

## Novel Water–Soluble Silicon(IV) Phthalocyanine for Photodynamic Therapy and Antimicrobial Inactivations

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Dedicated to Professor Dieter Wöhrle on the occasion of his 80th Anniversary

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The synthesis and characterization of axially bis(1,2,2,6,6-pentamethyl-4-piperidinol) silicon(IV) phthalocyanine (**3**) and its water-soluble cationic derivative (**3Q**) were defined in this study. Both newly synthesized silicon(IV) phthalocyanines were characterized by FT-IR, UV-Vis, <sup>1</sup>H NMR, MALDI-TOF and elemental analysis as well. Although the photophysical and photochemical properties of newly synthesized axially bis(1,2,2,6,6-pentamethyl-4-piperidinol) silicon(IV) phthalocyanine was investigated in DMSO, its water-soluble derivative was investigated in both DMSO and phosphate buffer saline (PBS). The fluorescence quenching behavior of the studied water-soluble silicon(IV) phthalocyanine (**3Q**) by the addition of 1,4-benzoquinone (BQ) was also described in DMSO. Besides, the binding behavior of **3Q**, it was examined by bovine serum albumin (BSA) protein in PBS for determination of transportation abilities of this phthalocyanine in the blood. Photodynamic activity of water-soluble silicon(IV) phthalocyanine (**3Q**) was tested in vitro on tumor cells and for inactivation of bacterial pathogens with promising photocytotoxicity for both PDT applications.

**Keywords:** Silicon phthalocyanine, photophysics, photochemistry, photodynamic therapy, pathogenic bacteria.

## Новые водорастворимый фталоцианинат кремния(IV): фотодинамическое применение для терапии и антибактериальной инактивации

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В работе приведены синтез и характеристика аксиального бис(1,2,2,6,6-пентаметил-4-пиперидинол) фталоцианината кремния(IV) (**3**) и его водорастворимого кватернизированного производного (**3Q**). Оба новых фталоцианината кремния(IV) охарактеризованы методами ИК, электронной, <sup>1</sup>H ЯМР спектроскопии, масс-спектрометрии и элементного анализа. Фотофизические и фотохимические свойства впервые синтезированного комплекса **3** были исследованы в ДМСО, а его водорастворимое производное было изучено как в ДМСО, так и в фосфатном буферном растворе. Было изучено гашение флуоресценции водорастворимого **3Q** в ДМСО при добавлении 1,4-бензохинона. Была оценена связывающая способность **3Q** с помощью белка бычьего сывороточного альбумина в фосфатном буферном растворе для определения способности этого фталоцианина

выполнять транспортную функцию в крови. *In vitro* на опухолевых клетках и патогенных бактериях протестирована фотодинамическая активность кватернизированного фталоцианината кремния(IV) (**3Q**), выявлена перспективная фотоцитотоксичность для применения в фотодинамической терапии.

**Ключевые слова:** Фталоцианинат кремния, фотофизика, фотохимия, фотодинамическая терапия, патогенные бактерии.

## Introduction

Photodynamic therapy (PDT) has been emerged as a hopeful treatment for the localized and superficial cancers, as well as for certain non-cancerous states and pathogens associated diseases.<sup>[1–3]</sup> PDT involves the combined action of a photosensitizer, light and oxygen to generate cytotoxic reactive oxygen species. The photosensitizers, which can be excited with light in the “therapeutic window” (630–850 nm) can transfer energy to surrounding oxygen to produce highly reactive oxygen species.<sup>[4,5]</sup> There are clinically approved porphyrins and phthalocyanines (Pcs), which underwent successful all stages of investigations and further they were certified for different cancer localizations.<sup>[6,7]</sup> The known as second generation PDT drug – Pcs possess great potential of their optical properties due to their long-wavelength absorption, low toxicity and ease of functionalization of the main skeleton.<sup>[8,9]</sup> One limitation of Pcs applications in solutions is that Pcs are characterized by very high tendency to aggregate, what reduces their photosensitizing ability through self-quenching.<sup>[10]</sup> The aggregation of phthalocyanines results in some difficulties in purification and characterization.<sup>[11]</sup> The lowering of aggregation behaviour can be achieved by the bulky groups to the central metals which have more than four coordination numbers.<sup>[12]</sup> Axially substituted phthalocyanines are much less likely to aggregate because of the steric hindrance of the non-planar substituents to the center atom.<sup>[13]</sup> It is known that unsubstituted phthalocyanines appear with low solubility and very high aggregation potential.<sup>[14]</sup> Though the axially di-substituted silicon(IV) phthalocyanines attract attention to study because they are not able to aggregate due to their non-planar molecular skeleton.

Photodynamic inactivation (PDI) features as another PDT application for treatment of pathogenic bacteria.<sup>[15]</sup> Nowadays is known that acute infections, associated with the resistance of pathogenic bacteria, have very low cure with the conventional therapeutic modality and PDI is an alternative successful approach towards bacterial drug-resistance.

This study reports the synthesis, characterization and photophysical properties of axially bis(1,2,2,6,6-pentamethyl-4-piperidinol)phthalocyaninosilicon(IV) and its water-soluble cationic derivative. In addition, the properties of the new water-soluble SiPc (**3Q**) were studied *in vitro* on cancer cell line and on pathogenic bacterial species. The results suggest that functionalization of phthalocyanine macrocycle with 1,2,2,6,6-pentamethyl-4-piperidinol leads to an improvement of the photophysical (absorption and fluorescence) and photochemical (singlet oxygen) properties. The photobiological *in vitro* studies with Si(IV)Pcs (**3** and **3Q**) against the HeLa (human cervical) cancer cell line and towards bacterial pathogens showed relatively high photocytotoxic efficacy which is probably due to quaternization.

Three pathogenic bacterial strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus mutans* were tested. The high photoinactivation was confirmed on two Gram-positive bacterial strains at concentration 3  $\mu\text{M}$  **3Q** at gentle irradiation dose (50  $\text{J}\cdot\text{cm}^{-2}$ ). The Gram-negative bacterium was also with relatively good inactivation with **3Q** but at much higher concentrations (over 10  $\mu\text{M}$ ). The results suggested that quaternization of silicon(IV) phthalocyanine (**3Q**) leads to a useful photosensitizer for PDT drug which is relatively effective in the both applications.

## Experimental

### Materials and Equipment

The used materials, equipments and the photophysical-chemical investigations, as well as photobiological studies are supplied as Supplementary Information.

