

## Investigation of the photophysical and photochemical behavior of substituted zinc phthalocyanines and their water-soluble quaternized derivatives

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**Abstract:** New nonperipherally and peripherally tetrasubstituted zinc(II) phthalocyanine complexes containing 1-benzylpiperidin-4-ol groups and their quaternized derivatives are reported in this work. These complexes were characterized successfully and their photophysical and photochemical properties were studied. These properties were determined in dimethylsulfoxide for all studied complexes and in water and water + Triton X-100 solutions for ionic Zn(II) phthalocyanines. All studied zinc(II) phthalocyanine complexes generated high singlet oxygen, especially in dimethylsulfoxide, and these properties make them potential photosensitizers for use in the photodynamic therapy of cancer. The effects of the position and quaternization of the substituents on the photophysical and photochemical properties were also examined. The fluorescence of the tetrasubstituted zinc(II) phthalocyanine complexes is effectively quenched by addition of 1,4-benzoquinone. Furthermore, the water soluble Zn(II) phthalocyanines can bind to bovine serum albumin, which is a blood plasma protein.

**Key words:** Phthalocyanine, quaternized, photodynamic therapy, photosensitizer, bovine serum albumin

### 1. Introduction

Phthalocyanines (Pcs) are a strategic class of second generation photosensitizers for photodynamic therapy (PDT) due to their high absorption at long wavelength, photoresistance, long excited triplet lifetime, higher singlet oxygen production, and ready clearing from the body once administered.<sup>1,2</sup> To date, second-generation photosensitizers such as phthalocyanines have been introduced into PDT in research and clinical trials.<sup>3,4</sup>

PDT is an alternative treatment method for cancer that involves the use of light in combination with a photosensitizer drug to produce singlet oxygen.<sup>5</sup> It enables selective treatment of the target malignant tumors because of favorable accumulation of photosensitizer. The light irradiation of tissue causes simultaneous excitation of the dye itself. The excited singlet state dye changes its spin to triplet state by intersystem crossing (ISC) and transfers the energy to oxygen molecules to generate highly reactive singlet oxygen. Following this, these cytotoxic reactions are concluded to damage the cancer cells.<sup>6,7</sup>

Because of the hydrophilic system of blood, water solubility is very important in drug injection.<sup>8</sup> Beside the water solubility, higher singlet oxygen generation is another important factor for dyes in PDT applications. Diamagnetic ions (Zn<sup>2+</sup>, Ga<sup>3+</sup>, and Si<sup>4+</sup>) enhance the singlet oxygen quantum yield and Pcs containing these ions in its cavity have been extensively investigated for PDT.<sup>9–11</sup>

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Bovine serum albumin (BSA) is one of the most widely studied groups of proteins due to the structural similarity with human serum albumin.<sup>12</sup> Serum albumin, the most abundant protein in the plasma, has a high ability to bind reversibly a large variety of compounds and determines the distribution of many drugs in the circulatory system.<sup>13–15</sup> It has been used to as an effective carrier for photosensitizers and anticancer drugs to improve selectivity.<sup>16</sup>

The target of this search is to obtain water-soluble ZnPc complexes for potential application in PDT. In this paper, the preparation of 1-benzylpiperidin-4-ol substituted nonionic (**6** and **7**) and ionic (**8** and **9**) zinc(II) phthalocyanines and investigation of their photophysical and photochemical properties are presented. The binding of the studied zinc(II) phthalocyanine complexes (**8** and **9**) to BSA protein was also investigated using fluorescence spectroscopy.

## 2. Results and discussion

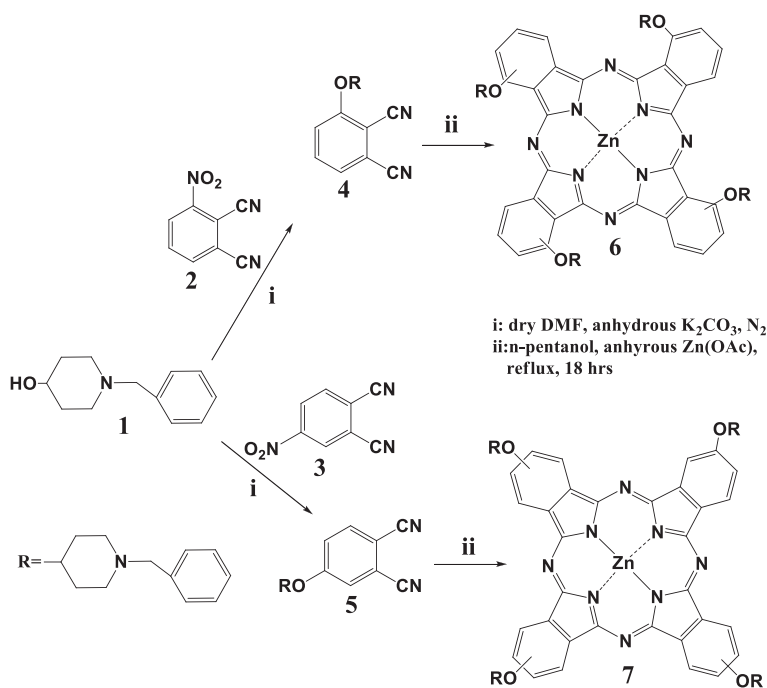
### 2.1. Synthesis and characterization

The synthetic pathway of the synthesis of new Zn(II)Pcs (**6**, **7**) and their quaternized derivatives (**8**, **9**) is given in Schemes 1 and 2, respectively. Compound **5** was synthesized according to the literature procedure and the substituted phthalonitrile derivative (**4**) was obtained by the reaction of compound **1** with compound **2**.<sup>17</sup> The substituted ZnPc complexes (**6**, **7**) were obtained by treatment of related phthalonitrile derivatives (**4** and **5**) with anhydrous  $\text{Zn}(\text{CH}_3\text{COO})_2$  in dry n-pentanol (Scheme 1).<sup>18</sup> The TLC was used for the purification of the crude products. Cationic ZnPcs (**8** and **9**) were obtained by the reactions of dimethylsulfate and compounds **6** and **7**, respectively (Scheme 2).<sup>19</sup> The unsubstituted Pc is not soluble in organic solvents or in water. The synthesized nonionic zinc (II) Pc complexes are soluble in most organic solvents and their quaternized cationic derivatives are soluble in water as well. The structures of these novel compounds were characterized by FT-IR,  $^1\text{H}$  NMR, UV-Vis, and  $^{13}\text{C}$  NMR spectroscopy; MALDI-TOF mass spectra; and elemental analysis. All spectral data were consistent with the proposed structures.

The FT-IR spectrum showed the formation of **4** with the appearance of vibration bands at 2223 ( $\text{C}\equiv\text{N}$ ), 1283  $\text{cm}^{-1}$  ( $\text{Ar}-\text{O}-\text{C}$ ). In the  $^1\text{H}$  NMR spectrum of compound **4**, the proton signal of the OH group of compound **1** disappeared as expected. In the  $^{13}\text{C}$  NMR spectrum of **4**, the signals of nitrile carbon atoms were observed at  $\delta = 115.33, 115.22$  ppm. The mass spectrum of compound **4** showed a molecular ion peak at  $m/z = 318.13$   $[\text{M} + \text{H}]^+$ .

In the IR spectra of compounds **6** and **7**, the vibrations of the nitrile groups of compounds **4** and **5** disappeared. The  $^1\text{H}$  NMR spectra of the ZnPcs (**6**, **7**) were obtained as expected (Figure S1 is given as an example for compound **7**). In the mass spectra of compounds **6** and **7**, the ion peaks were at 1335.91  $[\text{M} + \text{H}]^+$  (Figure S2A) and  $m/z = 1335.31$   $[\text{M} + \text{H}]^+$  (Figure S2B), respectively.

After reactions of zinc(II) phthalocyanines (**6** and **7**) with dimethylsulfate, the obtained quaternized cationic complexes were highly soluble in water. In the FT-IR spectra of **8** and **9**, strong vibrations at 1325  $\text{cm}^{-1}$  ( $\nu_{as}, \text{SO}_2$ ) and 1166  $\text{cm}^{-1}$  ( $\nu_{sym}, \text{SO}_2$ ) for **8** and at 1274  $\text{cm}^{-1}$  ( $\nu_{as}, \text{SO}_2$ ) and 1167  $\text{cm}^{-1}$  ( $\nu_{sym}, \text{SO}_2$ ) for **9** are indicative of the formation of quaternized complexes. The  $^1\text{H}$  NMR spectra of cationic Pc complexes did not give good results due to aggregation of these complexes in  $\text{D}_2\text{O}$ . The mass spectra of quaternized ZnPc complexes (**8** and **9**) confirmed the proposed structures, with the ion peaks at  $m/z: 349.75$   $[\text{M} + 1]^{4+}$  for **8** and 350.75  $[\text{M} + 2]^{4+}$  for **9** (Figures S3A and S3B, respectively).



