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Amino-functionalized water-soluble zinc phthalocyanines: Synthesis, photophysical, photochemical and protein binding properties

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ABSTRACT

Tetra-peripherally substituted symmetrical and low symmetrical Zn (II) phthalocyanines containing 2-[2-(2-ethoxyethoxy)ethoxy]-1-[2-((2-ethoxyethoxy)-ethoxy)ethoxymethyl] ethyloxy and [2-(tert-butoxycarbonyl)amino]ethoxy groups, as well as their deprotected amino-functionalized derivatives were synthesized for the first time. These novel zinc phthalocyanines were characterized by elemental analysis and spectroscopic methods including FT-IR, ^{1}H and ^{13}C NMR, MALDI-TOF, UV–vis. Their photophysical (fluorescence quantum yields and lifetimes) and photochemical (singlet oxygen and photodegradation quantum yields) properties were investigated in DMSO, in water and in water + triton X-100 solutions for comparison of solvents and aggregation effects. Effects of symmetries of the phthalocyanine molecules on these properties were also revealed. The fluorescence quenching of these Zn (II) phthalocyanines upon addition of 1,4-benzoquinone were examined in DMSO. Binding study of these phthalocyanine photosensitizers to bovine serum albumin one of the blood carrier proteins, was also examined in this study.

1. Introduction

Photodynamic therapy (PDT) is a therapeutic method for cancer treatment. This method requires a photosensitizer that accumulates selectively in the tumor tissue, exhibits good phototoxicity, low dark toxicity, high singlet oxygen quantum yield and absorption in the near-infrared region for deeper light penetration into the tissue and for destruction of tumor cells [1–4]. Treatment of cancer by PDT is promising, mainly because dangerous side effects in healthy tissues observed during chemotherapy and radiotherapy can be avoided if the photosensitizer is selectively accumulated in tumor tissues [4,5]. When designing a photosensitizer for photodynamic therapy, its water-solubility may be required. It facilitates the circulation of a photosensitizer in the bloodstream [6].

As phthalocyanines (Pcs) have convenient properties such as stronger absorption in the red visible region and higher performance at creating singlet oxygen, these compounds have been used as second generation photosensitizers for visible light [7,8]. Photochemistry of phthalocyanine compounds has been making rapid progress in the last decades, to optimize their properties. Recently, more efficient phthalocyanines have been synthesized for PDT applications. Especially, ZnPc derivatives exhibiting sufficient photophysical (triplet quantum yield and lifetime) and photochemical (singlet oxygen generation) properties are strong candidates as photosensitizers for PDT application [9]. Nowadays, researches are oriented toward the synthesis of targeting photosensitizers selectively localized in the tumors and eventually resulted in the third-generation of photosensitizer in PDT [10]. Amino groups have ability of strong interaction or covalent bonding with biological molecules. Hence, they are preferred functionalizations for preparation of PDT targeting photosensitizer [11]. Additionally, introduction of amino groups increases water-solubility of Pc macrocycle [12,13]. Pcs have nevertheless some disadvantages, as their aggregation tendency which reduces their photosensitizing activity or low solubility in water or polar solvents. To overcome these problems, possible strategies are the substitution of Pc with bulky and polar groups such as polyoxyethylene, or conjugation of Pc macrocycle to biomolecules [1,14,15].

Our ongoing investigations focus on the preparation of new water soluble phthalocyanine derivatives which have potential to be used as PDT agents [4,14]. For this purpose, tetra-peripherally substituted low symmetrical Zn (II) Pc derivatives substituted by bulky polyoxyethylene groups reducing aggregation, and by amino function for enhanced water solubility were designed and

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synthesized. In order to assess the potential of these newly synthesized phthalocyanines as PDT agents, their photophysical and photochemical properties were investigated, as well as their interaction with blood bovine serum albumin (BSA).

2. Experimental

2.1. Synthesis

4-{2-[2-(2-Ethoxyethoxy)ethoxy]-1-[2-((2-ethoxyethoxy)-ethoxy)ethoxymethyl] ethyloxy} phthalonitrile (**A**) [16], 4-{2′-[(*tert*-butoxycarbonyl)amino]ethoxy} phthalonitrile (**B**) [17] were prepared according to the literature.

