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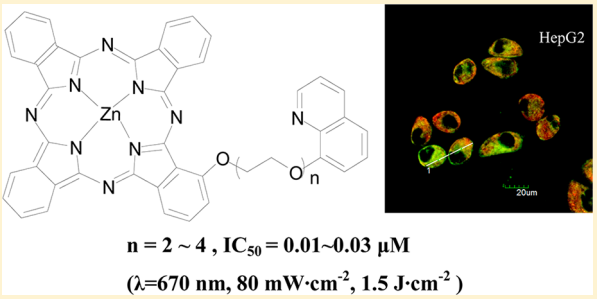
Synthesis and in Vitro Photodynamic Activity of Oligomeric Ethylene Glycol−Quinoline Substituted Zinc(II) Phthalocyanine Derivatives

Xiao Jia, Feng-Feng Yang, Jun Li, Jian-Yong Liu, and Jin-Ping Xue*

Fujian Engineering Research Center for Drug and Diagnoses, Treat of Photodynamic Therapy, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, China

Supporting Information

ABSTRACT: A new series of zinc(II) phthalocyanine derivatives have been synthesized and characterized. These macrocycles exhibited a sharp absorption band in the red visible region in DMF, which indicated that they were dissolved well and almost did not aggregate in this solvent. Compared with the unsubstituted zinc(II) phthalocyanine, all these phthalocyanines have a red-shifted Q-band (at 678−699 vs 670 nm) and exhibit a relatively weaker fluorescence emission and a higher efficiency at generating singlet oxygen. The monosubstituted photosensitizers also exhibit high photocytotoxicity toward HepG2 human hepatocarcinoma cells with IC_{50} values as low as 0.02−0.05 μM (λ = 670 nm, 80 mW·cm^{-2}, 1.5 J·cm^{-2}). The high photodynamic activities of these compounds are in accordance with their low aggregation tendency and high cellular uptake. Their structure−activity relationship was assessed by determining the photophysical properties, cellular uptake, and in vitro photodynamic activities of this series of compounds. As shown by confocal microscopy, monosubstituted phthalocyanines can target the mitochondria and lysosomes of the cells, and tetrasubstituted phthalocyanines tend to target the lysosomes of the cells.



INTRODUCTION

Photodynamic therapy (PDT) has emerged as a promising and noninvasive treatment for (against) malignant tumors and wet age-related macular degeneration. Fundamentally, the treatment utilizes the combination of a photosensitizer, light, and molecular oxygen to cause cellular and tissue damage, in which reactive oxygen species (ROS), generated through a series of photoinduced processes, is believed to be the major cytotoxic agent. For cancer treatment, PDT has several potential advantages including its noninvasive nature, tolerance of repeated doses, and high specificity that can be achieved through precise application of the light.[1−3]

Phthalocyanines are promising second-generation photosensitizers for PDT as a result of their strong absorption in tissue-penetrating red light and high efficiency of generating singlet oxygen.[4−7] However, one important problem related to phthalocyanine derivatives is their low solubility in several organic media and water. In addition, aggregation phenomena are observed and may have a strong influence on the efficiency of singlet oxygen production and then the bioavailability.[8] Quinoline derivatives have displayed a wide range of biological activity. Luis and his colleagues reported that 2-arylquinolines, particularly those containing methoxy substituents or no substituents in the quinoline skeleton, showed antiviral properties against human immunodeficiency syndrome (HIV).[9] Ridley and Hudson indicated that quinoline exhibited antimalarial property.[10] On the other hand, quinoline related chemical classes such as quinolines and naphthyridines are being exploited in cancer chemotherapy and a number of them are in different phases of clinical trials in recent years.[8,11,12] Shen et al. demonstrated some 8-hydroxyquinoline derivatives are potential anticancer drug candidates.[13] Vincent and his colleagues also discovered a new family of bis-8-hydroxyquinoline substituted benzylamines with proapoptotic activity in cancer cells.[14] It is very interesting that quinoline and its analogues have recently been examined for their modes of function in the inhibition of tyrosine kinases, proteasome, tubulin polymerization, and DNA repair.[15,16] Polyethylene glycols are known to be excellent pharmaceutical intermediates, which possess excellent biocompatibility and can minimize nonspecific uptake and enable specific tumor-targeting through the enhanced permeability and retention (EPR) effect.[17] Therefore, these hydrophilic moieties adding to the hydrophobic core will enhance the solubility and make the molecules amphiphilic, which is a desirable characteristic for efficient photosensitizers. Dennis et al. prepared a series of asymmetric methylated polyethylene glycol zinc(II) phthalocyanines, which proved to have high cytotoxic properties despite their water insolubility.[18] Free hydroxyl groups at the end of the polyoxochain were reported to considerably increase the water solubility of the phthalocyanine but have been found the lower photodynamic activity.[19] In addition, some photosensitizers such as lutetium texaphyrin involving the use of

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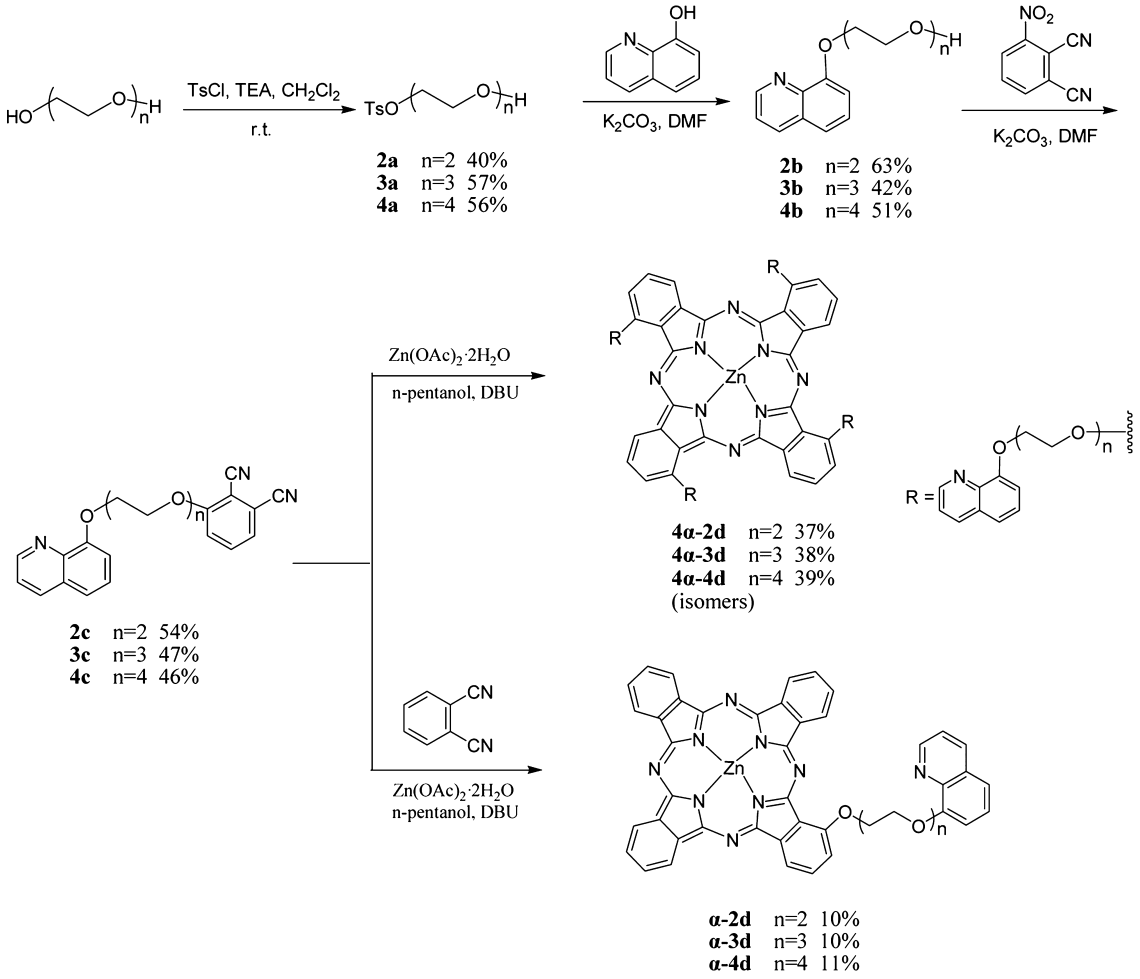
Scheme 1. Synthesis of α-Substituted Phthalocyanine Derivatives



polyethylene glycols derivatives as additive in formulations have been used in PDT clinical treatment.[20]

In this report, we describe a novel series of zinc(II) phthalocyanines substituted with oligomeric ethylene glycol and 8-hydroxyquinoline moieties. The two substituents reduce their aggregation tendency, thereby promoting the generation of singlet oxygen and resulting in higher photodynamic activities. In addition, we report the synthesis, spectroscopic and photophysical characteristics, and the in vitro photodynamic activities of these compounds.