**Scheme 1.** Synthetic procedure of nonionic ZnPcs substituted with 1-benzylpiperidin-4-ol groups.

## 2.2. Ground state electronic absorption and fluorescence spectra

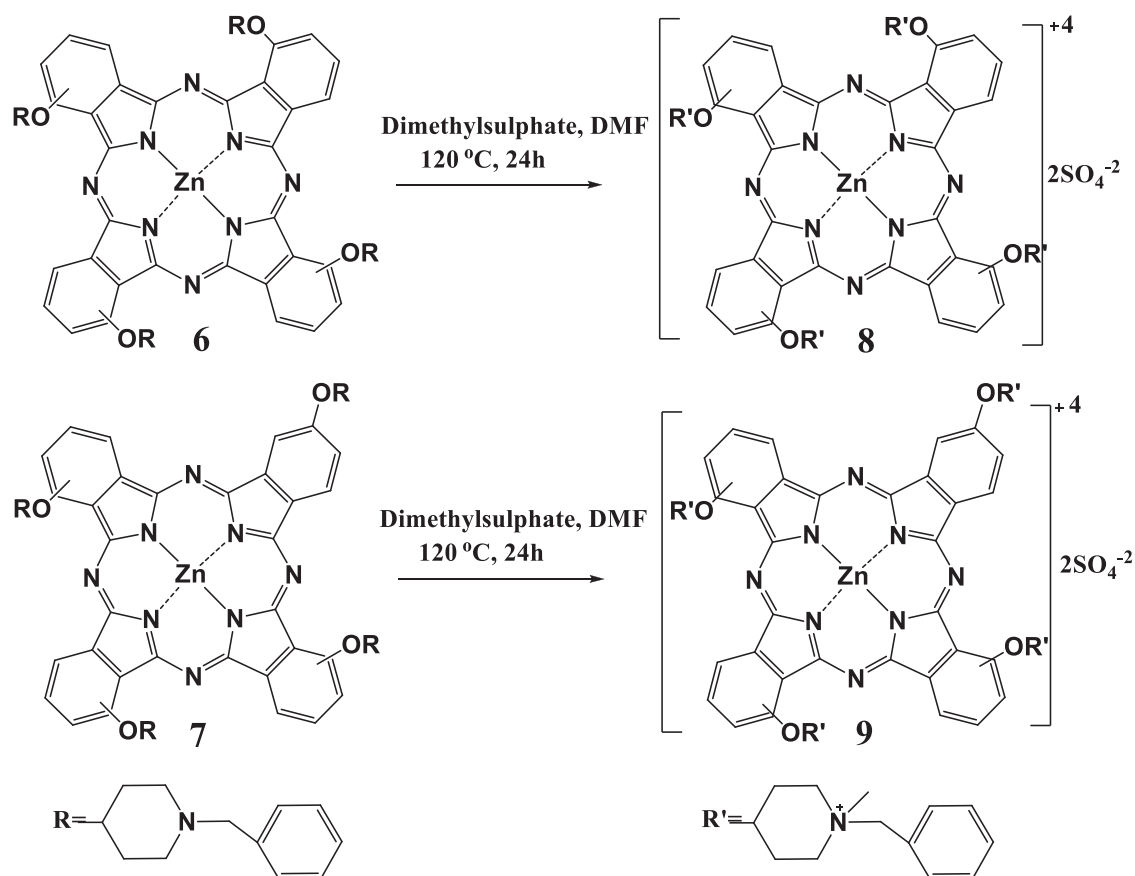
The electronic spectra of ZnPcs (**6–9**) showed sharp single Q bands in the visible region as a result of the monomeric behavior of typical metallated phthalocyanine complexes in DMSO (Table 1).<sup>20,21</sup>

**Table 1.** Absorption, excitation and emission spectral data for standard and studied ZnPcs.

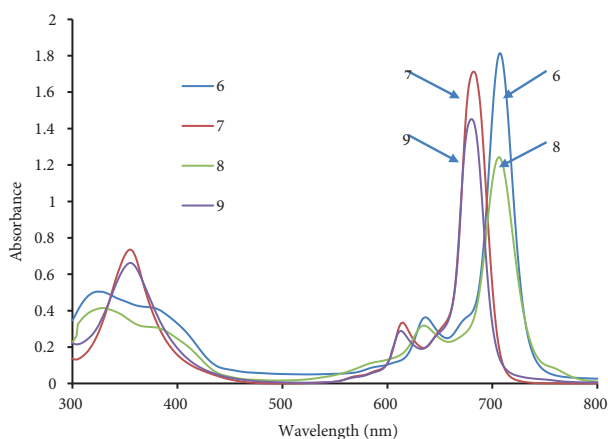
Compound	Solvent	Q band $\lambda_{max}$ (nm)	$\log \epsilon$	Excitation $\lambda_{Ex}$ (nm)	Emission $\lambda_{Ex}$ (nm)	Stokes shift $\Delta_{Stokes}$ (nm)
<b>6</b>	DMSO	707	5.21	709	719	10
<b>7</b>	DMSO	685	5.22	688	695	7
<b>8</b>	DMSO	707	4.96	709	719	10
	Water	709	4.67	710	722	12
	Water + TX	707	4.71	709	720	11
<b>9</b>	DMSO	685	5.15	686	695	9
	Water	640, 675	4.72	-	-	-
	Water + TX	685	5.05	685	695	10
ZnPc <sup>a</sup>	DMSO	672	5.14	672	682	10

<sup>a</sup>Data from reference<sup>21</sup>

Substitution of phthalocyanines at nonperipheral positions causes a bathochromic shift in the Q band.<sup>22–25</sup> When the results of the UV-Vis measurements of nonperipheral (**6** and **8**) and peripheral (**7** and **9**) tetrasubstituted ZnPc complexes are compared, the Q bands of compounds **6** and **8** are red shifted about 22 nm compared to compounds **7** and **9** in DMSO (Figure 1; Table 1). For the nonperipherally substituted Pc complexes **6** and **8**, the substituents are closer to the ring and therefore they lead to greater destabilization of the  $a_{1u}$  orbitals (HOMO) and result in larger bathochromic shifts.<sup>26</sup>



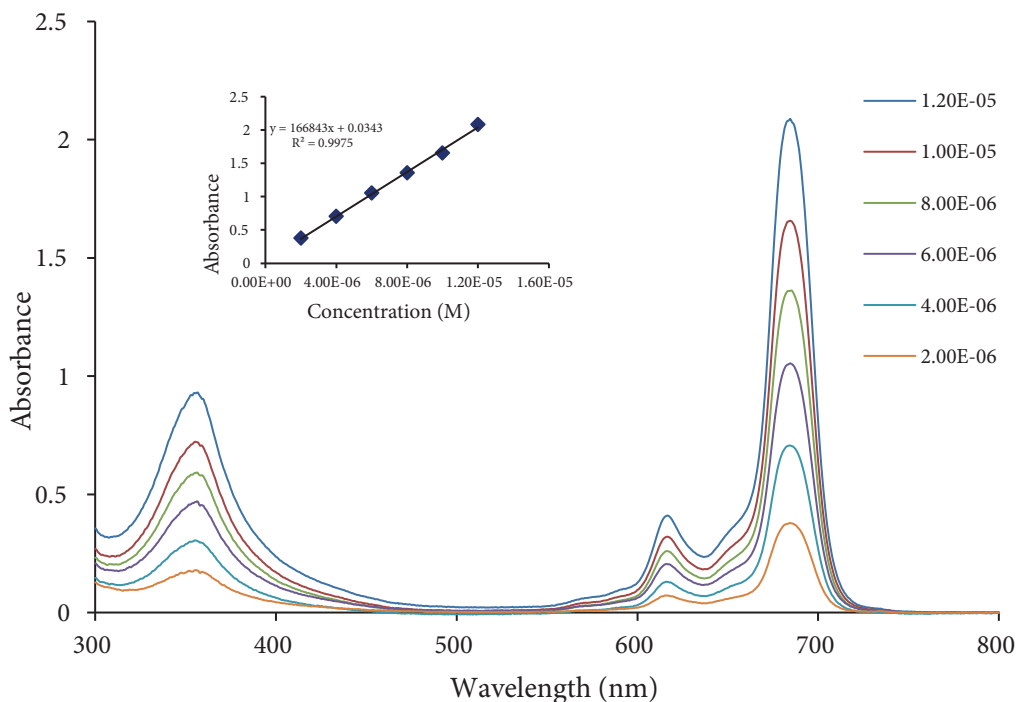
**Scheme 2.** Synthetic procedure of quaternized ZnPcs substituted with 1-benzylpiperidin-4-ol groups.



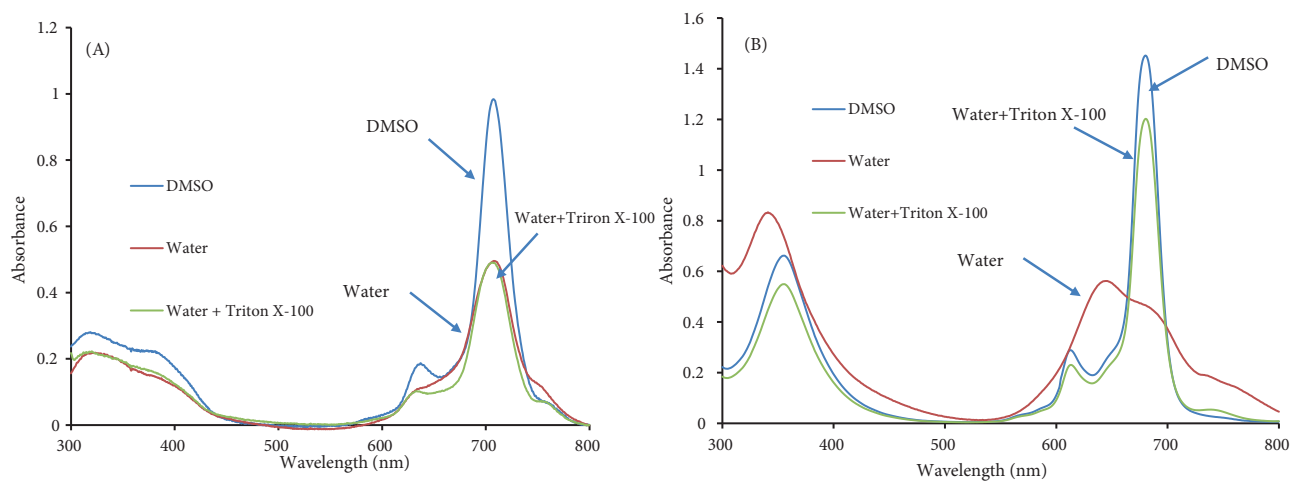
**Figure 1.** Electronic spectra of ZnPcs (**6–9**) in DMSO at  $1 \times 10^{-5}$  M concentration.

