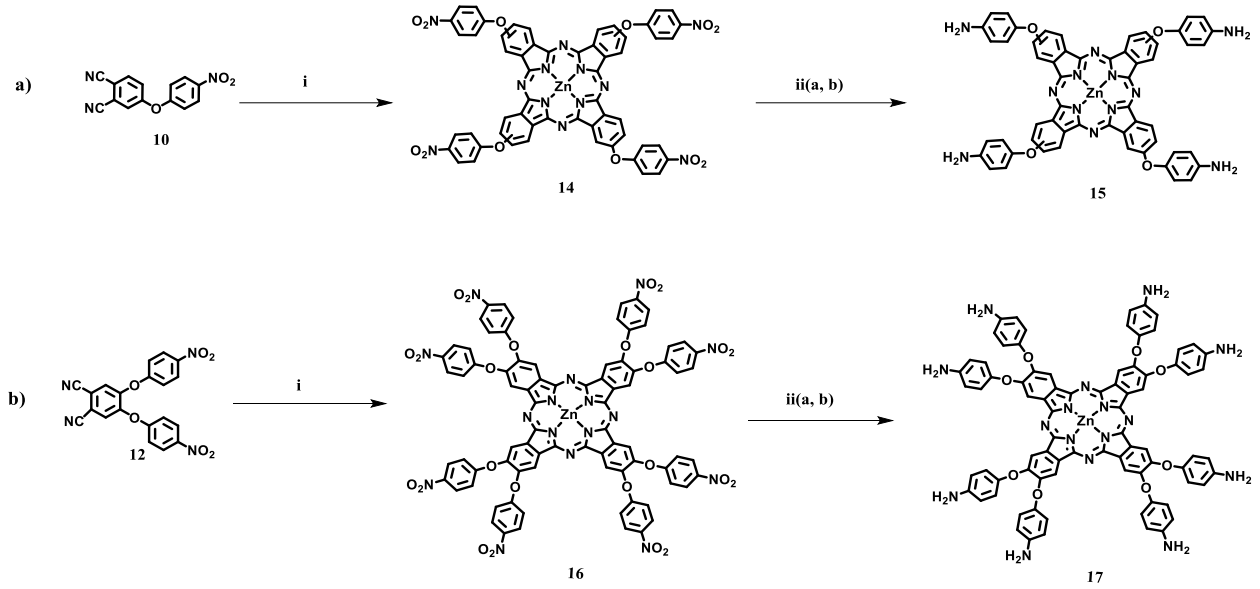
Bioconjugates of Zn(II)-phthalocyanine with amino acids tyrosine, phenylalanine, arginine and lysine are connected through a linkage group in the peripheral tetra- and octa-positions to ZnPc ring (Fig. 2.1.1). The resulting amide bond is between amino (aminophenoxy) group of phthalocyanine and carboxyl group from amino acids.



Scheme 2.1.1. Synthesis of tetra- and octa- amino acids substituted Zn(II)-phthalocyanines; 28-30% (tetra- and octa- ZnPcLys) and 44-68% for others.

The synthesis was carried out by following two synthetical pathways for preparation of tetra- octa- aminophenoxy substituted Zn(II)-phthalocyanine as the starting phthalocyanines for linkage with amino acids. Briefly, the synthesis was carried out from dinitriles with one or two aminophenoxy- substitution groups obtained by the nucleophilic displacement of nitro or dichloro substituted dinitriles. On the

next step, the phthalocyanine ring was obtained by a cyclotetramerization reaction followed the classical procedure of reflux in dry n-pentanol by addition of lithium cuttings to the reaction mixture or by direct way of addition of zinc salt and DBU as a catalyst. The obtained tetra- and octa nitrophenoxy substituted Zn(II)-phthalocyanines underwent a reduction reaction (Scheme 2.1.2). The starting phthalocyanines for linkage with amino acids are aminophenoxy Zn(II)-phthalocyanine complexes. The synthesis was carried following two synthetical approaches which involve the well-known starting dinitriles **10** and **12** for cyclotetramerization reactions and the next reduction reaction to the amino (aminophenoxy) substituted Zn(II) phthalocyanine derivatives.



Scheme 2.1.2. Synthesis of tetra- and octa substituted aminophenoxy Zn(II)-phthalocyanines. {(i) $Zn(OAc)_2$, DBU, 1-pentanol; (iia) dry DMF, H_2, Pd/C, 0 °C; (iib) DMF, $Na_2S.H_2O$}.

Zn(II)-phthalocyanines with nitrophenoxy substituents were purified on silica with THF as an eluent. The yields of the compounds **14** and **16** are relatively high {69% (14) and 82% (16)}. IR spectra show the characteristic bands at 3000 cm^{-1} for -CH aromatic groups; at 1586 cm^{-1} and 1340 cm^{-1} for $-NO_2$; 1515 cm^{-1} and 1483 cm^{-1} (Ar-C=C). ^{1}HNMR spectra show the signals of the aromatic protons in the region 7.30-8.35 ppm for **14** and 7.19-8.75 ppm for **16**. The absorption spectra have the maxima of the bands (λ_{max}) at 675 nm and 350 nm for **14** with relatively high value of ε (Q- band) ~10^5 M^{-1} cm^{-1}. The next reduction reaction is to

obtain the amino group of compounds **15** и **17** following different reaction procedures. The synthetic scheme involves one step procedure, but the difficulties are in isolation of the products, especially for compound **17** which may be due to extremely low solubility of the obtained product. The attempt to obtain metal-free phthalocyanine as a ligand molecule and then the formation of the complexes was made but they were not successful. This can be explained with a high reactivity of the amino groups with possible hydrogen bounding among the molecules.

The final step involves an activation of the carboxyl group of amino acids. The reaction was carried out with an excess of amino acids. Purifications were carried out with column chromatography with silica gel (SiO_2) and with a mixture of solvents $CHCl_3$: MeOH, which ration was different in dependance on the nature of the amino acid. The purified bioconjugates were obtained with yields between 44% - 68%, with an exception of bioconjugates with lysine which was with lower yields: ~ 30% for tetra- substituted TZnPcLys and ~ 28% for octa- substituted derivative OZnPcLys. The protected groups were removed in dry THF and trifluoracetic acid in a ration THF: TFA of 1:2 (v/v) or 1:4 (v/v).

The bioconjugates were characterized with ATR-IR spectra with the vibrations at 3290-3400 cm^{-1} (-NH), 3065-3306 cm^{-1} (-CH arom), 2851-2970 cm^{-1} (-CH aliph), 1600-1665 cm^{-1} (-NH), 1500-1507 cm^{-1} (-ArC=C), 1360-1400 cm^{-1}, 1200-1270 cm^{-1}, 1000-1095 cm^{-1} (Ar-O-Ar). ^{1}HNMR show the signals of aromatic protons in the region between 10.0-6.00 ppm and the signals of aliphatic protons between 4.00-1.00 ppm. The mass spectra were obtained on the spectrometer MALDI-TOF with different matrices namely DHB, CHCA and DIT. The mass of compounds was registered with an ion $[M]^+$; the others were obtained with two or more fragmentations $[M]^+$, $[M\text{-}C(CH_3)]^+$; $[M\text{-BocTyr(tBu)+2H}]^+$; $[M]^+$, [M- 2x OC-BocArg(Tos) – 2H]$^+$, [M- Phenyl-BocArg(Tos) – H]$^+$. The absorption spectra were recorded with the bands typical for phthalocyanine in DMSO with λ_{max} = 680-683 nm in in the red region and with λ_{max} = 350-365 nm in UVA region.

2.1.2. Physicochemical properties

The bioconjugates of Zn(II)-phthalocyanines with four and eight substituents of amino acids (tyrosine, phenylalanine, arginine and lysine) were studied in DMSO solutions for a concentration range (10^{-5} - 10^{-6} M). The absorption Q- bands have a typical symmetrical and high intensity band with λ_{max} = 680-683 nm in far red visible spectrum (Fig. 2.1.2). The phthalocyanines exist as monomeric molecules for concentrations 10^{-5} – 10^{-6} M in DMSO.

The absorption B-bands in UV region are with λ_{max} = 352-364 nm. The absorption properties are not differing for phthalocyanines with four and eight substituents of the selected amino acids (Table 2.1.1). The spectra in tris- buffer showed the typical bands for aggregation which was reported for other phthalocyanines with substitutions with amino acids [35, 36].

Table 2.1.1. Absorption properties of the bioconjugates Zn(II)- phthalocyanine with amino acids in DMSO **(10^{-5} M)**.

Tetra-AA Zn(II)Pc	Q band, λ_{max}, nm, (log ε)	B band, λ_{max}, nm, (log ε)	Octa-AA Zn(II)Pc	Q band, λ_{max}, nm, (log ε)	B band, λ_{max}, nm, (log ε)
TZnPcTyr	680 (3.02) 613 (-)	352 (-)	OZnPcTyr	680 (4.36) 613 (3.64)	364 (3.94)
TZnPcPhe	682 (4.23) 615 (3.60)	353 (3.93)	OZnPcPhe	683 (4.17) 616 (3.52)	357 (3.88)
TZnPcLys	683 (3.63) 618 (3.07)	356 (3.38)	OZnPcLys	681 (5.28) 613 (4.48)	360 (4.86)
TZnPcArg(Tos)	682 (4.27) 616 (3.66)	352 (3.97)	OZnPcArg(Tos)	682 (4.02) 616 (3.35)	355 (3.73)
ZnPc*	672				

*[35]

The formation of molecular associates is an undesirable phenomenon because the photo unactive molecules in solutions are not part of the photosensitization and the photodynamic action. In case of phthalocyanines there are two types of intermolecular interactions which are resulted in two kinds of non-active associates (H and J). The obtained coplanar structures are because of the interactions between the rings of the molecules and the noncovalent interactions of π electrons. As an effective approach of monomerization is the addition of detergents or other compounds with charge which provoke the opposite process namely to

stay the monomeric molecules in solution. This depends of several outer factors such as concentration, solvent's polarity, the additives and etc..

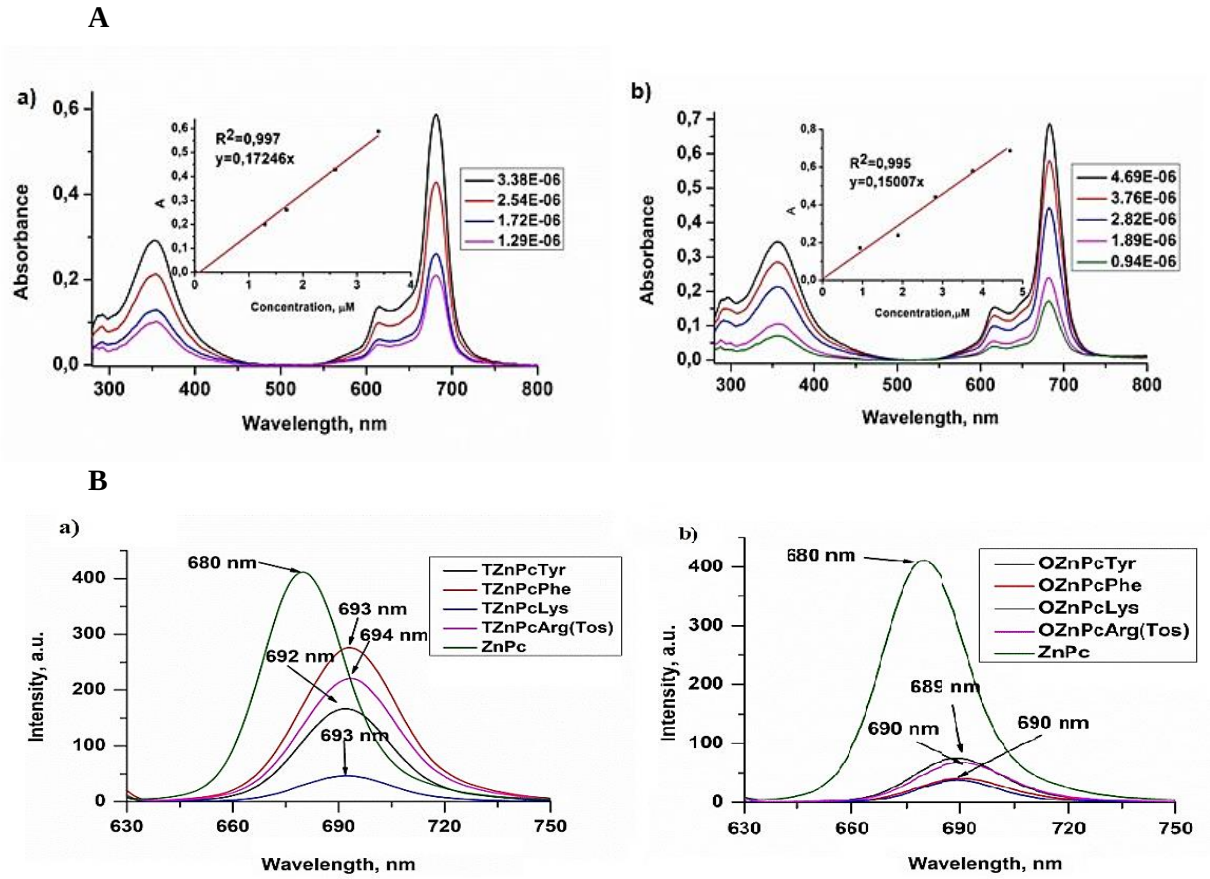


Figure 2.1.2. Absorption spectra for a range of concentrations (A) and the fluorescence spectra at exc: 615 nm (B) of a) tetra- and b) octa tyrosine substituted Zn(II) phthalocyanines in DMSO.

The phthalocyanines with substitution groups with positive charge is suitable a detergent with the opposite charge. Another option is an emulsion of 2% Chremophor EL (up to 5% of the total volume) which is accepted to solubilize the hydrophobic photosensitizers for clinical applications. The water-soluble lysine conjugate was evaluated with similar absorption spectra as for OZnPcTyr. A characteristic feature of the new bioconjugates is that the molar coefficients (ε > 1-3. 10^5 M^{-1} cm^{-1}) of the Q-bands at 680–683 nm are much higher than for the other similar compounds [32, 33]. It was found that the position of the fluorescent maximum is not in dependance on the spectra of excitation which means the homogenic solutions and characterizes without influence of the nature of the linked amino acid (Fig. 2.1.2, B). Both tetra- and octa bioconjugates with amino acids are registered with an intensive

fluorescence band which is typical for monomeric phthalocyanines. The fluorescent properties of the new bioconjugates are examined with the quantum yields and the lifetimes of fluorescence (Table 2.1.2).

