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Amphiphilic zinc phthalocyanine photosensitizers: synthesis, photophysicochemical properties and *in vitro* studies for photodynamic therapy†

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Peripherally and non-peripherally tetra-substituted zinc(II) phthalocyanines bearing 2-(2-{2-[3-(dimethylamino)phenoxy]ethoxy}ethoxy)ethoxy and 2-(2-{2-[3-(diethylamino)phenoxy]ethoxy}ethoxy)ethoxy groups (**2a**, **5a**, **3a** and **6a**) were synthesized by cyclotetramerization of the corresponding phthalonitriles (**2**, **5**, **3** and **6**). Their quaternized ionic derivatives (**2b**, **5b**, **3b** and **6b**) were also synthesized by the reaction of them with methyl iodide. The novel compounds were characterized by using standard spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR, UV-vis, mass and elemental analyses. The obtained quaternized phthalocyanines (**2b**, **5b**, **3b** and **6b**) showed amphiphilic behaviour with excellent solubility in both organic and aqueous solutions, which makes them potential photosensitizers for use in photodynamic therapy (PDT) of cancer. The photophysical (fluorescence quantum yields and lifetimes) and photochemical (singlet oxygen and photodegradation quantum yields) properties of these novel phthalocyanines were studied in DMSO for both non-ionic and ionic quaternized derivatives. However, these properties were examined in both DMSO and phosphate buffer solution (PBS) for quaternized ionic phthalocyanines. The effects of the positions of substituents (peripheral or non-peripheral) and the quaternization of the nitrogen atoms on the substituents about their photophysical and photochemical properties were also compared in this study. The bovine serum albumin (BSA) binding behaviours of the studied quaternized ionic zinc(II) phthalocyanines were also described in PBS solutions. The quaternized phthalocyanines (**2b**, **5b**, **3b** and **6b**) successfully displayed light-dependent photodamage in HeLa and HuH-7 cancer cells in photodynamic therapy treatment. The photosensitivity and the intensity of damage were found directly related to the concentration of the photosensitizers.

1. Introduction

Photodynamic therapy (PDT), a form of phototherapy using non-toxic light-sensitive compounds, is an early new technique for cancer treatment. PDT applications involve three key components: a photosensitizer, a light source and tissue oxygen.[1] Photosensitizers are exposed selectively to visible light in the presence of oxygen so they become toxic to diseased cells. PDT has a proven ability to kill microbial cells, including bacteria, fungi and viruses. It is used clinically to treat a wide range of medical conditions, including wet age-related macular degeneration and malignant cancers.[2] The photochemical interactions of sensitizer, light, and molecular oxygen produce singlet oxygen and other forms of active oxygen, such as peroxide, hydroxyl radical and superoxide anion. The resulting damage to organelles within malignant cells leads to tumour ablation. Membranous organelles, including mitochondria, plasma membrane, and lysosomes, have been suggested to be the main sites of PDT damage.[3,4] Apoptosis after PDT has been demonstrated *in vitro*[5] and *in vivo*.[6] For this therapy, dyes such as hematoporphyrin derivatives are used. A disadvantage of these dyes is that they are a mixture of various mostly unidentified compounds. Moreover, they absorb light at relatively short wavelengths that do not penetrate deeply into the tissue. To defeat these disadvantages, other dyes such as chlorins and phthalocyanines are tested.[7] Phthalocyanines and metallophthalocyanines have been studied extensively for many years, mostly for their use as dyes and catalysts. Phthalocyanines display cytotoxic effects when activated by light. Phthalocyanines are promoted to their excited states and generate singlet oxygen when irradiated by light.[8]

Phthalocyanines with many remarkable features have become commercially and technologically important dyes and

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pigments for many decades.[9,10] These properties enable us to use them for several applications in contemporary technologies such as colors for metal surfaces, laser light absorbers in optical data storage systems, gas sensors, solar cells and nonlinear optical and optical limiting devices.[11–15] Moreover, phthalocyanines are efficient photosensitizers in the photodynamic therapy of cancer (PDT);[16] this property results from their intense absorption in the red region between 600 and 800 nm with a greater penetration of tissue[17] and their high production capability of singlet oxygen.[18]

In this paper, we aimed at the synthesis of some novel amphiphilic zinc(II) phthalocyanine photosensitizers since the amphiphilicity of the photosensitizers is very important for PDT applications due to their solubility behavior in both aqueous and organic media. An ideal photosensitizer should be soluble in aqueous media for its transport in the blood vessel and in organic media for increasing the cell penetration. For this purpose, 2-(2-{2-[3-(dimethylamino)phenoxy]ethoxy}-ethoxy)ethanol and 2-(2-{2-[3-(diethylamino)phenoxy]ethoxy}-ethoxy)ethanol groups were preferred as substituents in this study because they contain ethoxy chains for enhanced organic solubility and quaternizable nitrogen atoms for water solubility. As a result, the synthesized novel quaternized ionic phthalocyanines (**2b**, **5b**, **3b** and **6b**) showed amphiphilic behavior as desired. The photophysical (fluorescence quantum yields and lifetimes) and photochemical (singlet oxygen and photodegradation quantum yields) properties of newly synthesized peripherally and non-peripherally tetra-substituted non-ionic zinc(II) phthalocyanines (**2a**, **5a**, **3a** and **6a**) and their quaternized ionic derivatives (**2b**, **5b**, **3b** and **6b**) were investigated in DMSO and in both DMSO and PBS, respectively. The bovine serum albumin (BSA) binding studies of water soluble zinc(II) phthalocyanines were also performed in PBS solutions. The *in vitro* studies were also performed for quaternized zinc (II) phthalocyanines (**2b**, **5b**, **3b** and **6b**) against HeLa and HuH-7 cancer cell lines for the determination of photodynamic activities of these photosensitizers.

2. Experimental section

2.1. Materials and methods

The used materials, equipment, and photophysical and photochemical parameters are given in ESI.†

2.2. Synthesis

2.2.1. 3-[2-(2-{2-[3-(Dimethylamino)phenoxy]ethoxy}ethoxy)-ethoxy]phthalonitrile (3). 2-(2-{2-[3-(Dimethylamino)phenoxy]-ethoxy}ethoxy)ethanol (**1**) (2 g, 7.43 mmol), 3-nitrophthalonitrile (1.28 g, 7.43 mmol) and K_2CO_3 (3.08 g, 22.29 mmol) in dry DMF (17 mL) were stirred at 60 °C for 4 days under a nitrogen atmosphere. Then, the reaction mixture was poured into water. The aqueous phase was extracted with chloroform (3 × 120 mL). The combined extracts were dried over anhydrous $MgSO_4$ and then filtered. The crude product was purified by passing through an aluminium oxide column using $CHCl_3$ as the eluent. Yield: 0.79 g (27%). IR (KBr pellet) ν (cm^{-1}): 3087 (Ar–H), 2920–2875 (Aliph. C–H), 2231 (C≡N), 1609, 1580, 1502, 1460, 1449, 1353, 1292, 1267, 1178, 1123, 1062, 1020, 998, 944, 811, 795, 754, 731, 687. ^{1}H NMR. ($CDCl_3$) δ (ppm): 7.59–7.52 (m, 2H, Ar–H), 7.25–7.21 (m, 2H, Ar–H), 7.01 (m, 1H, Ar–H), 6.21–6.11 (m, 2H, Ar–H), 4.16 (m, 4H, $-CH_2-O$), 3.99 (m, 2H, $-CH_2-O$), 3.81 (m, 4H, $-CH_2-O$), 3.65 (m, 2H, $-CH_2-O$), 2.83 (s, 6H, $-CH_3$). ^{13}C NMR. ($CDCl_3$: δ (ppm) 162.7, 161.4, 159.9, 151.8, 135.2, 129.9, 125.5, 117.8, 116.5, 115.8, 113.6, 106.0, 102.3, 99.8, 71.2, 70.9, 70.0, 69.8, 69.3, 67.3, 40.8. Calc. for $C_{22}H_{25}N_3O_4$: C 66.82, H 6.37, N 10.63%. Found: C 66.98, H 6.22, N 10.46%. MS (ESI): *m*/*z*: 396 $[M + H]^+$.

2.2.2. 3-[2-(2-{2-[3-(Diethylamino)phenoxy]ethoxy}ethoxy)-ethoxy]phthalonitrile (6). A similar preparation method to that of compound **3** was used to obtain compound **6** using a mixture of 3-nitrophthalonitrile (1.45 g, 8.41 mmol), 2-(2-{2-[3-(diethylamino)phenoxy]ethoxy}ethoxy)ethanol (**4**) (2.5 g 8.41 mmol), anhydrous K_2CO_3 (3.48 g, 25.25 mmol), and dry DMF (20 mL). Yield: 0.89 g (25%). IR (KBr pellet) ν (cm^{-1}): 3086 (Ar–H), 2928–2873 (Aliph. C–H), 2230 (C≡N), 1611, 1583, 1500, 1471, 1355, 1293, 1217, 1142, 1127, 1068, 1023, 988, 794, 755, 689. ^{1}H NMR. ($CDCl_3$): δ (ppm) 7.78–7.71 (m, 2H, Ar–H), 7.29 (m, 1H, Ar–H), 7.12 (t, 1H, Ar–H), 6.33–6.23 (m, 3H, Ar–H), 4.13 (t, 2H, $-CH_2-O$), 3.87 (t, 2H, $-CH_2-O$), 3.73–3.63 (m, 8H, $-CH_2-O$), 3.25 (q, 4H, CH_2-N), 1.14 (m, 6H, $-CH_3$). ^{13}C NMR. ($CDCl_3$) δ (ppm): 160.3, 160.2, 147.1, 135.5, 131.9, 130.3, 130.1, 123.2, 120.6, 118.5, 117.6, 114.6, 111.7, 105.6, 72.7, 71.0, 70.6, 70.1, 67.3, 62.0, 31.2, 12.8. Calc. for $C_{24}H_{29}N_3O_4$: C 68.06, H 6.90, N 9.92%. Found: C 68.24, H 6.75, N 10.08%. MS (ESI) *m*/*z*: 424 $[M + H]^+$.