### Synthesis

*Bis(1,2,2,6,6-pentamethyl-4-piperidinol)phthalocyaninosilicon(IV)* (**3**). Silicon phthalocyanine dichloride (**1**) (0.15 g, 0.245 mmol) and 1,2,2,6,6-pentamethyl-4-piperidinol (0.082 g, 0.490 mmol) (**2**) were dissolved in dry toluene (15 mL) and NaH (0.012 g, 0.490 mmol) was added to this mixture. The reaction mixture was refluxed for 24 h under argon atmosphere. After evaporation of the solvent in vacuum, the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  and filtered to separate insoluble impurities. Then it was purified by the column chromatography over aluminum oxide using solvent mixture  $\text{CH}_2\text{Cl}_2$ :ethanol (20:1) as an eluent. Yield: 50 mg (23 %). Found: C 70.77, H 6.45, N 15.97 %.  $\text{C}_{52}\text{H}_{56}\text{N}_{10}\text{O}_2\text{Si}$  requires C 70.88, H 6.41, N 15.90 %. MALDI-TOF  $m/z$ : calcd. 881.17; found: 851.02  $[\text{M}-2\text{CH}_3]^+$ , 798.07  $[\text{M}-6\text{CH}_3]^+$ , 711.96  $[\text{M}-\text{C}_{10}\text{H}_{20}\text{NO}]^+$ , 695.90  $[\text{M}-\text{C}_{11}\text{H}_{23}\text{NO}]^+$ . FT-IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3062 (aromatic-CH), 2922–2853 (aliphatic-CH), 1520 (C=C), 1428, 1290, 1121, 1078 (Si-O-C). UV-Vis (DMSO)  $\lambda_{\text{max}}$  (lg $\epsilon$ ) nm: 680 (4.72), 611 (3.91), 359 (4.31).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta_{\text{H}}$  ppm: 9.69 (s, 8H, Ar-H), 8.50 (s, 8H, Ar-H), 3.51 (s, 6H,  $-\text{CH}_3$ ), 2.36 (s, 2H,  $-\text{CH}$ ), 1.58–1.39 (m, 32H,  $-\text{CH}_2$ ,  $-\text{CH}_3$ ).

*Bis(N-methyl-1,2,2,6,6-pentamethyl-4-piperidinol)phthalocyaninosilicon(IV)* (**3Q**). Compound **3** (0.030 g, 0.034 mmol) was dissolved in chloroform (1.5 mL) and methyl iodide (0.024 g, 0.17 mmol) was added to this solution. The reaction mixture was stirred at room temperature under argon for 4 days. The product was filtered off, washed with chloroform, acetone, and then dried. Yield: 23 mg (58 %). Found: C 55.61, H 5.41, N 12.07 (%).  $\text{C}_{54}\text{H}_{62}\text{I}_2\text{N}_{10}\text{O}_2\text{Si}$  requires C 55.67, H 5.36, N 12.02 (%). MALDI-TOF-MS  $m/z$  calcd. 1165.03; found: 730.939  $[\text{M}(2\text{I})-12\text{CH}_3]^+$ , 559.058  $([\text{M}(2\text{I})+\text{CHCA}+\text{H}_2\text{O}]^{+2})/2$ . FT-IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3035 (aromatic-CH), 2919–2849 (aliphatic-CH), 1606, 1519 (C=C), 1429, 1336, 1163, 1127, 1080 (Si-O-C). UV-Vis (PBS)  $\lambda_{\text{max}}$  (lg $\epsilon$ ) nm: 688 (4.99), 620 (4.16), 353 (4.56).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ )  $\delta_{\text{H}}$  ppm: 9.75 (s, 8H, Ar-H), 8.60 (s, 8H, Ar-H), 1.93 (s, 6H,  $-\text{CH}_3$ ), 1.63 (s, 6H,  $-\text{CH}_3$ ), 0.07 (s, 24H,  $-\text{CH}_3$ ), -1.45 (br, 4H,  $-\text{CH}_2$ ), -2.07 (br, 4H,  $-\text{CH}_2$ ), -2.77 (s, 2H,  $-\text{CH}$ ).

## Results and Discussion

### Synthesis and Characterization

Silicon phthalocyanine dichloride (**1**, SiPc(Cl)<sub>2</sub>) was synthesized according to the literature.<sup>[16]</sup> Compound **3** was obtained from nucleophilic substitution reaction of 1,2,2,6,6-pentamethyl-4-piperidinol (**2**) with SiPc(Cl)<sub>2</sub> in dried toluene in the presence of NaH as a base at reflux for 24 h under argon atmosphere (Scheme 1). The product was purified by column chromatography over aluminum oxide using CH<sub>2</sub>Cl<sub>2</sub>:ethanol (20:1) solvent system as an eluent. Water-soluble axially disubstituted silicon(IV) phthalocyanine **3Q** was obtained by the reaction of corresponding phthalocyanine **3** with methyl iodide in CHCl<sub>3</sub> at room temperature (Scheme 1). The new water soluble silicon(IV) phthalocyanine (**3Q**) was filtered off, washed with chloroform, acetone and then dried. The structures of phthalocyanines **3** and **3Q** were characterized by FT-IR, UV-Vis, <sup>1</sup>H NMR spectroscopic methods, MALDI-TOF and elemental analysis as well.

In the FT-IR spectra of compounds **3** and **3Q**, the most decisive bands were observed at around 1078 and 1080 cm<sup>-1</sup> for Si-O-C bond for **3** and **3Q**, respectively. The spectra of **3** and **3Q** also showed peaks at 1520 and 1519 cm<sup>-1</sup> for -C=C- stretching; 3062 and 3035 cm<sup>-1</sup> for aromatic-CH stretching and 2922–2853 and 2919–2849 cm<sup>-1</sup> for aliphatic-CH stretching frequencies, respectively. A FT-IR spectrum of **3Q** was obtained without major change after quaternization of **3**.

<sup>1</sup>H NMR spectrum of **3** showed aromatic protons at 9.69 and 8.50 ppm as expected. On the other hand, the <sup>1</sup>H NMR spectrum of **3Q** exhibited characteristic signals for the aromatic protons at 9.75 and 8.60 ppm (Figure 1). Also, the peaks belonging to aliphatic CH protons in Si-O-CH bond were shifted to negative region (-2.77 ppm) in the <sup>1</sup>H NMR spectrum of **3Q** because of magnetic anisotropy of phthalocyanine ring.