Table 2.1.2. Fluorescent lifetime of bioconjugates of phthalocyanines with amino acids.

Tetra-AA Zn(II)Pc	Φ_F	τ_F (ns) λ_{exc} : 390 nm λ_{exc}: 405 nm	Octa-AA Zn(II)Pc	Φ_F	τ_F (ns) λ_{exc}: 390 nm λ_{exc}: 405 nm
TZnPcTyr	0.12	2.91	OZnPcTyr	0.040	2.03
TZnPcPhe	0.069	2.82	OZnPcPhe	0.018	2.67
TZnPcLys	0.047	2.85	OZnPcLys	0.030	-
TZnPcArg(Tos)	0.055	2.89	OZnPcArg(Tos)	0.038	2.56
ZnPc*	0.200	3.99			

*[35]

The low values of the fluorescent quantum yield ($\phi_{Fl} < 0.1$) were determined for all new bioconjugates, which can be an indication of possible loss of the absorbed energy by an intersystem crossing to the lower energy triplet excited state [37]. The connection of the substitution groups with fluorescent properties such as tyrosine is possible to contribute to the fluorescence of the phthalocyanine molecule. On the other hand, the bulky group of eight substituents can lead to a physical quenching of the emission. The nonradiative transitions of inner conversion singlet – singlet or triplet – singlet which experience the heat are not typical for photosensitizers. The structurally similar phthalocyanines linked to different amino acids are also evaluated with low fluorescence (< 0.2) as was reported before [35-38].

The phenomenon of negligible fluorescence capability of the highly conjugated molecule is due to the photoinduced electron transfer (PET). This effect is typical for compounds with amino groups or the other related derivative groups [39a-d]. The removal of the protective tertbutyl carbonyl (-Boc) groups resulted in higher electron density of the molecule. The quantum yields of fluorescence < 0.1 are observed also for both tetra- and octa aminophenoxy substituted Zn(II)-phthalocyanines.

As is well known the fluorescence lifetime of a photosensitizer determines the time interval the molecule is photoactive. The studied new bioconjugates with amino acids show the monoexponential curves which are typical for solutions of pure compound. The values of the fluorescent lifetimes ($\boldsymbol{\tau_F}$) are between 2.03 - 2.91 ns, which are approx. twice lower as compared to τ_F value of unsubstituted ZnPc (3.99 ns). The regioisomers in the studied solutions cannot be registered [36, 40]. The comparison of the data for τ_F of tetra and octa substituted conjugates showed that τ_F of tetra- (TZnPcAAs) are higher than for octa- (OZnPcAAs) bioconjugates as a consequence of the free amino- groups and the physical quenching of bulky substituents.

The ability of the new phthalocyanine conjugates with amino acids (tyrosine, phenylalanine, arginine, and lysine) to generate the reactive singlet oxygen was studied with an indirect photochemical method. The photooxidation of a molecule (DPBF) during the light exposure with a spectrum 635 nm or 660 nm was used. The collected data show that the quantum yields of singlet oxygen of tetra-substituted with tyrosine and phenylalanine phthalocyanines (TZnPcTyr and TZnPcPhe) have higher values then that for the related octa-substituted derivatives. Both arginine substituted bioconjugates TZnPcArg(Tos) and OZnPcArg(Tos) have equal values for the singlet oxygen quantum yields (Table 2.1.3).

Table 2.1.3. Singlet oxygen quantum yields of bioconjugates of phthalocyanine with amino acids (DMF).

Tetra-AA Zn(II)Pc	Φ_Δ	Octa-AA Zn(II)Pc	Φ_Δ
TZnPcTyr	0.63	OZnPcTyr	0.38
TZnPcPhe	0.71	OZnPcPhe	0.23
TZnPcLys	0.36	OZnPcLys	0.57
TZnPcArg(Tos)	0.39	OZnPcArg(Tos)	0.40

The photostability of the compounds which are evaluated as photosensitizers is of importance to ensure that the molecules are stable during the PDT irradiation protocol. The

molecular stability is determined by the changes in the absorption spectra during irradiation. The calculated values are summarized in Table 2.1.4. It is believed that the intermolecular interactions are the basis of the photostability which in case of phthalocyanines the substituents are in advance to this property [41].

Table 2.1.4. Photostability of the bioconjugates with amino acids at irradiation with 635 nm.

Tetra-AA Zn(II)Pcs	$\frac{dc}{dt}$, x 10^{10}	Octa-AA Zn(II)Pcs	$\frac{dc}{dt'}$ x 10^{10}
TZnPcTyr	3.10	OZnPcTyr	32.00
TZnPcPhe	5.63	OZnPcPhe	3.50
TZnPcLys	13.50	OZnPcLys	2.20
TZnPcArg(Tos)	1.53	OZnPcArg(Tos)	7.89
ZnPc	0.94		

The photostability results suggested the maximum speed of decrease of the extinction coefficient (ε) for the octa- tyrosine substituted complex (OZnPcTyr) followed by the tetra-lysine- substituted ZnPc.

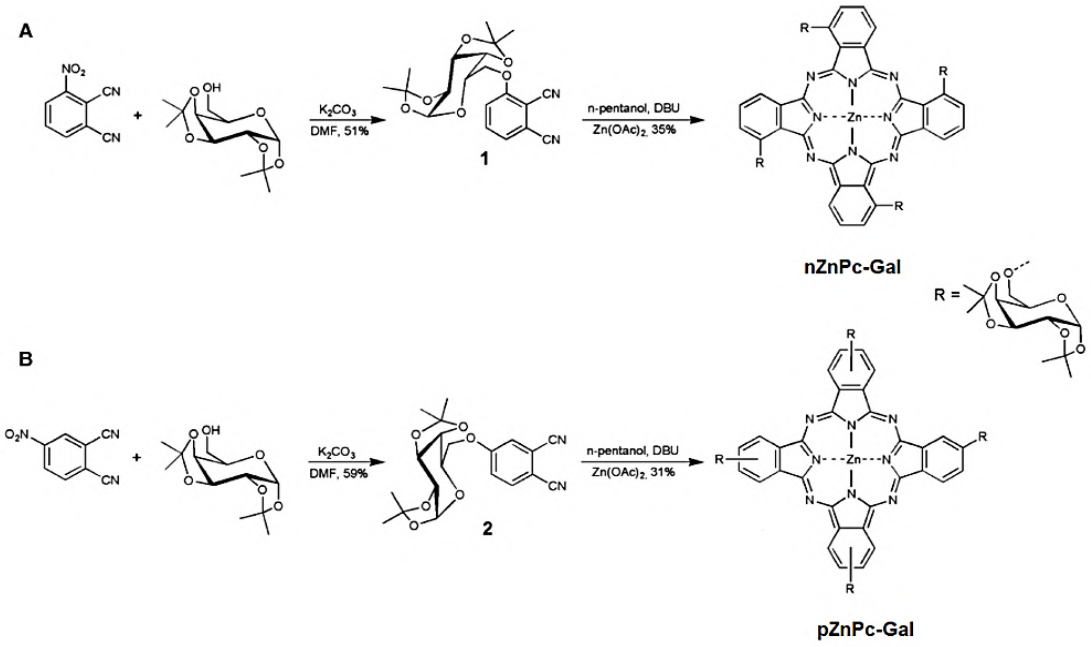
2.2. Bioconjugates of phthalocyanines with carbohydrates

The sugar moities linked to the photosensitizers are among the most studied biologically-active functinal groups with high impact to the efficacy of the new generation PDT drugs [43a-c, 44]. The initial goal was to obtain the derivatives with amphiphilic and even hydrophilic nature for studies of MPcs derivatives in solutions. After the first publication of *Maillard et al*., 1989 [45], the research interest about the phthalocyanines substituted with carbohydrates increased for a target specific antitumor PDT [46a-c]. The carbohydrates are preferable substituents for functionalization of phthalocyanines because as mention above of the improvement of solubility, but more likely because of the receptor-mediated selectivity [47, 48]. The carbohydrates are likely also to contribute on the main physicochemical properties of importance for the photosensitizers. Two kinds of bioconjugates of Zn(II)-

фталоцианини with substitutions on peripheral and non-peripheral positions of galactopyranose groups were prepared (Scheme 2.2.1). Bioconjugates with galactose are characterized with the physicochemical properties which are proper for the photosensitizers with PDT applications. The compounds were tested for the uptakes and selectivity of the photocytotoxic efficiency.

2.2.1. Synthesis and chemical properties

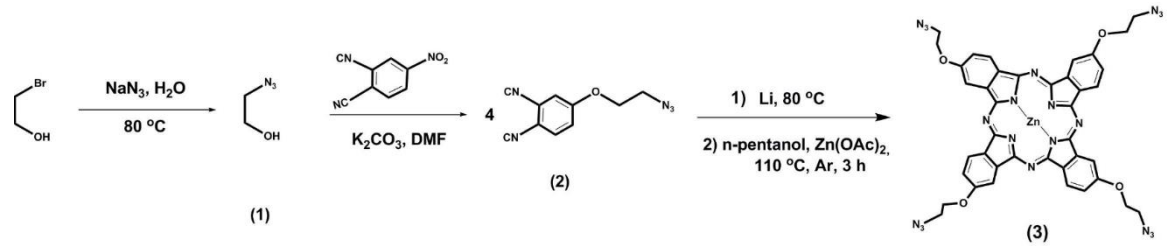
The synthetic procedure of tetra- galactopyranosyl substituted Zn(II)- phthalocyanines includes the glycosylation reaction by following the modified reaction scheme which was previously published [45-48]. The new step was to remove the protection groups as was firstly published for octa- substituted derivatives [48, c]. The procedure includes synthesis of the starting 3- or 4- nitro substituted phthalonitriles by following the well-developed procedure. The next step is the synthesis of substituted with a selected carbohydrate 3- and 4- dinitriles (**1** and **2**) according to Ref. [46, b]. This includes a nucleophilic substitution at the free -OH group of galactose as 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose.



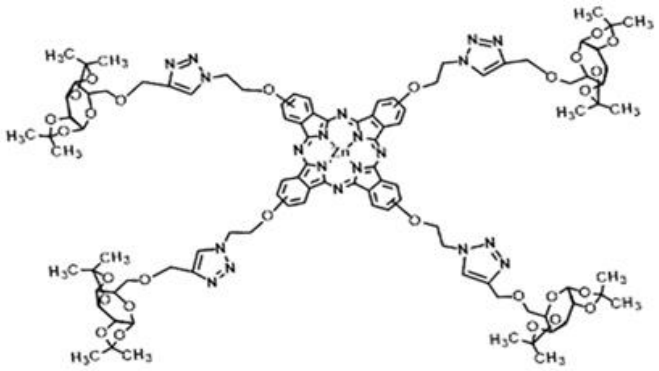
Scheme 2.2.1. Synthesis of tetra- galactopyranose substituted Zn(II)-phthalocyanines: A) in non-peripheral (**3**) and B) in peripheral positions (**4**). Yields: 30-31 %

The newly prepared compounds were dissolved in a minimal volume THF (or DCM) and TFA was added with the same volume. After dilution and vacuum evaporation, the product was taken from the bottom of the flask as fine precipitate with petroleum ether. The compounds were obtained after washing with several solvents without need of the additional purification and dried in Glass oven at 80 °C. The chemical analyses involved UV-vis, ^{1}H NMR, IR and MS spectrometry. The collected analytical data agree with the predicted structures. The obtained compounds as tetra- substituted phthalocyanines linked to galactose as functional groups are obtained as a mixture of positional isomers (regioisomers). The attempts for separation were not made because it is acceptable the compound to be a mixture of regioisomers. The used synthetic road for linkage of phthalocyanine to the carbohydrate moiety which is so called glycosylation owns to the nucleophilic displacement at hydroxyl group of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and NO_2 group of 3- or 4-phthalonitrile (Scheme 2.2.1). The reaction known as homo-cyclotetramerization, which means that the used dinitriles are of the same origin was published in the year 2006 by Hanack & Ziegler [48, b]. The reaction conditions include dimethylaminoethanol by boiling at 150 °C, without need of removal of per-O-benzylated glycosides groups which was not successful. This was the reason to initiate the acetylation method at much lower temperature of 100 °C in diaminoethanol and 1-butanol which results in the carbon linked phthalocyanines to glucose. A study announced the phthalocyanines linked by alkyl group with ribofuranose by palladium catalyzed Sonogashira reaction considered as not appropriate for applications in medicinal chemistry [49, 50]. The linkage of different substituents through triazole group appears an useful approach for functionalization of phthalocyanines [51a-c]. The reaction called on the name of the author R. Huisgen is 1,3-cycloaddition between alkyl and azido groups with addition (or without) of a Cu(I) catalyst [52-55].

Bioconjugate of Zn(II)-phthalocyanine with galactopyranose (**4.2**) was synthesized as a new product (Fig. 2.2.1). The reaction scheme was designed on the basis of the known chemical reactions (Scheme 2.2.2). The phthalocyanine for linkage was obtained as azidoethoxy substituted Zn(II) phthalocyanine. One hydroxyl group in the protected galactose was reacted to obtained alkyl group by a sodium hydride (NaH). The click reaction was done smoothly at RT but for approx. 36 hours.



Scheme 2.2.2. Tetra- azidoethoxy substituted Zn(II)-phthalocyanine for click chemistry; 30 %.



(**4.2**)

Fig. 2.2.1. Bioconjugate of Zn(II)-phthalocyanine with galactopyranose (4.2) linked via triazole ring.

The compounds were characterized by the means of the known analytical methods such as UV-Vis, ATR-IR and MALDI-TOF mass spectrometry. The peripherally substituted Zn(II)-phthalocyanines which was obtained with an ether bond (**4**) and with a through triazole group (**4.2**) have similarity in the IR spectra with a difference of the vibration at 1387 cm^{-1} of triazole ring. The spectrum of **4.2** has a band in the region between 1050 cm^{-1} and 1200 cm^{-1}, which are due to galactopyranose substitutions. The hydroxyl groups of galactoses obtained after removal of the protection groups in compound **4** have vibrations with low intensity in the range between 3200 cm^{-1} and 3360 cm^{-1}. The other characteristic bands are recorded at 2956 cm^{-1} and 2887 cm^{-1} of the aliphatic -CH groups of **4.2**; C-O-C group has a vibration at 1240 cm^{-1}. The MALDI-TOF spectrum has a signal with m/z: 2111.497 $[M]^+$ for **4.2**.