2.2.3. 2(3),9(10),16(17),23(24)-Tetrakis-(2-{2-[3-(dimethyl-amino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) (2a). A mixture of 4-[2-(2-{2-[3-(dimethylamino)phenoxy]-ethoxy}ethoxy)ethoxy]phthalonitrile **2** (0.40 g, 1.01 mmol), anhydrous zinc acetate (0.093 g, 0.5 mmol), anhydrous *n*-pentanol (4 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (5 drops) was immersed in an oil bath preheated to 160 °C and stirred at this temperature for 24 h under a nitrogen atmosphere. A dark green solution was poured into cold ethanol, left for half an hour and the crude product was filtered off and washed with hot ethanol and diethyl ether and dried in a vacuum. The green product was purified by passing through an aluminium oxide column using the $CHCl_3$: CH_3OH (100 : 4) solvent system as an eluent. Yield: 0.150 g (36%). IR (KBr pellet) ν (cm^{-1}): 3073 (Ar–H), 2919–2870 (Aliph. C–H), 1605, 1573, 1487, 1447, 1393, 1337, 1280, 1230, 1115, 1089, 1057, 997, 955, 820, 744, 685. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.22 (m, 4H, Ar–H), 7.54–7.48 (m, 8H, Ar–H), 6.76–6.73 (m, 8H, Ar–H), 6.13–6.10 (m, 8H, Ar–H), 4.19 (m, 16H, $-CH_2-O$), 3.94 (m, 16H, $-CH_2-O$), 3.85 (m, 16H, $-CH_2-O$), 2.84 (s, 24H, $-CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm): 160.6, 157.0, 152.2, 151.1, 131.2, 129.9, 129.5, 123.6, 123.5, 121.0, 106.1, 103.20, 100.1, 99.9, 71.3, 71.2, 70.1, 67.2, 40.5. UV-vis (DMSO) λ_{max} nm (log ε): 356 (4.86), 615 (4.50), 685 (5.17). Calc. for $C_{88}H_{100}N_{12}O_{16}Zn$: C 64.17, H 6.12, N 10.20%. Found: C 64.32, H 5.90, N 10.36%. MS (MALDI-TOF) *m*/*z*: 1648 $[M + H]^+$.

2.2.4. 1(4),8(11),15(18),22(25)-Tetrakis-(2-{2-[3-(dimethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) (3a). The synthetic method resembles that of compound **2a**. Yield: 0.191 g (46%). IR (KBr pellet) ν (cm^{-1}): 3070 (Ar–H), 2922–2869 (Aliph. C–H), 1601, 1574, 1487, 1446, 1332, 1231, 1117, 1063, 997, 882, 800, 742, 685. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.03–7.98 (m, 8H, Ar–H), 7.75 (m, 4H, Ar–H), 7.51 (m, 4H, Ar–H), 6.89 (m, 4H, Ar–H), 6.27–6.22 (m, 8H, Ar–H), 4.28 (m, 8H, $-CH_2-O$), 4.15 (m, 8H, $-CH_2-O$), 3.87 (m, 16H, $-CH_2-O$), 3.76 (m, 16H, $-CH_2-O$), 2.86 (s, 24H, $-CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm): 156.5, 142.9, 141.8, 138.1, 133.8, 132.4, 130.7, 129.6, 119.2, 111.4, 107.2, 106.5, 106.2, 106.0, 71.2, 71.1, 70.1, 67.3, 40.7. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 377 (4.57), 634 (4.48), 704 (5.18). Calc. for $C_{88}H_{100}N_{12}O_{16}Zn$: C 64.17, H 6.12, N 10.20%. Found: C 64.38, H 5.88, N 10.39%. MS (MALDI-TOF) m/z: 1648 $[M + H]^+$.

2.2.5. 2(3),9(10),16(17),23(24)-Tetrakis-(2-{2-[3-(trimethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) iodide (2b). Compound **2a** (0.05 g, 0.030 mmol) was dissolved in $CHCl_3$ (4 mL) and stirred with 3.2 mL of CH_3I at room temperature for 2 days. The green precipitate was filtered off and washed with chloroform, acetone and diethyl ether. The precipitate was dried *in vacuo*. Yield: 0.058 g (87%). IR (KBr pellet) ν (cm^{-1}): 3012 (Ar–H), 2903–2869 (Aliph. C–H), 1605, 1488, 1450, 1394, 1335, 1278, 1228, 1179, 1088, 1053, 944, 875, 773, 745, 685. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.77 (m, 4H, Ar–H), 7.77 (m, 4H, Ar–H), 7.56–7.45 (m, 16H, Ar–H), 7.17 (s, 4H, Ar–H), 4.25 (m, 8H, $-CH_2-O$), 4.09 (m, 8H, $-CH_2-O$), 3.88–3.78 (m, 32H, $-CH_2-O$), 3.34 (s, 36H, $-CH_3$). ^{13}C NMR (DMSO-d_6) δ (ppm): 161.1, 159.8, 152.5, 148.7, 140.4, 135.6, 131.3, 124.2, 124.2, 118.8, 115.9, 112.6, 108.4, 106.4, 70.6, 70.6, 69.8, 69.4, 68.7, 68.4, 56.9. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 357 (4.90), 614 (4.55), 683 (5.20). Calc. for $C_{92}H_{112}I_4N_{12}O_{16}Zn$: C 49.89, H 5.10, N 7.59%. Found: C 50.08, H 4.92, N 7.71%. MS (MALDI-TOF) m/z: 428 $[M + 2–4I]^{4+}$.

2.2.6. 1(4),8(11),15(18),22(25)-Tetrakis-(2-{2-[3-(trimethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) iodide (3b). The same synthetic method as that of compound **2b** was used to obtain compound **3b** by using a mixture of compound **3a** (0.040 g, 0.024 mmol), chloroform (3 mL) and methyl iodide (2.5 mL). Yield: 0.035 g (36%). IR (KBr pellet) ν (cm^{-1}): 3012 (Ar–H), 2918–2871 (Aliph. C–H), 1589, 1487, 1331, 1300, 1231, 1176, 1117, 1058, 943, 881, 802, 745, 685. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.18 (m, 4H, Ar–H), 7.85 (m, 4H, Ar–H), 7.42–7.17 (m, 20H, Ar–H), 4.09 (m, 16H, $-CH_2-O$), 3.83 (m, 16H, $-CH_2-O$), 3.61 (m, 16H, $-CH_2-O$), 3.31 (s, 36H, $-CH_3$). ^{13}C NMR (DMSO-d_6) δ (ppm): 158.3, 159.7, 150.2, 145.5, 140.6, 136.3, 135.5, 131.5, 123.9, 119.1, 117.4, 112.3, 107.5, 105.4, 71.8, 71.4, 70.2, 69.5, 68.2, 56.7. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 320 (4.69), 633 (4.55), 702 (5.23). Calc. for $C_{92}H_{112}I_4N_{12}O_{16}Zn$: C 49.89, H 5.10, N 7.59%. Found: C 50.10, H 4.94, N 7.74%. MS (MALDI-TOF) m/z: 428 $[M + 2–4I]^{4+}$.

2.2.7. 2(3),9(10),16(17),23(24)-Tetrakis-(2-{2-[3-(diethylamino)-phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) (5a). The same synthetic method as that of compound **2a** was used to obtain compound **5a** by using a mixture of compound **5** (0.4 g, 0.94 mmol), anhydrous $Zn(CH_3COO)_2$ (0.086 g, 0.47 mmol), anhydrous *n*-pentanol (4 mL) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (5 drops). Yield: 0.160 g (39%). IR (KBr pellet) ν (cm^{-1}): 3073 (Ar–H), 2965–2869 (Aliph. C–H), 1606, 1570, 1488, 1449, 1393, 1354, 1337, 1276, 1214, 1115, 1090, 957, 820, 743, 685. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.20–8.08 (m, 8H, Ar–H), 6.98–6.89 (m, 8H, Ar–H), 6.26–6.04 (m, 12H, Ar–H), 4.04 (m, 24H, $-CH_2-O$), 3.85–3.81 (m, 24H, $-CH_2-O$), 3.09 (m, 16H, $-CH_2-N$), 1.01 (m, 24H, $-CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm): 155.5, 154.3, 152.9, 147.8, 131.1, 130.5, 129.9, 127.5, 118.6, 113.8, 107.6, 105.4, 100.9, 99.1, 71.1, 70.2, 69.9, 44.4, 12.7. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 357 (4.92), 615 (4.55), 682 (5.22). Calc. for $C_{96}H_{116}N_{12}O_{12}Zn$: C 65.53, H 6.65, N 9.55%. Found: C 65.69, H 6.50, N 9.43%. MS (MALDI-TOF) m/z: 1759 $[M]^+$.