Finally, MALDI-TOF mass spectra were obtained in dihydroxybenzoic acid (DHB) for **3** and alpha-cyano-4-hydroxycinnamic acid (CHCA) for **3Q** as MALDI matrix

materials. Molecular ion peaks of compounds **3** and **3Q** were observed at  $m/z = 851.02$  as  $[M-2CH_3]^+$ ,  $798.07$  as  $[M-6CH_3]^+$ ,  $695.90$  as  $[M-C_{11}H_{23}NO]^+$  and  $m/z = 730.939$  as  $[M-(2I)-12CH_3]^+$ , and  $559.058$  as  $([M-(2I)+CHCA+H_2O]^{+2})/2$ , respectively. These molecular ion peaks in mass spectra support the predicted mass of these compounds.

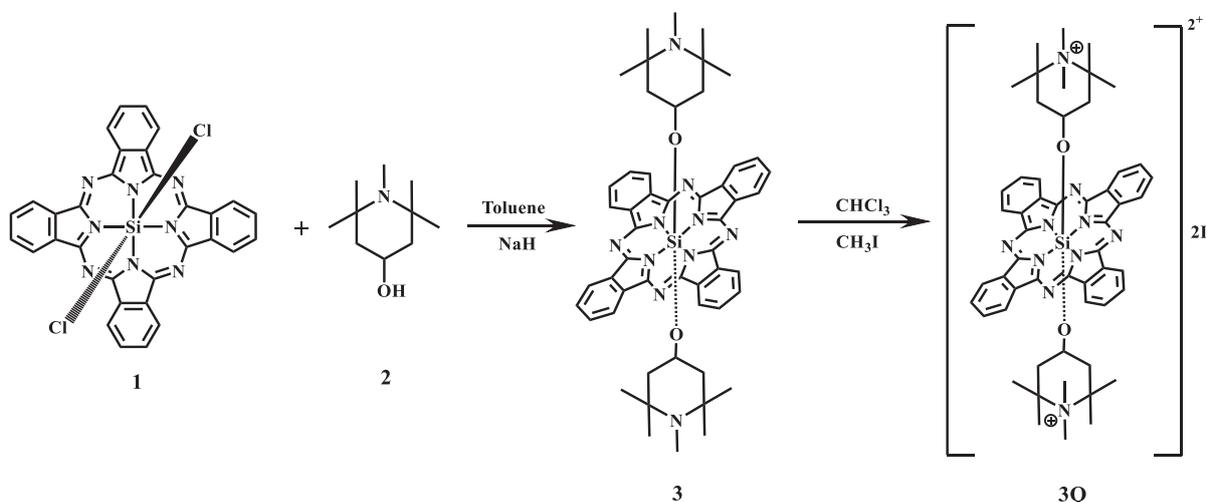
### Ground State Electronic Absorption and Fluorescence Spectra

The electronic absorption spectrum of the axially di-substituted silicon(IV) phthalocyanine compound **3** showed characteristic absorptions in the *Q*-band region with maxima at 680 nm in DMSO. Besides, the UV-Vis spectrum of the quaternized water-soluble silicon(IV) phthalocyanine (**3Q**) showed characteristic absorptions in the *Q*-band region with absorption maxima at 680 nm in DMSO and 688 nm in phosphate buffer solution (PBS). The *B*-bands were observed at around 360 nm for both phthalocyanines (**3** and **3Q**). The aggregation behaviors were investigated at different concentrations in DMSO for compound **3**, in both DMSO and PBS for compound **3Q** (Figure 2). The compound **3** did not show any aggregation tendency in DMSO, and its quaternized water-soluble derivative **3Q** formed monomeric molecules in solutions for both solvents (DMSO and PBS). Beer-Lambert law was obeyed for these compounds in the concentrations ranging between  $2 \cdot 10^{-6}$ – $1.2 \cdot 10^{-5}$  M.

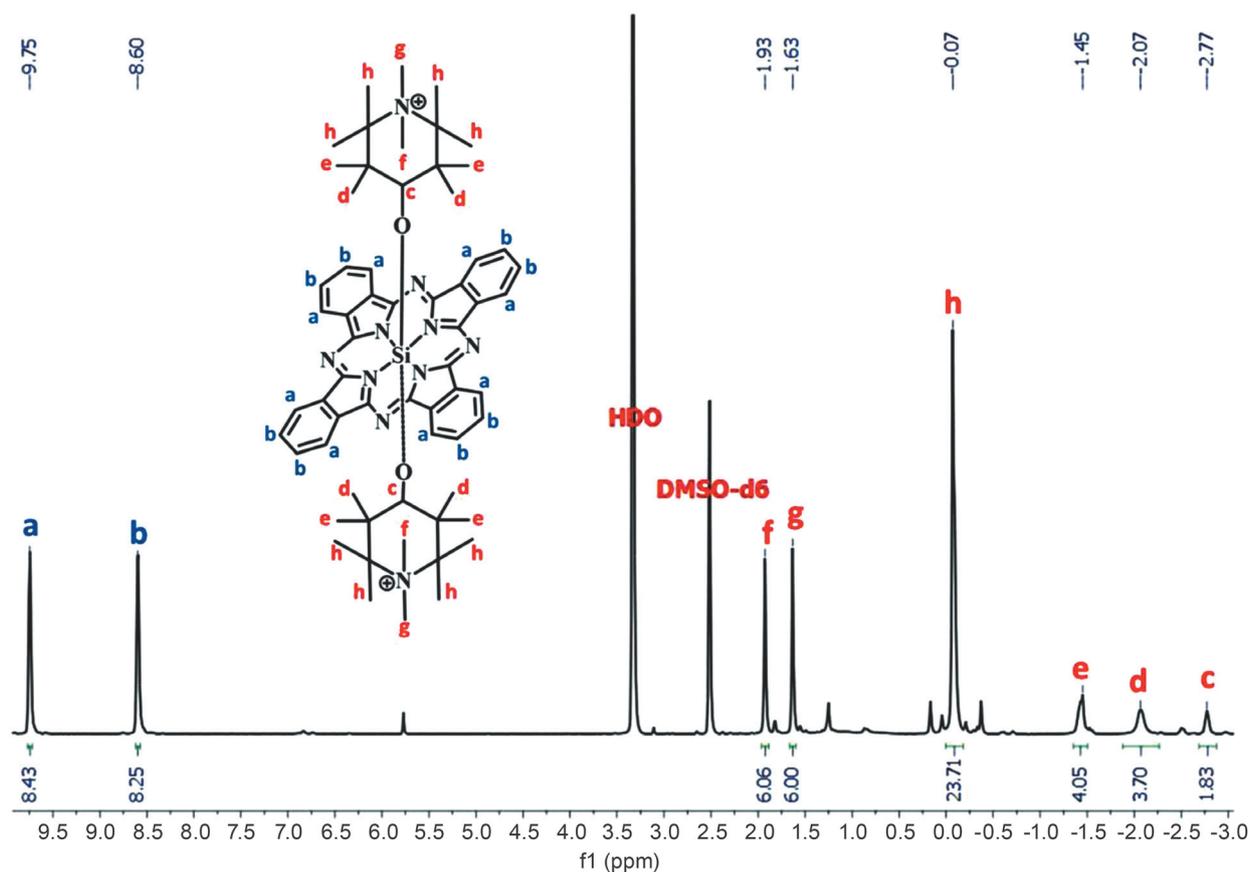
The results for fluorescence emission, absorption and excitation spectra of these axially disubstituted silicon(IV) phthalocyanines (**3** and **3Q**) are presented in Figure 3. The fluorescence emission maximum was observed at 684–685 nm for compounds **3** and **3Q** in DMSO. In addition, the fluorescence of water-soluble silicon(IV) phthalocyanine **3Q** was examined in PBS solutions with fluorescence emission maxima at 693 nm.