2.2.2. Photophysical and photochemical properties

The glycosylated phthalocyanine zinc complexes (**4** and **4.2**) prepared by the both approaches, showed the similar absorption spectra and only the position of the substitution

groups influences on the position of the maximum as observed for peripheral and non-peripheral derivatives. Both compounds are evaluated to be in the monomeric state in the studied concentration ranges as confirmed by the intensive and narrow Q-bands with maxima in the red region (682 nm for pGal-ZnPc; 702 nm for nGal-ZnPc) in DMSO solutions. The differences in the positions show the significant shifts as compared to ZnPc and the phthalocyanine derivatives. The absorption spectra of compounds without protective groups suggest that in solutions are formed the molecular associates. This phenomenon was also observed for the other studied carbohydrate substituted phthalocyanines in water solutions [56]. The free hydroxyl groups as is the case have tendency for protons interactions with formation of the stable associates. As it is known the molecular associates are typical for solutions at high concentration of phthalocyanines which depends on several external conditions such as solvents polarity, temperature, the structural features such as substitution groups and the nature of the coordinated metal ion [57]. The molecular associates are not able to participate in the photosensitization process. This is the reason that in the further studies the compounds were with protected galactose moiety. In addition, these derivatives have preferable amphiphilic nature and are likely to exist in solutions as monomeric molecules being photoactive for PDT applications.

The fluorescence spectra of the newly prepared phthalocyanines characterize with emission spectra with wide bands in the region 650-800 nm which does not overlap the fluorescence of the natural chromophores. The maximum of the fluorescence emission band of compounds **3** и **4** is bathochromically shifted to 691 nm (**4)** and to 707 nm (**3**) as compared to the absorption maxima, which is a negligible shift in the fluorescence spectrum of phthalocyanines. The proper values of the fluorescent quantum yields (Φ_F) such as 0.13±0.01 for peripheral p-GalZnPc (**4**) and lower for n-GalZnPc (**3**) such as 0.06±0.01 in DMSO were determined (Table 2.2.1). The spectra of the peripherally substituted derivatives **4** and **4.2** were recorded without differences due to the linkage groups.

The fluorescence lifetimes of galactosylated phthalocyanine derivatives are measured. As can be seen the obtained monoexponential curves suggest a monomolecular compound with lifetimes of 2.75 ns for a bioconjugate with non-peripheral substituents **3** and with 3.43 ns for compound with peripheral substituents **4**, respectively. As compared to ZnPc (3.99 ns), the measured values are a bit lower. The significant fraction of molecules can be deactivated by fluorescence after excitation [58]. The obtained values of fluorescent properties (Φ_F и τ_F) are characteristics of the monomeric molecules in solution.

Table 2.2.1. Photo-physicochemical properties of galactosylated Zn(II)Pcs.

ZnPc	Solvent	λ_{max} (Q) (nm)	λ_{max} (Fl.) (nm)	Φ_F (SD: 0.01-0.02)	ϕ_Δ	τ_F (ns)
n-GalZnPc, 3	DMSO	702	707	0.06	0.49	2.75
	DMF	698	701	0.11	0.52	
	MeOH	696	701	0.10		
	H_2O	696	702			
p-GalZnPc, 4	DMSO	683	691	0.13	0.26	3.43
	DMF	679	686	0.12	0.32	
	MeOH	675	684	0.10		
	H_2O	680	704			
ZnPc*	DMSO	672	683	0.18	0.67	3.99

*[35]

The singlet oxygen generation involves the process with an energy transfer from the triplet excited state of a molecule to oxygen which is the preferable transition for phthalocyanines. The singlet oxygen formation with participation of galactosylated ZnPcs was studied with a specific molecular probe (DPBF) and by irradiation with LED 635 nm or 665 nm. The reaction of photooxidation of the probe occurs due to the generated singlet oxygen in the experimental sample. The reference data for the singlet oxygen quantum yields (Φ_Δ) showed values of 0.26 and 0.49 with ZnPc as a standard [35]. There were determined lower values in DMSO in comparison to DMF (Table 2.2.1). The substituents of galactopyranose lower the quantum yields of the generated singlet oxygen maybe due to a physical quenching from the substitution groups.

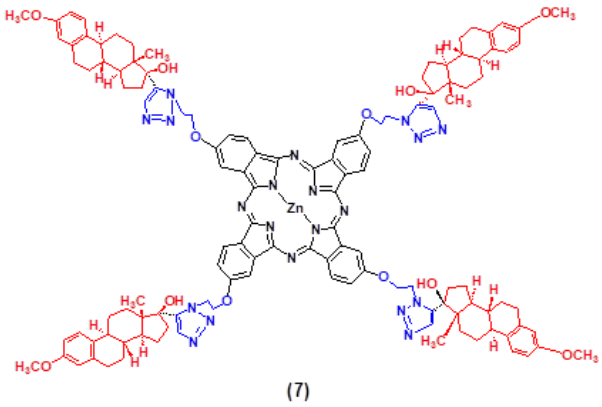
The photostability of the molecule during irradiation with a spectrum and light doses which are applicable for the photosensitizers was determined at the absorption maximum of the Q-bands. The obtained curves suggested the first order kinetic with a value of the constant 2.5 x 10^{-3} min^{-1} for **3** and **4**. The porphyrin derivatives with alkoxy groups were evaluated with constants that are higher than for phthalocyanines with the same substitution groups (2-4 x 10^{-2} min^{-1}) [59]. However, the higher photostability of porphyrins seems to be a disadvantage for biomedical applications because of the long-lasting release from cells and photosensitive associated side effects [60].

2.3. Bioconjugate of phthalocyanine with sterol

The structural features of the photosensitizers for photodynamic method appear a critical for the interactions with the cell membranes which is an essential property for the phototoxic effect [61]. The up-to-date knowledge about the structural characteristics of a proper photosensitizer selects the cationic charge, the amphiphilic nature as well as the substituents with proper bulky groups and with cell specificity or the biological functions as appropriate [62]. The cell wall of the pathogenic cells is known to contain in a high percentage of lipids as well as some sterols as compared to proteins and other bioorganic molecules. The natural sterols in cells are essential for rigidity, flexibility, and protection of membranes and with functionality of a membrane anchor. The sterols have a fundamental role in cell grown-up. It is well studied the delivery function phospholipids for drugs with different origin as well as their functionality to stimulate the interactions of the photoactive compounds with receptors of the disordered cells [63]. The sterols are evolutionary developed with the earth atmosphere and they are molecules which exist in oxygen medium. The pathogenic fungi contain ergosterols together with low quantity of cholesterol [64]. The cholesterol as a part of cell membranes is a molecular target for the oxidation reaction which lead to unfavorable conditions to human health [65, 66]. There are limited numbers of studies about the photosensitizers linked to steroids which were evaluated with very favorable properties for PDT applications [67].

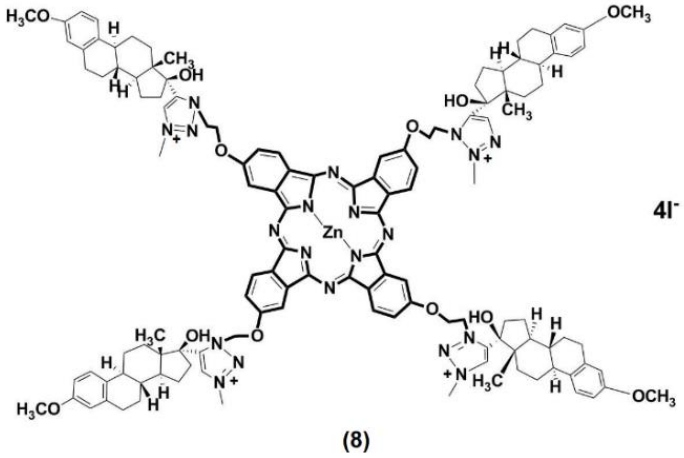
2.3.1. Synthesis and chemical properties

The new Zn(II)- phthalocyanines (Fig. 2.3.1) were prepared with four peripheral substitution groups after Click reaction with mestranol (**7**) and the cationic derivative (**8**). The linkage happens through 1,2,3- triazole ring which is suitable for quaternization reaction to obtain the cationic derivatives [68, 69]. The applied synthetic procedure starts with synthesis of 4- azidoethoxy substituted dinitrile which is a monomer for the cyclotetramerization reaction to obtain compound **3**.



(7)

Fig. 2.3.1. Bioconjugates of Zn(II)-phthalocyanine with mestranol (**7**) and the cationic derivative (**8**).



(8)

The synthetic sterols mestranol and ethinylestradiol are popular as parts of estrogen medication and it contains -C≡C- group which is suitable for linkage by Click cycloaddition reaction [70]. The bioconjugate **7** was synthesized in a cycloaddition reaction between azido-group of phthalocyanine and a sterol with a natural C ≡ C group, suitable for the linkage. This reaction was carried out by following the click procedure which includes the salts $CuSO_4$**.** $5H_2O$ (1 eqv.) and sodium ascorbate (6 eqv.) and a solvent mixture DMF/ DCM: H_2O (1:1:0.5) at room temperature. The reaction time continued between 24 and 36 hours as was controlled by TLC. Since the formed 1,2,3-triazole ring of compound **7** is suitable for quaternization the well-known procedure with MeI, RT, Ar, 6h was applied. The purification of the products was carried out on each step. The separation of the fractions after reaction to **7** was carried out on the column with Bio-beads SX-3 with DCM. The structure of the new compounds was analyzed by several methods: FT-IR, ^{1}H NMR, MALDI-TOF mass and UV-Vis spectroscopy. A vibration band at 2230 cm^{-1} which is a characteristic for C ≡ N groups of the starting 4-

azidoethoxy phthalonitrile **4** is not shown in IR spectrum of phthalocyanine. The new bands are shown at 3066 cm^{-1} for the aromatic CH group and between 2927-2856 cm^{-1} for the aliphatic CH groups. The formation of the conjugate with mestranol **7** was proven by the lack of the band typical for $-N_3$ at 2107 cm^{-1}. IR spectrum of the aromatic CH and the aliphatic CH groups have vibrations between 2952 - 2854 cm^{-1} and 1722 cm^{-1}, and 1609 cm^{-1}, 1577 cm^{-1}, respectively. The cationic derivative **8** showed the bands of the aromatic CH groups at 3165 cm^{-1} and 3019 cm^{-1}, and of the aliphatic CH groups between 2972-2754 cm^{-1}. The mass spectrum was obtained with a DIT MALDI matrix. The mass is registered m/z: 915.87 $[M]^+$ for **3**, m/z: 2163.318 $[M+3H]^+$ for **7**, and m/z: 560.200 $[M-4I]^{4+}$ for phthalocyanine **8** recorded as a single peak in MALDI-TOF spectra. ^{1}H-NMR spectrum of phthalocyanine **7** has the characteristic wide bands in the region between 4.04 ppm and 8.84 ppm for the aromatic protons. The signals which are describing the protons in the substitution groups are also registered. After the linkage of phthalocyanine with mestranol, the obtained ^{1}H-NMR spectra of **7** and **8** are not readable for the protons of the ring molecule which can be due to metal trails or aggregation of the molecules. The further attempts with different experimental conditions may be helpful.

2.3.2. Physicochemical properties

The absorption spectra of Zn(II)-phthalocyanine after linkage with mestranol (**7** and **8**) were measured in DMSO solutions in a concentration range (Fig. 2.3.2). The spectra show the intensive bands in the red region with a maximum at 684 nm (**7**) and 681 nm (**8)** in DMSO. The B-band has low intensity maximum in UVA region 330-365 nm, with a maximum at 350 nm (**7**) and 353 nm (**8**) respectively. For the studied concentration range both ZnPcs are monomeric compounds. The recorded fluorescence spectra of Zn(II)-phthalocyanines **7** and **8** with the maxima of the emission in the red region which are shifted to 694 nm (**7**) and 693 nm (**8**) as compared to the absorption maxima. The emission bands of the new derivatives were recorded with bathochromic shifts to the far-red region because of the substitution groups as was compared to the standard **ZnPc** (λ_{abs} = 672 nm and λ_{fl} = 680 nm, [35]). The fluorescence lifetime of the studied phthalocyanine complexes **7** and **8** was measured for a different excitation spectrum (360 nm, 610 nm and 660 nm). The recorded monoexponential curves are decreased with fluorescence quenching which suggests a homogenic solution.

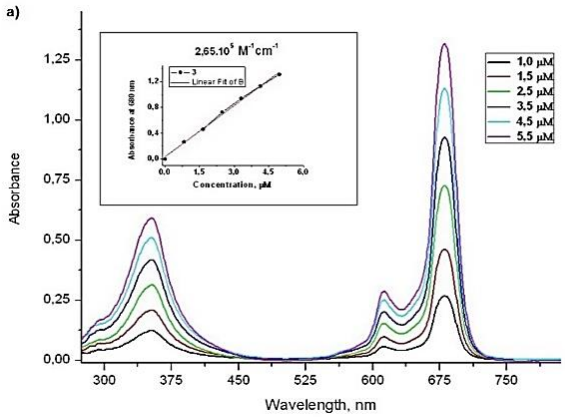
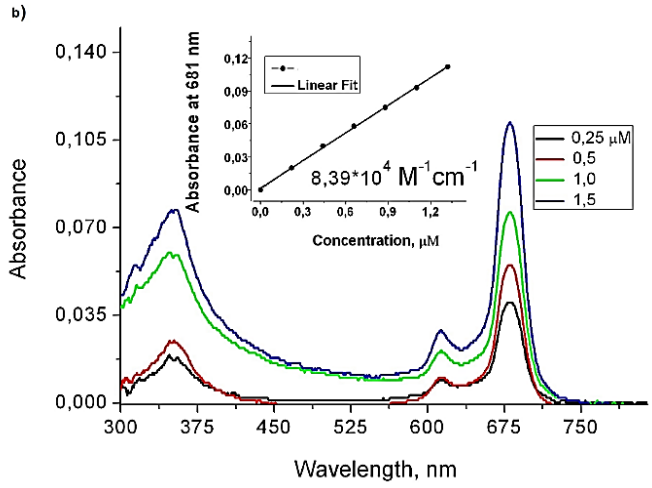
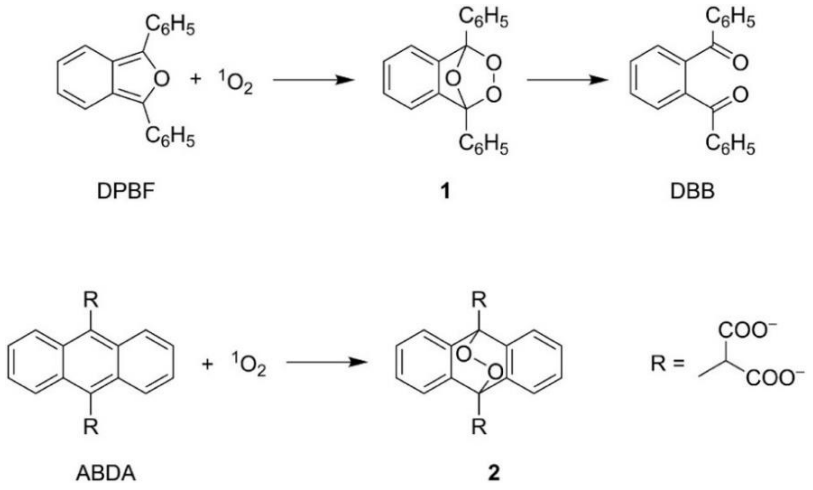


Fig. 2.3.2. Absorption spectra of phthalocyanines **7** (a) and **8** (b) in DMSO for a concentration range (Inset: Absorption changes at λ_{max} with the increase of concentration).