RESULTS AND DISCUSSION

Synthesis and Characterization. The synthesis of zinc(II) phthalocyanines is shown in Scheme 1 and Scheme 2. First, the alcohols were converted to the corresponding compounds **2a**−**4a** in triethylamine (TEA) and CH_2Cl_2 reaction systems. Then treatment of **2a**−**4a** with 8-hydroxyquinoline gave the substituted products **2b**−**4b**, which then underwent nucleophilic substitution with 3-nitrophthalonitrile or 4-nitrophthalonitrile to give **2c**−**4c**, **3c-1**, and **4c-1**. Heating these phthalonitriles in *n*-pentanol with $Zn(OAc)_2{\cdot}2H_2O$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) led to phthalocyanines **4α-2d**, **4α-3d**, **4α-4d**, **4β-3d**, **4β-4d**. Mixed cyclization of precursors **2c**−**4c** and **4c-1** with an excess of unsubstituted phthalonitrile (9 equiv) in the presence of $Zn(OAc)_2{\cdot}2H_2O$ and DBU in *n*-pentanol afforded the corresponding "3 + 1" unsymmetrical phthalocyanines **α-2d**, **α-3d**, **α-4d**, and **β-4d**. All these phthalocyanine derivatives could be purified readily by silica gel column chromatography in 10−48% yield. All the new compounds were characterized with ^{1}H NMR, HRMS, and IR.

Photophysical and Photochemical Properties. The electronic absorption spectra of phthalocyanines **α-2d**, **α-3d**, **α-4d**, **β-4d** and **4α-2d**, **4α-3d**, **4α-4d**, **4β-3d**, **4β-4d** in DMF were recorded. The spectra showed typical features of nonaggregated phthalocyanines. They displayed a Soret band at 320−350 nm, an intense and sharp Q-band at 672−699 nm, together with two vibronic bands at 610−650 nm. For all of these phthalocyanines, the Q-band strictly followed the Lambert−Beer law (up to 10 μM), indicating that the aggregation of these phthalocyanines is not significant in DMF (Figure S1−S8 in the Supporting Information). The electronic absorption spectra data for all phthalocyanines are summarized in Table 1. Compared with ZnPc (670 nm), all these analogues exhibit a red-shifted Q-band (672−699 nm). The α-substituted phthalocyanine further shifts its Q-band to 677 or 699 nm, while the β-substituted one has its Q-band at 672 or 679 nm. Compared with monosubstituted phthalocyanines, tetrasubstituted phthalocyanines have a further red-shift Q-band (at 699 nm vs 677 nm and at 679 nm vs 672 nm) (Figure S9−S18 in the Supporting Information). For **α-2d**, **α-3d**, **α-4d**, **β-4d**, **4β-3d**, **4β-4d** excited at 610 nm, it shows a fluorescence emission at 684−691 nm with a quantum yield (Φ_F) of 0.26−0.28 in DMF. For **4α-2d**, **4α-3d**, **4α-4d** excited at 630 nm, the fluorescence emission was red-shifted to 709−710 nm with an

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Scheme 2. Synthesis of β-Substituted Phthalocyanine Derivatives

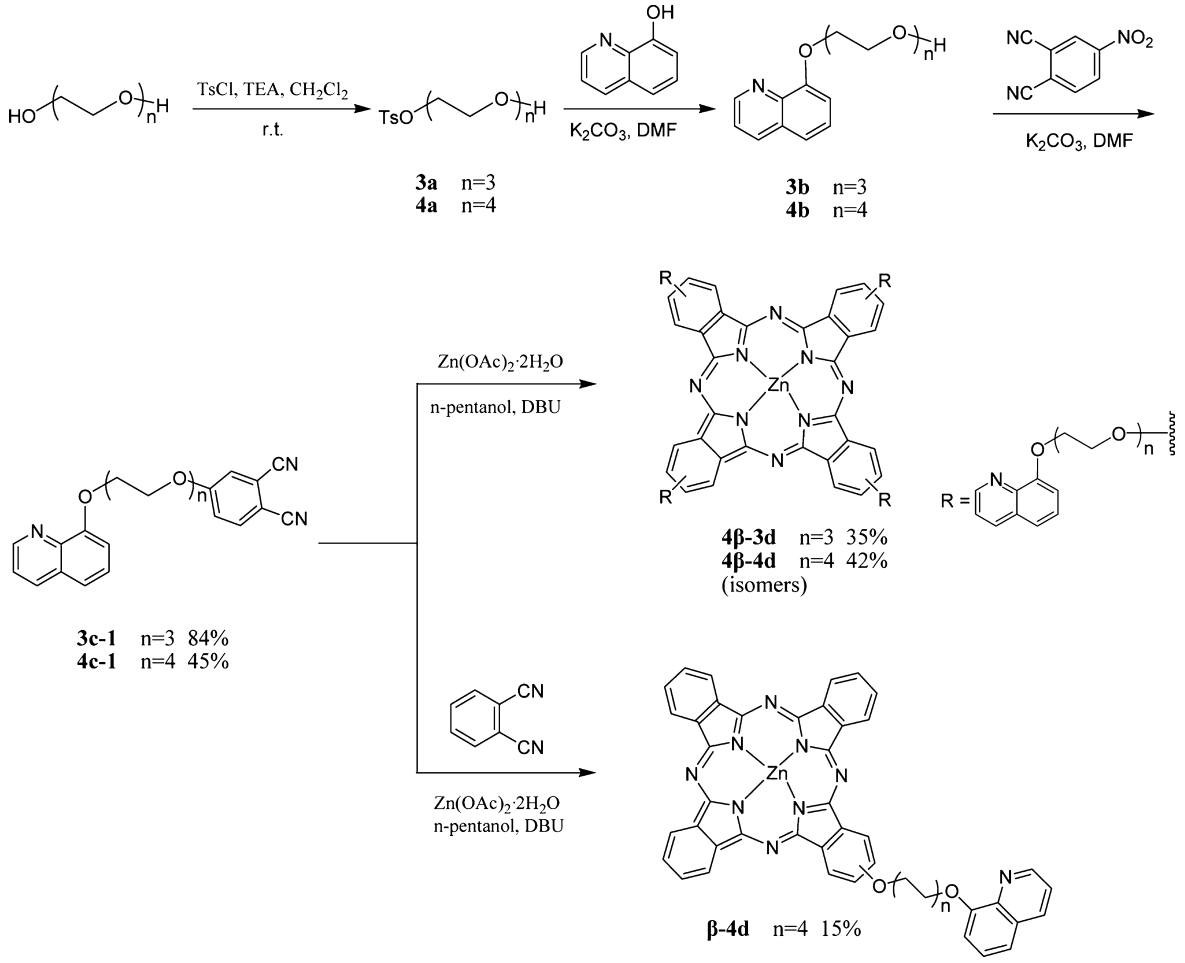

Table 1. Photophysical/Photochemical Data for All These Phthalocyanines

	λ_{max}^{abs} (nm) (log ε)		λ_{max}^{ex} (nm)		Φ_F [a]		Φ_Δ [b]
compd	in DMF	in medium	in DMF	in medium	in DMF	in medium	in DMF
α-2d[c]	677 (5.34)	682	685	691	0.24	0.19	0.63
α-3d[c]	677 (5.36)	681	684	693	0.24	0.19	0.66
α-4d[c]	677 (5.43)	681	686	690	0.24	0.21	0.68
β-4d[c]	672 (5.41)	676	680	682	0.26	0.18	0.62
4α-2d[d]	699 (5.43)	707	709	712	0.17	0.05	0.86
4α-3d[d]	699 (5.36)	708	709	717	0.17	0.06	0.86
4α-4d[d]	699 (5.34)	707	710	716	0.17	0.09	0.88
4β-3d[c]	679 (5.26)	687	691	693	0.26	0.05	0.58
4β-4d[c]	679 (5.28)	685	690	691	0.28	0.06	0.75

[a]Relative to ZnPc (Φ_F = 0.28). [b]Relative to ZnPc (Φ_Δ = 0.56). [c]Excited at 610 nm. [d]Excited at 630 nm.

even lower Φ_F of 0.17. It is likely that introduction of an electron-donor alkoxy group at the phthalocyanine macrocycle makes the Φ_F value smaller relative to ZnPc (Φ_F = 0.28)[21] as the energy gap decreases between the HOMO and the LUMO.