### Fluorescence Quantum Yields and Lifetimes

Phthalocyanine derivatives have an ideal fluorescence spectrum which is appropriate for imaging of the studied



**Scheme 1.** Synthesis pathway of axially di-substituted silicon(IV) phthalocyanine **3** and its water soluble derivative (**3Q**).



**Figure 1.**  $^1\text{H}$  NMR spectrum of quarternized axially di-substituted silicon(IV) phthalocyanine (**3Q**).

cells. Fluorescence quantum yields ( $\Phi_F$ ) and fluorescence lifetimes ( $\tau_F$ ) of the photosensitizer (Table 1) are important parameters for their potential applications in PDT. The fluorescence quantum yields ( $\Phi_F$ ) of the studied silicon(IV) phthalocyanine complexes are given Table 2. The fluorescence quantum yields ( $\Phi_F$ ) of the new phthalocyanines were

determined in DMSO (for **3** and **3Q**) and PBS for water-soluble derivative (for **3Q**). The fluorescence quantum yield was determined with a value 0.31 for compound **3** in DMSO, and for compound **3Q** with values of 0.26 and 0.25 in DMSO and PBS, respectively. The fluorescence lifetimes for the compound **3** (5.44 ns) in DMSO, for compound **3Q**

**Table 1.** Absorption, excitation and emission spectral data for **3** and **3Q**.

Compound	Solvent	Q band $\lambda_{\text{max}}$ <sup>a</sup> (nm)	(lg $\epsilon$ )	Excitation $\lambda_{\text{Ex}}$ (nm)	Emission $\lambda_{\text{Em}}$ (nm)	Stokes shift $\Delta_{\text{Stokes}}$ (nm)
<b>3</b>	DMSO	680	4.72	680	684	4
<b>3Q</b>	DMSO	680	5.04	680	685	5
	PBS	688	4.99	689	693	5
<b>SiPc(Cl)<sub>2</sub></b> <sup>a</sup>	DMSO	672, 701	4.71, 4.47	672	678	6

<sup>a</sup>Data from Ref.<sup>[17]</sup>

**Table 2.** Photophysical and photochemical data for **3** and **3Q** complexes.

Compound	Solvent	$\Phi_F$	$\tau_F$ (ns)	$\Phi_\Delta$
<b>3</b>	DMSO	0.31	5.44	0.31
<b>3Q</b>	DMSO	0.26	5.27	0.18
	PBS	0.25	4.94	0.15
<b>SiPc(Cl)<sub>2</sub></b>	DMSO	0.44 <sup>a</sup>	5.37 <sup>b</sup>	0.15 <sup>a</sup>

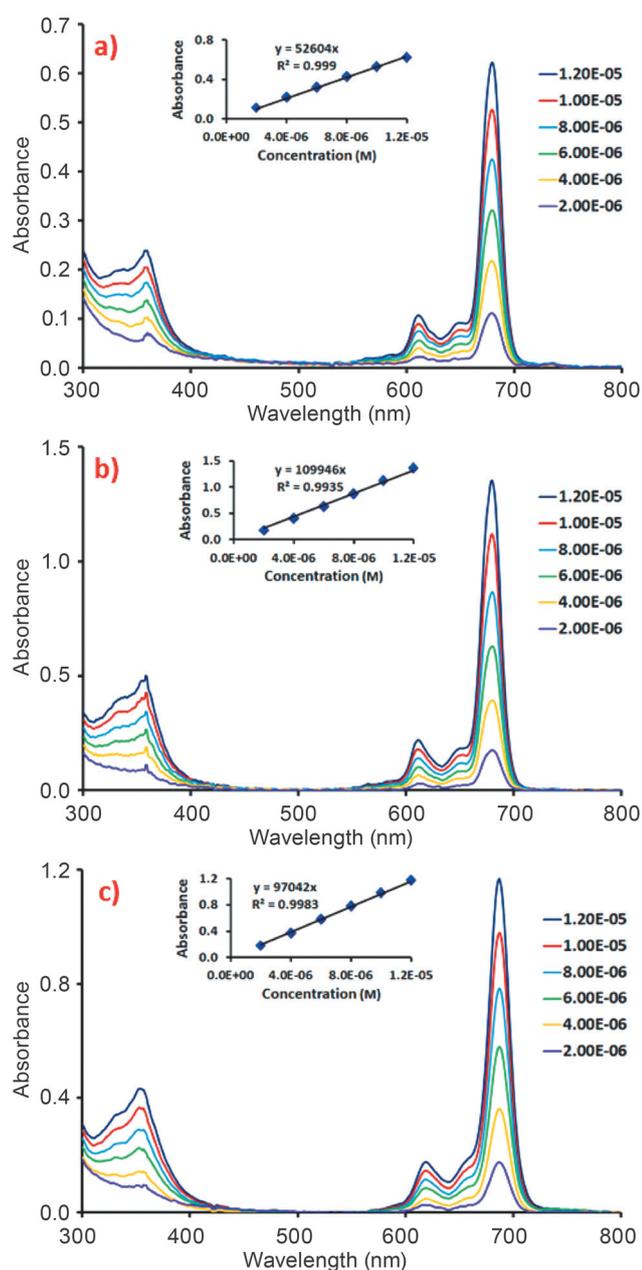
<sup>a</sup>Data from Ref.<sup>[18]</sup>

<sup>b</sup>Data from Ref.<sup>[19]</sup>

(5.27 ns and 4.94 ns) in DMSO and PBS were obtained by the time correlated single photon counting (TCSPC) technique by fitting the fluorescence decay data to a mono exponential function (Table 2 and Figure 4).