2.2. Peripherally tetra-substituted ZnPc derivatives (*Pc1–Pc5*): A_4-type ZnPc (*Pc1*), A_3B-type ZnPc (*Pc2*), A_2B_2-type ZnPc (*Pc3*), AB_3-type ZnPc (*Pc4*) and B_4-type ZnPc (*Pc5*) (Scheme 1)

Pc1–Pc5 were synthesized from condensation of a mixed condensation of phthalonitriles **A** and **B**.

A mixture of 4-{2-[2-(2-ethoxyethoxy)ethoxy]-1-[2-((2-ethoxyethoxy)-ethoxy)ethoxymethyl] ethyloxy} phthalonitrile (**A**) (2.70 g, 6 mmol), 4-{2-[(*tert*-butoxycarbonyl)amino]ethoxy} phthalonitrile (**B**) (0.57 g, 2 mmol), $Zn(CH_3CO_2)_2$ (0.75 mg, 4 mmol) and dimethylaminoethanol (DMAE) (6 mL) were stirred under argon atmosphere at 80 °C for 10 min. Then, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) (1 mL) was added and this reaction mixture was refluxed for 19 h under argon atmosphere, then cooled to room temperature. DMAE was removed by vacuum distillation. The waxy crude product was purified by column chromatography on silica gel (eluent ethanol/ethylacetate, 2:55). **Pc1–Pc5** with different molecular symmetries were isolated.

A_4-type Pc (Pc1): Yield 260 mg (9%). FT-IR spectrum (ν_{max}/cm^{-1}): 3066 (ArCH), 2973–2866 (Aliphatic CH), 1607 (C=C), 1486, 1391, 1259, 1104 (C—O—C). UV–vis (DMSO): λ_{max} nm ($\log\varepsilon$) 359 (4.59), 638 (4.55), 682 (5.02). ^{1}H NMR (DMSO-d_6): δ = 0.95 (m, 24H, CH_3), 3.26–3.94 (m, 96H, CH_2), 5.22 (m, 4H, CH), 7.80 (m, 4H, ArH), 8.91–8.98 (m, 4H, ArH), 9.16–9.26 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ = 160.71 (ArC), 153.37 (ArC), 152.68 (ArC), 140.53 (ArC), 140.43 (ArC), 131.86 (ArC), 124.17 (ArC), 108.35 (ArC), 77.52 (CH), 70.97 (CH_2), 70.58 (CH_2), 70.28 (CH_2), 69.61 (CH_2), 65.89 (CH_2), 55.32 (CH_2), 15.45 (CH_3). Calcd. for $C_{92}H_{136}N_8O_{28}Zn$: C%, 59.17; H%, 7.34; N%, 6.00. Found: C%, 59.25; H%, 7.05; N%, 6.10. MS (MALDI-TOF) *m*/*z*: Calc.: 1867.5; Found: 1868.7 $[M+H]^+$.

A_3B-type Pc (Pc2): Yield 305 mg (9%). FT-IR spectrum (ν_{max}/cm^{-1}): 3320 (NH), 3068 (ArCH), 2973–2867 (Aliphatic CH), 1715 (C=O), 1607(C=C), 1487, 1392, 1088 (C—O—C). UV–vis (DMSO): λ_{max} nm ($\log\varepsilon$) 358 (4.92), 615 (4.60), 682 (5.23). ^{1}H NMR (DMF-d_7): δ = 0.89–1.02 (m, 18H, CH_3), 1.50 (m, 9H, CH_3), 3.53–3.86 (m, 62H, CH_2), 4.07 (m, 12H, CH_2), 4.67 (t, 2H, CH_2), 5.33 (m, 3H, CH), 7.27 (b, 1H, NH), 7.42 (d, 1H, ArH), 7.93–7.99 (m, 3H, ArH), 9.01 (m, 1H, ArH), 9.08–9.12 (m, 3H, ArH), 9.30–9.39 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ = 169.22 (C=O), 163.84 (ArC), 135.67 (ArC), 125.27 (ArC), 124.96 (ArC), 124.15 (ArC), 122.02 (ArC), 110.31 (ArC), 110.00 (ArC), 77.55 (CH), 70.98–69.76 (O–CH_2), 65.86 (CH_2–NH), 28.74 (C($\underline{C}H_3$)$_3$), 15.49 (CH_2–$\underline{C}H_3$). Calcd. for $C_{84}H_{119}N_9O_{24}Zn$: C%, 59.20; H%, 7.04; N%, 7.40. Found: C%, 59.40; H%, 6.95; N%, 7.56. MS (MALDI-TOF) *m*/*z*: Calc.: 1704.3; Found: 1704.0 $[M]^+$.