To evaluate the photosensitizing efficiency of these phthalocyanines, their singlet oxygen quantum yields (Φ_Δ) were determined in DMF by a steady-state method with 1,3-diphenylisobenzofuran (DPBF) as the scavenger.[22] The variation of DPBF absorption was monitored using a UV−vis spectrophotometer. The absorbance at 415 nm gradually decreased (Figure S19−S24 in the Supporting Information), while there was no decrease in Q-band λ_{max}^{abs}. All the Φ_Δ values are summarized in Table 1. It can be seen that all of these monosubstituted phthalocyanines are efficient singlet oxygen generators having comparable quantum yields (Φ_Δ = 0.62−0.68), which is higher than that of ZnPc (Φ_Δ = 0.56), and tetra-α-substituted phthalocyanines exhibit higher ability to generate singlet oxygen with a singlet oxygen quantum yield of 0.86−0.88. As shown in Table 1, the Φ_Δ data are virtually the same for the analogues **α-2d**, **α-3d**, **α-4d**, **4α-2d**, **4α-3d**, and **4α-4d**, showing that the length of the chains does not affect the photochemical properties of phthalocyanine.

In Vitro Photodynamic Activities. The cytotoxic effects of these phthalocyanines in Cremophor EL emulsions were

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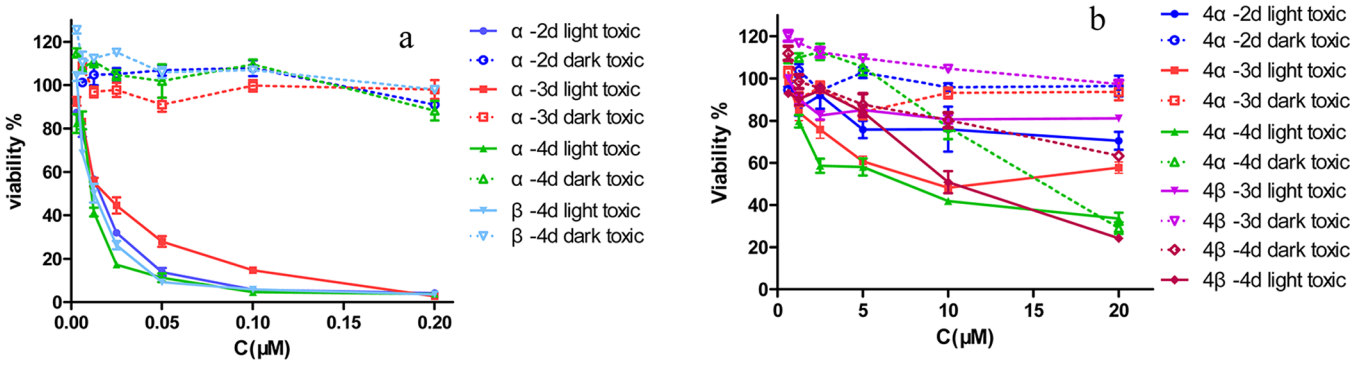

Figure 1. Cytotoxic effects of title compounds on HepG2 cells in the absence (closed symbols) and presence (open symbols) of light (λ = 670 nm, 80 mW·cm^{-2}, 1.5 J·cm^{-2}). Data are expressed as mean values ± standard error of the mean value (SEM) of three independent experiments, each performed in quadruplicate.

investigated against HepG2 human hepatocarcinoma cells. Figure 1 shows the dose-dependent survival curves for all these compounds. It can be seen that monosubstituted phthalocyanines are essentially noncytotoxic in the absence of light (up to 0.2 μM). However, upon illumination they have significantly high photocytotoxicity toward HepG2 cells with IC_{50} values, defined as the dye concentration required to kill 50% of the cells, as low as 0.01−0.03 μM (Figure 1a, Table 2) under a rather low light dose (λ = 670 nm, 80 mW·cm^{-2}, 1.5 J·cm^{-2}). Tetrasubstituted phthalocyanines show less photodynamic activity toward HepG2 cells (λ = 670 nm, 80 mW·cm^{-2}, 1.5 J·cm^{-2}). For **4*α*-2d**, **4*α*-3d**, **4*β*-3d**, they are almost noncytotoxic without light, but for **4*α*-4d** and **4*β*-4d**, they are cytotoxic in the absence of light for HepG2 cells (Figure 1b, Table 2). The IC_{50} value of ***α*-3d** is as low as 0.019 μM toward the HepG2 cells under a rather low light dose, which is 526-fold lower than that of **4*α*-3d**. The results show that monosubstituted phthalocyanines (***α*-2d**, ***α*-3d**, ***α*-4d**, and ***β*-4d**) have significantly high photocytotoxicity.

Table 2. IC_{50} for Phthalocyanines against HepG2 Cells (λ = 670 nm, 80 mW·cm^{-2}, 1.5 J·cm^{-2})

compd	IC_{50} (μM)	compd	IC_{50} (μM)
***α*-2d**	0.016	**4*α*-2d**	>40
***α*-3d**	0.019	**4*α*-3d**	10
***α*-4d**	0.027	**4*α*-4d**	8
***β*-4d**	0.012	**4*β*-4d**	10
		4*β*-3d	>40

The aggregation state of photosensitizers is an important factor relating to their photodynamic activities.[23] Usually, aggregation provides an efficient nonradiative relaxation pathway, thereby greatly shortening the excited-state lifetime, and the singlet oxygen quantum yield would decrease, eventually leading to photodynamic activity greatly reduced or no photodynamic activity. The aggregation behavior of phthalocyanines, formulated with Cremophor EL in the RPMI 1640 culture medium, was examined by absorption and fluorescence spectroscopic methods. As shown in Figure 2, monosubstituted phthalocyanines show a relatively sharp and intense Q-band and a relatively strong fluorescence emission. At the same time, tetra-*β*-substituted phthalocyanines have a significantly broadened Q-band and relatively weak fluorescence. The results suggest that monosubstituted phthalocyanines are not significantly aggregated under these conditions, which seems to be in accord with their high photocytotoxicity.

To account for the different photocytotoxicity of three phthalocyanines (***α*-3d**, **4*α*-3d**, and **4*β*-3d**), their cellular uptake was examined by fluorescence microscopy. As shown by the images captured by confocal laser scanning microscopy (Figure 3), three compounds could enter into the cells causing intracellular fluorescence after incubation for 24 h. After incubation with these phthalocyanines, SDS was used to lyse the cells and extract the phthalocyanines. The phthalocyanine concentrations inside the cells were quantified by measuring their fluorescence at 690 nm by using the fluorescence microscopy (excitation at 610 nm). The results are depicted in Figure 3 g, which shows that the cellular uptake of ***α*-3d** is only little higher than that of **4*α*-3d** and actually about 5-fold higher than that of **4*β*-3d**. The higher cellular uptake of ***α*-3d** is in accord with its higher photocytotoxicity.

We also investigate the subcellular localization of these phthalocyanines by confocal laser scanning microscopy. The

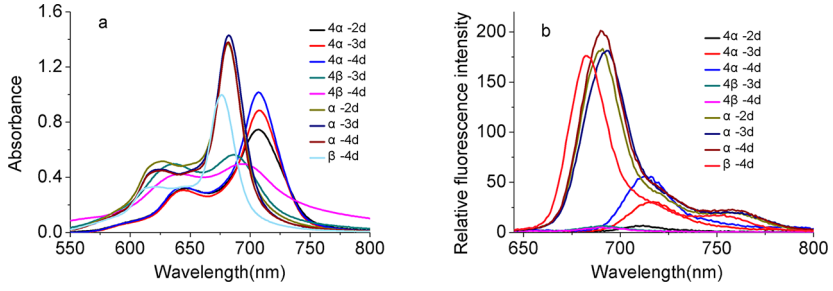

Figure 2. Electronic absorption spectra and fluorescence spectra of all new phthalocyanines, formulated with Cremophor EL, in the RPMI 1640 culture medium (all phthalocyanines at 8 μM).