This observation was confirmed for phthalocyanines **7** and **8** being in monomeric state for solutions with concentrations up to 10^{-5} M. The obtained values of the fluorescence lifetime are 3.25 ns (**7**) and 3.46 ns (**8**), as for the other phthalocyanines with substitution groups a bit lower than for the standard **ZnPc** (3.99 ns). Similarly, the other phthalocyanine complexes with substitution groups the fluorescence lifetime depends on the nature of the substituents and the coordinated metal ions [71]. This result suggests that the quaternized derivative **8** has a highest value (3.46 ns) which together with the cationic charge is an advantage for the efficacy of the photodynamic process.

The determination of the singlet oxygen was carried out with a model reaction with a singlet oxygen scavenger such as DPBF. The reaction consists of oxidation of DPBF by the generated in the medium singlet oxygen during irradiation (Scheme 2.3.3). This leads to a decrease of the absorption maximum at 417 nm (DMSO). The reaction control was performed based on the absorption spectra of compounds **7** and **8** before and after the photochemical reaction. The calculated quantum yields of singlet oxygen have values 0.51 (**7**) and 0.46 (**8**) which are high for phthalocyanines with substituents. The bioconjugate with mestranol after quaternization (**8**) shows a lower value of the quantum yield of the singlet oxygen generation possibly due to some physical quenching due to four methyl groups.



Scheme 2.3.2. The reactions of photooxidation of the probes DPBF in DMSO and ABDA in water by singlet oxygen [72].

2.4. Bioconjugates of phthalocyanines with parabens

The increasement of antibiotic resistance reinforced the research and development of new treatment modalities among each are the photosensitizers for PDT method which features as alternative treatment approach with successful therapeutic outcome. Among the first clinically approved complexes for PDT of cancer is a complex Si(IV)-phthalocyanine with axial substitution groups **Pc4** in USA [73, 74]. In Europe, phthalocyanine complexes of silicon have patented for applications as photosensitizers with the PDT method [75].

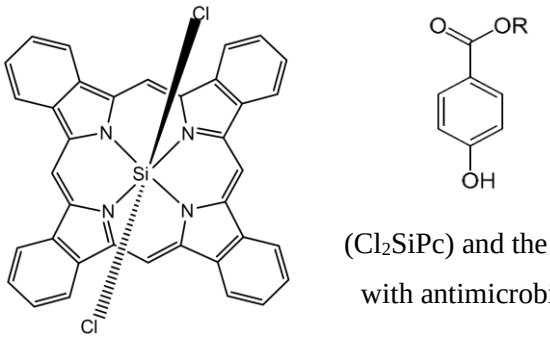
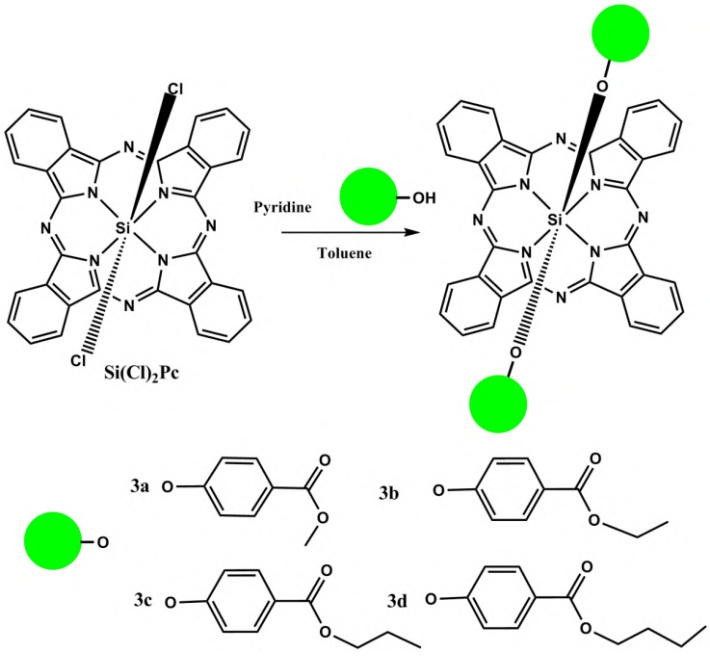


Fig. 2.4.1. Si(IV)-phthalocyanine (Cl_2SiPc) and the alkyl ester of p-hydroxybenzoic acid, both with antimicrobial activity, but with different mechanisms.

A proper approach for the creation of the effective structure of a photosensitizer appears the linkage of a photosensitizer such as $SiCl_2Pc$ with other compounds with antimicrobial activity. The esters of p-benzoic acid popular as parabens are well known as inhibitors of the microbial growth (Fig. 2.4.1). The parabens are one of the most used inhibitors which are additives in consumables and materials for medicine, cosmetics including the food stuff industry [76, 77].

2.4.1. Synthesis and chemical properties

Four new derivatives of Si(IV)-phthalocyanines with axial substitutions (R) of two methyl-, ethyl-, propyl- and butyl- paraben groups which are acting as inhibitors for pathogens are synthesized. There is a paper with an abstract in English from China authors about phthalocyanine linked to methylparaben [78]. The derivatives of Si(IV)-phthalocyanine with different alkyl-parabens were made with $SiCl_2Pc$ and substitution reaction on two axial positions (Scheme 2.4.1). The starting phthalocyanine is a commercial product but can be obtained by following the literature procedure [79]. On the next stage a nucleophilic reaction which includes boiling Cl_2SiPc in dry toluene and pyridine for substitution at two chloride atoms. The compounds are purified by column chromatography on silica gel (SiO_2) with a solvent mixture DCM: EtOH, (5: 0.1). The analyses (ATR-IR, ^1H NMR, ESI-MS and UV-Vis) were carried out with the new derivatives. The mass spectra obtained with ion peak for compound **3a**: 843.414 $[M+H]^+$; **3b**: 870.52 $[M]^+$; **3c:** 921.3292 $[M+Na]^+$ and **3d**: 940.7453 $[M+Na]^+$.



Scheme 4.2.1. Synthesis of Si(IV)-phthalocyanines with axial substituents of different parabens (**3a-3d**); 12-14 %.

Two Si(IV)-phthalocyanine derivatives with methylparaben 3**a** and butylparaben substituents **3d** are studied with their crystal structures which showed the molecular order in packages with two different structures namely a vertical chain and a zig-zag type (published: CCDC 976697 and CCDC 976698). The molecules are not interacted by hydrogen bonds but with two other intermolecular interactions **C-H···π** and **π-π** which served to stabilize the structure with a distance between molecules < **4.0 Å**.

2.4.2. Physicochemical properties

Four Si(IV)- phthalocyanines with axial substituents of different alkyl groups (**3a-3d**) are soluble in most of organic solvents (DCM, DMF, THF) but they have a limited solubility in DMSO. The photophysical studies were carried out in the above solvents for different concentrations. The absorption and fluorescent spectra were recorded in DMF solutions (Fig. 4.2.2). It is well documented that the formation of physical molecular associates lowers the photoactivity of a photosensitizer [35]. In the studied concentration range (10^{-5} – 2. 10^{-6} M) the molecules are monomers. The obtained absorption spectra of **3a, 3b**, **3c** and **3d** characterize with λ_{max} = 682 nm and with the maximum of UV bands at 355 nm (**3a-3d**). The results suggest that with an increasement of the hydrocarbon chain of the substituents, the values of the coefficient **ε** rise without an influence on the position of the absorption maximum of the compounds.

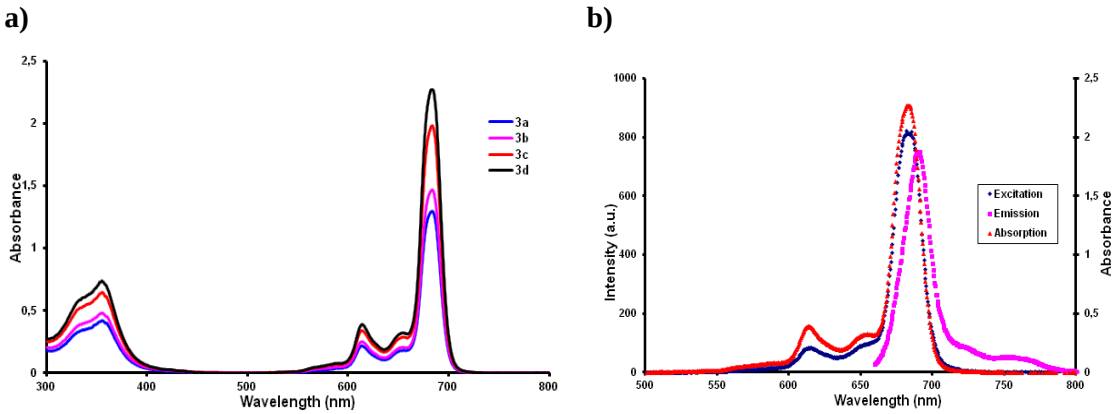


Fig. 4.2.2. Absorption spectra of phthalocyanines 3a-3d (10^{-5} M) in DMF (a) and (b) Absorption, emission, and excitation spectra of **3d** at exc: 650 nm.

The similar fluorescent spectra were recorded in DMF at excitation spectrum 650 nm as shown for SiPc, **3d** with butylparaben groups. As an example, it was shown the absorption, fluorescence, and excitation spectra of SiPc, **3d** with maximum of the fluorescence band at 690 nm (Fig. 4.2.2). The bathochromic shifts are obtained with similar values as for the used standard compound. The new phthalocyanine silicon complexes and derivatives with different parabens were studied with the fluorescence properties. The values show relatively high rates as compared to the standard ZnPc (Table 4.2.1). The fluorescent quantum yield (Φ_F) and fluorescent lifetime (τ_F) are of importance for the photosensitive compounds that are studied for PDT applications. The values of $\Phi_F > 0.2$ are evaluated as high because the fluorescence

is detectable and measurable in turbid media such as cell suspensions of pathogens and for the biofilms. The fluorescent quantum yield shows not dependency on the hydrocarbon chain of the axial substituents of the studied Si(IV)-phthalocyanine derivatives.

Table 4.2.1. Fluorescence properties of phthalocyanines 3a-3d in DMF.

Si(IV)Pc	Φ_F	τ_F (ns)	τ_0 (ns)
3a	0.30	7.11	2.73
3b	0.27	6.04	1.73
3c	0.27	5.54	1.96
3d	0.27	6.41	1.73
ZnPc	0.17	3.99	n.d.

*** [81]**

The exponential curves with two values of the fluorescence lifetime ($\boldsymbol{\tau_F}$) of **3a-3d** were recorded for DMF solutions at excitation with a spectrum 405 nm. This result suggests the formation of molecular associates in small quantity of **3a-3d** (10^{-5} M) in DMF. It is well studied the phenomenon of physical quenching of the singlet excited state of the monomolecular complexes due to aggregation in solution [80]. The fluorescence lifetime showed two values with higher percentage (96 % ± 1 %) of the molecules in monomeric state. The compounds were evaluated with $\boldsymbol{\tau_F}$ values in a wide range 7.11 nm and 5.54 ns in DMF. The monoexponential curves were recorded for compounds **3a-3d** in THF solutions with values between 6.2 nm and 5.8 ns. The dependance of slight decrease was determined between the size of carbohydrate chain and the values of $\boldsymbol{\tau_F}$ from methyl- (**3a)** to butyl-paraben substituted SiPc (**3d**). The highest value of $\boldsymbol{\tau_F}$ was obtained for compound **3a** in DMF as well as in THF (7.11 ns) in comparison to the other three compounds **3b-3d** with values of τ_0 = 6.41ns - 5.54 ns. The evaluation of the fluorescent properties by an application of different excitation spectra suggests the stable electron configuration of molecules without defeat of structural symmetry.

The photooxidation reaction between a molecular probe (DPBF) and a generated in the experimental cell during the red irradiation singlet oxygen was applied. The absorbance

typical for the probe (λ_{max} = 417 nm) was recorded to lower during photooxidation of DPBF. This depends on the quantity of the singlet oxygen molecules which can be formed during irradiation. The photosensitizer as a part of the photochemical reaction is possible to undergo the photooxidation by affecting the ring molecule which leads to structural changes with loss of structure [82]. Our observations suggest that during the measurements, the compounds are photostable towards the generated singlet oxygen. The structural stability of the studied compounds was controlled by the absorbances of **3a-3d** during the photosensitized oxidation reactions. The singlet oxygen quantum yields (Φ_Δ) are 0.46-0.48 which is higher than that for $SiCl_2Pc$, Φ_Δ= 0.28 in DMSO [83]. The longer hydrocarbon chain of different parabens showed no influence on the singlet oxygen quantum yields of phthalocyanines **3a-3d**.

The photosensitizer by irradiation with specific spectrum and exposed on oxygen can participate in the photosensitized reaction with undesirable structural changes of molecules [84]. The photostability of new SiPcs were determined by the quantum yield of photodegradation (Φ_d) of **3a-3d** by irradiation with an energy 18 mW. cm^{-2}. The results suggested a high photostability of the complexes with hydrocarbon chains with values (Φ_d) as followed: 0.18; 0.15 and 0.13, and an unusual value of 6.29 for **3a**.