2.2.8. 1(4),8(11),15(18),22(25)-Tetrakis-(2-{2-[3-(diethylamino)-phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) (6a). The synthetic method as that of compound **5a** was used to obtain compound **6a** by using a mixture of compound **6** (0.4 g, 0.94 mmol), anhydrous $Zn(CH_3COO)_2$ (0.086 g, 0.47 mmol), anhydrous *n*-pentanol (4 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (5 drops). Yield: 0.170 g (41%). IR (KBr pellet) ν (cm^{-1}): 3070 (Ar–H), 2924–2868 (Aliph. C–H), 1603, 1570, 1488, 1447, 1332, 1267, 1214, 1117, 1066, 986, 883, 800, 742, 686. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.03 (m, 4H, Ar–H), 7.54 (m, 4H, Ar–H), 6.99–6.97 (m, 8H, Ar–H), 6.21–6.17 (m, 12H, Ar–H), 4.01 (m, 16H, $-CH_2-O$), 3.75 (m, 32H, $-CH_2-O$), 3.24 (m, 16H, CH_2-N), 1.07 (m, 24H, $-CH_3$). ^{13}C NMR ($CDCl_3$ δ (ppm): 171.5, 169.0, 149.5, 149.2, 135.5, 130.6, 130.2, 130.0, 110.0, 105.4, 105.4, 100.8, 100.8, 99.3, 71.1, 70.2, 69.8, 67.1, 44.5, 12.8. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 311 (4.68), 634 (4.48), 705 (5.19). Calc. for $C_{96}H_{116}N_{12}O_{12}Zn$: C 65.53, H 6.65, N 9.55%. Found: C 65.72, H 6.47, N 9.40%. MS (MALDI-TOF) m/z: 1759 $[M]^+$.

2.2.9. 2(3),9(10),16(17),23(24)-Tetrakis-(2-{2-[3-(diethylmethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) iodide (5b). The synthetic method as that of compound **2b** was used to obtain compound **5b** by using a mixture of compound **5a** (0.040 g, 0.027 mmol), chloroform (4 mL) and methyl iodide (3 mL). Yield: 0.029 g (55%). IR (KBr pellet) ν (cm^{-1}): 3012 (Ar–H), 2903–2871 (Aliph. C–H), 1605, 1487, 1450, 1394, 1334, 1297, 1236, 1177, 1089, 1053, 954, 870, 827, 774, 746, 689. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.23 (m, 4H, Ar–H), 7.71 (m, 4H, Ar–H), 7.28–7.20 (m, 16H, Ar–H), 7.06 (m, 4H, Ar–H), 4.14 (m, 16H, $-CH_2-O$), 3.99 (m, 16H, $-CH_2-O$), 3.80 (m, 16H, $-CH_2-O$), 3.65 (m, 16H, CH_2-N), 3.52 (s, 12H, $N-CH_3$), 0.85 (m, 24H, CH_3). ^{13}C NMR (DMSO-d_6) δ (ppm): 159.2, 155.4, 151.4, 146.5, 140.8, 135.4, 134.6, 130.6, 124.6, 120.2, 118.7, 111.4, 107.9, 106.4, 71.9, 71.2, 70.5, 69.7, 68.8, 68.2, 56.2, 38.4, 13.7. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 357 (4.85), 615 (4.41), 683 (5.09). Calc. for $C_{100}H_{128}I_4N_{12}O_{16}Zn$: C 51.61, H 5.54, N 7.22%. Found: C 51.79, H 5.37, N 7.40%. MS (MALDI-TOF) m/z: 455 $[M + 1–4I]^{4+}$.

2.2.10. 1(4),8(11),15(18),22(25)-Tetrakis-(2-{2-[3-(diethylmethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalocyaninato zinc(II) iodide (6b). The synthetic method as that of compound **2b** was used to obtain compound **6b** by using a mixture of

compound **6a** (0.05 g, 0.028 mmol), chloroform (4 mL) and methyl iodide (3.1 mL). Yield: 0.05 g (76%). IR (KBr pellet) ν (cm^{-1}): 3022 (Ar–H), 2929–2869 (Aliph. C–H), 1588, 1487, 1448, 1393, 1331, 1258, 1232, 1174, 1116, 1062, 954, 870, 802, 744, 688. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.17 (m, 4H, Ar–H), 7.84 (m, 4H, Ar–H), 7.30–7.23 (m, 20H, Ar–H), 4.10 (m, 16H, –CH_2–O), 3.93 (m, 16H, –CH_2–O), 3.83 (m, 16H, –CH_2–O), 3.69 (m, 16H, CH_2–N), 3.60 (s, 12H, N–CH_3), 0.86 (m, 24H, CH_3). ^{13}C NMR (DMSO-d_6) δ (ppm): 162.2, 159.4, 156.2, 148.2, 141.7, 134.4, 133.5, 132.0, 124.8, 121.5, 119.7, 111.5, 108.2, 106.6, 71.9, 71.2, 70.6, 69.2, 68.5, 68.1, 56.1, 38.7, 13.4. UV-vis (DMSO) λ_{max} nm ($\log \varepsilon$): 321 (4.61), 634 (4.47), 703 (5.18). Calc. for $C_{100}H_{128}I_4N_{12}O_{16}Zn$: C 51.61, H 5.54, N 7.22%. Found: C 51.82, H 5.34, N 7.43%. MS (MALDI-TOF) m/z: 455 $[M + 1–4I]^{4+}$.

3. Results and discussion

3.1. Synthesis and characterization

The synthesis of new peripherally and non-peripherally tetra-substituted zinc(II) phthalocyanines (**2a**, **3a**, **5a** and **6a**) and their quaternized derivatives (**2b**, **3b**, **5b** and **6b**) is described in this work. The pathways for the synthesis of the studied phthalocyanines are given in Schemes 1, 2 and 3. Cyclotetramerization of the phthalonitrile derivatives substituted on 3 (compounds **3** and **6**) and 4 positions (compounds **2** and **5**) in the presence of anhydrous $Zn(CH_3COO)_2$ and a few drops of DBU as a strong base in *n*-pentanol at reflux temperature under a nitrogen atmosphere afforded the targeted non-peripherally (**3a** and **6a**) and peripherally (**2a** and **5a**) tetra-substituted zinc(II) phthalocyanines, respectively. The synthesized zinc(II) phthalocyanines (**2a**, **3a**, **5a** and **6a**) were treated with excess methyl iodide in chloroform at room temperature and the ionic zinc(II) phthalocyanine derivatives (**2b**, **3b**, **5b** and **6b**) were obtained by the quaternization of nitrogen atoms on the substituents. The quaternized products were soluble in DMF, DMSO and water, but not in chloroform. All newly synthesized compounds were characterized by UV-vis, FT-IR, ^{1}H NMR, ^{13}C NMR and MS spectroscopic data and elemental analysis. All obtained results were compatible with the proposed structures.