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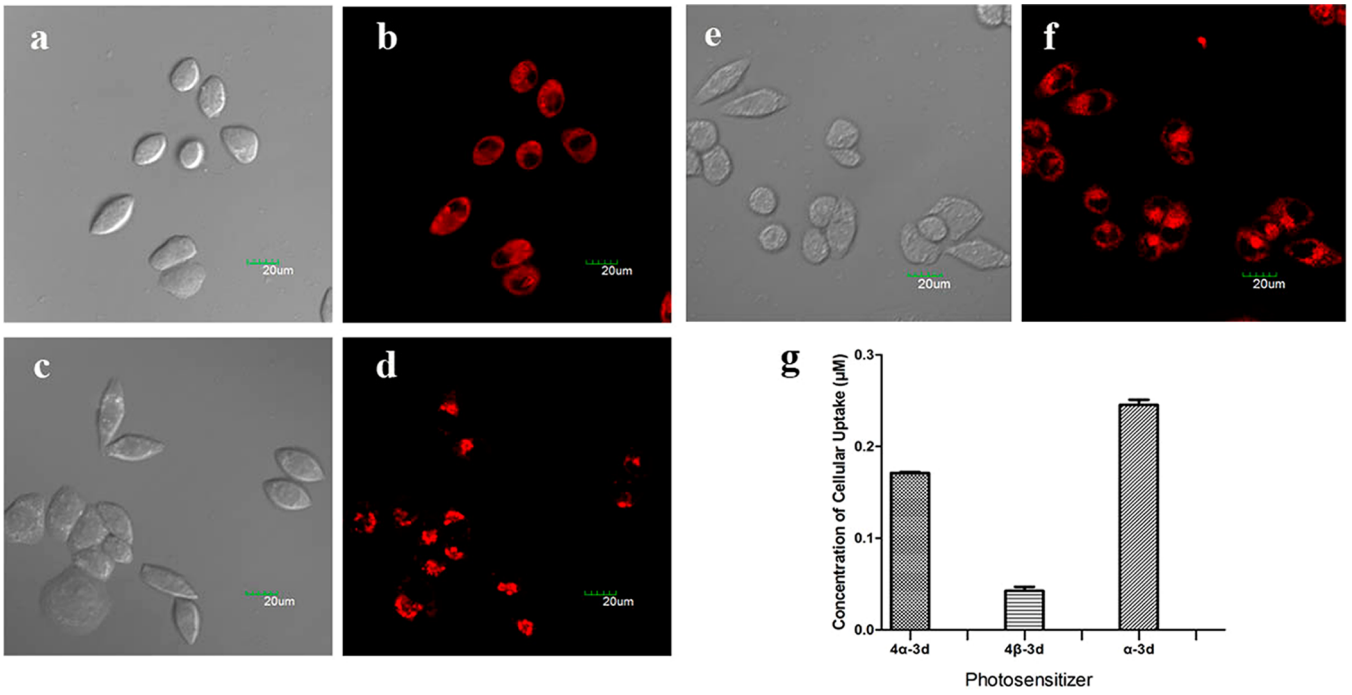

Figure 3. Confocal fluorescence images of HepG2 cells after incubation with ***α*-3d** (b) or **4*α*-3d** (d) or **4*β*-3d** (f) for 24 h (at 10 μM). The corresponding bright field images are given in the left column (a) for ***α*-3d**, (c) for **4*α*-3d**, and (e) for **4*β*-3d**. (f) Comparison of the percentage of cellular uptake of ***α*-3d**, **4*α*-3d**, and **4*β*-3d**. Data are expressed as mean value ± standard deviation (SD) of three independent experiments.

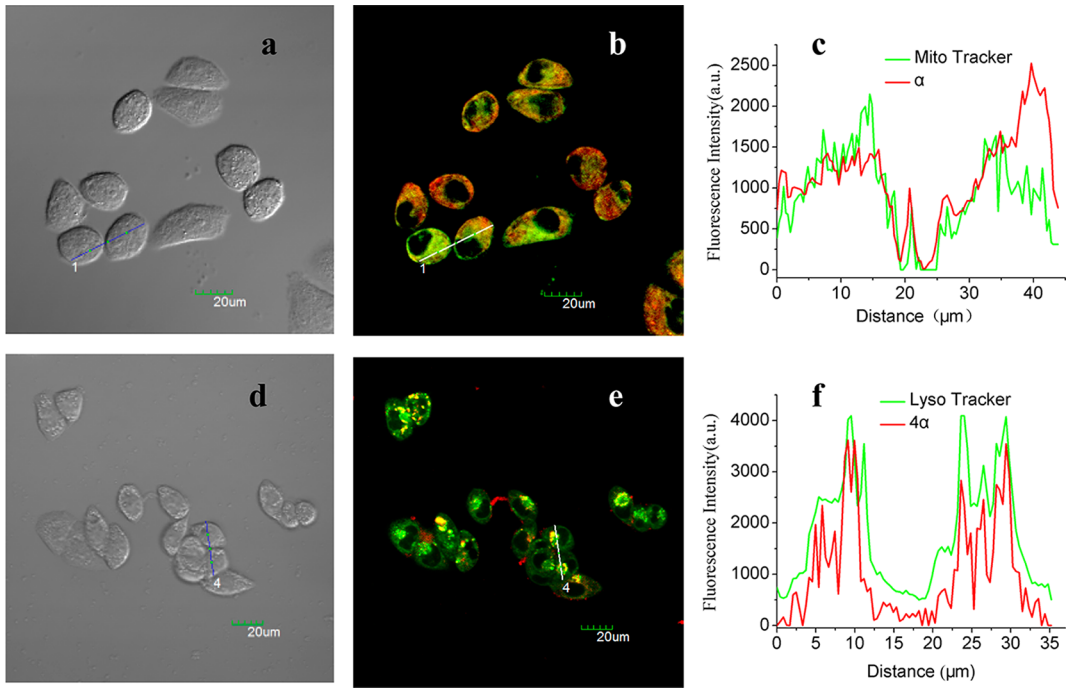

Figure 4. (a) Visualization of the bright field image. (b) Corresponding superimposed image of intracellular fluorescence of HepG2 using filter sets specific for ***α*-3d** (in red) and Mito Tracker (in green). (c) Fluorescence intensity profiles of ***α*-3d** (in red) and Mito Tracker (in green). (d) Visualization of the bright field image. (e) Corresponding superimposed image of intracellular fluorescence of HepG2 using filter sets specific for **4*α*-3d** (in red) and Lyso Tracker (in green). (f) Fluorescence intensity profiles of **4*α*-3d** (in red) and Lyso Tracker (in green).

cells were first incubated with the compound in the culture medium for 2 h and then stained with LysoTracker DND 26 or MitoTracker Green FM (for 10−20 min), which are specific dyes for lysosomes or mitochondria, respectively. As shown in Figure 4 and Figure S25 (in the Supporting Information), the fluorescence caused by the MitoTracker (excited at 488 nm, monitored at 500−570 nm) can superimpose with the fluorescence caused by ***α*-3d** (excited at 633 nm, monitored at 640−700 nm). The very similar fluorescence intensity line profiles of ***α*-3d** and MitoTracker traced along the green line in Figure 4 c also confirms that ***α*-3d** can target the mitochondria of the cells. Meanwhile the fluorescence images of ***α*-3d** could

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be merged with that of the LysoTracker (excited at 488 nm, monitored at 500−570 nm) (parts e and f of Figure S25 in the Supporting Information), showing that **α-3d** can target the lysosomes of the cells too. The fluorescence caused by the LysoTracker (excited at 488 nm, monitored at 500−570 nm) is superimposed with the fluorescence caused by **4α-3d** (or **4β-3d**) (excited at 633 nm, monitored at 640−700 nm) (Figure 4e or Figure S25k). The very similar fluorescence intensity line profiles of **4α-3d** (or **4β-3d**) and LysoTracker traced along the green line in Figure 4f (or Figure S25l)) also confirms that **4α-3d** (or **4β-3d**) can target the lysosomes of the cells. By contrast, the fluorescence images of **4α-3d** (or **4β-3d**) could not be merged with that of the MitoTracker (part c or part i of Figure S25 in the Supporting Information), showing that **4α-3d** (or **4β-3d**) is not localized in the mitochondria of the cells. The difference between mono- and tetrasubstituted phthalocyanine for subcellular localization may be explained by the varying degrees of the protonation of hydroxyquinoline of phthalocyanines caused by lower pH in lysosomes and by tetrasubstituted phthalocyanines becoming water-soluble and having difficulty crossing the membrane of lysosomes and being detained in lysosomes of the cells. It may explain the trend of photocytotoxicity observed for this series of compounds.

CONCLUSIONS

We have prepared and characterized a series of novel zinc(II) phthalocyanines substituted with quinolin-8-yloxy-poly-ethoxy at the peripheral positions and evaluated their in vitro photodynamic activities. All these monosubstituted phthalocyanines are less aggregated in the culture medium, and upon illumination they are highly photodynamic active toward HepG2 cells with IC_{50} values as low as about 0.02 μM, which is 450-fold lower than that of the tetrasubstituted phthalocyanines. As shown by confocal microscopy, monosubstituted phthalocyanines can target the mitochondria and lysosomes of the cells and tetrasubstituted phthalocyanines tend to target the lysosomes of the cells. The overall results show that these monosubstituted phthalocyanines are highly promising antitumor agents for photodynamic therapy.