Aggregation of MPc complexes is due to coplanar association of the rings progressing from monomer to dimer.<sup>27</sup> In DMSO, all complexes were monomeric and they did not show any aggregation. Beer's law was obeyed for the concentration range between  $2.0 \times 10^{-6}$  and  $1.2 \times 10^{-5}$  M (Figure 2, for complex **7** as an example). Complex **8** showed a small aggregation in water while it did not show aggregation in DMSO. Even after the addition of Triton-X 100, which is a surfactant used for the breaking of aggregates of complex **8**, there

was no significant change in the UV-spectra (Figure 3A). In water, the absorption spectra of complex **9** showed cofacial aggregation as judged by the nonvibrational peak at around 640 nm (Figure 3B). Complex **9** exhibited completely monomeric behavior with the addition of a small amount Triton-X 100. The Q band position of **9** in water (685 nm, in the presence of Triton-X 100) is almost the same as that in DMSO (Figure 3B).



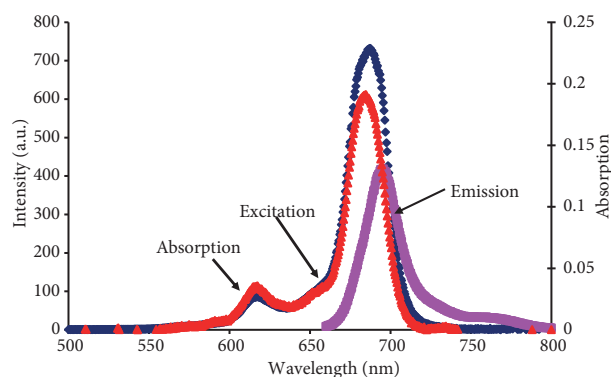
**Figure 2.** Electronic spectrum ZnPc (**7**) in DMSO at different concentrations.



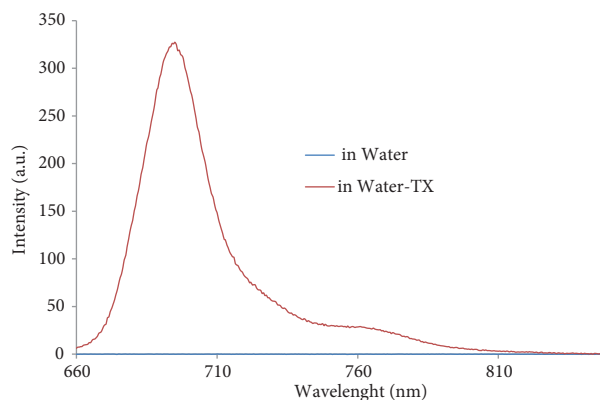
**Figure 3.** Electronic spectra of compounds **8** (A) and **9** (B) at  $1 \times 10^{-5}$  M concentration.

As the example of the studied ZnPc complexes, the absorption, fluorescence emission, and excitation spectra of complex **7** in DMSO are given in Figure 4 and the fluorescence emission data are given in Table 1. All studied ZnPc complexes (**6–9**) have similar fluorescence behavior in DMSO. For all ZnPc complexes, the fluorescence spectra were observed as mirror images of both the absorption and the excitation spectra,

and the excitation spectra were similar to the absorption spectra in DMSO. The complex **9** did not show fluorescence emission in water due to the aggregation of this complex in this solvent. The aggregation reduces the photoactivity of molecules through dissipation of energy and so aggregated MPc complexes do not display fluorescence properties.<sup>28</sup> The addition of the Triton-X 100 increased the intensity of the emission peak of complex **9** in water (Figure 5).



**Figure 4.** Absorption, excitation, and emission spectra of complex **7**. Excitation wavelength: 650 nm.



**Figure 5.** Fluorescence emission spectra of **9**. Excitation wavelength: 650 nm.

### 2.3. Fluorescence quantum yields and lifetimes

The obtained values of photophysical parameters for complexes **6–9** in DMSO, water, and water + Triton-X 100 are given in Table 2. In both DMSO and aqueous solutions (water and water + Triton X-100) the fluorescence quantum yield ( $\Phi_F$ ) values of ZnPc complexes **7** and **9** are higher than those of ZnPc complexes **6** and **8**, indicating that quenching of the excited singlet state by peripheral substitution is not as much as by nonperipheral substitution.<sup>19</sup> The  $\Phi_F$  values of the studied ZnPc complexes are lower than those of standard Zn(II)Pc in DMSO, which suggests that 1-benzylpiperidin-4-ol substituents quench fluorescence. Quaternized ZnPc complexes (**8** and **9**) exhibited lower  $\Phi_F$  values with respect to concerning nonionic ZnPc complexes (**6** and **7**) in DMSO, indicating that cationic ZnPcs are better fluorescence quenchers than nonionic derivatives. Comparing DMSO and water as solvents, complexes **8** and **9** had more fluorescence in DMSO as a result of their lower aggregation tendency. Addition of Triton-X 100 to the water solutions of complexes **8** and **9** increased the  $\Phi_F$  values of these complexes (Table 2).

The  $\tau_F$  values are longer for the peripherally substituted complexes (**7** and **9**) than their nonperipherally substituted analogues (**6** and **8**) in both DMSO and water + Triton-X 100. Fluorescence lifetime values of the quaternized complexes (**8** and **9**) increased with addition of Triton-X 100 with respect to the increasing fluorescence quantum yield due to the reduction of aggregation. For all studied complexes, the  $\tau_F$  values are lower than those of standard ZnPc in DMSO. In contrast to the  $\tau_F$  values, the natural radiative lifetimes ( $\tau_0$ ) of the MPc complexes (**6–9**) are higher than those of standard ZnPc. Larger  $\tau_0$  values were observed for the quaternized zinc Pc complexes (**8**, **9**) compared to the nonionic zinc Pc complexes (**6** and **7**) in DMSO. The rate constants for fluorescence ( $k_F$ ) values follow the same trends as their respective quantum yields ( $\Phi_F$ ). Complexes **7** and **9** showed larger  $k_F$  values than their corresponding nonperipherally substituted analogues (**6** and **8**) in DMSO (Table 2).

**Table 2.** Photophysical and photochemical data of standard and studied ZnPcs.

Compound	Solvent	$\Phi_F$	$\tau_F$ (ns)	$\tau_0$ (ns)	$^a k_F (s^{-1}) (\times 10^7)$	$\Phi_d$	$\Phi_\Delta$
<b>6</b>	DMSO	0.08	0.699	8.736	11.45	$1.92 \times 10^{-5}$	0.82
<b>7</b>	DMSO	0.148	1.207	8.158	12.26	$8.2 \times 10^{-5}$	0.48
<b>8</b>	DMSO	0.066	0.849	12.861	7.77	$3.4 \times 10^{-5}$	0.90
	Water	0.017	0.253	14.909	6.707	0.162	0.082
	Water + TX	0.16	2.957	18.484	5.410	0.072	0.38
<b>9</b>	DMSO	0.11	1.003	9.114	10.97	$9.79 \times 10^{-5}$	0.69
	Water	-	-	-	-	$8.96 \times 10^{-4}$	0.043
	Water + TX	0.44	4.924	11.191	8.93	0.031	0.31
<b>ZnPc<sup>b</sup></b>	DMSO	0.20	1.22	6.80	1.47	$2.61 \times 10^{-5}$	0.67

<sup>a</sup>The rate constant for fluorescence. Values calculated using  $k_F = \Phi_F/\tau_F$ .

<sup>b</sup>Data from reference<sup>21</sup>

#### 2.4. Singlet oxygen quantum yields

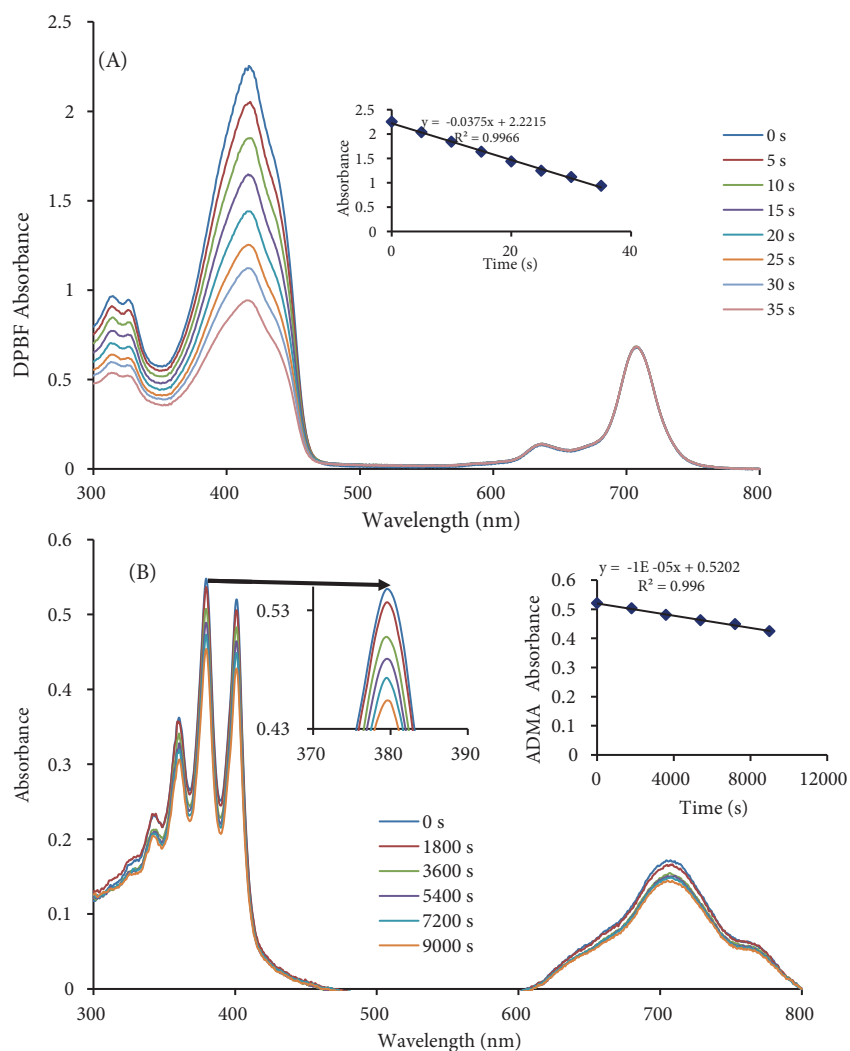
The amount of singlet oxygen produced by the excited dye is expressed by singlet oxygen quantum yield ( $\Phi_\Delta$ ). Singlet oxygen is formed by energy transfer from the triplet state of a photosensitizer to ground triplet state oxygen. Singlet oxygen producibility is the most consequential parameter of PDT.

