Synthesis, characterization and photodynamic activity of a new amphiphilic zinc phthalocyanine

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1. Introduction

Phthalocyanine (Pc) and its derivatives have attracted a great deal of interest due to their unique electronic, optical, and structural properties [1]. These unique properties of the phthalocyanine (Pc) macrocycle make it suitable for a wide range of applications. In addition to their traditional use as dyes and pigments, during recent decades phthalocyanines (Pcs) have been intensively studied due to their applications in many scientific areas such as optical data storage, chemical sensors, electrochromic agents, molecular metals, liquid crystals, solar energy conversion and as photosensitizers for photodynamic therapy of cancer (PDT) [1–3]. PDT appears as a promising treatment for certain malignant, premalignant, and non-malignant cancers [4]. In order to be used for PDT, sensitizers must have high photo-stability, high absorption coefficient, high selectivity to tumors, low dark toxicity, strong absorption in the region between 600 and 850 nm which offers high tissue penetration, long triplet state life time and high capability of photo-generation of singlet oxygen. Among the requirements for a successful photosensitizer, good solubility characteristics are also of paramount importance.

It is very well known that phthalocyanine derivatives satisfy the aforementioned conditions [5–12]. The hydrophilic phthalocyanines, especially sulfonated derivatives, have attracted much attention as second-generation photosensitizers for treatment of malignant tumors by photodynamic therapy (PDT) in the last two decades [13–20]. It has been also reported that water-soluble phthalocyanines containing aluminum or zinc as the central metal ions are most preferred as photosensitizers for PDT. In these dyes, the metal ion is diamagnetic and thus the efficiency of the central ion is unambiguous [21].

Because of the intermolecular interactions between the macrocycles, unsubstituted metal-free and metallophthalocyanines are insoluble or slightly soluble in common organic solvents and aqueous media, thereby minimizing their applications. While peripheral or nonperipheral substitution with alkyl, alkoxy, phenoxy and macrocylic groups leads to phthalocyanine products soluble in common organic solvents [22–26], the introduction of sulfonyl, carboxylic acid, or amino groups results in water-soluble metallophthalocyanines [27–33].

The size and the nature of the substituents are not the only criteria for the solubility of the substituted phthalocyanines; the change in symmetry caused by different substituents is also
important. Usually, octasubstituted phthalocyanines obtained from disubstituted phthalonitrile derivatives with two different substituents in the 4- and 5- positions are more soluble than the corresponding octa-substituted Pcs with identical substituents. The reason for enhanced solubility is most probably due to the formation of four constitutional isomers and the high dipole moment that results from the unsymmetrical arrangement of the substituents on the periphery [34–39]. In this sense, hexadeca-substituted phthalocyanines obtained from totally substituted phthalonitrile derivatives with tree different substituents have been expected to show similar behavior.

The nature of the substituents not only affects the solubility of the phthalocyanines but also their physical and chemical behavior. It has long been known that phthalocyanines bearing electron-donating substituents at the non-peripheral positions (1, 4, 8, 11, 15, 18, 22, 25) show some red-shift in the phthalocyanine chromophore, but only minor shifts appear as a consequence of substitution at the peripheral positions (2, 3, 9, 10, 16, 17, 23, 24) [40–43]. When compared to tetra- or octa-substituted phthalocyanines, the hexadeca-substituted phthalocyanines, especially those having three different substituents on each benzo group are relatively scarce [44].

Microwave synthesis can be effectively applied to any reaction scheme with the aim of creating faster reactions, improving yields, and producing cleaner chemistry. Traditional synthetic routes to phthalocyanines need long reaction times and very high temperatures. Synthesis of phthalocyanines that required many hours or even days to complete have been accomplished in minutes by using microwave energy [45].

In the present work, the preparation of novel hexadeca amphiphilic zinc phthalocyanine carrying three different substituents on each benzo group has been achieved by using microwave irradiation. As the introduction of phenoxy and hexyloxy substituents enhances the amphiphilic nature of the molecule, but also prevents its molecular aggregation in solution for steric reasons, only enhances the amphiphilic nature of the molecule, but also becomes amphiphilic in nature. The introduction of these groups not add hydrophilicity to the compounds; the metallo-phthalocyanines formation of four constitutional isomers and the high dipole moment that results from the unsymmetrical arrangement of the substituents in the hexadeca-substituted phthalocyanines, especially those having the hexadeca-substituted phthalocyanines, especially those having three different substituents on each benzo group are relatively scarce [44].