EXPERIMENTAL SECTION

Materials and Methods. All reactions were carried out under an atmosphere of nitrogen. *n*-Pentanol was distilled over sodium, and all other solvents and reagents were reagent grade and used as received. Compounds **2a**,[24] **3a**,[24] **4a**,[24] **2b**,[25] **3b**,[26] and **4b**[27] were prepared as previously described. ^{1}H NMR spectra were recorded on an AVANCE III 500 MHz spectrometer, in $CDCl_3$ unless otherwise stated. Spectra were referenced internally using the residual solvent (^{1}H: δ 7.26) resonances relative to $SiMe_4$. Electronic adsorption spectra were measured on a TU-1901 spectrometer, and fluorescence spectra were obtained on a Varian Cary eclipse spectrometer. IR spectra were measured on a Perkin-Elmer SP2000. HRMS spectra were obtained on ion trap mass spectrometry DECAX-30000 LCQ Deca XP mass spectrometer. The fluorescence quantum yields of the samples [$\Phi_{F(sample)}$] were determined by the equation $\Phi_{F(sample)} = (F_{sample}/F_{ref})(A_{ref}/A_{sample})(n_{sample2}/n_{ref2})\Phi_{F(ref)}$, where *F*, *A*, and *n* are the measured fluorescence (area under the emission peak), the absorbance at the excitation position, and the refractive index of the solvent, respectively. ZnPc in DMF was used as the reference [$\Phi_{F(ref)} = 0.28$].[21] The purity of all the new compounds was determined by HPLC analysis and was found to be ≥95%.

General Procedure for the Preparation of 2c, 3c, 4c, 3c-1, and 4c-1. A mixture of compounds **2b**−**4b** (1 equiv), 3-nitrophthalonitrile or 4-nitrophthalonitrile (1.1−1.5 equiv), and K_2CO_3 (3−5 equiv) in DMF (20−30 mL) was stirred at room temperature under an atmosphere of nitrogen for 48 h. The volatiles were removed in vacuo. Then the residue was mixed with water (100 mL) and extracted with CH_2Cl_2 (80 mL × 3). The combined organic extracts were dried over anhydrous $MgSO_4$. After evaporation under reduced pressure, the residue was purified by silica gel column chromatography using $CHCl_3$/EA/CH_3OH (20:30:1 v/v/v) as the eluent.

Phthalonitrile 2c. According to the general procedure, **2b** (3.09 g, 13.25 mmol) was treated with 3-nitrophthalonitrile (3.45 g, 19.93 mmol) and K_2CO_3 (5.56 g, 40.29 mmol) in DMF to give **2c** as a white solid (54%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (dd, *J* = 1.5, 4.0 Hz, 1 H, ArH), 8.13 (dd, *J* = 2.0, 8.5 Hz, 1 H, ArH), 7.48−7.39 (m, 4 H, ArH), 7.28 (d, *J* = 9.0 Hz, 1 H, ArH), 7.22 (d, *J* = 7.5 Hz, 1 H, ArH), 7.09 (d, *J* = 8.0 Hz, 1 H, ArH), 4.41 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.34 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.15 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.05 (t, *J* = 4.5 Hz, 2 H, CH_2). HRMS (ESI): *m*/*z* calcd for $C_{21}H_{17}N_3O_3$ [M + H]$^+$ 360.1342, found 360.1364. IR: υ(−CN) 2227 cm^{-1}; υ_{as}(−CH_2−) 2942 cm^{-1}, υ_s(−CH_2−) 2876 cm^{-1}; υ(Ar) 1581, 1499, 1470 cm^{-1}; υ(=CH) 3085 cm^{-1}; δ(=CH) 816 cm^{-1}; υ_{as}(C−O−C) 1111 cm^{-1}; υ(Ar−O−R) 1290 cm^{-1}; υ(C=N) 1613 cm^{-1}; υ(H−O−H) 3440 cm^{-1}.

Phthalonitrile 3c. According to the general procedure, **3b** (1.59 g, 5.72 mmol) was treated with 3-nitrophthalonitrile (1.48 g, 8.50 mmol) and K_2CO_3 (4.26 g, 30.65 mmol) in DMF to give **3c** as a white solid (47%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (dd, *J* = 1.5, 4.5 Hz, 1 H, ArH), 8.13 (dd, *J* = 1.5, 8.5 Hz, 1 H, ArH), 7.54 (t, *J* = 8.5 Hz, 1 H, ArH), 7.47−7.39 (m, 3 H, ArH), 7.27 (vd, *J* = 8.5 Hz, 2 H, ArH), 7.12 (d, *J* = 7.5 Hz, 1 H, ArH), 4.42 (t, *J* = 5.0 Hz, 2 H, CH_2), 4.27 (d, *J* = 4.5 Hz, 2 H, CH_2), 4.07 (t, *J* = 5.0 Hz, 2 H, CH_2), 3.93 (t, *J* = 5.0 Hz, 2 H, CH_2), 3.78 (s, 4 H, CH_2). HRMS (ESI): *m*/*z* calcd for $C_{23}H_{21}N_3O_4$ [M + H]$^+$ 404.1604, found 404.1614. IR: υ(−CN) 2229 cm^{-1}; υ_{as}(−CH_2−) 2904 cm^{-1}; υ_s(−CH_2−) 2872 cm^{-1}; υ(Ar) 1584, 1502, 1473 cm^{-1}; υ_{as}(C−O−C) 1103 cm^{-1}; υ(Ar−O−R) 1285 cm^{-1}; υ(H−O−H) 3567 cm^{-1}.

Phthalonitrile 4c. According to the general procedure, **4b** (6.23 g, 19.37 mmol) was treated with 3-nitrophthalonitrile (4.65 g, 26.86 mmol) and K_2CO_3 (8.93 g, 64.71 mmol) in DMF to give **4c** as a pale yellow oil (46%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (dd, *J* = 1.5, 4.0 Hz, 1 H, ArH), 8.12 (dd, *J* = 1.5, 8.5 Hz, 1 H, ArH), 7.56 (vt, *J* = 8.5 Hz, 1H, ArH), 7.46−7.37 (m, 3 H, ArH), 7.28−7.25 (m, 2 H, ArH), 7.09 (dd, *J* = 1.5, 8.0 Hz, 1 H, ArH), 4.39 (t, *J* = 5.0 Hz, 2 H, CH_2), 4.26 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.06 (t, *J* = 5.0 Hz, 2 H, CH_2), 3.89 (t, *J* = 5.0 Hz, 2 H, CH_2), 3.78−3.76 (m, 2 H, CH_2), 3.73−3.71 (m, 2 H, CH_2), 3.70−3.68 (m, 2 H, CH_2), 3.67−3.65 (m, 2 H, CH_2). HRMS (ESI): *m*/*z* calcd for $C_{25}H_{25}N_3O_5$ [M + H]$^+$ 448.1866, found 448.1905. IR: υ(−CN) 2230 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2876 cm^{-1}; υ(Ar) 1583, 1503, 1472 cm^{-1}; υ(=CH) 3086 cm^{-1}; δ(=CH) 732−824 cm^{-1}; υ_{as}(C−O−C) 1109 cm^{-1}; υ(Ar−O−R) 1294 cm^{-1}; υ(C=N) 1616 cm^{-1}; υ(H−O−H) 3430 cm^{-1}.

Phthalonitrile 3c-1. According to the general procedure, **3b** (2.45 g, 8.83 mmol) was treated with 4-nitrophthalonitrile (1.6 g, 9.24 mmol) and K_2CO_3 (3.6 g, 26.09 mmol) in DMF to give **3c-1** as a white solid (84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.90 (dd, *J* = 1.5, 4.5 Hz, 1 H, ArH), 8.14 (dd, *J* = 1.5, 8.0 Hz, 1 H, ArH), 7.58 (d, *J* = 9.0 Hz, 1 H, ArH), 7.47−7.40 (m, 3 H, ArH), 7.22 (d, *J* = 2.5 Hz, 1 H, ArH), 7.14 (dd, *J* = 3.0, 9.0 Hz, 1 H, ArH), 7.08 (d, *J* = 7.5 Hz, 1 H, ArH), 4.39 (t, *J* = 5.0 Hz, 2 H, CH_2), 4.17 (t, *J* = 4.5 Hz, 2 H, CH_2), 4.06 (t, *J* = 5.0 Hz, 2 H, CH_2), 3.88 (t, *J* = 4.5 Hz, 2 H, CH_2), 3.81−3.79 (m, 2 H, CH_2), 3.76−3.74 (m, 2 H, CH_2). HRMS (ESI): *m*/*z* calcd for $C_{23}H_{21}N_3O_4$ [M + H]$^+$ 404.1605, found 404.1621. IR: υ(−CN) 2226 cm^{-1}; υ_{as}(−CH_2−) 2929 cm^{-1}; υ_s(−CH_2−) 2881 cm^{-1}; υ(Ar) 1596, 1574 cm^{-1}; 1503 cm^{-1}; 1474 cm^{-1}; υ_{as}(C−O−C) 1126 cm^{-1}; υ(Ar−O−R) 1259 cm^{-1}; υ(H−O−H) 3401 cm^{-1}.