For the determination of the  $\Phi_\Delta$  values, 1,3-diphenylisobenzofuran (DPBF) and 9,10-antracenediylbis(methylene)dimalonic acid (ADMA) were used as singlet oxygen quenchers in DMSO and aqueous solution, respectively. The decreasing of the absorbance of these quenchers by light irradiation was monitored in their absorption spectra (Figure 6, as an example for complex **8** in DMSO and water solutions). The  $\Phi_\Delta$  values of all ZnPc complexes (**6–9**) were determined in both DMSO (for **6** and **7**) and in aqueous solutions (for **8** and **9**) and are given in Table 2. The  $\Phi_\Delta$  values of the studied ZnPcs are higher than those of standard ZnPc except for complex **7** in DMSO and the nonperipherally substituted complexes (**6** and **8**) have greater  $\Phi_\Delta$  values than the peripherally substituted complexes (**7** and **9**), suggesting that the higher  $\Phi_\Delta$  values of the nonperipherally substituted Pc complexes could be due to the smaller energy gap of the HOMO-LUMO of these complexes than that of the peripherally substituted ones. Water-soluble ionic ZnPcs (**8** and **9**) produced more singlet oxygen when compared to their corresponding nonionic forms (**6** and **7**) in DMSO, but the  $\Phi_\Delta$  values in aqueous solution were lower than in DMSO. The lower  $\Phi_\Delta$  values in water than in DMSO could be associated with aggregation behavior of these complexes in water. To better investigate the reason for this impairment, Triton-X 100 was added to the aqueous media to reduce the aggregation. In the presence of this surfactant, the  $\Phi_\Delta$  values of these complexes were higher, suggesting that the reason for the lower  $\Phi_\Delta$  values was the presence of the aggregated Pcs. Especially the values of nonperipherally substituted nonionic/ionic Pc complexes are better than average.<sup>28–31</sup>

#### 2.5. Photodegradation studies

Degradation of Pcs is identified by decrease in the intensities of the Q-bands during intense light irradiation. A photosensitizer should spend enough time in the body without degradation. On the other hand, it should be expelled from the body after completion of PDT activation in a minimum time in order to prevent side effects.

The  $\Phi_d$  values for the studied zinc(II) phthalocyanine complexes in DMSO (for **6** and **7**), water and water + Triton X-100 solutions (for **8** and **9**) are given in Table 2. Slight decreasing of the intensities of the absorption bands during irradiation showed that photodegradation occurred (Figure 7 for complex **8** as an example). MPc



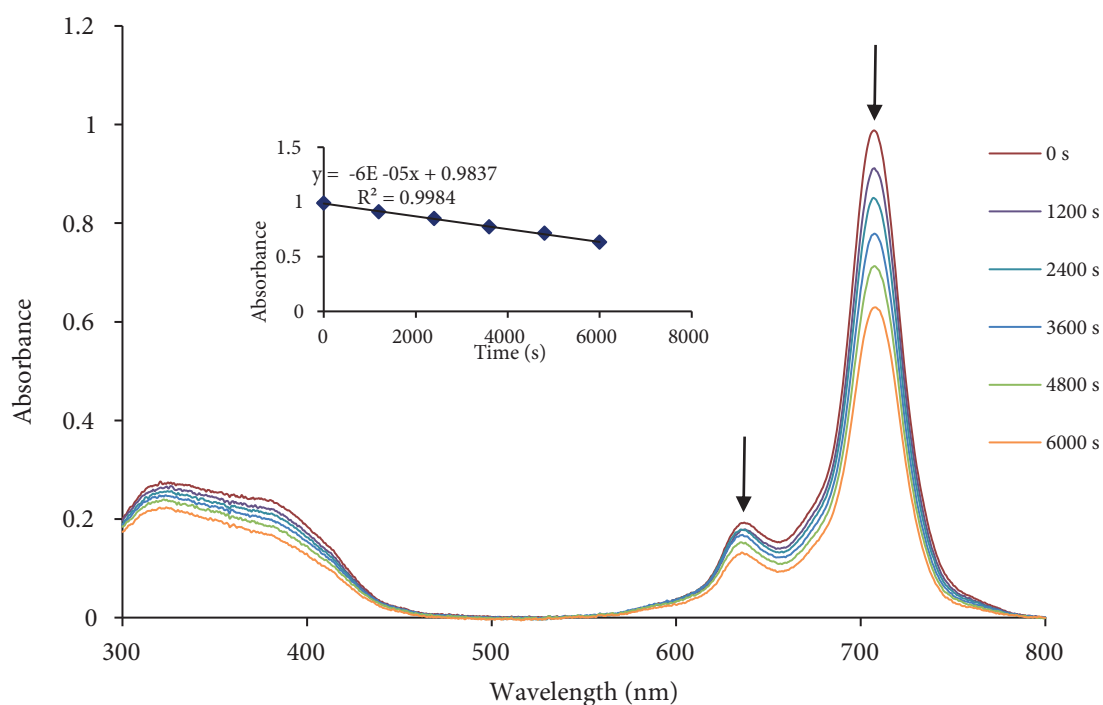
**Figure 6.** Absorption changes during the determination of singlet oxygen quantum yield of: **8** (A) in DMSO using DPBF as a singlet oxygen quencher and **8** (B) in water. (Inset: Plots of DPBF or ADMA absorbance versus time) Concentration:  $1 \times 10^{-5}$  M.

complexes (**6–9**) showed similar stability compared to other known ZnPc complexes in DMSO.<sup>28</sup> While typical values for stable phthalocyanines are of the order of  $10^{-6}$ , these values for unstable phthalocyanines are of the order of  $10^{-3}$ .<sup>32</sup> Nonionic ZnPc complexes (**6** and **7**) exhibited slightly lower  $\Phi_d$  values than their corresponding ionic ZnPc analogues (**8** and **9**) in DMSO, indicating that quaternization of ZnPcs decreased their stability. Furthermore, quaternized ZnPc complexes (**8** and **9**) were more stable in DMSO than in water.

## 2.6. Fluorescence quenching studies by 1,4-benzoquinone (BQ)

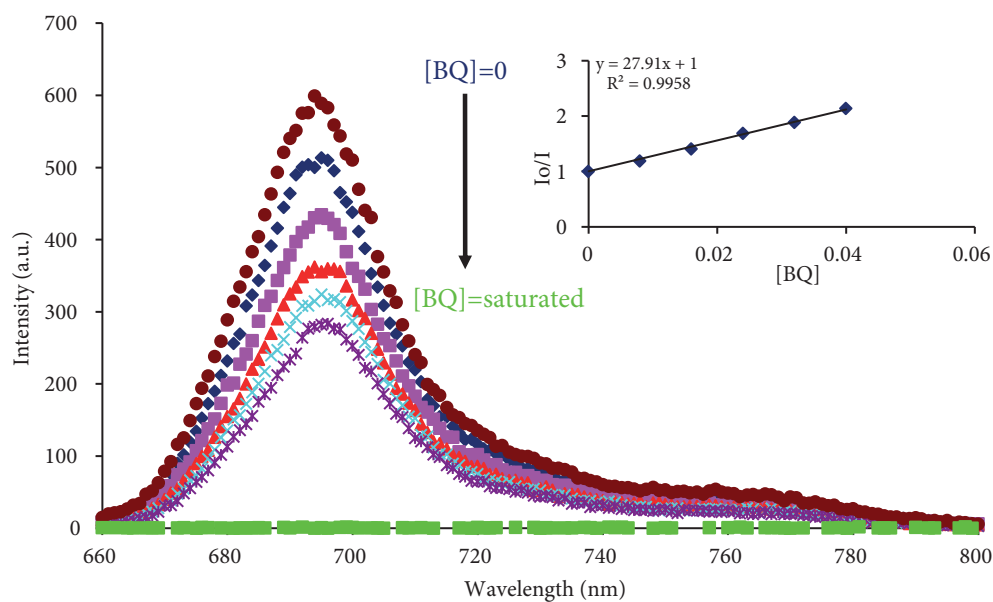
The fluorescence quenching of the zinc Pc complexes (**6–9**) by 1,4-benzoquinone (BQ) in DMSO was found to obey Stern–Volmer kinetics. Fluorescence quenching of MPc complexes by BQ is achieved via excited state electron transfer from the MPc to BQ as the energy of the lowest excited state for quinones is greater than the energy of the excited singlet state of MPc complexes.<sup>21,33</sup>





**Figure 7.** Absorption spectral changes during the investigation of the photodegradation quantum yield of compound **8** in DMSO. (Inset: plot of absorbance versus time).