### Singlet Oxygen Study

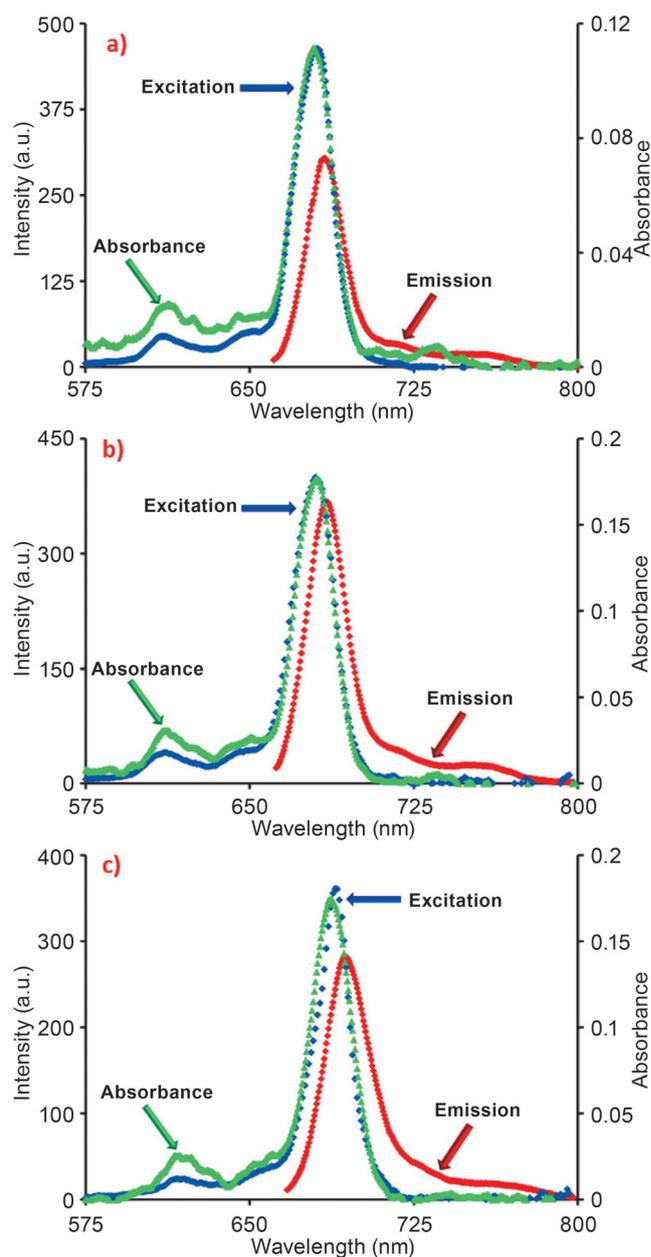
The singlet oxygen quantum yield ( $\Phi_\Delta$ ) defines the capability of the compounds as photosensitizers in PDT applications. The values were defined in DMSO for studied nonionic phthalocyanine (**3**) and in both DMSO



**Figure 2.** Aggregation behavior of **3** and **3Q** at different concentrations: (a) **3** in DMSO, (b) **3Q** in DMSO and (c) **3Q** in PBS.

and PBS solutions for its quaternized water-soluble derivative (**3Q**). The chemical process was used for definition of  $\Phi_{\Delta}$  values using 1,3-diphenylisobenzofuran (DPBF) and 9,10-antracenediyl-bis(methylene)dimalonic acid (ADMA) as singlet oxygen quenchers in organic and aqueous media, respectively. The reduction of DPBF (in DMSO) was control by absorbance at 417 nm. In case of ADMA the absorbance was measured at 380 nm in PBS (Figure 5). Obviously, both silicon(IV) phthalocyanines (**3** and **3Q**) are stable under irradiation without photodestruction of the molecule.

Table 2 presents the singlet oxygen quantum yields of both compounds **3** and **3Q** in DMSO and in PBS only

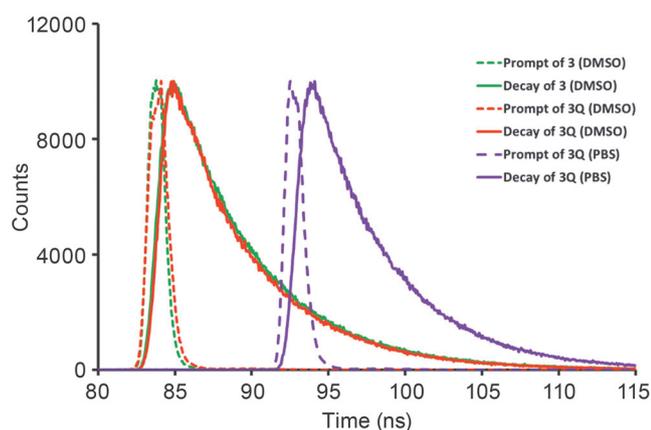


**Figure 3.** Absorption, excitation and emission spectra of compounds **3** and **3Q**: (a) **3** in DMSO, (b) **3Q** in DMSO and (c) **3Q** in PBS. Excitation wavelengths: 645 nm, 650 nm and 655 nm, respectively.

for compound **3Q**. The  $\Phi_{\Delta}$  value of the non-ionic SiPc (**3**) was found  $\Phi_{\Delta} = 0.31$  in DMSO. The ionic SiPc (**3Q**) showed similar  $\Phi_{\Delta}$  values in both solvents: DMSO –  $\Phi_{\Delta} = 0.18$  and PBS –  $\Phi_{\Delta} = 0.15$ .