A_2B_2-type Pc (Pc3): Yield 308 mg (10%). FT-IR spectrum (ν_{max}/cm^{-1}): 3345 (NH), 3066 (ArCH), 2972–2868 (Aliphatic CH), 1710 (C=O), 1607 (C=C), 1488, 1391, 1228, 1088 (C—O—C). UV–vis (DMSO): λ_{max} nm ($\log\varepsilon$) 357 (5.01), 615 (4.67), 682 (5.28). ^{1}H NMR (DMSO-d_6): δ = 0.93–1.04 (m, 12H, CH_3), 1.45–1.54 (t, 18H, CH_3), 3.36–4.28 (m, 52H, CH_2), 4.31–4.66 (b, 4H, CH_2), 5.05–5.47 (m, 2H, CH), 7.20–7.33 (m, 2H, NH), 7.47–9.24 (m, 12H, ArH). ^{13}C NMR (DMSO-d_6): δ = 156.42 (ArC), 140.23 (ArC), 131.82 (ArC), 131.42 (ArC), 124.05 (ArC), 123.80 (ArC), 119.37 (ArC), 118.38 (ArC), 78.42 (–$\underline{C}(CH_3)_3$), 77.35 (CH), 71.01 (CH_2), 70.73 (CH_2), 70.61 (CH_2), 70.46 (CH_2), 70.35 (CH_2), 69.64 (CH_2), 65.93 (CH_2), 28.76 (CH_3), 15.43 (CH_3). Calcd. for $C_{76}H_{102}N_{10}O_{20}Zn$: C%, 59.23; H%, 6.67; N%, 9.09. Found: C%, 58.95; H%, 6.45; N%, 9.18. MS (MALDI-TOF) *m*/*z*: Calc.: 1541.1; Found: 1541.3 $[M]^+$.

AB_3-type Pc (Pc4): Yield 185 mg (20%). FT-IR spectrum (ν_{max}/cm^{-1}): 3217 (NH), 3070 (ArCH), 2974–2868 (Aliphatic CH), 1717 (C=O), 1610 (C=C), 1484, 1353, 1284, 1094 (C—O—C). UV–vis (DMSO): λ_{max} nm ($\log\varepsilon$) 359 (4.67), 614 (4.31), 682 (4.94). ^{1}H NMR (DMF-d_7): δ = 0.81–1.61 (m, 33H, CH_3), 3.49–3.70 (m, 24H, CH_2), 3.70–3.88 (m, 12H, CH_2), 4.08 (b, 1H, CH), 4.62–4.70 (b, 3H, NH), 7.41–7.51 (m, 4H, ArH), 7.75–7.79 (m, 4H, ArH), 8.99–9.43 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ = 169.24 (C=O), 163.71 (ArC), 156.41 (ArC), 135.62 (ArC), 125.11 (ArC), 124.91 (ArC), 121.89 (ArC), 110.08 (ArC), 109.94 (ArC), 78.51(–$\underline{C}(CH_3)_3$), 71.08 (CH_2), 70.81 (CH_2), 70.64 (CH_2), 70.48 (CH_2), 70.20 (CH_2), 69.66 (CH_2), 66.09 (CH_2), 55.38 (CH_2), 28.66 (CH_3), 15.48 (CH_3). Calc. for $C_{68}H_{85}N_{11}O_{16}Zn$: C%, 59.27; H%, 6.22; N%, 11.18. Found: C%, 59.32; H%, 6.45; N%, 11.21. MS (MALDI-TOF) *m*/*z*: Calc.: 1377.8; Found: 1377.6 $[M]^+$.