Figure 8 shows the quenching of complex **7** by BQ as an example. The slope of the plot shown in the inset of the figure gives the Stern–Volmer constant ( $K_{SV}$ ) of this complex. Stern–Volmer plots for all studied complexes exhibit linear lines, depicting a diffusion-controlled quenching mechanism.



**Figure 8.** Fluorescence emission spectral changes of **7** ( $1 \times 10^{-5}$  M) during fluorescence quenching studies in DMSO.  $[BQ] = 0, 0.008, 0.016, 0.025, 0.032, 0.04$  M and saturated with BQ. (Inset: Stern–Volmer plots for BQ quenching of **7** in DMSO).

The  $K_{SV}$  and biomolecular quenching constant ( $K_q$ ) values of the ZnPcs (**6–9**) are listed in Table 3. The  $K_{SV}$  values of the tetrasubstituted ZnPc complexes (**6–9**) are lower than those of unsubstituted ZnPc. Furthermore, peripheral substituted zinc Pcs (**7** and **9**) showed higher  $K_{SV}$  values than the corresponding nonperipheral substituted ZnPcs (**6** and **8**) in DMSO. These results can be derived from the steric effect of the substituents on the nonperipheral positions of the phthalocyanine complexes. The observed  $k_q$  values of the studied ZnPc complexes are similar to the  $k_q$  values of unsubstituted zinc(II) phthalocyanine complex.

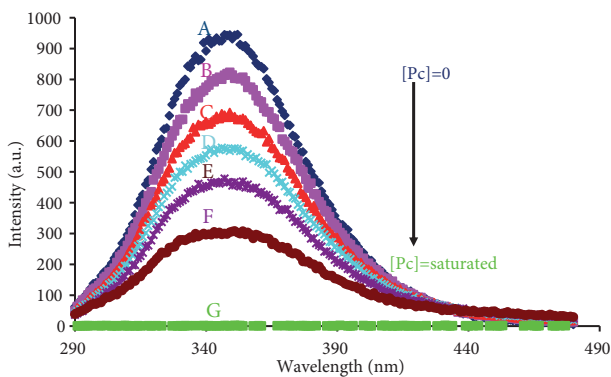
**Table 3.** Fluorescence quenching data for ZnPcs in DMSO.

Compound	$K_{SV}$ ( $M^{-1}$ )	$K_q/(dm^3 mol^{-1} s^{-1}) (\times 10^{10})$
<b>6</b>	22.08	3.16
<b>7</b>	27.91	2.31
<b>8</b>	17.52	2.06
<b>9</b>	26.03	2.59
ZnPc <sup>a</sup>	31.90	2.61

<sup>a</sup>Data from reference<sup>21</sup>

## 2.7. Interaction of cationic zinc(II) phthalocyanine complexes with BSA

Figure 9 shows the changes in the fluorescence emission spectra of BSA with the addition of different amounts of **9** (as an example) in water. The observed results suggest that the studied complexes readily bind to BSA, which demonstrated quenching of the fluorescence emission of BSA with the addition of these complexes.



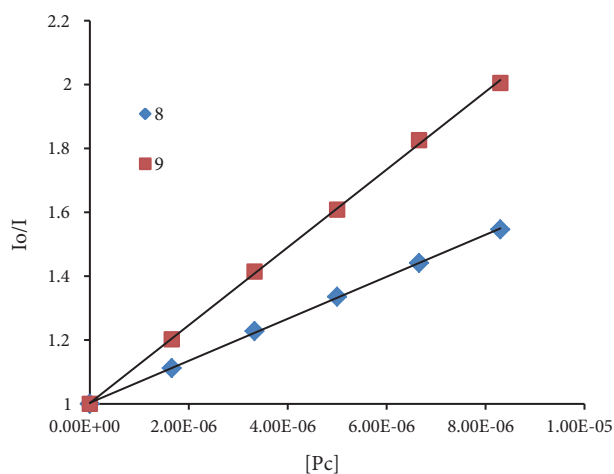
**Figure 9.** Fluorescence emission spectral changes in BSA ( $C = 1.00 \times 10^{-5}$  M) by the addition of varying concentrations of **9** in water.  $[9]$ : A = 0, B =  $1.66 \times 10^{-6}$ , C =  $3.33 \times 10^{-6}$ , D =  $5.00 \times 10^{-6}$ , E =  $6.66 \times 10^{-6}$ , F =  $8.33 \times 10^{-6}$  M, G = saturated with **9**.

Stern–Volmer quenching constants ( $K_{SV}$ ) were determined from the slopes of plots with respect to individual ZnPcs (Figure 10), listed in Table 4. The higher  $K_{SV}$  values for **9** indicate that fluorescence quenching of BSA is more effective for complex **9** than for complex **8** in water. This could be due to the steric effect of nonperipheral substituents of zinc(II) phthalocyanine complexes. The bimolecular quenching constants ( $k_q$ ) were determined for respective zinc(II) phthalocyanines using equation 9 (in supplementary information) and the approximate lifetime of BSA was 10 ns.<sup>34</sup> These  $k_q$  values are of the order of  $10^{13} M^{-1} s^{-1}$ , which exceed the proposed value of  $10^{10} M^{-1} s^{-1}$  for dynamic quenching, indicating that the quenching mechanism in this case is static.<sup>35</sup> The binding constants ( $k_b$ ) and binding stoichiometry ( $n$ ) values between the zinc(II)

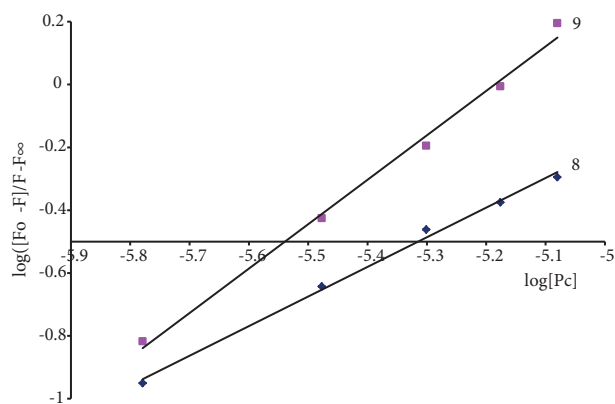
phthalocyanine complexes and BSA were also determined in water. The  $k_b$  and  $n$  values were obtained from the binding plot in Figure 11 and are listed in Table 4. The slope of the plots gave  $n$  values and the intercepts of these plots gave  $k_b$  values. The  $k_b$  and  $n$  values are typical of MPC-BSA interactions in aqueous solutions.<sup>13,34–36</sup> In this experiment, the obtained  $n$  values are close to 1, suggesting that the stoichiometric ratio of the ZnPCs and BSA in the formed complex is 1:1.

**Table 4.** Binding and fluorescence quenching data for interaction of BSA quaternized ZnPCs in water.

Compound	$K_{SV}^{BSA}/10^5$ ( $M^{-1}$ )	$k_q/10^{13}$ ( $M^{-1} s^{-1}$ )	$K_b/10^{-6}$ ( $M^{-1}$ )	N
<b>8</b>	0.64	0.64	4.84	0.94
<b>9</b>	1.87	1.87	2.88	1.41



**Figure 10.** Stern–Volmer plots of quaternized zinc phthalocyanines quenching of BSA in water.  $[BSA] = 1.00 \times 10^{-5}$  M in water.  $[Pc] = 0, 1.66 \times 10^{-6}, 3.33 \times 10^{-6}, 5.00 \times 10^{-6}, 6.66 \times 10^{-6}, 8.33 \times 10^{-6}$  M.



**Figure 11.** Determination of quaternized zinc phthalocyanine-BSA binding constant ( $k_b$ ) and number of binding sites ( $n$ ) on BSA.  $[BSA] = 1.00 \times 10^{-5}$  M and  $[Pc] = 0, 1.66 \times 10^{-6}, 3.33 \times 10^{-6}, 5.00 \times 10^{-6}, 6.66 \times 10^{-6}, 8.33 \times 10^{-6}$  M in water.

In this paper, we described the synthesis of novel nonperipheral and peripheral tetrasubstituted zinc(II) phthalocyanine complexes bearing 1-benzylpiperidin-4-ol substituents (**6** and **7**) and their water soluble cationic quaternized complexes (**8** and **9**). All studied zinc Pc complexes were characterized. The photophysical and photochemical properties of these complexes were also investigated in DMSO for all complexes (**6–9**) and in aqueous (water or water + Triton X-100) solutions for quaternized cationic complexes (**8** and **9**). The effects of the position of the substituents and quaternization of these substituents on the photophysical and photochemical parameters of these complexes were reported. The values obtained for these complexes were also compared with those of unsubstituted ZnPc, which is used as standard. The nonionic and ionic zinc(II) Pc complexes were found to be monomeric in DMSO. The quaternized complexes exhibited excellent solubility in water. While the peripherally substituted complexes formed highly aggregated species, the nonperipherally substituted complexes formed mainly monomeric species. Furthermore, the effect of DMSO or water on the photophysical and photochemical properties of the quaternized complexes was also investigated. These studied zinc(II) Pc complexes have high singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) in DMSO, ranging from 0.48 to 0.90, which

makes them potential photosensitizers to be used in the PDT of cancer. The fluorescence of the tetrasubstituted zinc Pc complexes is effectively quenched by the addition of BQ. Furthermore, this study also presented that the water soluble zinc(II) phthalocyanines strongly bind to blood plasma proteins such as BSA.