#### Fluorescence Quenching Studies by 1,4-Benzoquinone (BQ)

The fluorescence quenching of **3Q** by 1,4-benzoquinone (BQ) was investigated in DMSO and the results were found to obey Stern-Volmer kinetics. The decrease of the emission spectra due to quenching by BQ is shown in Figure 6 and the slope of the plots in Figure 6 shows Stern-Volmer



**Figure 4.** Time correlated single photon counting (TCSPC) fluorescence decay curves of the compounds **3** and **3Q**.

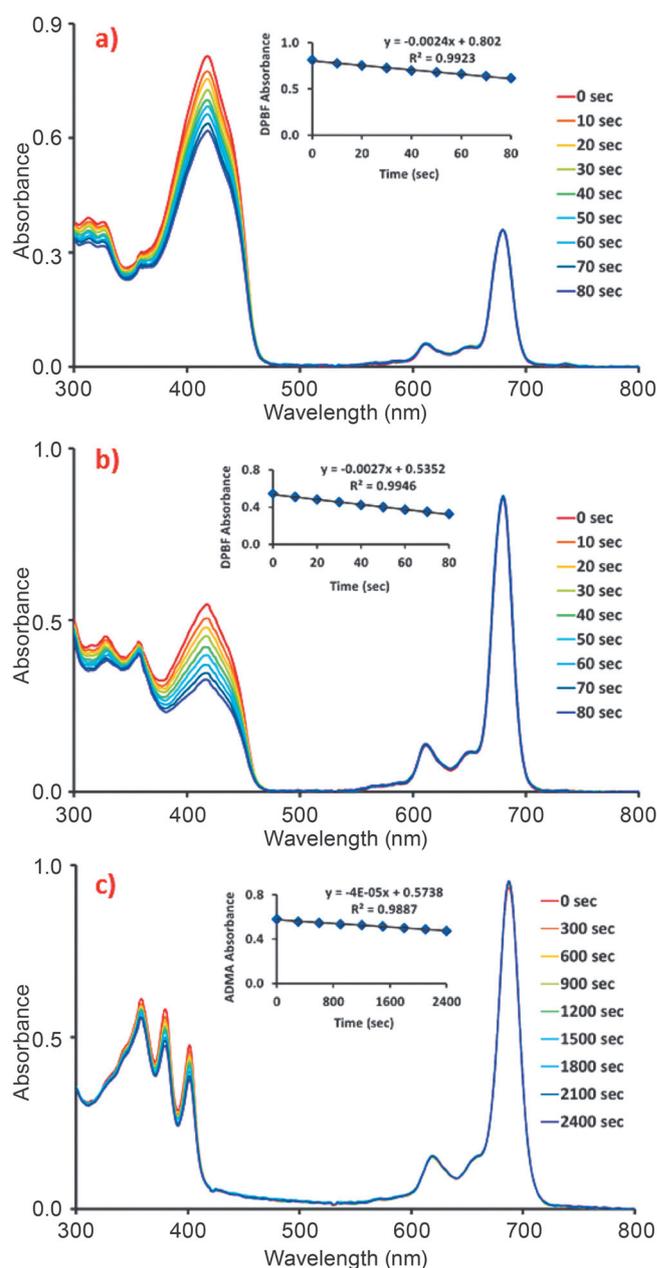
constant ( $K_{SV}$ ) value of  $14.62 \text{ M}^{-1}$ . This value was lower than those of the unsubstituted zinc(II) phthalocyanine ( $K_{SV} = 31.90 \text{ M}^{-1}$ ) in DMSO.<sup>[20,21]</sup> The bimolecular quenching constant ( $k_q$ ) value was found to be close to the diffusion-controlled limits ( $\sim 10^{10} \text{ M}^{-1}\cdot\text{s}^{-1}$ ) which is in agreement with the Einstein-Smoluchowski approximation at room temperature for diffusion-controlled bimolecular interactions.<sup>[22]</sup> The  $k_q$  value was found to be  $2.77\cdot 10^{10} \text{ M}^{-1}\cdot\text{s}^{-1}$  for **3Q** which was the same as for unsubstituted zinc(II) phthalocyanine ( $2.61\cdot 10^{10} \text{ M}^{-1}\cdot\text{s}^{-1}$ ).

#### Binding Studies of Water-Soluble Silicon(IV) Phthalocyanine **3Q** by Bovine Serum Albumin (BSA) Protein

Bovine serum albumin (BSA) protein is known to have an efficient drug delivery function in the blood. The solutions of **3Q** at five different concentrations ( $0, 1.66\cdot 10^{-6}, 3.33\cdot 10^{-6}, 5.00\cdot 10^{-6}, 6.66\cdot 10^{-6}, 8.33\cdot 10^{-6} \text{ M}$ ) were prepared and added to  $3.0\cdot 10^{-5} \text{ M}$  solution of BSA in PBS. The sample with BSA was excited at  $280 \text{ nm}$  and fluorescence emission spectra were recorded between  $290 \text{ nm}$  and  $500 \text{ nm}$ . Figure 7 shows the fluorescence emission spectra of BSA solution by addition of **3Q** in PBS. The fluorescence emission band of BSA at  $348 \text{ nm}$  decreases by the addition of **3Q**. The Stern-Volmer quenching constant ( $K_{SV}$ ) was determined from Figure 7 and the  $K_{SV}$  value was found to be  $3.96\cdot 10^5 \text{ M}^{-1}$ .

#### In vitro Investigations on Tumor Cells

Photocytotoxicity assays were carried out on human cervical cancer (HeLa) cancer lines. Figure 8 shows the survival curve of the studied cell lines for different concentrations of both SiPcs (**3** and **3Q**) and for two different doses ( $1$  and  $2 \text{ J}\cdot\text{cm}^{-2}$ ). The dark incubation of HeLa cells with **3** and **3Q** for  $24 \text{ h}$  with concentrations between  $0$  to  $10 \mu\text{M}$  did not show any toxicity (Figure 8). The observed cell survival after treatment with compounds **3** and **3Q** ( $0.5\mu\text{M}$ ) was  $80\%$  and  $64\%$ , respectively. At the highest concentration ( $10 \mu\text{M}$ ) a high photodynamic effect was observed by the non-ionized derivative (**3**) with  $15\%$  cell survival. However, the high photocytotoxicity was observed with ionized derivative at