2. Experimental

2.1. Chemicals and instrumentation

IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR (ATR sampling accessory) spectrophotometer, electronic spectra on a Scinco S-3100 spectrophotometer. 1H-NMR spectra were recorded on Agilent VNMRS 500 MHz and the spectrum was referenced internally by using the residual solvent resonances (δ = 2.49 ppm for DMSO-d6 and δ = 7.26 for CDCl3 in 1H NMR). Mass spectra were measured on a Micromass Quatro LC/ULTIMA LC-MS/MS spectrometer. Single mode reactor (CEM DISCOVER SP) were used for microwave heating. All reagents and solvents were of reagent grade obtained from commercial suppliers. The homogeneity of the products was tested in each step by TLC. The solvents were stored over molecular sieves. Anhydrous potassium carbonate (K2CO3) was finely ground and dried at 100 °C. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (1) was purchased from Sigma–Aldrich. TritonX-100 were purchased from Fluka. 4,5-Dichloro-3,6-dihydroxyphthalonitrile (2) were prepared according to reported procedure [46]. The singlet oxygen generation measurements were carried out using an Optizem UV spectrophotometer and 1,3-Diphenylisobenzofuran (DPBF) was purchased from Aldrich.

2.2. Synthesis

2.2.1. 4,5-dichloro-3,6-bis(hexyloxy)phthalonitrile (3)

4,5-Dichloro-3,6-dihydroxyphthalonitrile (3.69 g, 16.1 mmol) was dissolved in aqueous solution of KOH (1.9M, 22 mL) and then tetrabutylammonium bromide (1.04 g, 3.22 mmol) and 1-iodohexane (8.43 g, 39.8 mmol) were added. The reaction mixture was heated under reflux under nitrogen for 6 h and then cooled to room temperature. The upper organic layer was collected, dried in vacuo and purified by column chromatography (silica gel,CHCl3-petroleum ether 2:1). Light yellow waxy product was soluble in CHCl3, CH2Cl2, THF, DMF and DMSO Yield: 12.5 g (80%), FTIR, cm−1 2955–2870 (C–H aliphatic), 2236 (C≡N), 1246, 1236 (Ar–O–R), 1H NMR (400 MHz, CDCl3): δ, ppm 4.14 (4H, t), 1.81 (4H, quin), 1.44 (4H, quin), 1.23–1.32 (8H, m), 0.84 (6H, t), MS: m/z (%) 396 [M]+. Anal. calc. for C20H26N2Cl2O2 : C, 60.46; H, 6.60; N, 7.05; found C, 60.35; H, 6.52; N, 7.15.

2.2.2. 4-(2-chloro-4,5-dicyano-3,6-bis(hexyloxy)phenoxy)benzenesulfonic acid (4)

Compound 3 (1 g, 2.5 mmol) was dissolved in DMSO (8 mL) at 45 °C and sodium 4-hydroxybenzenesulfonate (0.87 g, 5.5 mmol) was added. After stirring for 15 min, 1.4 g finely ground anhydrous K2CO3 (10 mmol) was added portionwise in 2 h with efficient stirring. The reaction mixture was stirred under nitrogen at 45 °C for further 48 h. After being cooled to room temperature, the mixture was poured into of 1M HCL (100 mL). After completion of the precipitation approximately in 24 h, the crude product was collected by vacuum filtration and washed with cold ethanol. The purification of the crude product was accomplished by column chromatography on silica gel as the stationary phase using CH2Cl2/Ethanol 2:1 as the eluent. White waxy product was soluble in methanol, CHCl3, CH2Cl2, DMF and DMSO. Yield: 0.50 g, %37. FTIR, cm−1 2955, 2938, 2870 (R–H), 2236 (CN), 1246, 1236 (R–O–Ar), 1H-NMR (500 MHz, DMSO-d6): δ, ppm 7.56 (2H, d), 6.90 (2H, d), 4.2 (2H, t), 4.09 (2H, t), 1.77 (2H, quin), 1.52 (2H, quin), 1.46 (2H, quin), 1.33–1.13 (10H, m), 0.86 (3H, t), 0.81 (3H, t), 13-NMR 156.30, 155.64, 152.53, 149.34, 143.14, 130.44, 127.95, 114.58, 113.45, 109.21, 107.44, 75.99, 31.30, 31.15, 29.84, 26.9, 25.22, 25.08, 22.43, 22.36, 14.31, 14.27, MS: m/z 533.96 [M]+. Anal. calc. for C32H24N2O6S: C, 58.36; H, 5.84; N, 5.24; found C, 58.31; H, 5.86; N, 5.22%.