### 3. Experimental

#### 3.1. Synthesis

##### 3.1.1. 3-[(1-Benzylpiperidin-4-yl)oxy]phthalonitrile (4)

1-Benzylpiperidin-4-ol (**1**) (1.59 g, 8.33 mmol) and 3-nitrophthalonitrile (**2**) (1.44 g, 8.33 mmol) were dissolved in dry DMF. Then anhydrous  $K_2CO_3$  (11.50 g, 83.37 mmol) was added over 2 h. The reaction media were stirred under  $N_2$  at 25 °C for 7 days. After the completeness of the reaction that was controlled by TLC, the mixture was poured into ice-water and the precipitate was filtered and washed with distilled water. The pure product was obtained by crystallization in ethanol. Yield: 1.32 g (50%), mp: 105–107 °C. IR  $v_{max}/cm^{-1}$ : 3082 (Ar–H), 2950–2828 (Aliph. C–H), 2223 ( $C\equiv N$ ), 1582–1470 ( $C=C$ ), 1283 (Ar–O–C), 1043 (N–CH<sub>2</sub>), 797, 749. <sup>1</sup>H NMR ( $CDCl_3$ ) ( $\delta$ : ppm): 7.06 (t,  $J = 6.4$  Hz, 1H, Ar–H), 7.32–7.30 (m, 5H, Ar–H), 7.25–7.21 (m, 2H, Ar–H), 4.58–4.56 (m, 1H, CH–CH<sub>2</sub>), 3.54 (s, 2H, CH<sub>2</sub>–Ar), 2.71 (t,  $J = 8$  Hz, 2H, CH<sub>2</sub>–N), 2.41 (t,  $J = 8$  Hz, 2H, CH<sub>2</sub>–N), 2.00–1.98 (m, 2H, CH<sub>2</sub>–CH), 1.93–1.91 (m, 2H, CH<sub>2</sub>–CH). <sup>13</sup>C NMR ( $CDCl_3$ ) ( $\delta$ : ppm): 160.25, 138.47, 129.01, 128.24, 127.08, 125.03, 118.07, 117.23, 115.33, 115.22, 113.24, 105.93, 75.11, 62.86 (2C), 49.56, 30.53 (2C). Elemental analysis: calcd (%) for  $C_{20}H_{19}N_3O$ : C, 75.69; H, 6.03; N, 13.24. Found: C, 75.66; H, 6.00; N, 13.30. MS ( $ES^+$ ):  $m/z$ : calcd. 317.38; found 318.13 [ $M + H$ ]<sup>+</sup>.

##### 3.1.2. 1(4), 8(11), 15(18), 22 (23)-Tekrakis-[1-benzylpiperidin-4-yl] oxy] phthalocyaninato zinc(II) (6)

3-[(1-Benzylpiperidin-4-yl)oxy]phthalonitrile (**4**) (0.60 g, 1.89 mmol) was dissolved in n-pentanol and anhydrous  $Zn(CH_3COO)_2$  (0.17 g, 0.95 mmol) was added to the media. The mixture was refluxed for 18 h. Then n-hexane was added and the precipitate was filtered. Purification of this novel compound was achieved by a two-step chromatographic procedure. Firstly silica gel column chromatography (DCM as eluting solvent) and then preparative thin layer chromatography (TLC) (DCM/ethanol (10:2) solvent system) were used. Yield: 160 mg (26%); UV/Vis (DMSO):  $\lambda_{max}$  ( $\log \epsilon$ ) 323 (4.57), 379 (4.60), 638 (4.47), 707 (5.25); IR  $v_{max}/cm^{-1}$ : 3064 (Ar–H), 2941–2800 (Aliph. C–H), 1583–1452 ( $C=C$ ), 1229 (Ar–O–C), 1040 (N–CH<sub>2</sub>), 720, 697; <sup>1</sup>H NMR ( $CDCl_3$ ) ( $\delta$ : ppm): 9.85–9.47 (m, 4H, Pc–H), 9.59–9.47 (m, 4H, Pc–H), 9.33–9.26 (m, 4H, Pc–H), 8.98–8.95 (m, 16H, Ar–H), 7.25–7.14 (m, 4H, Ar–H), 5.41–5.38 (m, 4H, CH–CH<sub>2</sub>), 4.87 (s, 8H, CH<sub>2</sub>–Ar), 3.62–3.58 (m, 16H, CH<sub>2</sub>–N), 3.01–2.89 (m, 16H CH<sub>2</sub>–CH); <sup>13</sup>C NMR ( $CDCl_3$ ) ( $\delta$ : ppm): 170.20, 156.80, 143.20, 141.10, 137.02, 131.73, 129.75, 128.36, 126.73, 118.82, 118.19, 116.39, 79.88, 64.82, 64.62, 57.67, 33.47, 32.64; Elemental analysis: calcd (%) for  $C_{80}H_{76}N_{12}O_4Zn$ : C, 71.98; H, 5.74; N, 12.59. Found: C, 71.96; H, 5.79; N, 12.55; MALDI-TOF-MS:  $m/z$ : calcd. 1334.95; found 1335.91 [ $M + H$ ]<sup>+</sup>.

##### 3.1.3. 2(3), 9(10), 16(17), 23(24)-Tetrakis-[1-benzylpiperidin-4-yl] oxy] phthalocyaninato zinc(II) (7)

The synthetic procedure for compound **6** was used except 4-[(1-benzylpiperidin-4-yl)oxy]phthalonitrile (**5**) instead of **4** was employed. The amounts of the reagents were 4-[(1-benzylpiperidin-4-yl)oxy]phthalonitrile (**5**)

(0.60 g, 1.89 mmol), anhydrous  $\text{Zn}(\text{CH}_3\text{COO})_2$  (0.17 g, 0.95 mmol). Solvent system for TLC; dichloromethane/ethanol (10:1) Yield: 190 mg (31%); UV/Vis (DMSO):  $\lambda_{max}$  ( $\log \varepsilon$ ) 358 (4.88), 618 (4.49), 685 (5.21); IR  $\nu_{max}/\text{cm}^{-1}$ : 3060 (Ar-H), 2925–2803 (Aliph. C-H), 1605–1452 (C=C), 1225 (Ar-O-C), 1043 (N-CH<sub>2</sub>), 746; <sup>1</sup>H NMR ( $\text{CDCl}_3$ ) ( $\delta$ :ppm): 8.91–8.87 (m, 4H, Pc-H), 8.66–8.55 (m, 4H, Pc-H), 7.55–7.48 (m, 4H, Pc-H), 7.33 (m, 8H, Ar-H), 7.21 (t, 8H, Ar-H), 7.12 (t, 4H, Ar-H), 4.90–4.88 (m, 4H, CH-CH<sub>2</sub>), 3.53 (s, 8H, CH<sub>2</sub>-Ar), 2.85–2.83 (m, 8H, CH<sub>2</sub>-N), 2.43–2.40 (m, 8H, CH<sub>2</sub>-N), 2.26–2.24 (m, 8H CH<sub>2</sub>-CH), 2.03–2.00 (m, 8H CH<sub>2</sub>-CH); <sup>13</sup>C NMR ( $\text{CDCl}_3$ ) ( $\delta$ : ppm): 161.35, 141.07, 139.02, 133.42, 130.51, 129.81, 128.52, 126.74, 125.22, 120.30, 119.83, 109.34, 75.25, 68.78, 52.59, 35.94, 33.23; Elemental analysis: calcd (%) C<sub>80</sub>H<sub>76</sub>N<sub>12</sub>O<sub>4</sub>Zn: C, 71.98; H, 5.74; N, 12.59. Found: C, 72.02; H, 5.71; N, 12.64; MALDI-TOF-MS: m/z: calcd. 1334.95; found 1335.31 [M + H]<sup>+</sup>.

### 3.1.4. 1(4), 8(11), 15(18), 22(23)-Tekrakis-[N-methyl-(1-benzylpiperidin-4-yl) oxyphthalocyaninato] zinc(II) sulfate (8)

Compound **6** (0.06 g, 0.0045 mmol) was heated to 120 °C in dry DMF (3 mL) and a high equivalent amount of dimethylsulfate (0.1 mL) was added to the media. The mixture was stirred at 120 °C for 18 h. After cooling to room temperature, the reaction mixture was precipitated by addition of acetone. The green solid product was filtered and washed with acetone, dichloromethane, ethanol, n-hexane, and diethyl ether. Yield: 74 mg (93%); UV/Vis (DMSO):  $\lambda_{max}$  ( $\log \varepsilon$ ) 320 (4.43), 384 (4.32), 639 (4.25), 707 (4.98); IR:  $\nu_{max}/\text{cm}^{-1}$  3036 (Ar-CH), 2907–2760 (Aliph. C-H), 1589–1480 (C=C), 1325 (S=O,  $\nu_{as}$ ), 1217 (Ar-O-C), 1116 (S=O,  $\nu_{sym}$ ), 1002 (N-CH<sub>2</sub>), 858, 743, 695; Elemental analysis: calcd (%) for C<sub>84</sub>H<sub>88</sub>N<sub>12</sub>O<sub>12</sub>S<sub>2</sub>Zn: C 63.57, H 5.59, N 10.59; found: C 63.55, H 6.03, N 10.54; MALDI-TOF-MS: m/z: calcd. 1395.08; found 349.75 [M + 1]<sup>4+</sup>.