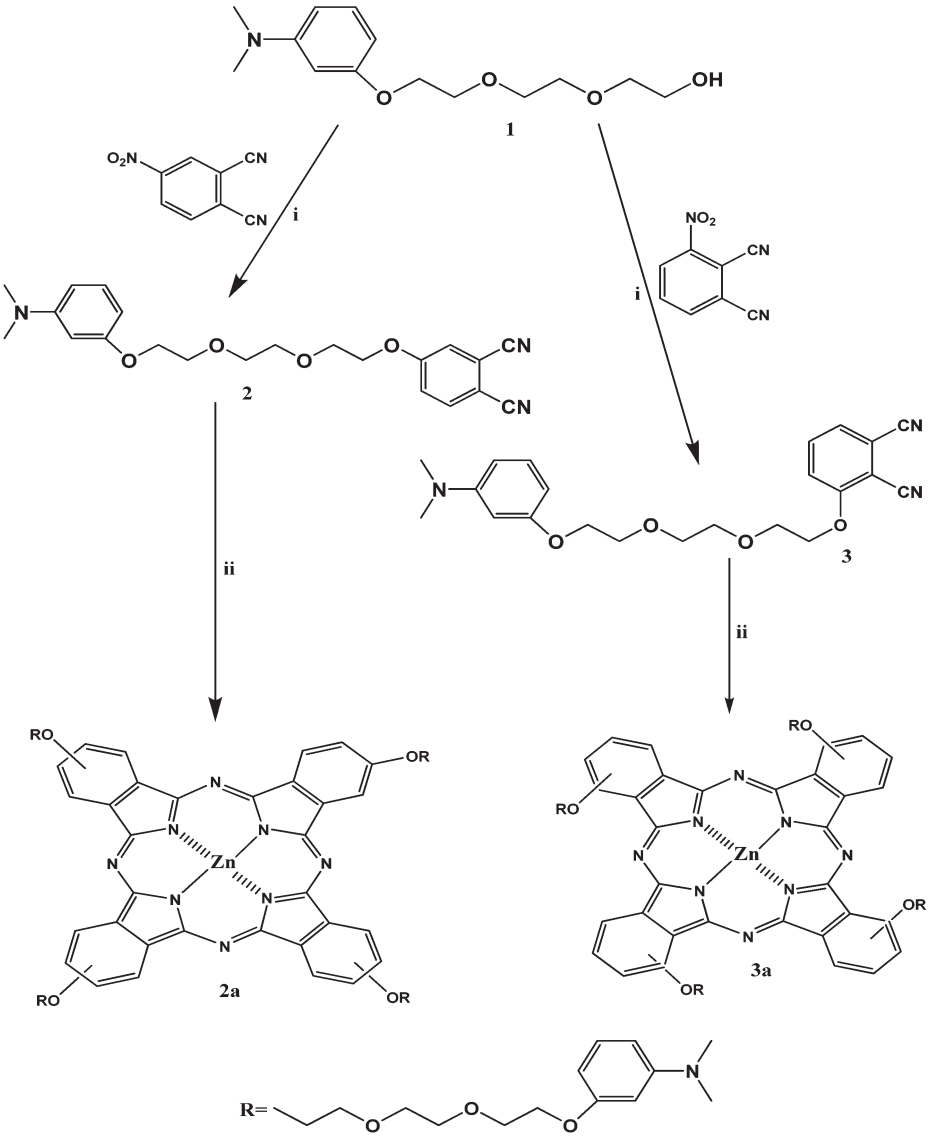
In the FT-IR spectra of zinc(II) phthalocyanines (**2a** and **5a**), cyclotetramerization of 4-[2-(2-{2-[3-(dimethylamino)phenoxy]-ethoxy}ethoxy)ethoxy]phthalonitrile (**2**) and 4-[2-(2-{2-[3-(diethylamino)phenoxy]ethoxy}ethoxy)ethoxy]phthalonitrile (**5**) to Pcs (**2a** and **5a**) was confirmed by the disappearance of the sharp –C≡N vibrations at 2229 cm^{-1} (for **2**) and 2230 cm^{-1} (for **5**). On the other hand, the FT-IR spectra of zinc(II) phthalocyanines (**2a** and **5a**) are very similar. In the ^{1}H-NMR spectra of peripherally tetra-substituted zinc(II) phthalocyanines (**2a** and **5a**), the aromatic protons appeared in the range between 8.22–6.10 ppm (for complex **2a**) and 8.20–6.04 ppm (for complex **5a**). The ^{1}H NMR spectra of zinc phthalocyanine complexes (**2a** and **5a**) in $CDCl_3$ exhibited the expected chemical shifts. In the MALDI-TOF mass spectra of compounds **2a** and **5a**, the molecular ion peaks were observed at m/z = 1648 as $[M + H]^+$ and 1759 as $[M]^+$, respectively, which confirmed the proposed structures.

In the FT-IR spectra of phthalonitrile derivatives **3** and **6**, the characteristic C≡N stretching vibrations were observed at 2231 and 2230 cm^{-1}, respectively. These sharp peaks disappeared after the reaction of cyclotetramerization. On the other hand, the studied zinc(II) phthalocyanines (**3a** and **6a**) gave very similar FT-IR spectra. In the ^{1}H NMR spectra of peripherally tetra-substituted zinc(II) phthalocyanines (**3a** and **6a**), the aromatic protons appeared in the range between 8.03–6.22 ppm (for complex **3a**) and 8.03–6.17 ppm (for complex **6a**). Aliphatic protons of these phthalocyanine complexes were observed between 4.28 and 2.86 ppm for complex **3a** and between 4.01 and 1.07 ppm for complex **6a**. The ^{1}H NMR spectra of non-peripherally tetra-substituted zinc(II) phthalocyanines (**3a** and **6a**) in $CDCl_3$ harmonize with the ^{1}H NMR spectra of peripherally tetra-substituted zinc(II) phthalocyanines (**2a** and **5a**). In the MALDI-TOF mass spectra of these compounds (**3a** and **6a**), the molecular ion peaks were observed at m/z = 1648 as $[M + H]^+$ and 1759 as $[M]^+$ supporting the proposed formula for these compounds. No major change in the FT-IR spectra was observed after quaternization of zinc phthalocyanine compounds. The NMR spectra of quaternized compounds **2b**, **3b**, **5b** and **6b** showed more unresolved patterns compared to non-quaternized derivatives. These complexes **2b**, **3b**, **5b** and **6b** showed the phthalocyanine ring protons integrating for a total of 28 protons. The MALDI-TOF mass spectra of quaternized compounds **2b**, **3b**, **5b** and **6b** showed molecular ion peaks at m/z = 428 $[M + 2–4I]^{4+}$, 428 $[M + 2–4I]^{4+}$, 455 $[M + 1–4I]^{4+}$ and 455 $[M + 1–4I]^{4+}$, respectively, supporting the proposed formula for these compounds.

3.2. Photophysical and photochemical studies

3.2.1. Ground state electronic absorption spectra. The ground state electronic absorption behaviors of the phthalocyanine compounds are usually examined by UV-vis spectroscopy. This spectroscopy is a quite useful method for the characterization of phthalocyanine compounds. Phthalocyanine derivatives show two absorption bands: the first one, known as Q, is observed in the visible region of the spectrum at around 600–750 nm due to the $\pi \rightarrow \pi^*$ transitions from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) of the phthalocyanine ring and the other one, known as B band, is observed in the ultraviolet region of the spectrum at around 300–450 nm arising from deeper π levels → LUMO.[19]

In this study, the electronic spectra of the studied peripherally (**2a** and **5a**) and non-peripherally (**3a** and **6a**) substituted zinc(II) phthalocyanine complexes and their quaternized derivatives (**2b**, **5b**, **3b** and **6b**) showed characteristic absorption in the Q band region at around 680–700 nm in DMSO, Table 1. The spectra of these phthalocyanines showed monomeric behavior in the DMSO evidenced by the formation of a single (narrow) Q band. Changing the variety of the substituents did not significantly affect the electronic spectra of the studied zinc(II) phthalocyanine compounds (Fig. 1). The elec-

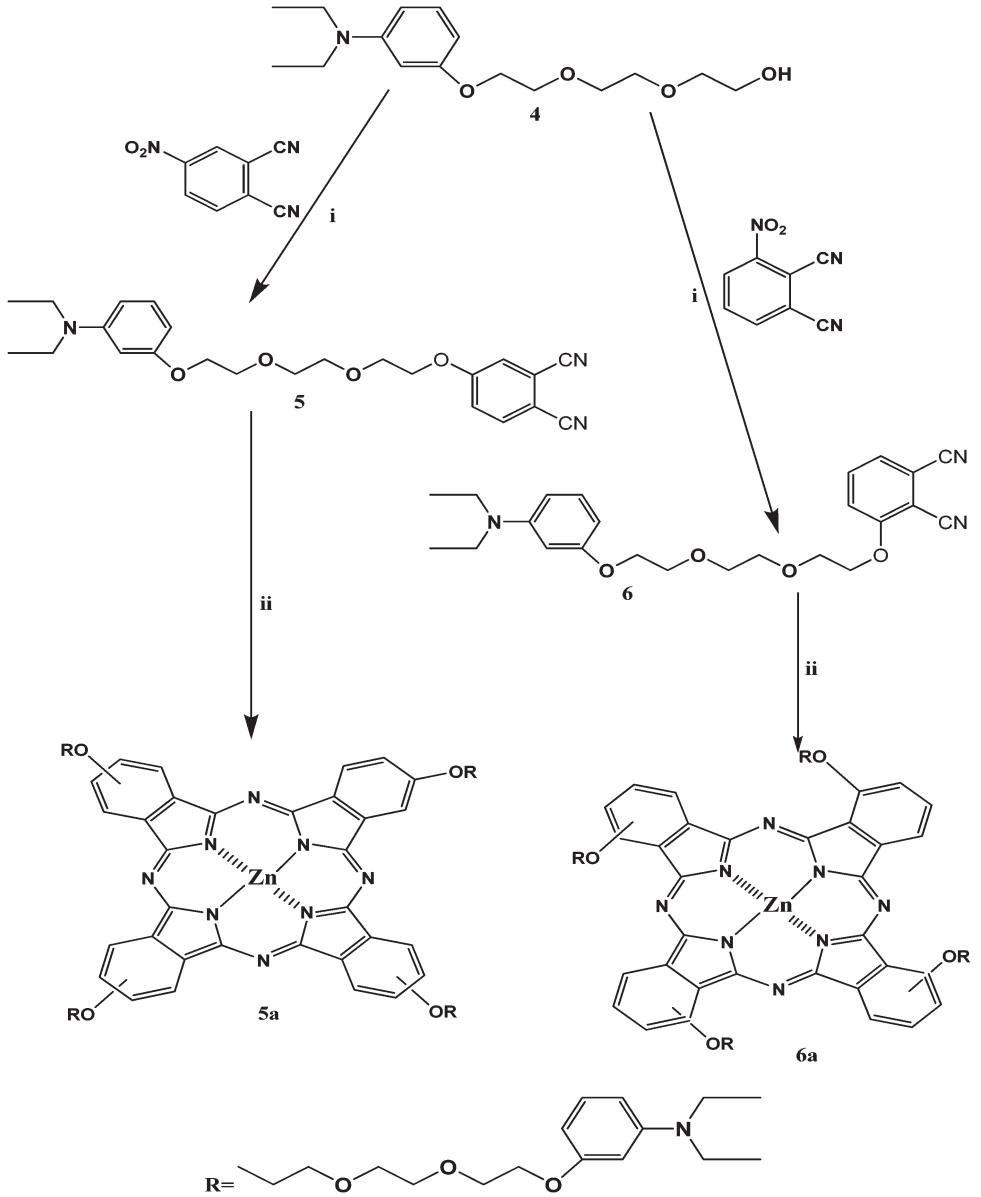


Scheme 1 The synthesis of the zinc(II) phthalocyanines **2a** and **3a**. (i) K_2CO_3, N_2, DMF. (ii) $Zn(CH_3COO)_2$, *n*-pentanol, DBU, 160 °C.

tronic absorption spectra of the studied zinc(II) phthalocyanines were also measured in DMSO at different concentrations for the determination of the aggregation behavior of these phthalocyanines depending on the concentration. The Lambert–Beer law was obeyed for all of these compounds at concentrations ranging from 1.2×10^{-5} to 2×10^{-6} M. All substituted zinc(II) phthalocyanine complexes did not show any aggregation at this concentration range in DMSO.