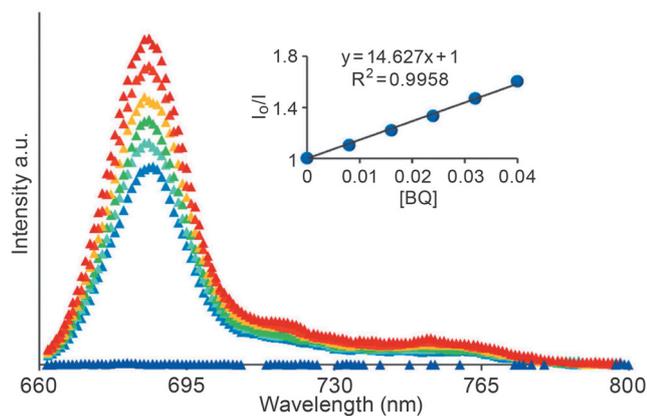


**Figure 5.** Absorbance changes during the determination of singlet oxygen quantum yield. This determination was recorded for (a) compound **3** in DMSO, (b) **3Q** in DMSO and (c) **3Q** in PBS at a concentration of  $1.0\cdot 10^{-5} \text{ M}$  (inset: plot of DPBF or ADMA absorbances versus irradiation time).

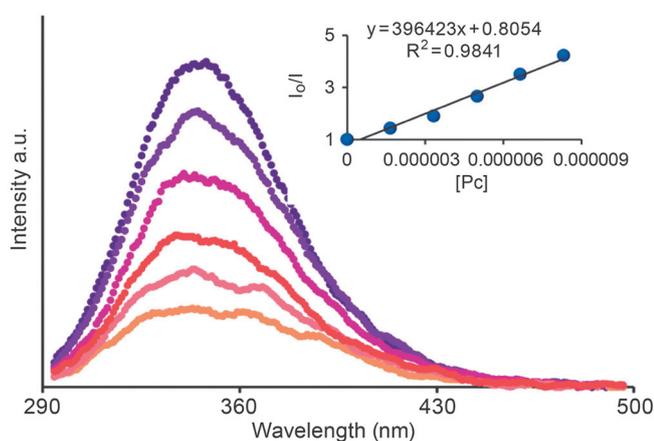
the lowest concentration of  $0.5 \mu\text{M}$  for **3Q** which was less effective than **3** in the highest concentration of  $10 \mu\text{M}$ . This observation can be explained by ionic interaction of ionized derivative in the aqueous media.

#### In vitro Study on Pathogenic Bacteria

The water-soluble cationic SiPc (**3Q**) was studied on three bacterial species namely the Gram-positive *S. aureus* and *S. mutans*, and the Gram-negative *P. aeruginosa*. The samples of suspensions with cell densities approx.  $10^6$ – $10^7 \text{ CFU mL}^{-1}$  were treated for evaluation of photodynamic inactivation capacity of new compound (Figure 9). As

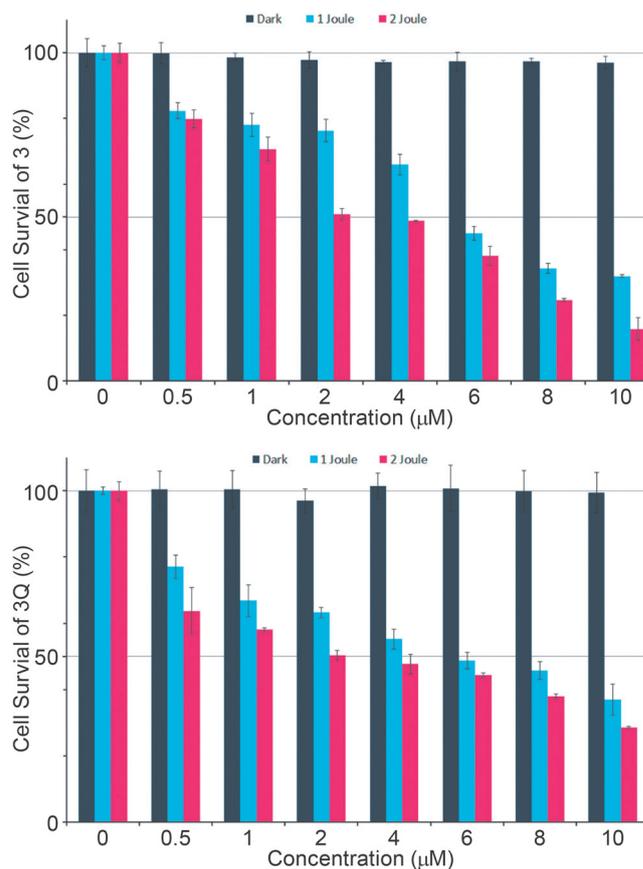


**Figure 6.** Fluorescence emission changes of quaternized silicon(IV) phthalocyanine (**3Q**) by the addition of different concentrations of BQ in DMSO. [BQ] = 0, 0.008, 0.016, 0.024, 0.032, 0.040 M.

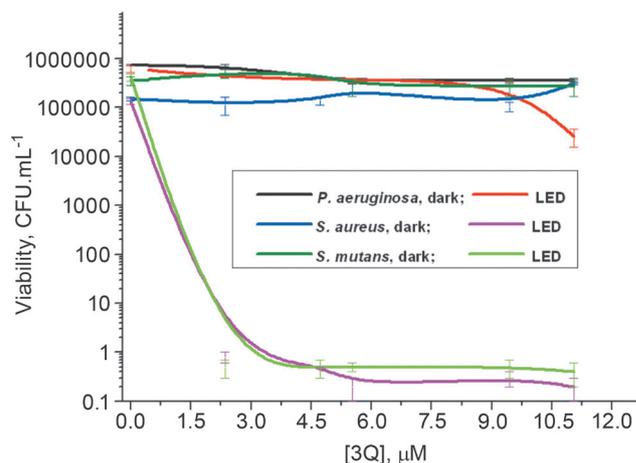


**Figure 7.** Fluorescence emission spectral changes of BSA by addition of different concentrations of **3Q** in PBS solution. [BSA] =  $3.00 \cdot 10^{-5}$  and [Pc] = 0,  $1.66 \cdot 10^{-6}$ ,  $3.33 \cdot 10^{-6}$ ,  $5.00 \cdot 10^{-6}$ ,  $6.66 \cdot 10^{-6}$ ,  $8.33 \cdot 10^{-6}$  M.