3. PHTHALOCYANINE HYBRIDES

Photocatalytic action of titanium dioxide and natural sun light is an effect which is well-documented and is commonly applied for disinfection and cleansing of the industrial waters and natural pools [85 a-d]. The next more effective usage is to combine the different metal oxides as additives in surface building materials for prevention and control of pathogens' contaminations of the environment [86]. Presently, TiO_2 is a part of technology for water purification but as an antimicrobial substance the efficacy is still not well explored for a common application. A possible reason is the limitation which is observed in the spectrum of the natural light which covers only 3 % of the absorption spectrum of the known metal oxides and the complex structures by additions of oxides which influence the photocatalytic action but not the spectra of irradiation [87]. Foster et al. [88] described the efficiency of TiO_2 in inactivation of the main forms of pathogenic microorganisms by its action of a photocatalyst which are suitable for control and prevention of spreading of infections. The idea was to combine both mechanisms namely the photosensitization and the photocatalysis which can allow the usage of daily light with the spectrum which covers the absorption of both substances to produce reactive species of different origin.

3.1. Phthalocyanines adsorbed on titanium dioxide.

The new photoactive hybrid was prepared based on the adsorbed ZnPc on the crystal structure of TiO_2 as anatase (25 nm). The appropriate compound for this approach is a hydrophobic Zn(II)-phthalocyanine with four peripheral dodecylpyridyloxy groups (ZnPcDo) which was previously synthesized [89]. It is particularly important to achieve a good adhesion of ZnPcDo with nanoparticles of TiO_2. The suspension of TiO_2 (~ 1 $g.L^{-1}$) in ethanol was prepared by stirring and ZnPc in pyridine was added stepwise. The reaction vessel is placed in ultrasound bath by heating and under argon.

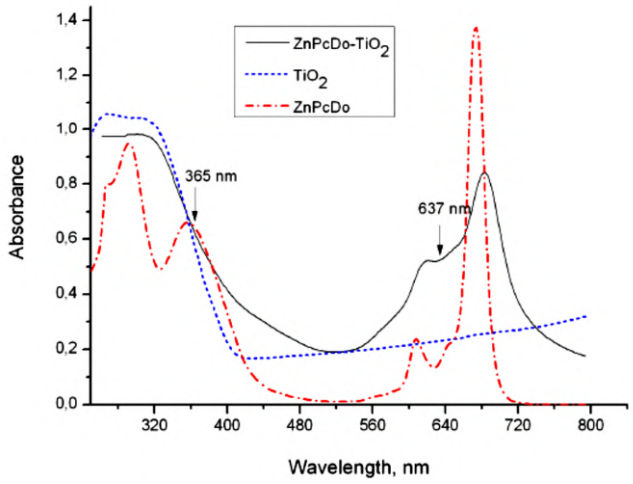


Fig. 3.1.1. Absorption spectra of the composite ZnPcDo-TiO_2 and the pure anatase as solids, and ZnPcDo (10^{-5} M) in DMSO.

The reaction control was carried out by absorption measurements till the absorption remained stable. Then the suspension was filtered and washed till transparency or with not any absorptivity of the filtrate. The hybrid material as light green product was dried at 80 °C. The hybrid material was characterized by the main physicochemical properties. Absorption refraction spectra of Ti_2O and Ti_2O-ZnPcDo were measured as for solids. The absorption spectrum of phthalocyanine ZnPcDo was recorded for DMSO solution (Fig. 3.1.1). The spectrum of the new hybrid material is characterized with a broad band of absorption in the visible spectrum (550-800 nm) and in UV range (250-400 nm). The absorbance in the visible spectral range has two maxima which are at 619 nm and 683 nm, both bathochromically shifted as compared to the maxima of the phthalocyanine alone (610 nm and 674 nm). The characteristic in the spectrum of anatase is a broad band which is covering the UV spectral region with strong absorption up to 400 nm. The hydrophobic phthalocyanine ZnPcDo leads to an expansion of the absorbance to the visible region, which allows the optimal usage of the daily sun light for photodesinfection and decontamination of the natural water supplies. The recorded ATR-IR spectra from the solid state of the nanoparticles TiO_2 and for a composite after adsorption of ZnPcDo showed characteristic vibration at 721 cm^{-1}. The ring molecule

has the bands at 1579 cm^{-1} and at 1497 cm^{-1} for C=N groups. Two strong signals at 1394 cm^{-1} and 1466 cm^{-1} are characteristic for C-N and C-C groups of aromatic structure. The spectra of the composite do not show the vibration bands of the phthalocyanine. IR spectrum of the pure TiO_2 as anatase has signal at 497 cm^{-1}. The vibrations at 2853 cm^{-1} and 2959 cm^{-1} are characteristic for CH_2 groups. The methyl groups of the carbohydrate chain are described with a band at 2925 cm^{-1}. The spectrum shows the bands which are typical for aliphatic chain with position at 2852 cm^{-1} and 2959 cm^{-1}. The characteristic bands of phthalocyanine ring adsorbed on the nanoparticles cannot be show due to mask of the surface from the particles. The inclusion of water molecules in the structure of anatase can also mask the typical vibration bands of the ring molecule.

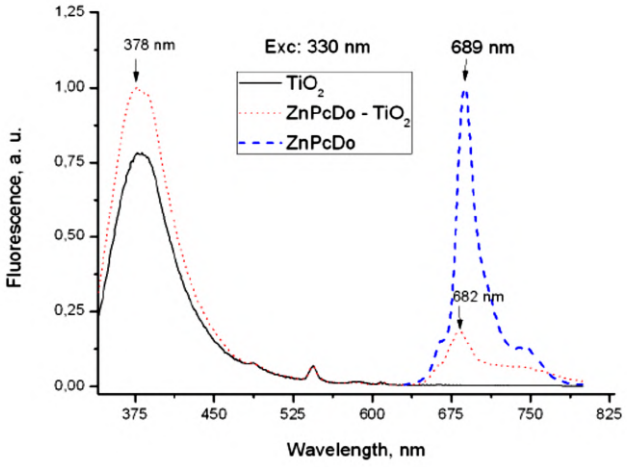


Fig. 3.1.2. Fluorescent spectra of the solids of TiO_2, ZnPcDo-TiO_2 and for solution ZnPcDo in DMSO (exc: 330 nm).

The fluorescent spectrum of ZnPcDo in DMSO showed the typical band of fluorescent emission with λ_{max} = 679 nm which is bathochromically shifted as compared to the absorption λ_{max} = 674 nm (Fig. 3.1.2). The hybrid material with phthalocyanine has low intensity band in the red spectrum with maximum at 682 nm. The high intensity fluorescence was recorded in the UV region with a maximum at 375 nm. The results suggest that the newly prepared hybrid structure is suitable as for photoinactivation of pathogenic species also for visualization of localization in pathogenic species. The fluorescence of the hydride was observed by fluorescence using the CLSM equipment. The obtained images of TiO_2 - ZnPcDo in water suspension and as a layer on the glass showed the typical fluorescence in the red region (660-800 nm) which is characteristic for phthalocyanines. This observation serves as a proof that the attachment of ZnPcDo to titanium dioxide happens in the photoactive monomeric state with intensive fluorescent signal. The new hybrid with properties of photocatalyst and photosensitizer was tested as effective for photodynamic inactivation of pathogenic bacterial species with resistance to the conventional treatments.

3.2. Phthalocyanine hybrids with polymeric brushes.

The hydrophobic interactions between ZnPc molecules and polymer serve as a basis to obtain new hybrid material of molecules with different origin. This approach for conjugation is useful for lipophilic photosensitizers such as unsubstituted Zn(II)-phthalocyanine (ZnPc) and with substitutions with long hydrocarbon chains. This allows preparation of "water-soluble" phthalocyanine which can be directly applied to the target pathogenic species in PDT experiments. As is well studied the active drug release depends on the medium which in most cases can vary in the acidity, the polarity and the surroundings of natural enzymes which can serve as a target specific approach for a proper uptake of photoactive molecules into the target pathogenic or tumor cells [90, 91]. The hydrophobic ZnPc can be dissolved in biocompatible organic solvent (DMSO) in monomeric state, but the addition of some carrier system for incorporation of a photosensitizer is preferable for biomedical applications.

The well-known example for incorporations of the active molecules is the recently introduced biopolymers such as polymeric brushes [92 a, b]. The characteristic feature of nanocontainers with amphiphilic nature allows to incorporate the phthalocyanine owning to the hydrophobic nature of molecule. The used polymeric brushes are synthesized and characterized in the Institute of Macromolecular Chemistry, Russia Academy of Sciences.

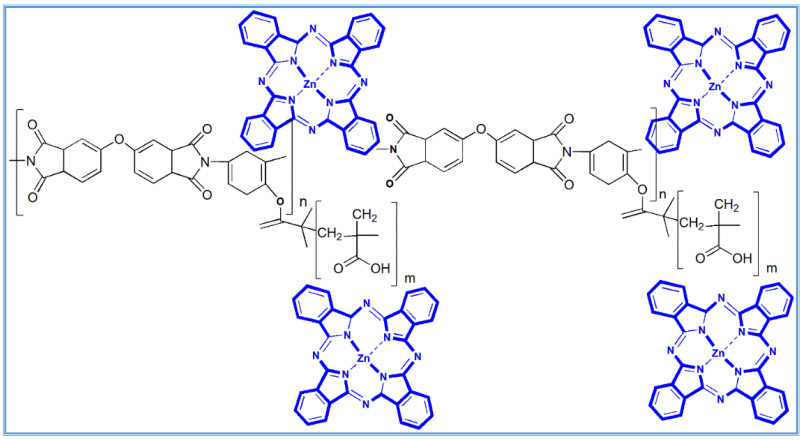


Fig. 3.2.1. The possible configuration of ZnPc with polymeric brushes: PAT2: n = 53 (P1, Mw / Mn = 2,14 of GPC), m = 183 (PMAA Mw / Mn = 1,5 of GPC); PAT3: n = 53 (P1, Mw / Mn = 2,14 of GPC), m = 155 (PMAA Mw / Mn = 1,36 of GPC).

The polymers consist of polymeric backbone with long polymeric side chains with high density of the molecular fragments. The backbone is hydrophobic and suitable for interactions with hydrophobic phthalocyanine **ZnPc** (Fig. 3.2.1). The side chains characterized with hydrophilicity due to carboxylic groups and they can react on outer factors with adoption of their properties to the target membranes. One typical example is the acidity which is typical for tumor and other diseases conditions (pH 6 - 6.5). In this condition the side chains undergo

the conformational changes to adapt the molecule to the cell membranes. As it as expected, the fluorescence emission of ZnPc (690 nm) was not registered in PBS buffer (pH 7.4) but the addition of the polymers serves as a container of the phthalocyanine which turns to fluoresce with a typical high intensity emission spectrum in the red region. This phenomenon allows a fast route to prove the monomeric photoactive state of phthalocyanine dye incorporated in polymeric brushes.

4. PHTHALOCYANINES TOWARDS PATHOGENS WITH RESISTANCE

The studies of the key photobiological results such as the uptakes, the localization, and the antimicrobial efficacy of the phthalocyanine derivatives as photodynamic sensitizers towards pathogenic species and tumor cell are summarized. The discussions and conclusions about the relationship between the chemical structure and the most important photobiological functions are presented.

Currently a limited number of phthalocyanine complexes are clinically approved on different stages of evaluation and only a few have been applied for curative usages with PDT method [93]. Nevertheless, the long lasting history of development and clinical assessment, the approved phthalocyanines possess disadvantages which diminish their PDT efficacy. The most critical properties of the approved drugs are a low selectivity and the lack of specificity of the uptakes and some dark toxicity for ZnPc which diminish the activity [94, 95].

In our research studies, the photodynamic effect was observed in dependance on the uptakes and localization of phthalocyanine derivative in bacterial strains of the Gram (+) *Staphylococcus* and *Streptococcus,* which belong to pathogens causing bacteremia. The most widely imposed specie is *Staphylococcus aureus* (MRSA) which is normally existed on skin and breathing system. Another pathogenic bacterium *Streptococcus mutans* is a cariogenic strain which occurs in mouth. The bacterial strains *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Aerumonas hydrophila (A. hydrophila),* and *Salmonella enteritidis* have characteristic of a Gram (-) species which can cause acute infections with bacteremia. The PDT studies were carried out also on a fungal strain of *Candida albicans* (*C. albicans*).

4.1. PHOTODYNAMIC INACTIVATION OF PATHOGENIC BACTERIA AND FUNGUS

The photodynamic inactivation capability is a result of the oxidation consequences on the level of the cellular membranes as they consist of biomolecules as was reported for lipids, proteins, RNA for the bacterium *Escherichia coli* [101].

4.1.1. *In vitro* studies with cationic phthalocyanine complexes

The studies of the antimicrobial activity of cationic MPc complexes of Lu(III), Sn(IV) and Pd(II) with peripheral and non-peripheral positions of the substitution groups (**1.1**) were carried out on representative Gram (-) bacterial strain *P. aeruginosa* and an yeast *C. albicans* as suspensions with cell density of approx. 10^6 CFU.mL^{-1}. In a real situation, the pathogens tend to form biofilms, so that the efficacy of the studied phthalocyanines were also tested on samples of bacterial and fungal biofilms grown on a glass slide. The results obtained for the complexes of Lu(III)Pcs after incubation with concentrations between 1 μM - 30 μM and irradiation with spectra at 635 nm or 665 nm (LEDs) with a light dose of 50 J. cm^{-1} and power density of 60 mW. cm^{-1} are shown in Fig. 4.1.1. The studied lutetium complexes are not toxic in dark which can be considered as not toxic effect due to the metal ions. As a result of the optimal physicochemical properties the full photodynamic inactivation was observed but at relatively high concentrations for photosensitizers which are 20 μM LuPc, **3a** (*P. aeruginosa*) and 30 μM (*C. albicans*). Considering the metal ion, the non-peripheral complexes Lu(III)Pc, **3a** and Pd(II)Pc, **7a** were evaluated with higher efficacy (> log 3) at lower concentrations for **7a** as compared to tin complex Sn(IV)Pc, **5a**, at similar experimental conditions.

The peripheral complex Lu(III)Pc, **4a** showed inactivation effect but at relatively high concentrations than the observed for zinc complex (**ZnPcMe)** with the same substitution groups which was previously tested on different Gram (-) bacterial species [96]. The relatively high photoactivity with full inactivation of several typical dental pathogens were observed at concentrations 3-6 μM for complexes of Ga(III), Al(III), Si(IV) with peripheral substitution of methylpyridyloxy groups. The study of group of T. Nyokong [97] of two In(III)Pcs showed that tetra- substituted is more active than mono- substituted InPc towards *E. coli*. This suggests that the number of positive charges is more important than the molecular symmetry.