In the UV-vis spectra of peripherally substituted phthalocyanines **2a** and **5a** in DMSO, the Q band absorption was observed at 685 and 682 nm, respectively. In addition, B band absorption of these phthalocyanines (**2a** and **5a**) was observed at 359 and 358 nm, respectively. On the other hand, non-peripherally substituted phthalocyanines **3a** and **6a** showed intense Q band absorption at 704 and 705 nm, respectively in DMSO. B band absorption of compounds **3a** and **6a** was also observed at 312, 380 nm and 314, 374 nm, respectively. The ground state electronic spectra of the quaternized zinc phthalocyanines **2b**, **3b**, **5b** and **6b** showed characteristic absorption in the Q band region at 683 nm for **2b**, 702 nm for **3b**, 683 nm for **5b** and 703 nm for **6b** in DMSO. B band absorption was observed at 356, 373, 358 and 372 nm, respectively.

3.2.2. Aggregation studies. In this study, the newly synthesized zinc(II) phthalocyanines (**2a**, **3a**, **5a** and **6a**) showed good solubility in many organic solvents. The quaternized zinc (II) phthalocyanines (**2b**, **3b**, **5b** and **6b**) showed solubility in polar organic solvents such as DMSO and DMF, and water as well. The electronic absorption spectra of the studied zinc(II) phthalocyanine compounds were studied in different solvents (Fig. 2 as an example for compound **2a** and its quaternized derivative **2b**) for the investigation of aggregation behavior of these phthalocyanines in different solvents.

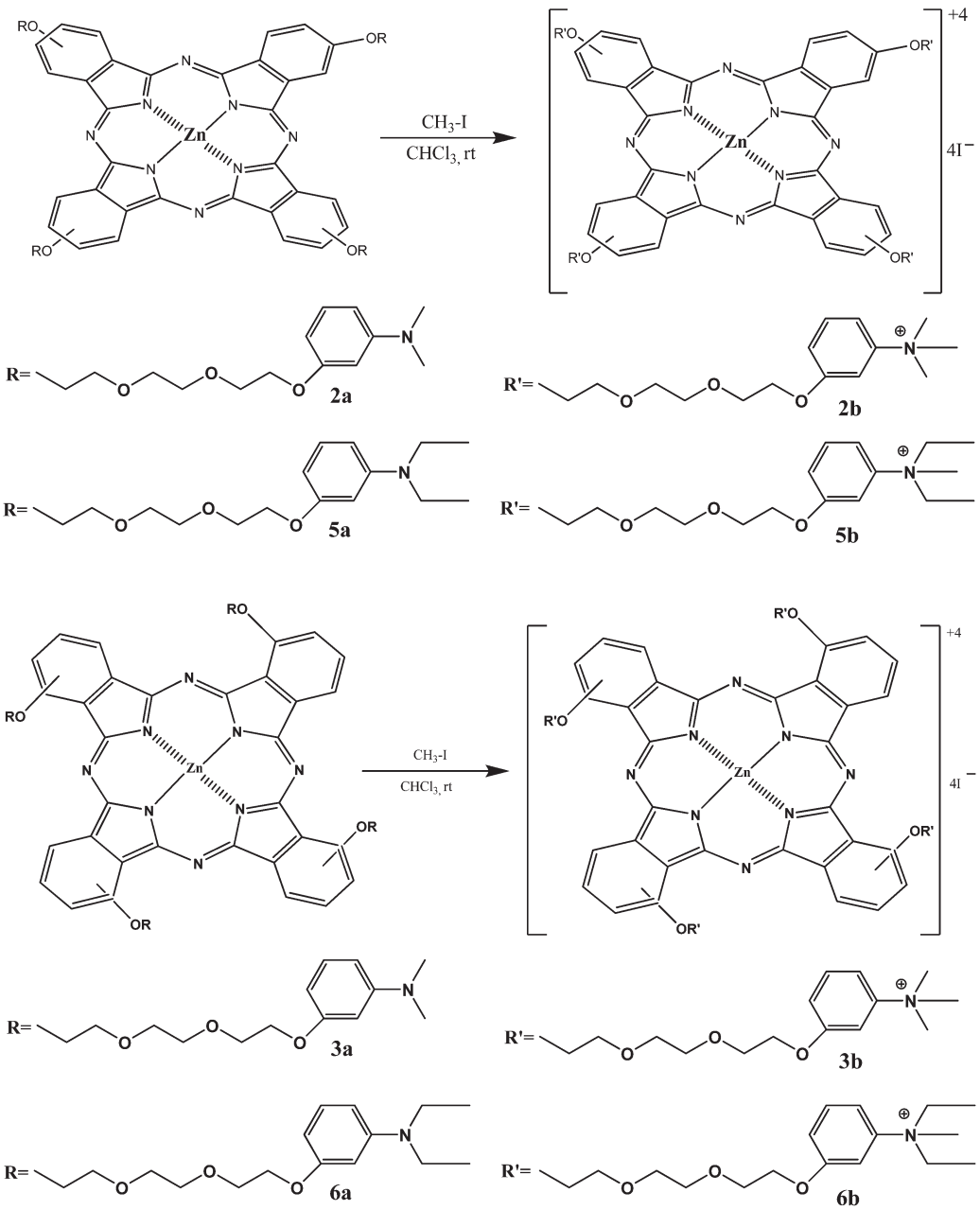


Scheme 2 The synthesis of the zinc(II) phthalocyanines **5a** and **6a**. (i) K_2CO_3, N_2, DMF. (ii) $Zn(CH_3COO)_2$, *n*-pentanol, DBU, 160 °C.

The UV-vis spectra of non-ionic zinc(II) phthalocyanine derivatives (**2a**, **3a**, **5a** and **6a**) showed monomeric behavior as evidenced by a single (narrow) Q band in organic solvents including DMSO, DMF, THF, chloroform and dichloromethane. On the other hand, peripheral and non-peripheral quaternized ionic zinc(II) phthalocyanines showed broad peaks at the Q band region in PBS solutions (Fig. 3b as an example for compounds **5b** and **6b**) due to aggregation of these phthalocyanines in aqueous solution. The addition of a surfactant, Triton X-100, to the aggregated aqueous solution of these complexes gave monomeric peaks at Q regions as a result of disaggregation (Fig. 3a as an example of compound **5b**).

3.2.3. Fluorescence spectra. Newly synthesized zinc(II) phthalocyanine complexes' fluorescence emission, absorption and excitation spectra are given in Fig. 4 for compound **5b** as an example. These spectra were recorded in DMSO and all substituted zinc(II) phthalocyanine complexes showed similar fluorescence spectra in this solvent.

Fluorescence emission and excitation maxima are listed in Table 1. The observed Stokes' shifts were within the region observed for typical zinc(II) phthalocyanine complexes. The excitation spectra were similar to absorption spectra and both were mirror images of the emission spectra for all studied zinc (II) phthalocyanine complexes suggesting that the molecules did not show any degradation during excitation in DMSO. The fluorescence behaviors of quaternized zinc(II) phthalocyanines were also examined in PBS solutions but they did not show any fluorescence in this medium. It could be due to the formation of aggregates among the zinc phthalocyanine molecules in PBS solution.



Scheme 3 The synthesis of the quaternized zinc(II) phthalocyanines **2b**, **3b**, **5b** and **6b**.