2.2.3. 2,9(10),16(17),23(24)-tetra-chloro1,4,8,11,15,18,22,25-octahexyloxy 3,9(10),16(17),23(24)-tetra-(4-sulfonylphenoxy)phthalocyaninatozinc(II) (5)

A mixture of 4-(2-chloro-4,5-dicyano-3,6-bis(hexyloxy)phenoxy)benzenesulfonic acid (0.100 g, 0.187 mmol), anhydrous Zn(CH3COO)2 (0.043 g, 0.235 mmol) and DBU (0.2 mmol), in n-pentanol (2 mL) was irradiated with microwave at 150 °C in a sealed glass tube for 25 min. After cooling to room temperature, the green mixture was precipitated by adding diethyl ether and it was filtered. Acetic acid (5 mL) was poured into the product and it was stirred for 15 min and again precipitated with diethyl ether. The resulting dark green crude product was purified in two steps: first, column chromatography by using silica gel as stationary phase and methanol as eluent. Then preparative chromatography was applied by using silica gel as stationary phase and 2/3 DC/Ethanol as eluent. The isomeric mixture of phthalocyanines was soluble in ethanol, methanol, DMF and DMSO. Yield: 0.088 g, 16% m.p., >200 °C, FTIR, cm−1 2955, 2938, 2870 (R–H), 1236, 1246 (R–O–Ar), 1546 (Ar). UV–Vis λmax (nm) Methanol: 724, 324 1H-NMR
(500 MHz, DMSO-d$_6$): $\delta$, ppm 7.64–7.58 (8H, m), 7.08–7.00 (8H, m), 5.06–4.86 (16H, m), 2.14–1.67 (16H, m), 1.61–0.69 (72H, m), MS: m/ z 2206 [M + H]$^+$.

Analytical calculations for C$_{104}$H$_{124}$N$_8$Cl$_4$O$_{24}$S$_4$Zn: C, 56.63; H, 5.67; N, 5.08; found C, 56.51; H, 5.63; N, 5.12%.

2.3. Singlet oxygen detection

The singlet oxygen detection measurement was carried out in 2-propanol and diphenylisobenzofuran (DPBF) was used as the singlet oxygen trap. The experiment was performed in such a way that the requirements of both the red light and phthalocyanine sensitizer were unequivocally established and the irradiation was measured at 725 nm with an LED lamp. The reactions were followed spectrophotometrically by observing the decrease in the 415 nm absorption peak of DPBF as a function of irradiation time.

2.4. The investigation of the in vitro PDT activity of the photosensitizer

A standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to quantify cytotoxicity [47]. The human HepG2 liver adenocarcinoma cells were cultured in DMEM (Dulbecco’s modified Eagles Medium) with 10% fetal bovine serum, 2 mM L-glutamine, 100 mg/mL penicillin and 100 mg/mL streptomycin at 37 °C in a humidified incubator containing 5% CO$_2$.