The main conclusion of the studies is that replacement of zinc with another diamagnetic ion with a high atom number such as lutetium or tin led to an improvement of optical physicochemical properties of the MPc complexes but not as expected with an impact on the photoinactivation capability towards pathogenic cells.

The size of *C. albicans* is rounded to oval 3-8 μm and based on the actual fluorescent microscopy, both allows the precise evaluation of the localization of the studied MPc by the means of a confocal laser scanning microscopy (CLSM). The early-stage biofilms (48 h) of the species *P. aeruginosa* and *C. albicans* were evaluated with a measurable fluorescence emission signal of the incubated LuPc, **3a** (Fig. 4.1.1).

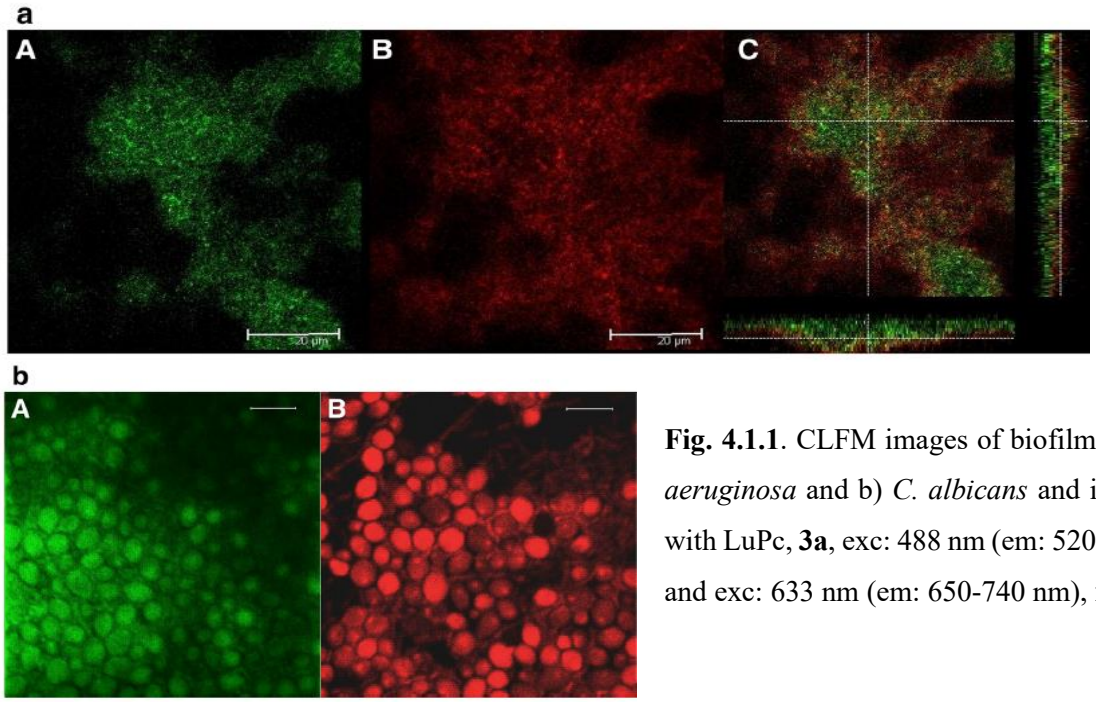
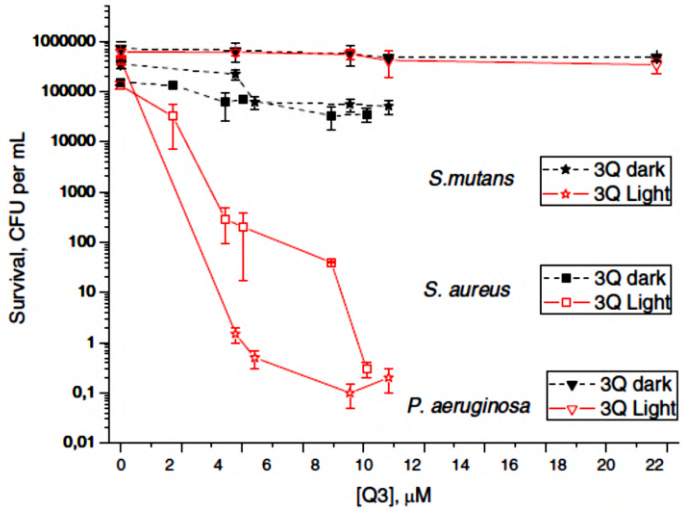


Fig. 4.1.1. CLFM images of biofilms of a) *P. aeruginosa* and b) *C. albicans* and incubated with LuPc, **3a**, exc: 488 nm (em: 520-580 nm) and exc: 633 nm (em: 650-740 nm), x 40.

The native fluorescence (**A**) which is in a spectral region 520 - 580 nm showed that the studied compound **3a** with a red spectrum 660 - 740 nm (**B**) is localized partly in the biofilm but also in the loose pockets as seen by the overlapped layers (**C**). In case of fungus *C. albicans* is seen that **3a** is localized in the membranes but also inside the fungal cells which is typical for the cells without viability. The depth of penetration of the studied MPc into formed biomass was evaluated by following both fluorescent signals of the native fluorophores vs. the studied MPcs. The scanning on y-axis provided an information about the penetration depth into biofilms for cationic MPcs which was shown with the full penetration into the biomass and only parts of biofilm was observed with a limited penetration.

The photodynamic inactivation was observed on the suspension of pathogenic strain methicillin resistant *S. aureus* (*MRSA*) with a density of 10^6 CFU. mL^{-1} for 10 μM PdPc, **7a** and a spectrum of irradiation with a LED 665 nm and light dose of 60 $J.cm^{-2}$ for an energy of 50 mW. cm^{-2}. The compounds are evaluated for concentrations up to 12 μM. There is a dependance of the inactivation efficacy on the concentrations up to 5-6 μM with the viability value below 3 logs.

The photoinactivation studies with a cationic silicon complex **3Q** (2.1) were carried out on three pathogenic bacterial strains *S. aureus*; *S. mutans* and *P. aeruginosa* as a suspension with cell density ~10^6 CFU mL^{-1} (Fig. 4.1.3).



Фиг. 4.1.3. Survival of bacteria in a suspension (10^6 CFU.mL^{-1}) after incubation with a range of concentration of Si(IV)Pc, **3Q** and irradiation with LED 635 nm (50 J. cm^{-2}).

Si(IV)-phthalocyanine derivatives (**3Q** and **4Q**) were evaluated without dark toxicity for a wide concentration range. After irradiation **3Q** showed high efficiency on the Gram (+) bacteria (> log 3) at concentrations above 5 µM. The full inactivation capacity was observed for concentrations 9 and 10 µM. The Gram (-) strain characterizes with high resistance to any inactivation's due to the complex structure of bacterial cell wall. The results showed not inactivation response up to 22 µM **3Q**. There is a dependance on the nature of the bacterial strain for its susceptibility to PDT action.

The experimental results with differently substituted silicon phthalocyanines on methicillin resistant *S. aureus* suggested that the complex with bulky groups on axial positions (**3Q**) is more effective than the starting Cl_2SiPc without substituents and as compared with SiPc, 2 from our previous studies with four peripheral methylpyridyloxy groups [9]. The peripheral substituents in SiPc seems more appropriate as molecular probe with its high fluorescence signal for optical detection. This for example can be useful for biofilm characterization and for the drug localization into the target pathogens. The scanning of the *MRSA* biofilm incubated with **4Q**, slide by slide with a thickness of 0.150–0.200 µm showed the penetration depth of the studied SiPc between 8-11 µm which suggested the full penetration into the formed biomass. The high photodynamic activity of both cationic SiPcs complexes (**3Q** and **4Q**) was observed on the studied pathogenic strains of Gram (+) bacteria *Streptococcus mutans* and *Staphylococcus aureus*, with an inactivation capability at very low concentrations of compound [**4Q**] = 3 µM. On the resistant Gram (-) strain *P. aeruginosa* both silicon complexes showed potential for inactivation but at relatively high concentrations (> 10 µM).

Presently, in the emergency cases of bacteremia the phenothiazine dyes such as methylene blue (MB) and toluidine blue (TBO) are well accepted. In the former time these dyes were used for the antibacterial action without light (in veterinary medicine) and nowadays these dyes are still actual photosensitizers because of the wide absorption spectrum favorable for irradiation with a white light [99 a, b].

4.1.2. *In vitro* study of zwitterionic and cationic Zn(II)-phthalocyanines

A comparative photodynamic inactivation study was carried out with four water-soluble zwitterionic Zn(II)-phthalocyanines with four or eight substitution groups of oxypyridine or mercaptopyridine 2-(N-propane sulfonic acid (ZnPc1-4). The photoinactivation studies with ZnPc1-4 showed a full inactivation of the pathogenic bacteria *E. faecalis* and *P. aeruginosa* with ZnPc**3** which is octa- substituted complex at concentration of 6 μM. Both tetra- peripheral substituted ZnPc**1** and ZnPc**2** complexes showed similar values but for the non-peripheral the inactivation effect was not observed. The Gram (-) strain is not responded for the treatment even at extremely high ZnPc1-4 concentrations of 30 μM. The studies suggest that photodynamic inactivation with the peripherally substituted zinc complexes is more effective than that for the non-peripheral ZnPc4 complex (> 3 log). The cytotoxicity study on the cell line Balb/c 3T3 showed that ZnPc1-4 are not toxic in the dark but after irradiation with PDT light dose and energy (50 J. cm^{-2} and 60 mW. cm^{-2}) only for octa- substituted complex ZnPc3 the photocytotoxicity was not observed.

Cationic Zn (II)-phthalocyanines with four or eight methylpyridyloxy groups on peripheral or non-peripheral positions were studied for evaluation of structure – PDT activity towards pathogenic bacteria. In this group of phthalocyanines the maximal efficacy showed the complexes with four and eight peripheral methylpyridyloxy groups with oxygen (ZnPc**1**) as well as with sulfur (ZnPc**4**) as the linkage atoms.

The general conclusion of these studies with emphases the dependence of the efficacy on the charges, the position, and the linkage atom of the substitution groups to ZnPc macrocycle and the influence on the uptakes and the photodynamic efficacy. The zwitterionic ZnPc3 and a cationic ZnPc1 complexes have high uptakes in pathogenic cells and high selectivity of the photocytotoxicity of the normal versus pathogenic cells.

4.1.3. Uptakes and phototoxicity of bioconjugates of Zn(II)-phthalocyanine

The photoinactivation studies with bioconjugates of Zn(II)-phthalocyanine with different amino acids were carried out with suspensions of fungus *Candida albicans* (~ 10^7 CFU.mL^{-1}) following the well-developed procedure for testing the new photosensitizers. The protocol included several steps, starting with an incubation of the studied ZnPcs with tetra- and octa- phenylalanine ZnPcs (TZnPcPhe, OZnPcPhe), with arginine (TZnPcArg, OZnArg) and with lysine groups (TZnPcLys, OZnPcLys) in a concentration of 10 μM by irradiation with a light source LED 665 nm with irradiation dose 50 J.cm^{-2} and the power density of 60-100 mW. cm^{-2}. The results showed not dark toxicity and after irradiation the inactivation effect was observed (Fig. 4.1.4). As seen, there is not photoinactivation of the complexes before linkage of amino acids as was obtained for the complexes with zinc with the same substitutions. The irradiation led to a significant inactivation with tetra- phenylalanine substituted complex (TZnPcPhe).

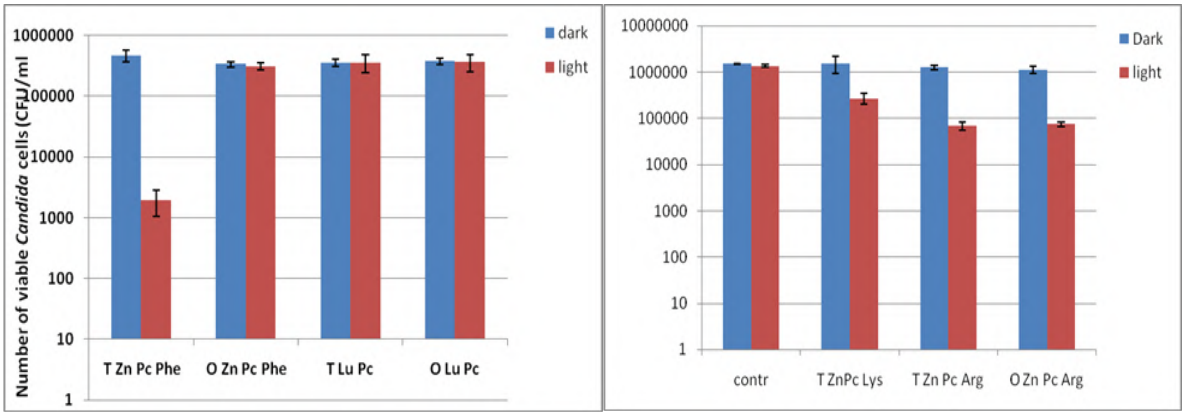


Fig. 4.1.4. Photocytotoxicity of *C. albicans* after incubation with phthalocyanines' bioconjugates with amino acids at irradiation (LED 665 nm, PDT group) and without irradiation (dark control).

The newly prepared bioconjugates containing phthalocyanine molecule and cationic amino acids in physiological conditions were studied for the uptake and localization based on their fluorescence property. The results were obtained by usage of two methodologies, namely the chemical extractions of the studied derivative after incubation into the pathogenic cells and the fluorescence measurements of the collected samples; by a direct fluorescence imaging which visualized the location of a photosensitizer in the fungal cells.

The cell wall of yeast *Candida albicans* characterizes with a property of an ion exchanger, with the exceptions of the positively charged ions and molecules such as proteins with a binding possibility. This feature was taken into consideration for creation of a structure of a relatively ideal photosensitizer. The possible interactions between photosensitive molecules of new derivatives are hydrophobic because of the Pc ring and additionally through the hydrogen bonds due to the free amino groups of the substituents. Here, should be included the cationic charge of lysine and arginine at physiological medium which can contribute to the interactions with membranes and to allow the saturation with significant amounts of MPcs. The cationic phthalocyanine complexes of Lu(III), Sn(IV) and Pd(II) showed high uptake in the pathogens as was observed in our systematic work on model and clinical pathogenic strains. The procedure with variations is described in our published papers on the PDT topic [8, 9]. The fluorescence spectra are recorded in the region between 670 - 800 nm and based on the calibration curves the numbers of bounded molecules per cell were calculated (Fig. 4.1.5).