3.2.4. Fluorescence quantum yields and lifetimes. After exciting the photosensitizer an orbital electron from a higher quantum state relaxes to the ground state, releasing a photon of light that consists of fluorescence. The fluorescence quantum yield (Φ_F) determines the efficiency of the fluorescence process. The photon number is defined as the ratio of this value to the number of absorbed photons emitted.[20]

The fluorescence quantum yield (Φ_F) and fluorescence lifetime values of the studied zinc(II) phthalocyanines were measured in DMSO solutions. The Φ_F values of the studied zinc(II) phthalocyanine compounds (**2a** and **b**, **3a** and **b**, **5a** and **b**, **6a** and **b**) ranged from 0.07 to 0.27 in DMSO, Table 2. The Φ_F values of all substituted zinc(II) phthalocyanines (**2a** and **b**, **3a** and **b**, **5a** and **b**, **6a** and **b**) were compared with the unsubstituted zinc(II) Pc (**Std-ZnPc**) in DMSO, Table 2. When compared to the studied zinc(II) Pc compounds, the quaternization of the zinc(II) phthalocyanines increased the Φ_F values. It could be suggested that the lone pair of electrons of the nitrogen atoms on the substituents in non-quaternized complexes (**2a**, **3a**, **5a** and **6a**) were engaged when they quaternized. In addition, Φ_F values of peripherally substituted zinc(II) phthalocyanine compounds (**2b** and **5b**) were found to be equal to unsubstituted Zn(II) phthalocyanine. The non-peripherally substituted compound (**3b**) is higher than other studied

Table 1 Absorption, excitation and emission spectral data for unsubstituted and substituted zinc(II) phthalocyanine complexes in DMSO and PBS solutions

Compound	Solvent	Q band λ_{max} (nm)	Log ε	Excitation λ_{Ex} (nm)	Emission λ_{Em} (nm)	Stokes shift Δ_{Stokes} (nm)
2a	DMSO	685	5.17	688	697	9
2b	DMSO	683	5.20	689	700	11
	PBS	634, 685	4.63, 4.99	—	—	—
3a	DMSO	704	5.18	706	712	6
3b	DMSO	702	5.23	706	711	5
	PBS	655, 710	4.93, 5.15	—	—	—
5a	DMSO	682	5.22	682	694	12
5b	DMSO	683	5.09	683	694	11
	PBS	646, 683	4.64, 4.97	—	—	—
6a	DMSO	705	5.19	701	710	9
6b	DMSO	703	5.18	701	710	9
	PBS	655, 710	4.64, 4.78	—	—	—
Std ZnPc	DMSO[a]	672	5.14	672	682	10

[a] Data from ref. 25.

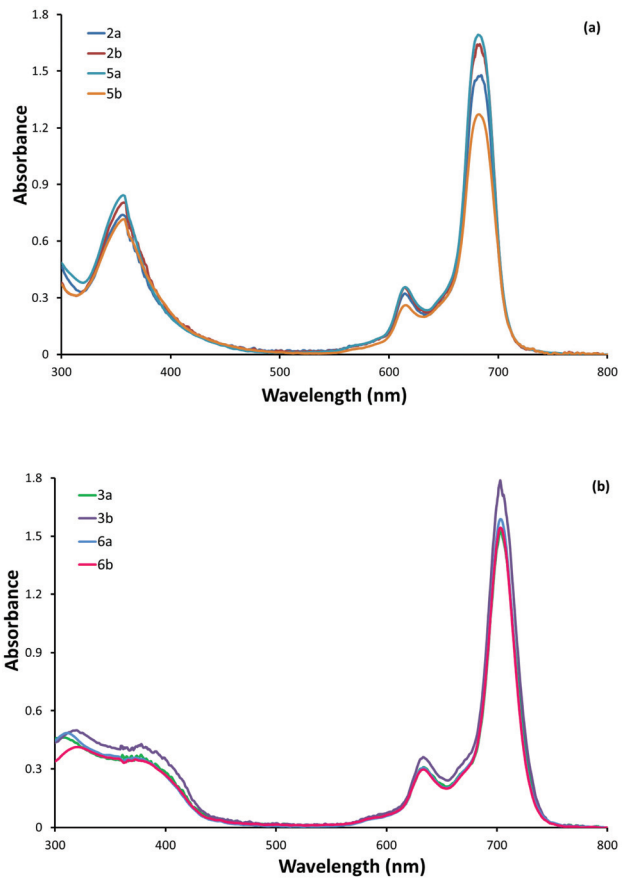


Fig. 1 Electronic absorption spectra of the compounds (a) **2a** and **b**, **5a** and **b** and (b) **3a** and **b**, **6a** and **b** in DMSO. Concentration = 1.00×10^{-5} M.

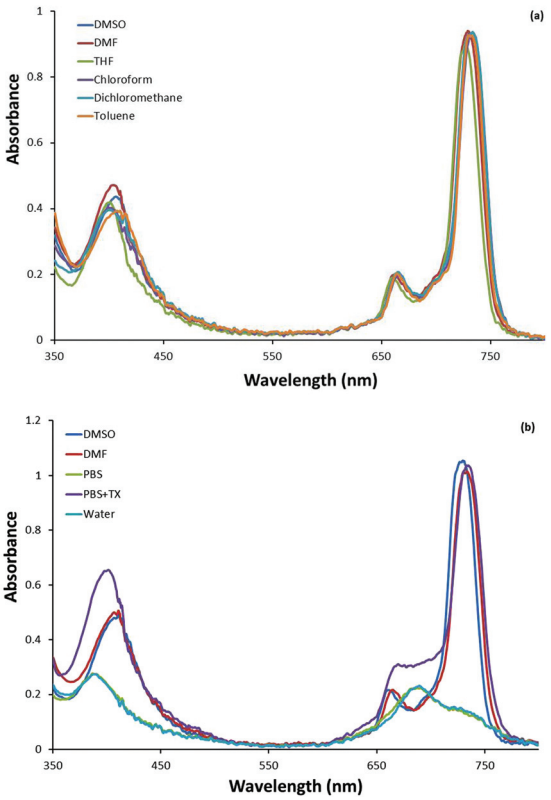


Fig. 2 Electronic absorption spectra of: (a) **2a** and (b) **2b** in different solvents. Concentration = 1.00×10^{-5} M.

zinc(II) phthalocyanines and unsubstituted Zn(II) phthalocyanine as well. These results suggest that the substituents' type and position on the phthalocyanine framework and quaternization of the substituents affect the Φ_F values of these compounds.

Fluorescence lifetime (τ_F) refers to the average time a molecule stays in its excited state before it returns to its ground state by emitting.[20] Fluorescence lifetime values (τ_F) of the studied zinc(II) phthalocyanines were measured by a time correlated single photon counting (TCSPC) method in DMSO

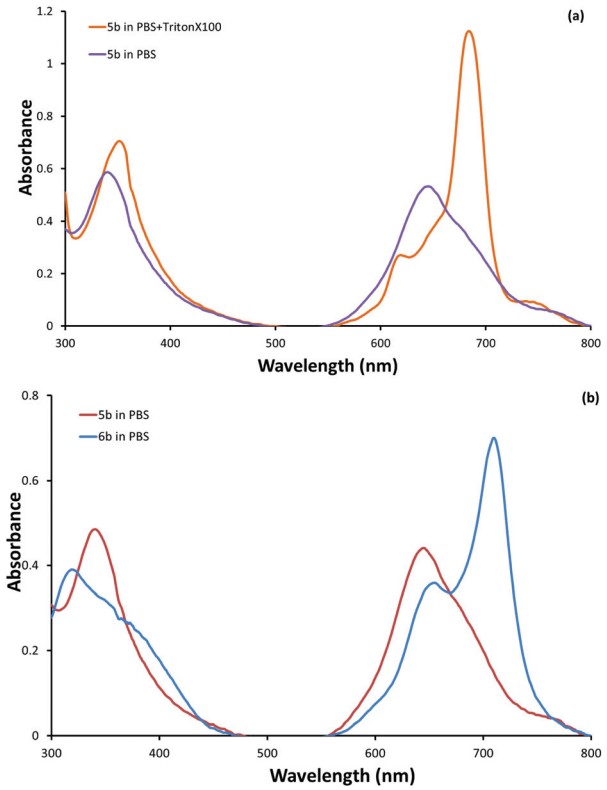


Fig. 3 Electronic absorption spectra of: (a) **5b** in PBS and after the addition of Triton X-100 (1%, v/v) to PBS solution and (b) **5b** and **6b** in PBS solution. Concentration = 1.00×10^{-5} M.

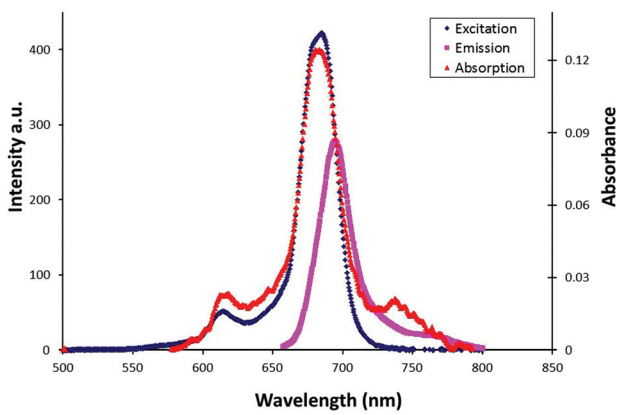


Fig. 4 Absorption, excitation and emission spectra for compound **5b** in DMSO. Excitation wavelength = 645 nm.

solutions. Fluorescence lifetime spectra are given in Fig. 5 for compound **3b** as an example. The peripherally substituted zinc (II) phthalocyanines showed lower τ_F values than unsubstituted Zn(II) phthalocyanine. But the non-peripherally substituted counterparts showed higher τ_F values than unsubstituted Zn(II) phthalocyanine, except for compound **3a** which showed slightly lower τ_F values. On the other hand, the non-peripherally substituted zinc(II) phthalocyanine compounds (**3a**, **6a**, **3b** and **6b**) showed higher τ_F values compared to the peripherally substituted zinc(II) phthalocyanine compounds (**2a**, **5a**, **2b** and **5b**) in DMSO (Table 2), suggesting more quenching of zinc(II) phthalocyanine compounds by peripheral substitution. Actually the position of substituents has an effect on the τ_F values of substituted zinc(II) Pc compounds.