3. Results and discussion

3.1. Synthesis and characterization

The synthesis of the isomeric phthalocyanines 2,9(10),16(17), 23(24)-tetrachloro-1,4,8,11,15,18,22,25-octahexyloxy-3,9(10),16(17), 23(24)-tetra-(4-sulfonylphenoxy)-phthalocyaninatozinc(II) (5) is shown in the Scheme. The important precursor for this work is 4,5-dichloro-3,6-bis(hexyloxy)phthalonitrile (3). Although this compound was reported earlier [46], we have used a different route to achieve higher yield and more reproducible results. The first step for the precursor 3 was reduction of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1) with Na$_2$S$_2$O$_5$ to obtain 4,5-dichloro-3,6-dihydroxyphthalonitrile (2). After this step, we have preferred its alkylation with 1-iodohexane to obtain 3 similar to that reported for the butyl derivative [48], rather than Mitsunobu reaction where triphenylphosphine and diisopropylazodicarboxylate have been used [46]. The new dinitrile compound, namely 4-(2-chloro-4,5-dicyano-3,6-bis(hexyloxy)phenoxy) benzenesulfonic acid (4) was prepared from 3 by substitution of one of the chloro groups of 3 with sodium 4-hydroxybenzenesulfonate in dry DMSO under an N$_2$ atmosphere (Scheme 1). Optimal results were obtained by using K$_2$CO$_3$ as the base at 45 °C in 48 h. Even when the ratio of 3/sodium 4-hydroxybenzenesulfonate dihydrate was taken to be 1:5, only one of the two chloro groups was exchanged with sodium 4-hydroxybenzenesulfonate and 4 was obtained. Our efforts to realize displacement of both chloro groups by changing the reaction conditions (e.g. reaction time, strength of the base, temperature, etc.) have not resulted with any product other than 4. Such a phenomena has been also reported for the reaction of 1,2-dichloro-4,5-dicyanobenzene with bulky diethylmalonate to give 1-chloro-3,4-dicyano-6-(1,1-dicarbethoxymethyl)benzene [35].

Cyclotetramerization of 4 with anhydrous zinc salt (Zn(CH$_3$COO)$_2$) and DBU by microwave irradiation in n-pentanol, led to the formation of the zinc phthalocyanine 5 (Scheme 1). The purification of newly synthesized zinc phthalocyanine 5 was achieved by column chromatography using silica gel as stationary phase and methanol as eluent, then silica gel preparative chromatography by using CH$_2$Cl$_2$/.
ethanol 2:3 mixture as eluent. Phthalonitrile derivatives containing three different substituted groups afford an isomeric mixture of phthalocyanine derivatives. In all cases, a mixture of four possible structural isomers is obtained. We expect that zinc phthalocyanine 5 was obtained as a statistical mixture of four regioisomers owing to the various possible positions of the phenoxysulfonic acid and chloro side-chains relative to one another (Scheme 1). The four isomers can be obtained with molecular symmetries $D_{2h}$, $C_{4h}$, $C_{2v}$, and $C_{5}$ in ratio of 1:1:2:4. Up to now, the successful separation of these four isomers with common column chromatography or by recrystallization has not been reported in the literature. No attempt has been made to separate the isomers of 5. As the introduction of phenoxy and hexyloxy substituents enhances the lipophilicity, and the sulfonic acid substituents give hydrophilic character to the compound, the zincphthalocyanine 5 possesses amphiphilic character. Also, the introduction of these bulky substituents are expected to be efficient in hindering the aggregation of macrocycle [36–39,49–56]. This green phthalocyanine compound was soluble in ethanol, methanol, DMF and DMSO. MALDI-TOF MS spectrum of compound of 5 shows molecular ion peak at $m/z = 2206 [M + H]^+$. 

In the $^1$H NMR spectra of the phthalocyanine precursor 4 displayed two doublets for the aromatic protons at 7.56 ppm and 6.9 ppm integrating for 2 protons each. Because phthalonitrile derivative is asymmetric two resolved triplets were observed for Ar–O–CH$_2$ protons at 4.2 and 4.09 ppm integrating for 2 protons each. Three resolved quintets were observed for second and one of the third –CH$_2$– protons in the hexyloxy groups at 1.77, 1.52, 1.46 ppm integrating 2, 2, 2 for each. For the remaining –CH$_3$– protons overlapped multiplets were observed between 1.33 and 1.13 ppm integrating for 10 protons. For the two terminal methyl protons overlapped multiplets were observed at 0.86 and 0.81 ppm integrating 3 and 3 for each. $^1$H NMR investigation of the zinc(II) phthalocyanine 5 provided the expected chemical shifts for the structure. The $^1$H NMR spectra of zinc phthalocyanine showed complex patterns because it is a mixture of constitutional isomers. The signals observed for aromatic protons as multiplets between 7.64 and 7.58 ppm, 7.08–7.0 ppm. The O–CH$_2$ protons of hexyloxy group were observed as overlapped multiplets, 5.1–4.79, and remaining protons of hexyloxy groups were observed between 2.17–1.03 ppm and 0.84–0.66 ppm. The signals from the SO$_3$H group were not observed in the $^1$H NMR spectra of compound 4 and 5 due to fast chemical exchange between this group.