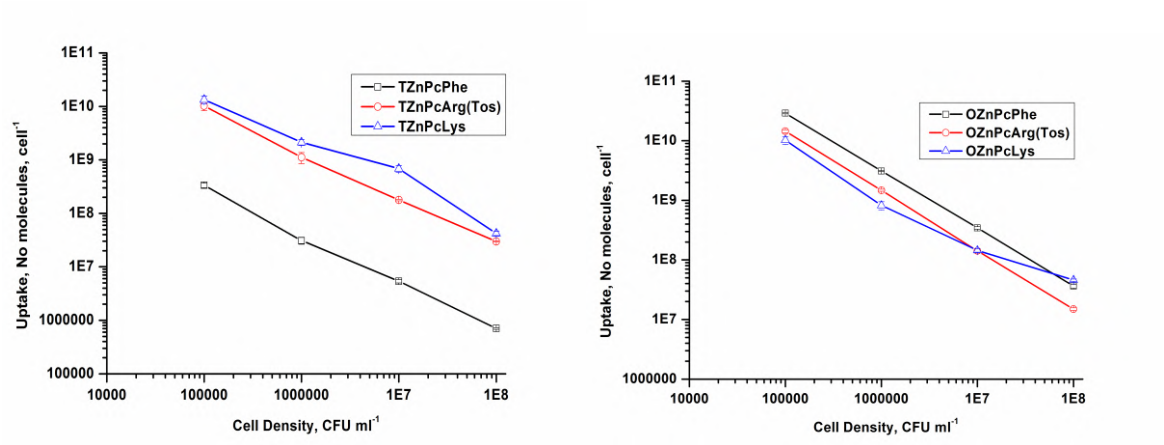


Fig. 4.1.5. Uptake of the conjugates of phthalocyanines with amino acids (5.5 μM) in *C. albicans* cells for suspensions in a concentration range. The data are presented as an average value ±SD (n=3).

The results suggested relatively high uptakes of tetra- and octa- arginine and lysine substituted Zn(II)-phthalocyanine derivatives as compared to phenylalanine with two order of magnitude lower values. The decreasing of the number of molecules/ one cell with the increasement of the cell density (10^5-10^8 CFU. mL^{-1}) was observed for all tested pathogenic strains. This phenomenon was firstly reported by T. Demidova & M. Hamblin, 2005 for *Escherichia coli* [100]. The obtained results confirmed that the uptakes are in dependence on the density of cell suspension together with the structure of the phthalocyanine derivative.

4.1.4. Phototoxicity of phthalocyanine adsorbed on titanium dioxide.

The photoinactivations potential of hydrophobic phthalocyanine (ZnPcDo) adsorbed on titanium dioxide as anatase (TiO_2, 25 nm) was observed on two representative pathogenic strains, namely a Gram (+) strain *Staphylococcus aureus* (*MRSA*) and a Gram (-) strain *Salmonella enteritidis* (Fig. 4.1.6). The effect was evaluated for two light sources (UVA lamp 346 nm and LED 643 nm) of exposure from a single light source or from both.

The hydrophobic derivative ZnPcDo applied from DMSO solutions (< 5 % DMSO from a total volume) showed not inactivation efficacy towards the Gram (-) bacterium strain. ZnPcDo adsorbed on titanium dioxide and by irradiation with UVA showed full inactivation or with combination with red light the photoinactivation increase with ~ 5 log loss of viability (Fig. 4.1.6).

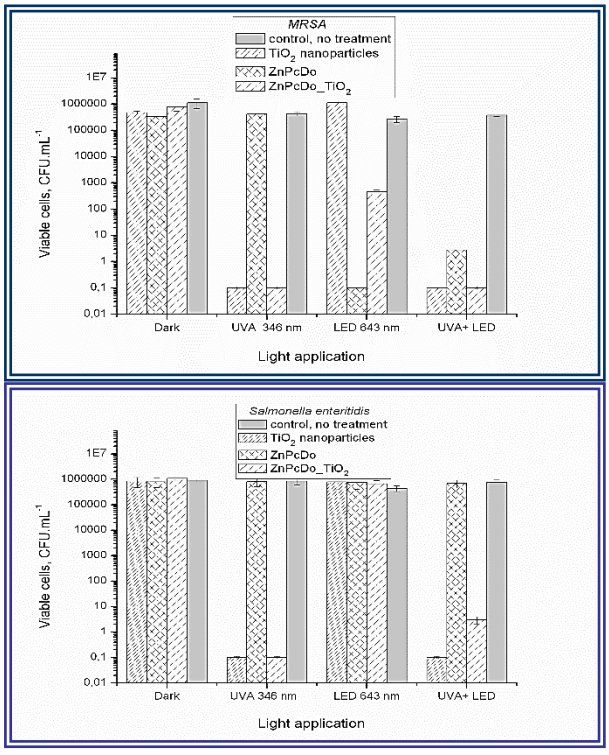


Fig. 4.1.6. Photodynamic inactivation with hydrophobic phthalocyanine adsorbed on TiO_2 (1 g. L^{-1}) by irradiation with UVA 364 nm, LED 643 nm and both UVA 364 nm + LED 643 nm of *MRSA* (a) and *S. enteritidis* (b). The ingredients were studied at the same conditions.

The irradiation with red visible spectrum (643 nm) of the composite ZnPcDo-TiO_2 showed not photoactivity to Gram(-) pathogen which can be due to the effect of physical reflection without absorption of red light from the hybrid suspension. The simultaneous irradiation leads to lower efficacy than only UVA spectrum which is suitable for both parts of the composite. In case of *MRSA* strain the obtained results show the high efficiency with TiO_2 and irradiation with UV source (346 nm); for ZnPcDo and red LED 643 nm; as for the simultaneous exposure with UVA+LED for the hybrid ZnPcDo+Ti_2O. In the experiments with a group of Zn(II)-phthalocyanines only *dodecyl* pyridyloxy ZnPcDo showed dark toxicity on a *A. hydrophila* strain. The same compound ZnPcDo after adsorption on TiO_2 has not dark toxicity.

The main conclusion from the brief in vitro experiments is that the newly prepared hybrid based on the hydrophobic and toxic in the dark phthalocyanine ZnPcDo with an advantage of broadening the spectrum of the applied irradiation and inactivation occurs by two possible mechanisms, and in hybrid, the dark toxicity of the phthalocyanine (which belongs to the long hydrocarbon chain) is diminished. Relating to the sun light spectrum, the composite can be useful for the development of environmentally friendly photocatalysts with application for photodesinfection and decontamination of natural and industrial water resources.

4.2. PDT TOWARDS VIRUSES

The studies of antivirus activity of the metal phthalocyanine complexes together with the photodynamic method have been part of the research studies since last thirty years [102-105]. The rare investigations focus on the photodesinfection of blood products, as for example is the study with Al(III)-phthalocyanine complexes with efficacy for inactivation of viruses (Sindbis virus, VSV and HIV-1) contaminating the blood products [102]. The related structurally cationic porphyrin derivatives {Tri-P(4)} were also evaluated with a high inactivation efficiency for viruses: bovine viral diarrhea virus (BVDV), VSV, HIV-1 and pseudorabies virus [103].

During the time of working on these experiments (during 2010 and a few further years) with Drs. Nikolaeva-Glomb and M. Remichkova, the information about the relationship photosensitizer's structure vs. PDT efficacy was almost not known. The aim of our studies was to compare the efficacy of two phthalocyanines differing in charges namely the cationic Zn(II)-phthalocyanine (ZnPcMe) and anionic sulfonated Zn(II)-phthalocyanine (ZnPcS) on viruses which belong to different taxonomic groups (BVDV, HSV-1 and VV). The characteristic of these viruses is that they have a lipid layer or lipid envelope and are named enveloped viruses. The second group of viruses under investigations are so called the non-enveloped viruses such as Coxsackievirus B1 and human adenovirus type 5. The characteristic of enveloped viruses suggests a high sensitivity of these forms to photodynamic action as was obtained for HSV-1 virus for mild irradiation. *Smetana et al*., [104] described the phthalocyanine derivatives with different substitution groups having non-uniform efficacy towards herpes virus which was explained with the role of substituents to the mechanism of inactivation. The photoinactivation of enveloped viruses can be explained by the interruption of the lipid layer with tend to stop the interaction of the virus with hosting mammalian cells. The strain Vaccinia virus was inactivated with a dark cytotoxic effect. Considering the charges

of the studied phthalocyanines, it was noticed a difference with significance for the strain BVDV with two times higher efficiency of anionic ZnPcS than cationic ZnPcMe. There was not inactivation efficacy for the NDV strain. The group of viruses without lipid layer was not inactivated with PDT procedure which maybe a result of the lack of the target such as the lipid layer. According to other authors [105] the non-enveloped viruses are not sensible to phthalocyanines and PDT due to protein Capsid and the genome as a target for the photoinactivation.

A comparative study was carried out with two cationic phthalocyanine complexes of gallium and indium (Ga4 и In4) with a structure as for zinc phthalocyanine (ZnPcMe), and octa- methylpyridyloxy substituted Ga(III)-phthalocyanine (Ga8). Costa et al., 2012 [106 a, b] reported that the enveloped viruses are inactivated easier than the non-enveloped because of the proper target such as this layer and the efficacy of inactivation depended on the virus strain (forms). The obtained results of our studies suggested a high photoinactivation of some of the tested viruses such as BVDV, but without effect on the popular during this time influenza virus A(H3N2). The successful inactivation of the first strain was published before in the work of Ben-Hur et al., 2000 [107], but the results on photodynamic inactivation of flu virus was not published till the year 2017, maybe because they still not responded to PDT action. The phthalocyanine derivatives together with PDT method feature as effective approach to be use in decontamination of the blood products, the photodesinfection of surfaces and instruments for medicine, and for antiviral safety control of the environment [108, 109]. Moreover, since the late months of 2019, the accidental situation with a new strain of a coronavirus SARS-Cov-2 arises the emergency conditions to keep under control the pathogen challenges for human being life in the threatening environment.

The conclusion from these studies is that the lipid layer appears a possible target for inactivation of enveloped viruses by a mechanism of the photosensitized oxidation. The non-enveloped viruses as a relatively resistant forms towards existing therapeutics can be inactivated by PDT with phthalocyanines as was shown for complex with gallium. The results with human adenovirus are in support to the PDT method as a promising alternative approach for treatment of resistant species.

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IV. CONTRIBUTIONS

1. New phthalocyanine derivatives were synthesized as complexes of the traditional metals of *zinc*, *silicon* and *palladium* and the atypical metal ions such as *lutetium*, *tin* and *nickel*, not known for phthalocyanines with PDT applications. The newly synthesized phthalocyanine derivatives can be divided according to the structural features in two main groups which are included:

1.1. According to *the coordinated metal ions*:

These are complexes of Lu(III), Sn(IV), Pd(II), and Ni(II). Thece complexes have pyridyloxy groups in peripheral and non-peripheral positions which after methylation are converted to the water-soluble cationic phthalocyanines.

The complexes of the typical metals for phthalocyanines such as Zn(II) and Si(IV) are prepared as derivatives with numerous different substitution groups.

1.2. According to *the biologically active substitution groups:*

There are bioconjugates with functionality by itself and by the chemical linkage to phthalocyanines with aims such as the increasement of ability to interactions with cell wall, the improvement of the cell uptake and the selectivity to the disordered cells. The newly prepared original Zn(II)-phthalocyanine derivatives are functionalized with the following biologically-active units:

- Amino acids: *tyrosine, phenylalanine, arginine* and *lysine*;
- Carbohydrates: *galactose* (*furanose)*;
- Sterols: *mestranol (cholesterol)*;
- Inhibitors: *methyl-, ethyl-, propyl- and butyl parabens*.

1.3. Phthalocyanine hybrid materials with *titanium dioxide* and *polymers*.

2. The original synthetic schemes are developed based on the well-known synthetic reactions and procedures for phthalocyanines which are modified accordingly to prepare the new derivatives. These include the chemical substitution of the phthalocyanine macromolecule on peripheral positions by the several linkage groups and possible chemical bonds:

1) with amino acids through aminophenoxy groups by dehydration and amide bond;
2) with carbohydrates or parabens by direct linkage with ether bond;
3) with carbohydrates and sterols linked through 1,2,3-triazole with Click reaction;

The applied synthetic procedures for synthesis of new phthalocyanine derivatives included the follow approaches:

1) the linkage to the monomer 3- or 4- substituted phthalonitrile and next cyclotetramerization reaction;

2) the linkage to the possible peripheral (four and eight) positions and non-peripheral four positions of phthalocyanine ring molecule;

3) the nucleophilic substitution at silicon of Si(IV)-phthalocyanine complex Cl_2Si(IV)Pc with chromophore of bulky groups and the inhibitors like parabens which are in use because of the antibacterial properties.

3. New phthalocyanine derivatives were examined with the main photophysical and photochemical properties which are related to the PDT method, by using self-configured set-up developed especially for the spectra of absorption and fluorescence of phthalocyanine dyes. The obtained values of the photo- physicochemical properties agree with the structural characteristics of the presented phthalocyanines with values suggesting their potential as photosensitizers for PDT method.

4. The pharmacological characteristics of new phthalocyanine derivatives such as the uptakes and localization were determined based on the specifically developed procedure which included chemical extraction and fluorescence detection of phthalocyanines in pathogenic cells. This method is valuable for compounds with detectable fluorescence and emission spectra which is not overlap the spectrum of natural chromophores (MPcs, em: 660 – 850 nm). The relatively high uptakes were evaluated for the tested resistant and multi-resistant strains of the Gram(+) and more likely for the Gram(-) bacterial pathogens and for a fungus strain *Candida albicans*. The localization of phthalocyanine derivatives were determined on pathogenic biofilms based on the fluorescence property (CLSM). A high selectivity was observed for galactosylated and for tyrosine substituted Zn(II)-phthalocyanines on *Candida*.