Table 2 Photophysical and photochemical data of unsubstituted and substituted zinc(II) phthalocyanine complexes in DMSO and PBS solutions

Compound	Solvent	Φ_F	τ_F (ns)	Φ_d	Φ_Δ
2a	DMSO	0.11	2.81	1.42×10^{-5}	0.64
2b	DMSO	0.20	1.86	0.58×10^{-5}	0.71
	PBS	—	—	8.63×10^{-5}	0.29
3a	DMSO	0.14	3.71	1.27×10^{-5}	0.56
3b	DMSO	0.27	5.42	0.77×10^{-5}	0.68
	PBS	—	—	346×10^{-3}	0.25
5a	DMSO	0.14	2.93	1.87×10^{-5}	0.42
5b	DMSO	0.20	0.08	2.70×10^{-4}	0.62
	PBS	—	—	7.66×10^{-5}	0.27
6a	DMSO	0.07	4.23	6.45×10^{-5}	0.76
6b	DMSO	0.12	5.62	6.25×10^{-5}	0.87
	PBS	—	—	381×10^{-5}	0.30
Std ZnPc	DMSO	0.20[a]	3.99	2.61×10^{-5} [b]	0.67[c]

[a] Data from ref. 21. [b] Data from ref. 25. [c] Data from ref. 26.

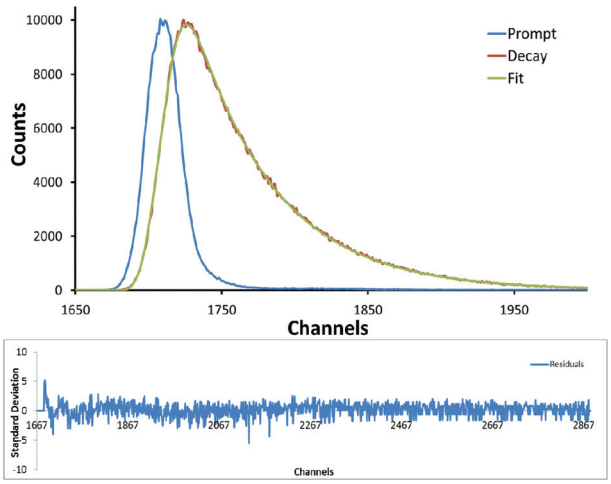


Fig. 5 Time correlated single photon counting (TCSPC) fluorescence decay curve of **3b** in DMSO.

3.2.5. Singlet oxygen quantum yields. During the PDT process, the energy was transferred between the triplet state of photosensitizers and ground state molecular oxygen that formed the singlet oxygen ($^1O_2$). This transfer must be as efficient as possible to generate a large amount of singlet oxygen. The generating amount of singlet oxygen is quantified by the singlet oxygen quantum yield (Φ_Δ), a parameter giving an indication of the potential of molecules to be used as photosensitizers in applications where singlet oxygen is required in PDT applications. The singlet oxygen quantum yield (Φ_Δ) corresponds to the number of singlet oxygen mole-

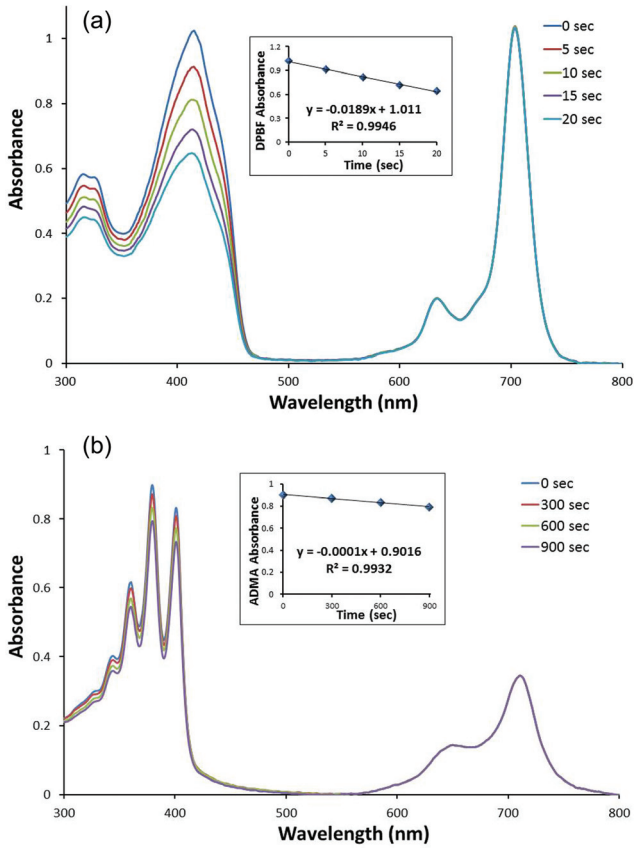


Fig. 6 Absorbance changes during the determination of the singlet oxygen quantum yield. This determination was for compound **6b** (a) in DMSO and (b) in PBS at a concentration of 1.0×10^{-5} M (inset: plot of DPBF or ADMA absorbances *versus* irradiation time).

cules generated by one photon absorbed by a photosensitizer.[20]

In this study, the singlet oxygen quantum yield (Φ_Δ) values were determined by a chemical method using singlet oxygen quenchers in DMSO for non-ionic zinc(II) phthalocyanine complexes and in both DMSO and PBS for ionic quaternized derivatives. 1,3-Diphenylisobenzofuran (DPBF) and 9,10-anthracenediyl-bis(methylene)dimalonoic acid (ADMA) were used as singlet oxygen quenchers for the determination of singlet oxygen quantum yields in DMSO and PBS, respectively. The decrease of DPBF absorbance at 417 nm or ADMA absorbance at 380 nm was monitored using a UV-vis spectrophotometer (Fig. 6 for compound **6b** as an example). The Q band intensities of the studied zinc(II) phthalocyanines did not exhibit any changes during the Φ_Δ determination, which indicates that the studied phthalocyanines were not degraded during singlet oxygen studies. The Φ_Δ values of the substituted zinc(II) phthalocyanines in DMSO are given in Table 2. The Φ_Δ values were increased in DMSO with quaternization of the compounds. Compound **6b** has shown the highest Φ_Δ value in all the studied zinc(II) Pc compounds in DMSO. Especially, the Φ_Δ values of quaternized zinc(II) phthalocyanine derivatives are relatively higher than their non-ionic counterparts in DMSO.

The Φ_Δ values for quaternized ionic zinc(II) phthalocyanines were measured in both DMSO and PBS solutions and the results were compared in Table 2. Low Φ_Δ values were observed in PBS compared to DMSO. This is explained by the fact that singlet oxygen absorbs light at 1270 nm and water also absorbs light at around this wavelength while DMSO exhibits little absorption in this region,[22] resulting in the large Φ_Δ values that were observed in DMSO compared to PBS solution in this study.

3.2.6. Photodegradation studies. Light irradiation causes the degradation of the molecule. Photodegradation can be used to determine the stability of compounds. The determination of photodegradation is particularly important for compounds which are intended for use in photocatalytic applications. Photodegradation degree can be determined using photodegradation quantum yield (Φ_d). Photodegradation depends on the structure of the compound, the light intensity, the solvent and the concentration.[20]

The spectral changes for all tetra-substituted complexes during light irradiation confirmed that photodegradation occurred without phototransformation (Fig. 7 as an example for **5a** in DMSO). A 300 W General Electric quartz line lamp with a power density of 18 mW cm^{-2} was used as a light source. The Φ_d values are of the order of 10^{-5} and similar phthalocyanine derivatives have different metals and substituents on the phthalocyanine ring.[22] Table 2 shows that the Φ_d values of all the studied zinc(II) phthalocyanine compounds (**2a** and **b**, **3a** and **b**, **5a** and **b**, **6a** and **b**) are similar to unsubstituted zinc(II) phthalocyanine.

3.2.7. Binding of water soluble quaternized zinc(II) phthalocyanine derivatives to BSA protein. The binding of quaternized zinc(II) phthalocyanine derivatives (**2b**, **3b**, **5b** and **6b**) to BSA was studied by spectrofluorometry at room temperature in a PBS solution. An aqueous solution of BSA (fixed concentration) was titrated with varying concentrations of the

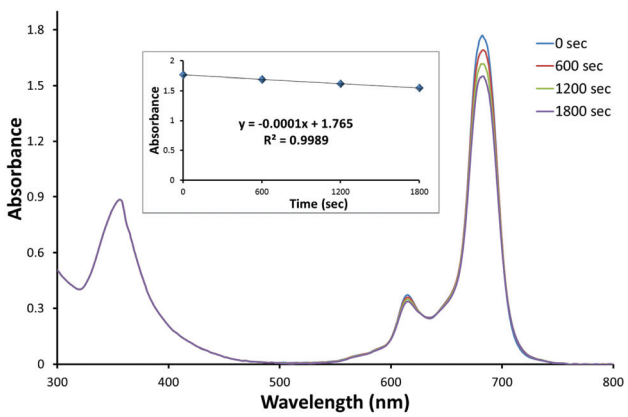


Fig. 7 Absorbance changes during the photodegradation study of **5a** in DMSO showing the decrease of the Q band at 600 s intervals (inset: plot of Q band absorbance *versus* time).