### 3.1.5. 2(3), 9(10), 16(17), 23(24)-Tetrakis-[N-methyl-(1-benzylpiperidin-4-yl) oxy] phthalocyaninat]zinc (II) sulfate (9)

The synthetic procedure for **8** was used except **7** was employed instead of **6**. The amounts of the reagents were compound **7** (0.06 g, 0.0045 mmol), excess dimethylsulfate (0.1 mL). Yield: 71 mg (89%); UV/Vis (DMSO):  $\lambda_{max}$  ( $\log \varepsilon$ ) 358 (4.82), 618 (4.41), 685 (5.15); IR:  $\nu_{max}/\text{cm}^{-1}$  3030 (Ar-CH), 2982–2871 (Aliph. C-H), 1605–1475 (C=C), 1274 (S=O,  $\nu_{as}$ ), 1224 (Ar-O-C), 1167 (S=O,  $\nu_{sym}$ ), 1045 (N-CH<sub>2</sub>), 765, 747, 670; Elemental analysis: calcd (%) for C<sub>84</sub>H<sub>88</sub>N<sub>12</sub>O<sub>12</sub>S<sub>2</sub>Zn: C 63.57, H 5.59, N 10.59; found: C 64.01, H 5.54, N 10.57; MALDI-TOF-MS: m/z: calcd. 1395.08; found 350.75 [M + 2]<sup>4+</sup>.

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# SUPPLEMENTARY INFORMATION

## **Investigation of photophysical and photochemical behavior of substituted zinc phthalocyanines and their water-soluble quaternized derivatives**

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## 1. Materials

Zinc(II) phthalocyanine (ZnPc), 1,3-diphenylisobenzofuran (DPBF), and 1-benzylpiperidin-4-ol were purchased from Aldrich. Zinc(II) acetate,  $K_2CO_3$ , dimethylsulfate (DMS), and 9,10-antracenediyl-bis(methylene) dimalonic acid (ADMA) was purchased from Fluka. Dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were dried as described by Perrin and Armarego before use.<sup>1</sup> Methanol, n-hexane, chloroform ( $CHCl_3$ ), dichloromethane (DCM), tetrahydrofuran (THF), acetone, and ethanol were freshly distilled. Mixed sulfonated zinc phthalocyanine ( $ZnPcS_{mix}$ ),<sup>2</sup> 3-nitrophthalonitrile,<sup>3</sup> and 4-nitrophthalonitrile<sup>4</sup> were prepared and purified by reported procedures.

## 2. Equipment

Absorption spectra in the UV-vis region were recorded with a Shimadzu 2101 UV spectrophotometer. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1-cm pathlength cuvettes at room temperature. FT-IR spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer. Positive ion and linear mode MALDI-MS of complexes were obtained in dihydroxybenzoic acid as MALDI matrix using a nitrogen laser accumulating 50 laser shots using a Bruker Microflex LT MALDI-TOF mass spectrometer.  $^1H$  and  $^{13}C$  NMR spectra were recorded in  $CDCl_3$  solutions on a Varian 500 MHz spectrometer. Elemental analyses were obtained with a Thermo Finnigan Flash 1112 Instrument. Photo-irradiations were done using a General Electric quartz line lamp (300 W). A 600-nm glass cut-off filter (Intor) and a water filter were used to filter off ultraviolet and infrared radiations, respectively. An interference filter (Intor, 670 nm with a band width of 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX 5100 (Molelectron) power meter.



### 3. Photophysical parameters

#### 3.1. Fluorescence quantum yields and lifetimes

Fluorescence quantum yields ( $\Phi_F$ ) were determined by the comparative method using Eq. (1).<sup>5</sup>

$$\Phi_F = \Phi_F(\text{Std}) \frac{F \cdot A_{\text{Std}} \cdot n^2}{F_{\text{Std}} \cdot A \cdot n_{\text{Std}}^2}, \quad (1)$$

where  $F$  and  $F_{\text{Std}}$  are the areas under the fluorescence emission curves of the samples (6–9) and the standard, respectively.  $A$  and  $A_{\text{Std}}$  are the respective absorbances of the samples and standard at the excitation wavelengths, respectively.  $n^2$  and  $n_{\text{Std}}^2$  are the refractive indices of solvents used for the sample and standard, respectively. Unsubstituted ZnPc (in DMSO) ( $\Phi_F = 0.20$ ) was employed as the standard. The absorbance of the solutions at the excitation wavelength ranged between 0.04 and 0.05.<sup>6</sup>

Natural radiative life times ( $\tau_0$ ) were determined using the program PhotochemCAD, which uses the Strickler–Berg equation. The fluorescence lifetimes ( $\tau_F$ ) were evaluated using Eq. (2).<sup>7</sup>

$$\Phi_F = \frac{\tau_F}{\tau_0} \quad (2)$$

### 4. Photochemical parameters

#### 4.1. Singlet oxygen quantum yields

Singlet oxygen quantum yield ( $\Phi_\Delta$ ) determinations were carried out using the experimental set-up described above in the literature.<sup>8,9</sup> Typically, a 3-mL portion of the respective unsubstituted, nonperipheral, and peripheral tetrasubstituted zinc(II) phthalocyanine (6–9) solutions ( $C = 1 \times 10^{-5}$  M) containing the singlet oxygen quencher was irradiated in the Q band region with the photo-irradiation set-up described in the references.<sup>8,9</sup> Singlet oxygen quantum yields ( $\Phi_\Delta$ ) were determined in air using the relative method with

unsubstituted ZnPc (in DMSO) or ZnPcS<sub>mix</sub> (in aqueous media) as references. DPBF and ADMA were used as chemical quenchers for singlet oxygen determination in DMSO and aqueous media, respectively. Eq. (3) was employed for the calculations:

$$\Phi_{\Delta} = \Phi_{\Delta}^{\text{Std}} \frac{R \cdot I_{\text{abs}}^{\text{Std}}}{R^{\text{Std}} \cdot I_{\text{abs}}}, \quad (3)$$

where  $\Phi_{\Delta}^{\text{Std}}$  is the singlet oxygen quantum yield for the standard unsubstituted ZnPc ( $\Phi_{\Delta}^{\text{Std}} = 0.67$  in DMSO)<sup>10</sup> or ZnPcS<sub>mix</sub> ( $\Phi_{\Delta}^{\text{Std}} = 0.45$  in aqueous media).<sup>11</sup> R and R<sub>Std</sub> are the DPBF (or ADMA) photobleaching rates in the presence of the respective samples (6–9) and standards, respectively. I<sub>abs</sub> and I<sub>abs</sub><sup>Std</sup> are the rates of light absorption by the samples (6–9) and standards, respectively. To avoid chain reactions induced by DPBF (or ADMA) in the presence of singlet oxygen,<sup>12</sup> the concentration of quenchers (DPBF or ADMA) was lowered to  $\sim 3 \times 10^{-5}$  M. Solutions of sensitizer (C =  $1 \times 10^{-5}$  M) containing DPBF (or ADMA) were prepared in the dark and irradiated in the Q band region using the photoirradiation set-up. DPBF degradation at 417 nm and ADMA degradation at 380 nm were monitored. The light intensity  $6.75 \times 10^{15}$  photons s<sup>-1</sup> cm<sup>-2</sup> was used for  $\Phi_{\Delta}$  determinations.

#### 4.2. Photodegradation quantum yields

Photodegradation quantum yield ( $\Phi_d$ ) determinations were carried out using the experimental set-up described in the literature.<sup>8,9</sup> Photodegradation quantum yields were determined using Eq. (4),

$$\Phi_d = \frac{(C_0 - C_t) \cdot V \cdot N_A}{I_{\text{abs}} \cdot S \cdot t}, \quad (4)$$

where C<sub>0</sub> and C<sub>t</sub> are the sample (6–9) concentrations before and after irradiation, respectively, V is the reaction volume, N<sub>A</sub> is the Avogadro's constant, S is the irradiated cell area, t is the irradiation time, and I<sub>abs</sub> is the overlap integral of the radiation source light intensity and the

absorption of the samples (6–9). A light intensity of  $2.25 \times 10^{16}$  photons  $s^{-1} cm^{-2}$  was employed for  $\Phi_d$  determinations.

### 5. Fluorescence quenching by 1,4-benzoquinone (BQ)

Fluorescence quenching experiments on the nonperipheral and peripheral tetrasubstituted ZnPc derivatives (6–9) were carried out by the addition of different concentrations of BQ to a fixed concentration of the complexes, and the concentrations of BQ in the resulting mixtures were 0, 0.008, 0.016, 0.024, 0.032, and 0.040 M. The fluorescence spectra of tetrasubstituted ZnPc derivatives (6–9) at each BQ concentration were recorded, and changes in fluorescence intensity related to BQ concentration by the Stern–Volmer equation<sup>13</sup> as shown in Eq. (5):

$$\frac{I_0}{I} = 1 + K_{SV} [BQ], \quad (5)$$

where  $I_0$  and  $I$  are the fluorescence intensities of fluorophore in the absence and presence of quencher, respectively.  $[BQ]$  is the concentration of the quencher and  $K_{SV}$  is the Stern–Volmer constant, which is the product of the bimolecular quenching constant ( $k_q$ ) and  $\tau_F$  and is expressed in Eq. (6),

$$K_{SV} = k_q \cdot \tau_F \quad (6)$$

The ratios of  $I_0/I$  were calculated and plotted against  $[BQ]$  according to Eq. (5), and  $K_{SV}$  was determined from the slope.