Phthalonitrile 4c-1. According to the general procedure, **4b** (7.42 g, 23.09 mmol) was treated with 4-nitrophthalonitrile (5.97 g, 34.48 mmol) and K_2CO_3 (6.45 g, 46.74 mmol) in DMF to give **4c-1** as a pale yellow oil (45%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92−8.91 (m, 1 H, ArH), 8.13 (dd, *J* = 1.5, 8.5 Hz, 1 H, ArH), 7.62 (d, *J* = 9.0 Hz, 1 H, ArH), 7.46−7.39 (m, 3 H, ArH), 7.23 (d, *J* = 2.5 Hz, 1 H, ArH), 7.15 (dd, J = 2.5, 8.5 Hz, 1 H, ArH), 7.11−7.10 (m, 1 H, ArH), 4.41 (t,

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J = 5.0 Hz, 2 H, CH_2), 4.16 (t, J = 4.5 Hz, 2 H, CH_2), 4.06 (t, J = 5.0 Hz, 2 H, CH_2), 3.86 (t, J = 4.5 Hz, 2 H, CH_2), 3.79−3.77 (m, 2 H, CH_2), 3.70−3.68 (m, 6 H, CH_2). HRMS(ESI): m/z calcd for $C_{25}H_{25}N_3O_5$ $[M + H]^+$ 448.1866, found 448.1809. IR: υ(−CN) 2230 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2876 cm^{-1}; υ(Ar) 1598, 1503, 1472 cm^{-1}; υ(=CH) 3086 cm^{-1}; δ(=CH) 732−816 cm^{-1}; υ_{as}(C−O−C) 1107 cm^{-1}; υ(Ar−O−R) 1257 cm^{-1}; υ(H−O−H) 3436 cm^{-1}.

General Procedure for the Preparation of Phthalocyanines α-2d, α-3d, α-4d, and β-4d. A mixture of phthalonitriles **4a−c** and **4c-1** (1 equiv), phthalonitrile (9 equiv), and $Zn(OAc)_2{\cdot}2H_2O$ (5 equiv) in *n*-pentanol (15 mL) was heated to 100 °C. Then a small amount of DBU (0.5 mL) was added. The mixture was stirred at 150 °C for 12 h. After a brief cooling, the volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (30:1 v/v) as the eluent. The crude product was further purified by recrystallization from a mixture of THF and hexane to give the product as a blue solid.

Phthalocyanine α-2d. According to the above procedure, phthalonitrile **2c** (0.18 g, 0.50 mmol) was treated with unsubstituted phthalonitrile (0.58 g, 4.52 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.88 g, 4.00 mmol) to give **α-2d** as a blue solid (0.04 g, 10%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.33−9.24 (m, 6 H, Pc-H_α), 8.88 (d, J = 8.0 Hz, 1 H, Pc-H_α), 8.75 (s, 1 H, ArH), 8.22−8.02 (m, 8 H, Pc-H_β), 7.70 (d, J = 8.0 Hz, 1 H, ArH), 7.43−7.40 (m, 1 H, ArH), 7.32−7.26 (m, 2 H, ArH), 7.15 (d, J = 7.5 Hz, 1 H, ArH), 4.92 (s, 2 H, CH_2), 4.54 (t, J = 4.5 Hz, 2 H, CH_2), 4.50 (t, J = 4.5 Hz, 2 H, CH_2), 4.43 (t, J = 4.5 Hz, 2 H, CH_2). HRMS (ESI): m/z calcd for $C_{45}H_{29}N_9O_3Zn$ $[M + H]^+$ 808.1757, found 808.1671. IR: υ(Pc) 1648, 1588, 1488, 1452 cm^{-1}; 736; υ_{as}(−CH_2−) 2921 cm^{-1}; υ_s(−CH_2−) 2852 cm^{-1}; υ_{as}(C−O−C) 1114 cm^{-1}; υ_s(Ar−O−R) 1265 cm^{-1}; υ(C−N) 1332 cm^{-1}; υ(H−O−H) 3437 cm^{-1}.

Phthalocyanine α-3d. According to the above procedure, phthalonitrile **3c** (0.20 g, 0.50 mmol) was treated with unsubstituted phthalonitrile (0.58 g, 4.49 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.54 g, 2.45 mmol) to give **α-3d** as a blue solid (0.04 g, 10%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.21−9.08 (m, 3 H, Pc-H_α), 9.02−8.98 (m, 2 H, Pc-H_α), 8.97 (d, J = 2.0 Hz, 1 H, Pc-H_α), 8.69−8.68 (m, 1 H, Pc-H_α), 8.63 (d, J = 7.0 Hz, 1 H, Pc-H_β), 8.16−8.13 (m, 5 H, Pc-H_β), 8.10−8.03 (m, 2 H, Pc-H_β), 7.87 (t, J = 8.0 Hz, 1 H, ArH), 7.47 (d, J = 8.0 Hz, 1 H, ArH), 7.37−7.34 (m, 1 H, ArH), 7.30−7.24 (m, 2 H, ArH), 6.91 (dd, J = 1.5 Hz, 7.5 Hz, 1 H, ArH), 4.75 (br s, 2 H, CH_2), 4.34 (t, J = 4.0 Hz, 2 H, CH_2), 4.10−4.07 (m, 4 H, CH_2), 3.88 (t, J = 4.5 Hz, 2 H, CH_2), 3.85 (t, J = 4.5 Hz, 2 H, CH_2). HRMS (ESI): m/z calcd for $C_{47}H_{33}N_9O_4Zn$ $[M + H]^+$ 852.2019, found 852.2002. IR: υ(Pc)1599, 1488, 1456, 736 cm^{-1}; υ_{as}(−CH_2−) 2924 cm^{-1}; υ_s(−CH_2−) 2868 cm^{-1}; υ_{as}(C−O−C) 1115 cm^{-1}; υ_s(Ar−O−R) 1257 cm^{-1}; υ(C−N) 1332 cm^{-1}; υ(H−O−H) 3430 cm^{-1}.

Phthalocyanine α-4d. According to the above procedure, phthalonitrile **4c** (0.36 g, 0.80 mmol) was treated with unsubstituted phthalonitrile (0.92 g, 7.19 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.88 g, 3.99 mmol) to give **α-4d** as a blue solid (0.08 g, 11%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.40−9.38 (m, 6 H, Pc-H_α), 9.00 (d, J = 7.0 Hz, 1 H, Pc-H_α), 8.71−8.70 (m, 1 H, ArH), 8.24−8.22 (m, 6 H, Pc-H_β), 8.14−8.11 (m, 2 H, Pc-H_β), 7.73 (d, J = 8.0 Hz, 1 H, ArH), 7.39−7.36 (m, 1 H, ArH), 7.30 (d, J = 8.0 Hz, 1 H, ArH), 7.24 (t, J = 8.0 Hz, 1 H, ArH), 6.87 (d, J = 8.0 Hz, 1 H, ArH), 4.87 (vt, J = 4.0 Hz, 2 H, CH_2), 4.39 (t, J = 4.5 Hz, 2 H, CH_2), 4.07 (t, J = 4.5 Hz, 2 H, CH_2), 4.00 (t, J = 4.5 Hz, 2 H, CH_2), 3.76 (t, J = 4.5 Hz, 2 H, CH_2), 3.68 (t, J = 4.5 Hz, 2 H, CH_2), 3.60−3.58 (m, 2 H, CH_2), 3.55−3.53(m, 2 H, CH_2). HRMS (ESI): m/z calcd for $C_{49}H_{37}N_9O_5Zn$ $[M + H]^+$ 896.2281, found 896.2210. IR: υ(Pc) 1642, 1589, 1488, 1456, 745 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2870 cm^{-1}; υ_{as}(C−O−C) 1095 cm^{-1}; υ_s(Ar−O−R) 1256 cm^{-1}; υ(C−N) 1331 cm^{-1}; υ(H−O−H) 3426 cm^{-1}.