B_4-type Pc (Pc5): Yield 153 mg (25%). FT-IR spectrum (ν_{max}/cm^{-1}): 3322 (NH), 3064 (ArCH), 2964–2831 (Aliphatic CH), 1701 (C=O), 1608 (C=C), 1489, 1391, 1259, 1090 (C—O—C). UV–vis (DMSO): λ_{max} nm ($\log\varepsilon$) 357 (4.64), 614 (4.27), 680 (4.91). ^{1}H NMR (DMSO-d_6): δ = 1.39–1.52 (m, 36H, C (CH_3)$_3$), 3.49–3.81 (m, 8H, CH_2N), 4.25–4.65 (m, 8H, CH_2O), 7.04–7.3 (b, 4H, NH), 7.35 (m, 4H, ArH), 7.80 (m, 4H, ArH), 8.51–9.10 (m, 4H, ArH). ^{13}C NMR (DMSO-d_6): δ = 160.91 (ArC), 163.91 (ArC), 156.12 (ArC), 135.62 (C=O), 125.22 (ArC), 124.92 (ArC), 120.75 (ArC), 124.168 (ArC), 108.80 (ArC), 78.48 (CH_2), 78.32 (CH_3), 67.92 (CH_2), 28.72 (CH_3), 28.62 (CH_3). Calcd. for $C_{60}H_{68}N_{12}O_{12}Zn$: C%, 57.62; H%, 5.80; N%, 13.44. Found: C%, 58.01; H%, 5.25; N%, 13.85. MS (MALDI-TOF) *m*/*z*: Calc.: 1214.6; Found: 1215.9 $[M+H]^+$.

2.2.1. Deprotected ZnPc derivatives (*Pc2a–Pc5a*) (Scheme 1)

The same procedure was employed to deprotection of amino group on the **Pc2–Pc5** compounds and experimental details were given for only **Pc2a**.

A_3B-type mono amino containing ZnPc (Pc2a): **Pc2** (30 mg, 0.0176 mmol) was dissolved in CH_2Cl_2 (2 mL) and trifluoroacetic acid (TFA) (1 mL) was added to the mixture at 0 °C. The reaction mixture was stirred at room temperature for 2 h and then the solvent was removed by vacuum distillation. The residue was treated with 2 M NaOH and extracted with ethyl acetate. Organic phase extracted with distillated water and then dried over anhydrous sodium sulfate. After filtered off and evaporating the solvent, the monoamino-substituted Pc derivative was obtained to yield 25 mg (88.55%). FT-IR spectrum (ν_{max}/cm^{-1}): 3420 (NH), 3070 (ArCH), 2963–2867 (Aliphatic CH), 1606 (C=C), 1488 (NH), 1397 ($C(CH_3)_3$, 1229, 1090 (C—O—C). ^{1}H NMR (DMF-d_7): δ = 1.04–1.32 (m, 18H, CH_3), 1.41–1.46 (s, 2H, NH_2), 3.52–3.62/3.68–374/3.75–3.92/3.94–4.08/4.10–4.46 (m, 72H, CH_2), 5.37–5.62 (b, 4H, CH_2), 7.97–8.18 (bm, 3H, CH), 8.8.60–10.25 (m, 12H, ArH). ^{13}C NMR (DMF-d_7): δ = 154.26 (ArC), 141.24 (ArC), 132.47 (ArC), 124.24 (ArC), 119.23 (ArC), 116.09 (ArC), 115.71 (ArC), 108.29 (ArC), 78.48 (CH–O), 71.33–70.37–69.38 (–CH_2), 65.88 (CH_2–NH), 16.85 (–CH_3). Calcd. for $C_{79}H_{111}N_9O_{22}Zn$: C%, 59.18; H%, 6.97; N%, 7.86. Found: C%, 59.52; H%, 6.75; N%, 7.68. MS (MALDI-TOF) *m*/*z*: Calc.: 1604.1; Found: 1606.8 $[M+2H]^{2+}$.

A_2B_2-type di amino containing ZnPc (Pc3a): Yield: 17.5 mg (73.3%). FT-IR spectrum (ν_{max}/cm^{-1}): 3262 (NH), 3066 (ArCH), 2968–2867 (Aliphatic CH), 1606 (C=C), 1487, 1393, 1227, 1087 (C—O—C). ^{1}H NMR (DMF-d_7): δ = 0.95–1.07 (m, 12H, CH_3), 1.10–1.27