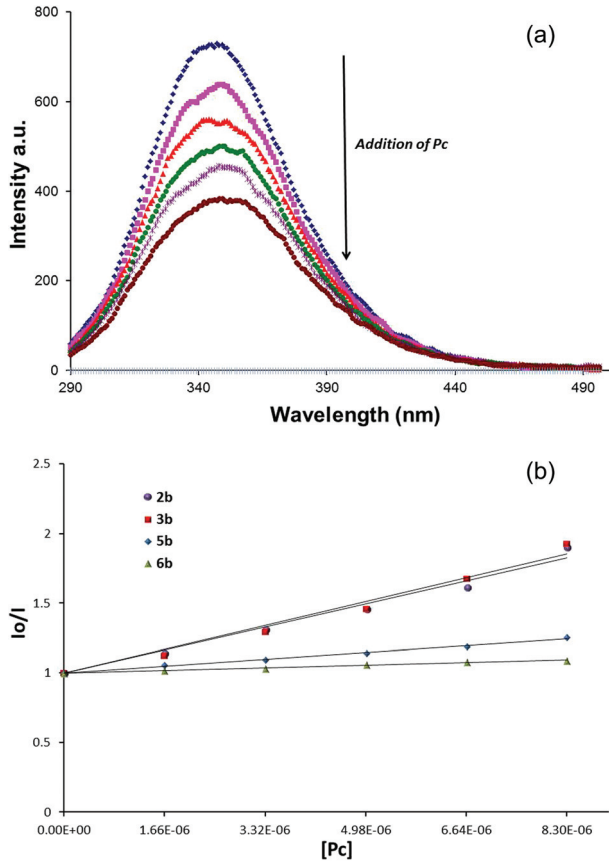


Fig. 8 (a) Fluorescence emission spectral changes of BSA by the addition of different concentrations of **2b** in PBS solution. (b) Stern–Volmer plots for the quenching of BSA by quaternized complexes (**2b**, **3b**, **5b**, and **6b**) in PBS solution. [BSA] = 3.00×10^{-5} M and [Pc] = 0, 1.66×10^{-6}, 3.33×10^{-6}, 5.00×10^{-6}, 6.66×10^{-6}, 8.33×10^{-6} M.

respective zinc(II) phthalocyanine solutions. BSA was excited at 280 nm and fluorescence emission spectra were recorded between 290 nm and 500 nm.

Fig. 8a shows the fluorescence emission spectra of BSA solution by the addition of **2b** in PBS as an example. The fluorescence emission band at 348 nm was decreased by the addition of zinc(II) phthalocyanine solutions due to the interaction of the phthalocyanine molecules with tryptophan residues on BSA protein. BSA and quaternized zinc(II) phthalocyanines showed reciprocated fluorescence quenching on the other; hence it was possible to determine Stern–Volmer quenching constants (K_{SV}). The slopes of the plots shown in Fig. 8b gave K_{SV} values as listed in Table 3. The K_{SV} values of quaternized ionic zinc(II) phthalocyanine (**2b**, **3b**, **5b** and **6b**) were of the order of 10^5 and these values were similar to phthalocyanine derivatives having different metals and substituents on the phthalocyanine ring.[23]

The bimolecular quenching constant (k_q) values of quaternized zinc(II) phthalocyanines were determined using eqn (5) given in the ESI.† These values were of the order of 10^{13} M^{-1} s^{-1}, which exceeds the proposed value for diffusion-controlled

Table 3 Binding and fluorescence quenching data for the interaction of BSA with quaternized zinc(II) phthalocyanines in PBS solution

Compound	$K_{SV}/10^5$ (M^{-1})	$k_q/10^{13}$ (M^{-1} s^{-1})
2b	0.99	5.3
3b	1.03	1.9
5b	0.29	0.9
6b	0.11	0.3

(dynamic) quenching (10^{10} M^{-1} s^{-1} according to the Einstein–Smoluchowski approximation) at room temperature.[24] This is an indication that the mechanism of BSA quenching by quaternized zinc(II) phthalocyanines is not diffusion controlled.

3.2.8. ***In vitro*** **studies.** Fig. 9 shows the survival of HeLa and HuH-7 cells following illumination with 2 J cm^{-2} of 690 nm (±10 nm) light after uptake of quaternized zinc(II) phthalocyanine molecules (**2b**, **3b**, **5b** and **6b**). Cell survival appears to be dose-dependent and there is not observed any dark toxicity with 2 J cm^{-2} irradiation.

In this study, photodamage to the studied cell lines occurred after PDT. In the presence of each phthalocyanine, cell survival decreased following irradiation while the number of cells remained the same without irradiation. The concentration-dependent dark cytotoxicity was investigated in both HeLa and HuH-7 cells exposed to increased concentrations of each studied phthalocyanine (up to 20 μM) for 24 h in the dark. The survival curves for photosensitized cells are given in Fig. 9. Among the studied phthalocyanines, **5b** showed a similar photodynamic effect in both cell lines at the lowest concentration; both cell lines' survival degree was 50% (Fig. 9c). In addition, only **5b** showed the highest photodynamic effect in HuH-7 cells after 2 J cm^{-2} irradiation (Fig. 9c). On the other hand, **6b** showed the highest photodynamic effect in HeLa cells after 2 J cm^{-2} irradiation (Fig. 9b). PDT

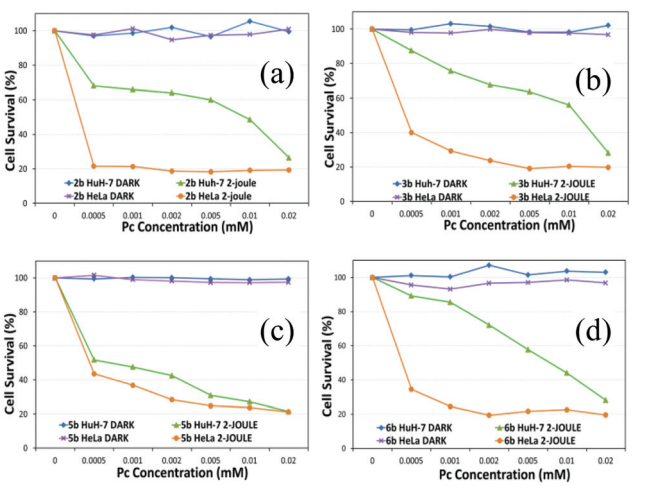


Fig. 9 Survival of HeLa and HuH-7 cells following illumination with 2 J cm^{-2} of 690 nm (±10 nm) light after 24 h with various concentrations for: (a) **2b**, (b) **3b**, (c) **5b** and (d) **6b**. Each data represents the mean ± SD for three experiments.

combined with water soluble zinc(II) phthalocyanine sensitizers resulted in the death of both HuH-7 and HeLa cells under different conditions (Fig. 9a–d). However, the photodamage was also dependent on the used irradiation dose according to the results of this study. Viability studies have shown that optimum phototoxic effects such as the illumination dose or the concentration of phthalocyanines were tested. However, following illumination with 1 J cm^{-2}, 50% cell survival in HeLa cells was observed at a phthalocyanine concentration of 0.5–1 μM, and in HuH-7 cells at a phthalocyanine concentration of only above 10 μM (Fig. S5†). According to these results, the HuH-7 cell line is more resistant than the HeLa cell line to the studied quaternized zinc(II) phthalocyanine compounds.

4. Conclusion

In conclusion, this work describes the synthesis, characterization, photophysical and photochemical properties of new peripherally and non-peripherally tetra-substituted zinc(II) phthalocyanine complexes (**2a**, **3a**, **5a** and **6a**) and their amphiphilic quaternized derivatives (**2b**, **3b**, **5b** and **6b**). The newly synthesized phthalocyanines were characterized by a combination of UV-vis, FT-IR, ^{1}H NMR, ^{13}C NMR and MS spectroscopic data and elemental analysis as well. The photophysical (fluorescence quantum yields and lifetimes) and photochemical properties (singlet oxygen and photodegradation quantum yields) of tetra-substituted zinc(II) Pc compounds were investigated in DMSO for non-ionic complexes and in both DMSO and PBS solutions for quaternized ionic complexes. The obtained data were compared according to the effects of the position of the substituents on the phthalocyanine framework (peripheral or non-peripheral), quaternization of phthalocyanines under nitrogen atoms on the substituents and the used solvents (DMSO or PBS). The binding behavior of the studied zinc(II) phthalocyanines (**2b**, **3b**, **5b**, and **6b**) to BSA protein was also examined in PBS solution. It was shown that these compounds were bound to BSA from tryptophan residues on the BSA protein. The studied zinc(II) phthalocyanines, especially quaternized derivatives, generated highly singlet oxygen molecules. The high singlet oxygen generation capability of the quaternized zinc(II) Pc compounds (**2b**, **3b**, **5b**, and **6b**) makes them good candidates for the treatment of cancer by PDT. For this reason, the *in vitro* photodynamic activities of these quaternized phthalocyanines against HeLa and HuH-7 cancer cell lines were determined. All the studied quaternized phthalocyanines showed PDT activity to the studied cancer cell lines. Especially compound **5b** showed the highest activity against both cell lines.

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