Phthalocyanine β-4d. According to the above procedure, phthalonitrile **4c-1** (0.51 g, 1.14 mmol) was treated with unsubstituted phthalonitrile (1.15 g, 8.98 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (1.09 g, 4.97 mmol) to give **β-4d** as a blue solid (0.15 g, 15%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.22−9.16 (m, 3 H, Pc-H_α), 9.11 (d, J = 7.0 Hz, 1 H, Pc-H_α), 9.06 (d, J = 7.0 Hz, 1 H, Pc-H_α), 9.03 (d, J = 7.0 Hz, 1 H, Pc-H_α), 8.8−8.79 (m, 1 H, Pc-H_α), 8.76 (d, J = 8.5 Hz, 1 H, Pc-H_α), 8.34 (s, 1 H, ArH), 8.21−8.04 (m, 7 H, Pc-H_β), 7.52−7.50 (m, 1 H, ArH), 7.45−7.42 (m, 1 H, ArH), 7.38−7.12 (m, 2 H, ArH), 7.11−7.09 (m, 1 H, ArH), 4.57 (s, 2 H, CH_2), 4.23 (t, J = 4.5 Hz, 2 H, CH_2), 4.07 (t, J = 4.5 Hz, 2 H, CH_2), 3.88 (t, J = 4.5 Hz, 2 H, CH_2), 3.83−3.81 (m, 2 H, CH_2), 3.74−3.72 (m, 4 H, CH_2), 3.70−3.68 3.83−3.81 (m, 2 H, CH_2). HRMS(ESI): m/z calcd for $C_{49}H_{37}N_9O_5Zn$ $[M + H]^+$ 896.2281, found 896.2211. IR: υ(Pc) 1503, 1486 cm^{-1}; υ_{as}(−CH_2−) 2920 cm^{-1}; υ_s(−CH_2−) 2867 cm^{-1}; υ_{as}(C−O−C) 1060 cm^{-1}; υ_s(Ar−O−R) 1240 cm^{-1}; υ(C−N) 1334 cm^{-1}; υ(H−O−H) 3443 cm^{-1}.

General Procedure for the Preparation of Phthalocyanines 4α-2d, 4α-3d, 4α-4d, 4β-3d, 4β-4d. A mixture of phthalonitriles **4a−c**, **3c-1**, and **4c-1** (1 equiv) and $Zn(OAc)_2{\cdot}2H_2O$ (2 equiv) in *n*-pentanol (15 mL) was heated to 100 °C. Then a small amount of DBU (0.5 mL) was added. The mixture was stirred at 150 °C for 12 h. After a brief cooling, the volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (15:1 v/v) as the eluent. The crude product was further purified by recrystallization from a mixture of $CHCl_3$ and hexane to give the product as a green solid.

Phthalocyanine 4α-2d. According to the above procedure, phthalonitrile **2c** (0.86 g, 2.4 mmol) was treated with $Zn(OAc)_2{\cdot}2H_2O$ (0.26 g, 1.21 mmol) to give **4α-2d** as a green solid (0.33 g, 37%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.94−8.80 (m, 4 H, Pc-H_α), 8.79−8.66 (m, 4 H, Pc- H_β), 8.19−8.18 (m, 2 H, Pc-H_β), 8.09 (t, J = 7.0 Hz, 2 H, Pc-H_β), 8.06 (br s, 2 H, ArH), 7.92 (br s, 2 H, ArH), 7.69−7.68 (m, 4 H, ArH), 7.46−7.43 (m, 2 H, ArH), 7.36−7.26 (m, 8 H, ArH), 7.24−7.14 (m, 4 H, ArH), 6.94 (vt, J = 6.0 Hz, 2 H, ArH), 5.19 (br s, 4 H, CH_2), 4.89 (br s, 4 H, CH_2), 4.52−4.48 (m, 8 H, CH_2), 4.42−4.39 (m, 4 H, CH_2), 4.28 (br s, 4 H, CH_2), 4.23 (br s, 4 H, CH_2), 4.04 (br s, 4 H, CH_2). HRMS (ESI): m/z calcd for $C_{84}H_{68}N_{12}O_{12}Zn$ $[M + H]^+$ 1501.4449, found 1501.3867. IR: υ(Pc) 1586, 1501, 1448, 739 cm^{-1}; υ_{as}(−CH_2−) 2921 cm^{-1}; υ_s(−CH_2−) 2868 cm^{-1}; υ_{as}(C−O−C) 1080 cm^{-1}; υ_s(Ar−O−R) 1263 cm^{-1}; υ(C−N) 1334 cm^{-1}; υ(H−O−H) 3435 cm^{-1}.

Phthalocyanine 4α-3d. According to the above procedure, phthalonitrile **3c** (1.02 g, 2.53 mmol) was treated with $Zn(OAc)_2{\cdot}2H_2O$ (0.26 g, 1.17 mmol) to give **4α-3d** as a green solid (0.40 g, 38%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.05−9.03 (m, 2 H, Pc-H_α), 8.96−8.92 (m, 2 H, Pc-H_α), 8.71−8.68 (m, 4 H, ArH), 8.13−8.06 (m, 8 H, Pc-H_β), 7.78−7.70 (m, 2 H, ArH), 7.65−7.56 (m, 2 H, ArH), 7.38−7.24 (m, 12 H, ArH), 6.91−6.83 (m, 4 H, ArH), 5.15−5.10 (m, 4 H, CH_2), 4.84−4.80 (m, 4 H, CH_2), 4.39 (br s, 4 H, CH_2), 4.18−4.15 (m, 4 H, CH_2), 4.09 (br s, 8 H, CH_2), 3.96 (br s, 4 H, CH_2), 3.87 (vt, J = 3.5 Hz, 8 H, CH_2), 3.76−3.75 (t, J = 4.0 Hz, 4 H, CH_2), 3.71 (br s, 4 H, CH_2), 3.62 (br s, 4 H, CH_2). HRMS (ESI): m/z calcd for $C_{92}H_{84}N_{12}O_{16}Zn$ $[M + H]^+$ 1677.5492, found 1677.5356. IR: υ(Pc) 1588, 1501, 1489, 1450 cm^{-1}; υ_{as}(−CH_2−) 2920 cm^{-1}; υ_s(−CH_2−) 2868 cm^{-1}; υ_{as}(C−O−C) 1096 cm^{-1}; υ_s(Ar−O−R) 1264 cm^{-1}; υ(C−N) 1335 cm^{-1}; υ(H−O−H) 3435 cm^{-1}.

Phthalocyanine 4α-4d. According to the above procedure, phthalonitrile **4c** (1.13 g, 2.53 mmol) was treated with $Zn(OAc)_2{\cdot}2H_2O$ (0.29 g, 1.31 mmol) to give **4α-4d** as a green solid (0.46 g, 39%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (br s, 2 H, Pc-H_α), 8.91 (br s, 2 H, Pc-H_α), 8.71 (s, 4 H, Pc-H_β), 8.15 (d, J = 8.0 Hz, 4 H, Pc-H_β), 8.07 (s, 4 H, ArH), 7.79−7.74 (m, 2 H, ArH), 7.68−7.67 (m, 2 H, ArH), 7.41−7.38 (m, 4 H, ArH), 7.33−7.26 (m, 8 H, ArH), 6.91 (d, J = 7.0 Hz, 4 H, ArH), 5.11 (br s, 4 H, CH_2), 4.81−4.78 (m, 4 H, CH_2), 4.35 (s, 4 H, CH_2), 4.13 (s, 4 H, CH_2), 4.03−3.99 (m, 12 H, CH_2), 3.74−3.70 (m, 12 H, CH_2), 3.64 (d, J = 4.0 Hz, 4 H, CH_2), 3.63−3.54(m, 8 H, CH_2), 3.51(s, 4 H, CH_2), 3.43(s, 8 H, CH_2). HRMS (ESI): m/z calcd for $C_{100}H_{100}N_{12}O_{20}Zn$ $[M + H]^+$ 1853.6541, found 1853.6202. IR: υ(Pc) 1641, 1586, 1501, 1488, 1448, 745 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2867 cm^{-1}; υ_{as}(C−O−C) 1081 cm^{-1}; υ_s(Ar−O−R) 1263 cm^{-1}; υ(C−N) 1333 cm^{-1}; υ(H−O−H) 3427 cm^{-1}.

Phthalocyanine 4β-3d. According to the above procedure, phthalonitrile **3c-1** (0.85 g, 2.11 mmol) was treated with $Zn(OAc)_2{\cdot}2H_2O$ (0.23 g, 1.04 mmol) to give **4β-3d** as a green solid (0.31 g, 35%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.98−8.92 (m, 4 H,

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Pc-H_α), 8.85−8.84 (m, 4 H, Pc-H_α), 8.59−8.52 (m, 4 H, Pc-H_β), 8.22−8.20 (m, 4 H, ArH), 7.65−7.56 (m, 4 H, ArH), 7.54−7.43 (m, 4 H, ArH), 7.42−7.37 (m, 8 H, ArH), 7.16−7.13 (m, 4 H, ArH), 4.61 (br s, 4 H, CH_2), 4.54(br s, 4 H, CH_2), 4.32−4.29 (m, 8 H, CH_2), 4.08 (br s, 4 H, CH_2), 4.01−3.93 (m, 12 H, CH_2), 3.84 (s, 8 H, CH_2), 3.79 (s, 8 H, CH_2). HRMS (ESI): m/z calcd for $C_{92}H_{84}N_{12}O_{16}Zn$ $[M + H]^+$ 1677.5492, found 1677.5499. IR: υ(Pc) 1606, 1571, 1488, 1450 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2869 cm^{-1}; υ_{as}(C−O−C) 1096 cm^{-1}; υ_s(Ar−O−R) 1263 cm^{-1}; υ(C−N) 1317 cm^{-1}; υ(H−O−H) 3418 cm^{-1}.