can be seen **3Q** is not toxic in the dark but after irradiation showed relatively high photocytotoxicity for concentrations over  $3 \mu\text{M}$  in case of the treated two Gram-positive strains. The similar results were obtained in our previously work with quaternized SiPc axially substituted with alpha, alpha-diphenyl-4-pyridylmethanol.<sup>[23]</sup> Moreover, the photoinactivation of these two Gram-positive *S. aureus* and *S. mutans* bacteria shows that the photocytotoxicity occurs even at lower concentrations ( $3\text{--}5 \mu\text{M}$ ). Thus, suggests that **3Q** is a suitable photosensitizer for photodynamic inactivation of pathogenic bacteria from the Gram-positive class. The species of the Gram-positive bacteria such as the studied *S. aureus* and *S. mutans* have a cell wall which is much more susceptible to photosensitization. The bacterial cell wall in case of Gram-negative bacterium is more complicated and more difficult to destroy. It is well studied the high resistance to any treatment of the Gram-negative bacteria.<sup>[24]</sup> The studied *P. aeruginosa* was also successable to photodynamic



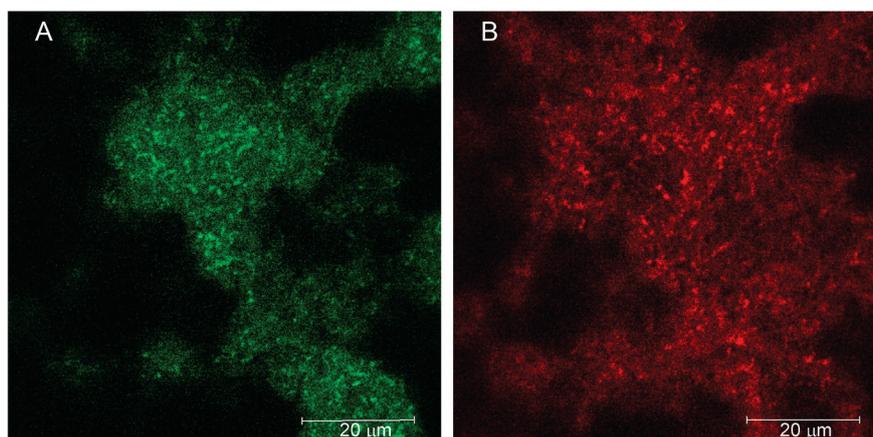
**Figure 8.** Survival fractions of HeLa tumor cells in suspension for  $0.5\text{--}10 \mu\text{M}$  Si(IV)Pcs (**3** and **3Q**) at gentle light doses ( $1$  and  $2 \text{ J}\cdot\text{cm}^{-2}$ ).



**Figure 9.** Viability of the studied bacterial strains (*Ps. aeruginosa*, MRSA and *S. mutans*) after incubation with **3Q** on the dark and after irradiation with red LED.

inactivation but at higher **3Q** concentrations ( $> 10 \mu\text{M}$ ). The inactivation was minor with lower than  $3 \log$  photoinactivation of *P. aeruginosa* as is shown in Figure 9.

The new **3Q** has higher PDI efficacy as compared to the axial propylparaben substituted SiPc which was recently studied by us.<sup>[25]</sup> Obviously, quaternization is more



**Figure 10.** Confocal fluorescence images of an early stage biofilm incubated with **3Q** for 1.5 hour at excitation with lasers: A – 488 nm (green), and B – 633 nm (red).

favorable as a structure than the *N*-containing substituents. The present study of photoinactivation of pathogenic bacterial species also suggests that the cationic phthalocyanines are more effective because of the positive charge and the interaction with the membranes.<sup>[25]</sup> The further achievement of this interaction is the easier induction of photoinactivation by disrupting the cellular wall. The Gram-negative bacteria can develop the high level of resistance to PDT as compared to the Gram-positive species because of the reduced binding capability which results in negligible singlet oxygen saturation in the target cells surrounding.

#### Localization of **3Q** in MRSA Biofilms

The fluorescence intensity of SiPc **3Q** was used for observation of its localization in an early stage MRSA biofilm. The thicknesses between 0.150–0.200 μm were scanned after incubation with **3Q**. The transmission channel was also applied to show the parameters of the studied biofilms with thicknesses between 8–11 μm. The natural chromophores have fluorescence emission which is located faraway from the fluorescence spectrum of the phthalocyanine compounds.<sup>[26]</sup> This allows to image the bacterial biofilms so that to distinguish from the native fluorescence of the cell chromophores.<sup>[27]</sup> The laser scanning of the biomass ( $\lambda_{exc} = 488$  nm;  $\lambda_{em} = 500$ –580 nm) showed the native 48-h biofilm as is shown in Figure 10A (green channel). The collected samples were evaluated at excitation laser  $\lambda_{exc} = 633$  nm to obtain the fluorescence emission spectrum  $\lambda_{em} = 660$ –740 nm for detection of compound **3Q** (Figure 10B, red channel). The localization and the penetration of **3Q** throughout the whole biomass were evaluated also by transmittance mode. The comparison of the images (green versus red channels) suggested that for an early stage biofilm the compound is located also in the channels and in the biomass.

#### Conclusions

In this work, two new phthalocyanines, namely, axially bis(1,2,2,6,6-pentamethyl-4-piperidinol) silicon(IV) phthalocyanine (**3**) and its water-soluble derivative (**3Q**)

were synthesized. They were characterized by the known spectroscopic techniques FT-IR, <sup>1</sup>H NMR, UV-Vis, mass and elemental analysis as well. The photophysical studies of **3** and **3Q** in DMSO showed similar position of the absorption and fluorescence maxima for non-ionized derivative (**3**) and in DMSO and PBS for quaternized derivative (**3Q**). Also, the fluorescence quenching behavior of water-soluble phthalocyanine **3Q** by the addition of 1,4-benzoquinone suggests the high potential as a photosensitizer. The study with bovine serum albumin (BSA) with silicon(IV) phthalocyanine (**3Q**) showed a promising binding behavior of the new photosensitizer **3Q**. Furthermore, *in vitro* studies of SiPcs (**3** and **3Q**) against cervical cancer cells (HeLa) showed good PDT activity. The bacterial pathogens among the Gram-positive bacteria *Streptococcus mutans*, and *Staphylococcus aureus* showed high capability to be photoinactivated with **3Q**. A Gram-negative *Pseudomonas aeruginosa* bacterium was photoinactivated at higher concentrations over 10 μM (~3log). The obtained results are essential for further development of novel silicon(IV) phthalocyanine derivatives for photodynamic therapy applications.

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