### 6. Binding of water soluble cationic zinc (II) phthalocyanine complexes with BSA

The binding properties of the samples (6–9) to BSA were studied by spectrofluorometry at room temperature. A PBS solution of water (fixed concentration) was titrated with varying concentrations of the respective samples (6–9) solutions in water. BSA was excited at 280 nm and fluorescence recorded between 290 and 500 nm. The steady diminution in BSA fluorescence with increase in cationic zinc Pc concentrations was noted and used in the

determination of the binding constants and the number of binding sites on BSA, according to Eq. (7),<sup>14–16</sup>

$$\log \left[ \frac{(F_0 - F)}{(F - F_\infty)} \right] = \log K_b + n \log [Pc], \quad (7)$$

where  $F_0$  and  $F$  are the fluorescence intensities of BSA in the absence and presence of cationic Pc complexes (6–9), respectively;  $F_\infty$  is the fluorescence intensity of BSA saturated with cationic Pc complexes;  $K_b$  is the binding constant;  $n$  is the number of binding sites on a BSA molecule; and  $[Pc]$  is the concentration of cationic Pc complexes. Plots of  $\log \left[ \frac{(F_0 - F)}{(F - F_\infty)} \right]$  against  $\log [Pc]$  would provide the values of  $n$  (from the slope) and  $K_b$  (from the intercept).

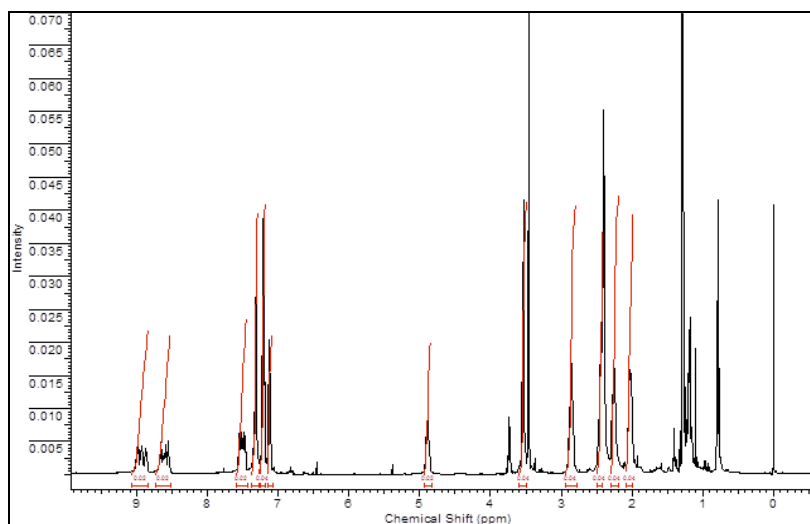
The changes in BSA fluorescence intensity were related to cationic Pc concentrations by the Stern–Volmer relationship (Eq. (8)):

$$\frac{F_0^{BSA}}{F^{BSA}} = 1 + K_{SV}^{BSA} [Pc] \quad (8)$$

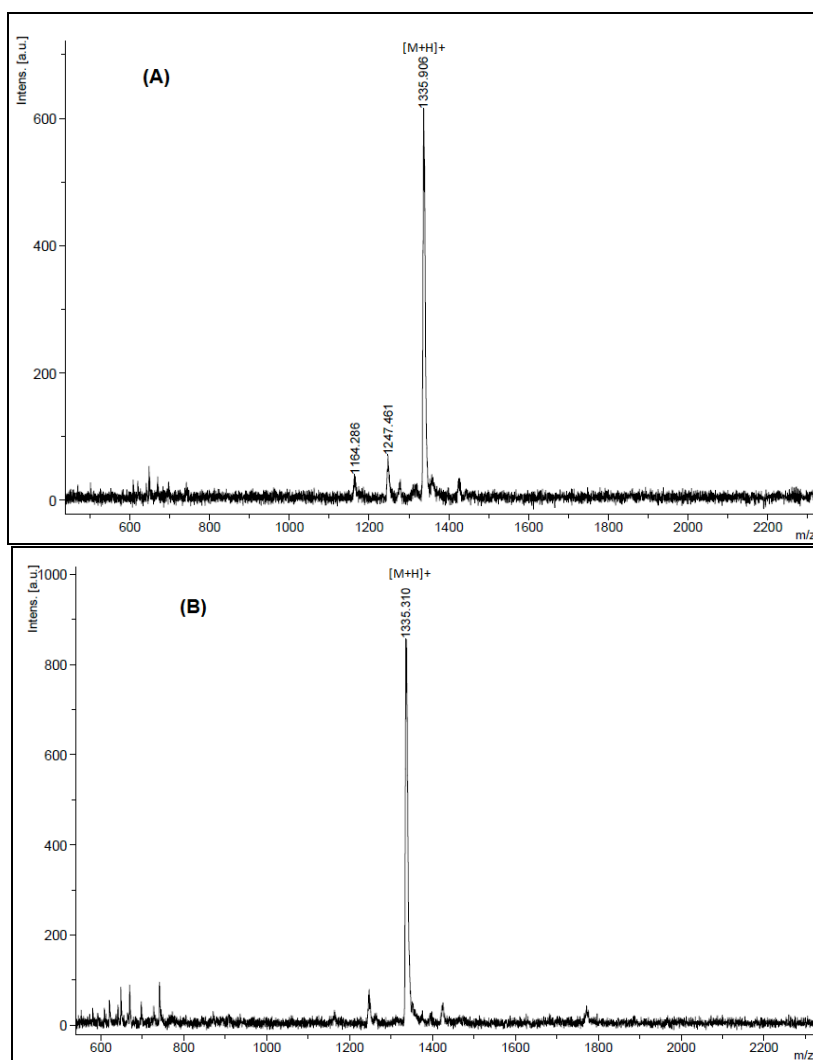
and  $k_{SV}^{BSA}$  is given by Eq. (9):

$$K_{SV}^{BSA} = k_q \tau_{F(BSA)}, \quad (9)$$

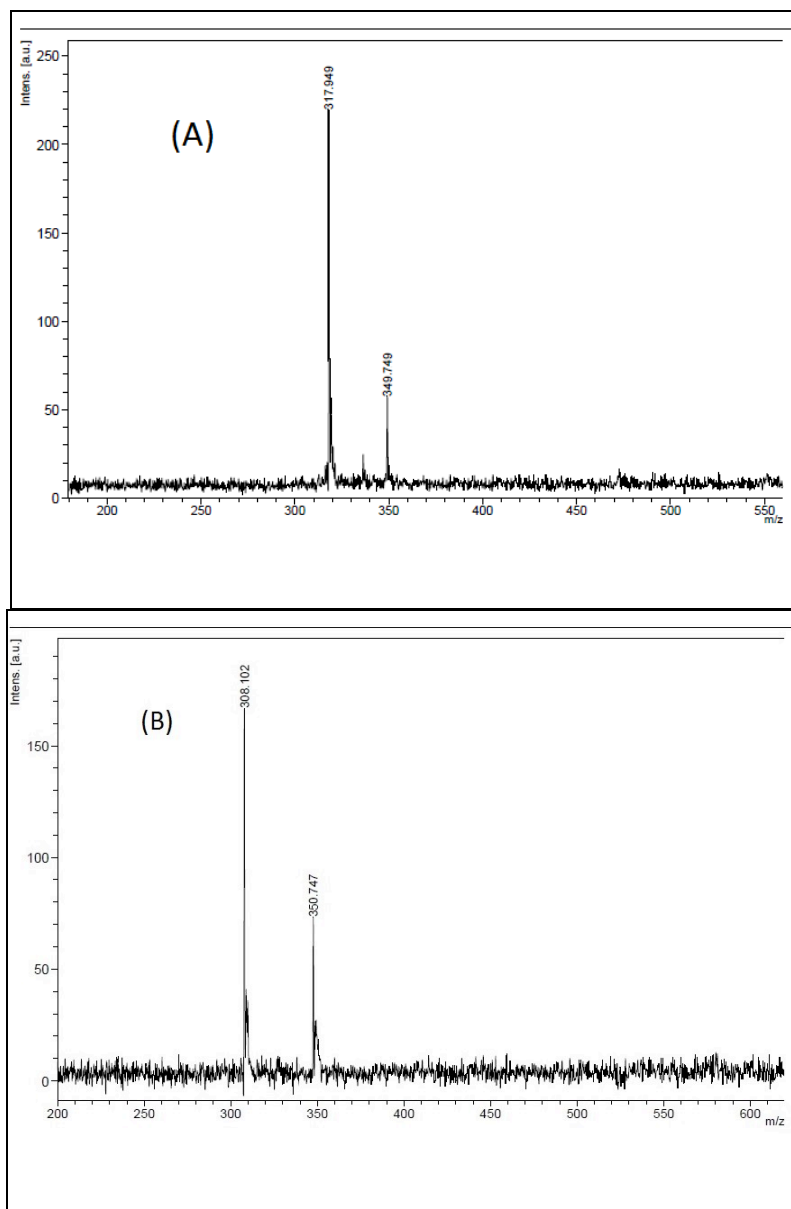
where  $F_0^{BSA}$  and  $F^{BSA}$  are the fluorescence intensities of BSA in the absence and presence of cationic Pc complexes (6–9), respectively;  $K_{SV}^{BSA}$ , the Stern–Volmer quenching constant;  $k_q$ , the bimolecular quenching constant; and  $\tau_{F(BSA)}$ , the fluorescence lifetime of BSA.  $\tau_{F(BSA)}$  is known to be 10 ns; thus from the values of  $K_{SV}^{BSA}$  obtained from the plots of  $F_0^{BSA} / F^{BSA}$  versus  $[Pc]$ , the value of  $k_q$  may be determined (Eq. (9)).<sup>17–19</sup>



**Figure S1.**  $^1\text{H}$ -NMR spectrum of compound **7** in  $\text{CDCl}_3$ .



**Figure S2.** Mass spectra of complex **6** (A), complex **7** (B).



**Figure S3.** Mass spectra of complex **8** (A), complex **9** (B).

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