Phthalocyanine 4β-4d. According to the above procedure, phthalonitrile **4c-1** (1.40 g, 3.13 mmol) was treated with Zn-$(OAc)_2{\cdot}2H_2O$ (0.35 g, 1.59 mmol) to give **4β-4d** as a green solid (0.61 g, 42%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.98−8.93 (m, 4 H, Pc-H_α), 8.81−8.80 (m, 4 H, Pc-H_α), 8.57−8.51 (m, 4 H, Pc-H_β), 8.20−8.18 (m, 4 H, ArH), 7.65−7.59 (m, 4 H, ArH), 7.57−7.56 (m, 4 H, ArH), 7.45−7.09 (m, 8 H, ArH), 7.09−7.08 (m, 4 H, ArH), 4.60 (br s, 4 H, CH_2), 4.53 (br s, 4 H, CH_2), 4.22 (d, J = 3.5 Hz, 8 H, CH_2), 4.05 (br s, 4 H, CH_2), 3.98 (br s, 4 H, CH_2), 3.87−3.84(m, 8 H, CH_2), 3.79−3.78(m, 4 H, CH_2), 3.73−3.62(m, 28 H, CH_2). HRMS (ESI): m/z calcd for $C_{100}H_{100}N_{12}O_{20}Zn$ $[M + H]^+$ 1853.6541, found 1853.6550. IR: υ(Pc) 1605, 1570, 1488, 1449 cm^{-1}; υ_{as}(−CH_2−) 2917 cm^{-1}; υ_s(−CH_2−) 2867 cm^{-1}; υ_{as}(C−O−C) 1093 cm^{-1}; υ_s(Ar−O−R) 1234 cm^{-1}; υ(C−N) 1317 cm^{-1}; υ(H−O−H) 3435 cm^{-1}.

Singlet Oxygen Quantum Yields. Singlet oxygen quantum yield (Φ_Δ) values were determined by comparative method using 1,3-diphenylisobenzofuran (DPBF) as singlet oxygen chemical quencher in DMF[28] (eq 1 in air)

$$\Phi_\Delta = \Phi_\Delta^{std}\frac{kI_{abs}^{std}}{k^{std}I_{abs}} \quad (1)$$

where Φ_Δ^{std} is the singlet oxygen quantum yield for the ZnPc standard[29] (Φ_Δ^{std} = 0.56 in DMF); k and k^{std} are the DPBF photobleaching rates in the presence of compound **4** and reference, respectively; and I_{abs} and I_{abs}^{std} are the rates of light absorption by synthetic phthalocyanines and reference substance. The degradation of the solutions was monitored at 415 nm, and DPBF concentrations were reduced to 0.1 mmol·L^{-1}.

Cell Culture. HepG2 cells (from ATCC) were maintained in RPMI medium 1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and Primocin antibiotic (Invitrogen). The cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. All the compounds required the preparation of a concentrated DMSO stock solution (5% CEL), which were then diluted with medium, and the final DMSO concentration was 1% in medium.

Cytotoxicity Assay. To assess the cytotoxic effect of the phthalocyanines, about 1.0 × 10^4 HepG2 cells per well in the culture medium were seeded in 96-multiwell plates and incubated at 37 °C for 24 h in a humidified 5% CO_2 atmosphere. Phthalocyanines were first dissolved in DMF to give 10 mM solutions, which were diluted to 1 mM with the culture medium in the presence of 0.5% Cremophor EL. These served as the stock solutions for the following in vitro studies. For cytotoxicity studies, the solutions were further diluted with the culture medium. The cells, after being rinsed with phosphate buffered saline (PBS), were incubated with 100 μL of the diluted phthalocyanine solutions for 2 h at 37 °C under 5% CO_2 . The cells were then rinsed again with PBS and refed with 100 μL of the culture medium before being illuminated at ambient temperature. For dark cytotoxicity, phthalocyanines were diluted and added to tetraplicate wells. After 24 h, the added compounds were removed by fresh medium and were incubated for another 24 h. The cell survival was assessed using the MTT assay. For light cytotoxicity, after incubation with phthalocyanines for about 24 h, the cells were exposed to light (λ = 670 nm) at a dose of 1.5 J·cm^{-2} and then incubated again for 24 h, and finally the MTT cell viability assay was performed and each experiment was performed in triplicate.

Cell viability was determined by means of the colormetric MTT assay.[30] After illumination, the cells were incubated at 37 °C under 5% CO_2 overnight. An MTT (Sigma) solution in PBS (3 mg mL^{-1}, 50 μL) was added to each well followed by incubation for 2 h under the same environment. A solution of sodium dodecyl sulfate (SDS; Sigma, 10% by weight, 50 μL) was then added to each well. The plate was incubated in an oven at 60 °C for 30 min, and then 80 μL of isopropyl alcohol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 s before the absorbance at 540 nm at each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the following equation: % viability = $[\sum(A_i/A_{control} \times 100)]/n$, where A_i is the absorbance of the ith data (i = 1, 2, ..., n), $A_{control}$ is the average absorbance of the control wells in which the phthalocyanine was absent, and n (=4) is the number of data points.

Intracellular Localization. Approximately 1.0 × 10^5 HepG2 cells in the culture medium (1 mL) were plated on a culture dish and incubated for 24 h. Then the medium was replaced by fresh medium with 10 μM phthalocyanines and incubated for another 24 h. After incubation, the cells were rinsed with physiological saline and incubated with 1 mL MitoTracker Green or LysoTracker (40 nM for MitoTracker Green and 75 nm for LysoTracker in the medium) for 20 or 60 min. Then the cells were rinsed with physiological saline again. The subcellular localization of these phthalocyanines was revealed by comparing the intracellular fluorescence images caused by the MitoTracker Green (or LysoTracker) and these compounds. Both MitroTracker and LysoTracker were excited at 488 nm and monitored at 500−570 nm, while phthalocyanines were excited at 633 nm and monitored at 640−700 nm.

Cellular Uptake. About 3.0 × 10^6 HepG2 cells in the culture medium (2 mL) were seeded on a Petri dish (diameter of 35 mm) and incubated overnight at 37 °C under 5% CO_2. The medium was removed, and the cells were rinsed with PBS (2 mL).The cells were then incubated with a solution of phthalocyanine **α-3d** or **4α-3d** or **4β-3d** in the medium (10 μM, 2 mL) for 24 h under the same conditions. The solution was then removed, and the cells were rinsed with PBS (2 mL) and then harvested by 0.25% trypsin−EDTA (Invitrogen, 500 μL), followed by quenching of the trypsin with medium (500 μL). The solution was transferred to 1.5 mL centrifuge tubes and centrifuged at 2400 rpm for 3 min. The pellet was then washed with PBS (1 mL), and the suspension was centrifuged again. After removal of the PBS, the cells were lysed with DMF (1 mL). The mixture was sonicated for 20 min and then centrifuged again. The supernatants were transferred for UV−vis spectroscopic measurements. The absorbance at 684, 709, and 691 nm for phthalocyanines **α-3d**, **4α-3d**, and **4β-3d**, respectively, was compared with the respective calibration curves to give the uptake concentrations. Each experiment was repeated three times.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Absorption and fluorescence spectra/data for zinc(II) phthalocyanines synthesized in this paper in DMF or DMEM, comparison of the rate of decay of DPBF sensitized by phthalocyanines or ZnPc in DMF, results of subcellular localization studies, and ^{1}H NMR and HRMS spectra of all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +86-591-83758209. Fax: +86-591-87892632. E-mail: xuejinping66@fzu.edu.cn.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CEL, Cremophor EL series; DPBF, 1,3-diphenylisobenzofuran; EPR, enhanced permeability and retention; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PDT, photodynamic therapy; TEA, triethylamine; RPMI, Roswell Park Memorial Institute; SD, standard deviation; ref, reference; SEM, standard error of the mean value; ZnPc, zinc(II) phthalocyanine; Φ_F, fluorescence quantum yield; Φ_Δ, singlet oxygen quantum yield

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