5. Original *in vitro* protocols for photobiological experiments with pathogenic microorganisms, bacteria, fungi, and viruses, and for tumor – normal cells were developed which are applicable for studies of photosensitizers with similar structures. The important conditions for *in vitro* studies are determined. These are the range of concentration for PDT on cells with phthalocyanine derivatives (0.1 - 20 μM), the range of applicable light doses (12 - 60 J. cm^{-2}) and power density of the applied irradiation (50 - 100 $mW.cm^{-2}$) without heating or other undesirable effects so that the obtained conditions are applicable for variety of pathogenic species studied for efficiency of their inactivation with the photodynamic method.

SUMMARY OF THE OPTIMAL PROPERTIES OBTAINED WITH NEW PHTHALOCYANINE DERIVATIVES

MPcs (DMSO)	Abs. λ_{max}, nm	Fl. λ_{max}, nm	τ_{fl}	ϕ_{fl}	ϕ_{Δ}	Conc. range for phototoxic effect of log 3
Lu(III)Pc, 3a	685	721	2.24	0.012	0.35	10 - 20 µM
Sn(IV)Pc, 6a	681	707	1.86	0.090	0.43	5 - 10 µM
Pd(II)Pc, 8a	676	684	3.14	0.160	0.68	10 - 12 µM
Si(IV)Pc, Q3 (PBS)	680 (688)	685 (693)	5.27 (4.94)	0.260 (0.250)	0.18 (0.15)	4 - 8 µM
Si(IV)Pc, 3a	683	690	7.11	0.300	0.47	5 - 10 µM
Si(IV)Pc, 3d	682	690	6.41	0,270	0.48	5 - 10 µM
TZn(II)Pc-TYR	680	692	2.92	0.120	0.63	5 µM
TZn(II)Pc-PHE	682	692	2.82	0.070	0.71	5 µM
OZn(II)Pc-ARG	682	692	2.56	0.040	0.40	10 µM
OZn(II)Pc LYS	681	690	n.d.	0.030	0.57	10 µM
nZn(II)PcGal	702	707	2.75	0.060	0.49	6 µM
Zn(II)Pc, 8	681	693	3.47	0.080	0.46	n.d.
Zn(II)Pc	671	680	3.99	0.200	0.67	Dark toxicity

V. ANEXES

1. List of publications

1. Mantareva*, V.; Kussovski, V.; Angelov, I., Cationic Metal Phthalocyanines as Effective Photosensitizers towards Pathogenic Microorganisms. In: *Photosensitizers: Types, Uses and Selected Research, Series: Chemistry Research and Applications*, Cody Whitmire (ed.), 2016, (24 pages). (Book Chapter)

2. Syuleyman, M.; Angelov, I.; Mitrev, Y.; Durmus, M.; **Mantareva*, V**., Cationic amino acids linked to Zn(II) phthalocyanines for photodynamic therapy: Synthesis and effects on physicochemical properties, *J. Photochem. Photobiol. A*, 2020, 396, 112555. (**Q1**)

Citates: **3**

3. Aliosman, M.; Goksel, M.; **Mantareva*, V**.; Stoineva, I.; Durmus, M., Tyrosine conjugated zinc(II) phthalocyanine for photodynamic therapy: Synthesis and photophysicochemical properties, *J. Photochem. Photobio*l. *A*, 2017, 334, 101. (**Q1**)

Citates: **11**

4. Taskin, G. C.; Durmus, M.; Yuksel, F.; **Mantareva, V.****; Kussovski, V.; Angelov, I.; Atilla, D., Axially paraben substituted silicon(IV) phthalocyanines towards dental pathogen Streptococcus mutans: Synthesis, photophysical, photochemical and in vitro properties, *J.Photochem. Photobiol. A*, 2015, 306, 31-40. (**Q1**)

Citates: **22**

5. Omeroglu, I.; Kaya, E.N.; Goksel, M.; Kussovski, V.; **Mantareva, V.**; Durmus, M., Axially substituted silicon(IV) phthalocyanine and its quaternized derivative as photosensitizers towards tumor cells and bacterial pathogens, *Bioorg. Med. Chem*., 2017, 25, 5415. (**Q1**)

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6. Aliosman, M.; Angelov, I.; Mitrev, Y.; Iliev, I.; Durmus, M.; **Mantareva*, V.,** Novel Zn(II) phthalocyanine with tyrosine moieties for photodynamic therapy: Synthesis and comparative study of light-associated properties, *POLYHEDRON*, 2019, 162, 121-128. (**Q2**)

Citates: **5**

7. Mantareva*, V.; Durmus, M.; Aliosman, M.; Stoineva, I.; Angelov, I., Lutetium(III) acetate phthalocyanines for photodynamic therapy applications: Synthesis and photophysicochemical properties, *Photodiag. Photodyn. Ther*., 2016, 14, 98-103. (**Q2**)

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9. Mantareva*, V.; Gol, C.; Kussovski, V.; Durmus, M.; Angelov, I. Impact of water-soluble zwitterionic Zn(II) phthalocyanines against pathogenic bacteria, *Z. Naturforsch. C*, 2019, 74(7-8), 183-191. (**Q3**) **Citates**: **3**

10. Omeroglu, I.; Goksel, M.; Kussovski, V.; **Mantareva*, V**.; Durmus, M., Novel Water-Soluble Silicon(IV) Phthalocyanines for Photodynamic Therapy and Antimicrobial Inactivations, *Macroheterocycles*, 2020, 12(3), 255-264. (**Q3**) **Citates**: **5**

11. Kussovski, V.; **Mantareva*, V**.; Durmus, M.; Angelov,I., Quaternized Zn(II) phthalocyanines for photodynamic strategy against resistant periodontal bacteria, *Z. Naturforsch. C*, 2018, 73(5-6), 221-228. (**Q3**)

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Citates: **9**

13. Nikolaeva-Glomb, L.; Mukova,L.; Nikolova,N.; Kussovski,V.; Doumanova,L.; **Mantareva, V**.; Angelov,I.; Wohrle,D.; Galabov, A.S., Photodynamic Effect of some Phthalocyanines on Enveloped and Naked Viruses, *Acta Virologica*, 2017, 61, 341-346. (**Q3**)

Citates: **7**

14. Angelov, I.; Kril, A.; Dimitrov, R.; Borisova, E.; Avramov, L.; **Mantareva, V.**, Light enhancement of in vitro antitumor activity of galactosylated phthalocyanines. *Photonics & Lasers in Medicine*, 5, De Gruyter, 2016, 1-18. (**Q3**)

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15. Mantareva*, V.; Eneva, I.; Kussovski, V.; Borisova, E.; Angelov, I., Antimicrobial photodisinfection with Zn(II) phthalocyanine adsorbed on TiO2 upon UVA and red irradiation, *Proc. of SPIE*, 9447, 2015, 94470W1-9. (**SJR**)

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Citates: **17**

17. Semyachkina-Glushkovskaya, O.; Borisova, E.; **Mantareva, V**.; Angelov, I.; Eneva, I.; Terskov, A.; Mamedova, A.; Shirokov, A.; Khorovodov, A.; Klimova, M.; Agranovich, I.; Blohina, I.;Lezhnev, N.;Kurths, J., Photodynamic Opening of the Blood–Brain Barrier Using Different Photosensitizers in Mice, *Appl. Sciences*, 2020, 10 (33), 1-12. (**Q1)**

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18. Eneva, I.; Aliosman, M.; Angelov, I.; Popov, K.; **Mantareva*, V**., Mono-ring phthalocyanine complexes of large ions Lu^{3+} and Sn^{4+}: synthesis and comparison of photophysical properties, *Bulg. Chem. Comm*., Special Issue D, 2017, 246-252. (**Q4**)

Citates: **1**

19. Borisova, E.; Genova, Ts.; Yakimanski, A.; **Mantareva, V**.; Angelov, I.; Gisbrecht, A.; Khorovodov, Al.; Agranovich, I.; Klimova, M.; Semyachkina-Glushkovskaya, O., Conjugation of Zn (II) phthalocyanine with polymeric brushes for improved photodiagnostics and photodynamic therapy, *Proc. SPIE*, 11457, 2020, 114570R-1-5 (**SJR**).

20. Mantareva*, V.; Aliosman, M.; Durmus, M.; Angelov, I., Amino acids substituted phthalocyanine complexes: an overview on the synthetic approaches and UV-vis properties related to photodynamic applications *Bulg. Chem. Comm*., Special Issue J, 50, 2018, 185-192. (**Q4**)

21. Aliosman, M.; Eneva, I.Z.; Stoineva, I.B.; Durmus, M.; **Mantareva, V**., Aminophenoxy-substituted zinc(II) phthalocyanines as basic photosensitizers for conjugation with biologically active moieties via amide bond, *Bulg. Chem. Comm*., Special Issue E, 2017, 79-85. (**Q4**)

2. Citates

"Sonix.bas.bg/bg": **128** citations of the publications included in the thesis, as was checked till April, 2021.

3. List of scientific projects

***Project 1*:** The contract in the competition «Fundamental science» NFSI, **КП-06-Н29/11**, 18.12.2018 with a title: „Phthalocyanine photosensitizers towards microbial resistance "; leader of the project: Assoc. Prof. Vanya Mantareva, **2018 г. – 2022.**

Project 2: The collaboration project between Bulgarian Academy of Sciences and Turkish foundation **TUBITAK**, Turkey, title: „Water-soluble silicon phthalocyanines for fluorescent diagnosis and photodynamic therapy"; leader of the project: Assoc. Prof. Vanya Mantareva, **2013 г. – 2016.**

Project 3: The contract in the competition «Fundamental science» NFSI, **B02/9/2014** with a partner IOCCP, title «The research Centre of Biophotonics»; the project leader: Prof. D. Sci. Latchezar Avramov, **2014 г. – 2017.**

Project 4: The contract in the competition «Fundamental science» NFSI **КП-06-Н26/11,** with a title: „Inovative photodynamic methods for effect on glioblastoma tumor cells", Hospital „St. Ivan Rilski", leader: Assoc. Prof. Krasimir Minkin, **2018 г. – 2022.**

Project 5: The contract in the competition «Fundamental science» NFSI, **B02/9/2014** with a partner IOCCP, title «The research Centre of Biophotonics - 2»; the project leader: Prof. D. Sci. Latchezar Avramov, **2019 – 2022.**

4. Short list of participation in the scientific meetings (2016-2020 г.)

1. Vanya Mantareva, Yavor Mitrev, Mahmut Durmus, Ivan Angelov, Veselin Kussovski, Bioconjugates of phthalocyanine complexes with steroid moieties towards pathogens, ***Eight International Conference on Radiation in Various Fields of Research*** (RAD-8), 20-24 July, 2020, virtual conference.
2. Vanya Mantareva, Ivan Angelov, Adriana Slavova-Kazakova, Ekaterina Borisova, Mahmut Durmus, Aleksandar Gisbrecht, Vesselin Kussovski, BIOCONJUGATES OF PHTHALOCYANINE WITH STEROID UNITS FOR PHOTODYNAMIC THERAPY,

XXI International Conference and School on Quantum Electronics: "Laser Physics and Applications", 21-24. Sept., 2020, virtual meeting.

3. V. Mantareva, Aliosman, M., Angelov, I., Mitrev, Y., Kussovski, V., Durmus, M., Gisbrecht, A., Biologically active phthalocyanines for target-specific Antimicrobial PDT (Доклад) - ***17th International Congress on Photobiology 18th Congress of the European Society for Photobiology***, 25-30.08.2019, Barcelona, Spain.

4. V. Mantareva, Aliosman, M., Angelov, I., Durmush, M., Kussovski, V.. Impact of substituents to phthalocyanines for targeted antimicrobial PDT, ***The 4th GTU Photodynamic Day***, Istanbul, Turkey, 24-25.04.2019.

5. V. Mantareva, Kussovski, V., Gisbrecht, A., Najdenski, H., Orozova, P.. Antimicrobial Photodynamic Therapy for Control of Farm Fishes Pathogens (Постер), ***17th International Congress on Photobiology 18th Congress of the European Society for Photobiology***, 25-30.08.2019, Barcelona, Spain.

6. V. Mantareva, Slavova-Kazakova, A., Aliosman, M., Mitrev, Y., Durmus, M., Angelov,I., PHOTOOXIDATION OF CHOLESTEROL via PHOTODYNAMIC ACTION OF PHTHALOCYANINE COMPLEXES AND LIGHT (Постер) - *[17.09.2019]*, ***3rd International Conference on Bio-antioxidants*** (BIO-ANTIOXIDANTS 2019, Nessebar, Bilgaria, 17.09.2019 - 21.09.2019.

7. Vanya Mantareva, Cem Gol,Vesselin Kussovski, Mahmut Durmuş, Ivan Angelov Impact of water-soluble zwitterionic Zn(II) phthalocyanines against pathogenic bacteria, (Доклад) ***3rd GTU Photodynamic Day***, 09, May, 2018, Gebze, Turkey.

8. V. Mantareva, I. Angelov, A. Yakimanski, I. Eneva, E. Borisova, Phthalocyanine-conjugates with polymeric brushes for photodynamic therapy applications, ***17th Congress European Society for Photobiology***, 04-08. Sept. 2017, Pisa, Italy.

9. M. Aliosman, M. Durmus, I. Angelov, V. Mantareva, Zn(II) phthalocyanines functionalized with amino acids for PDT applications, ***17th Congress European Society for Photobiology***, 04-08.Sept. 2017, Pisa, Italy.

10. V. Mantareva, I. Angelov, M. Aliosman, I. Soineva, V. Kussovski, An overview on the impact of cationic phthalocyanine complexes for inactivation of drug-resistant microorganisms, ***PDT and PD UPDATE***; 24-28. Oct. 2016, Nancy, France

11. M. Aliosman, V. Kussovski, I. Stoineva, I. Angelov, M. Durmus, V. Mantareva, Lutetium (III) acetate phthalocyanines and LED 660 nm irradiation for effective inactivation of MRSA, ***19th International Conference and School on Quantum Electronics***, 26-30. Sept. 2016, Sozopol, Bulgaria.

12. I. Eneva, V. Mantareva, E. Borisova, V. Kussovski, L. Avramov, I. Angelov, TiO2 and ZnO-conjugated phthalocyanines: nanoparticals for inactivation of wastewater bacterial strains, ***19th International Conference and School on Quantum Electronics***, 26-30. Sept. 2016, Sozopol, Bulgaria.

5. Other

Scientific advisor of the PhD student: Meliha Bahri Aliosman (Syuleyman), with starting date: 01.01.2014 according to the file № РД-09-12/ 30.01.2014 and the Certificate № РД-09-103/ 08.05.2019 with the time of presentation 19.07.2019 for a PhD diploma.

NOTES