Fig. 1. Absorption spectra of ZnPc (5) in methanol in different concentrations.

Aggregation of phthalocyanines in polar media is a well-known fact in phthalocyanine chemistry [57]. The photosensitizing potential of phthalocyanines has been intensely affected as a result of aggregation. Therefore it is meaningful to examine the aggregation behavior of the zinc phthalocyanine. Its aggregation has been investigated in methanol over a concentration range of 2 μM–12 μM and extinction coefficient remained constant as followed from Beer–Lambert law graph seen in Fig. 1. It confirms that zinc phthalocyanine exists as monomeric species in methanol in the concentration range studied.

The aggregation tendency of zinc phthalocyanine was also studied in water by addition of surfactant Triton X-100. It is well known that addition of this reagent diminishes the aggregation tendency of pcs in aqueous medium [37]. As shown in Fig. 2 no appreciable change in Q absorption maximum has been observed after addition of TritonX-100, so we might conclude that these phthalocyanine molecules are already in monomeric form in water without any need to diminish aggregation with any reagent. Hexyloxy groups in nonperipheral positions do not possess free rotation around the macrocycle and they are constrained to one face of the macrocycle; this prevents face to face arrangement of the planar macrocycles to aggregate [58,59].

Fig. 2. Absorption spectra of ZnPc (5) in water — addition of one drop Triton X-100.

Fig. 3. The change in the absorbance spectrum of 1,3-diphenylisobenzofuran (DPBF) and dye mixture on irradiation with LED light source at 725 nm.
2.5 mW cm$^{-2}$ and kept either in the dark (back row), or under 3 h irradiation with an LED at 242 nm. Seed HepG2 cells were either seeded in 96-well plates with varying concentration of dye, or under 3 h irradiation with an LED at 242 nm. Percent viability as determined by a standard MTT assay with HepG2 cells is shown in Fig. 4.

3.2. Singlet oxygen generation

It has previously been reported that singlet oxygen generation was detectable by monitoring the reaction of DPBF by UV spectrophotometry. By following the disappearance of the 415 nm absorbance band of DPBF at the initial concentration of 50 μM in the presence of varied concentrations from 2 to 40 μM of dye, it was shown that singlet oxygen generation occurred during the reaction [60] (Fig. 3). No degradation of the DPBF trap was observed either in the dark or in aerated solution.

3.3. In vitro PDT effect of the photosensitizer

The positive results on singlet oxygen generation reported in Section 3.2 encouraged us to test the potential of the new phthalocyanine as a sensitizer on HepG2 cells. When the cells were kept in the dark either with or without the sensitizer, no change in viability was observed. In the presence of the dye (varying concentration from 2 to 40 μg/mL), when the cells were irradiated with an LED source at 725 nm for 3 h, followed by 24 h of incubation, a significant decrease in cell viability was observed. At the concentration of 20 μg/mL of the sensitizer, the cell viability was decreased to 42% (Fig. 4). Cell viability (%) in here was calculated as optical density (OD) of treated cells/OD of nontreated cells $\times 100$.

4. Conclusion

The present work describes the synthesis, characterization and in vitro PDT activity of a new hexadeca substituted zinc phthalocyanine. The synthesized phthalocyanine complex shows excellent solubility in polar organic solvents such as methanol, DMSO, DMF. It was also demonstrated that this new phthalocyanine dye is a very efficient singlet oxygen generator. In addition, this soluble photosensitizer displayed photoinduced cytotoxicity under low flow rate LED irradiation.

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References


