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Core-modified porphyrins. Part 6: Effects of lipophilicity and core structures on physicochemical and biological properties in vitro

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Abstract—Thiaporphyrins **2**–**8** were prepared as analogues of 5,20-diphenyl-10,15-bis[4-(carboxymethyleneoxy)-phenyl]-21,23- dithiaporphyrin (**1**) to examine the effect of structural modifications: substituent changes in meso aryl groups of dithiaporphyrins with one water-solubilizing group (**2**–**5**), dihydroxylation of a pyrrole double bond and reduction to dihydroxychlorins (**6** and **7**), and the removal of two meso aryl groups to give unsubstituted meso positions (**8**). The impact of these structural modifications was measured in both physicochemical (UV spectra, generation of singlet oxygen, lipophilicity, and aggregate formation) and biological properties (dark toxicity and phototoxicity, cellular uptake, and subcellular localization). Mono-functionalized porphyrins had much higher lipophilicity than di-functionalized porphyrin **1** and, consequently, formed more aggregates in aqueous media. The formation of aggregates might lower the efficiency of lipophilic porphyrins as photosensitizers. Interestingly, dihydroxylation of a core pyrrole group in the dithiaporphyrin core did not affect either the absorption spectrum or the efficiency for generating singlet oxygen. The phototoxicity of dihydroxydithiachlorins mainly depended on their intracellular uptake. The potent phototoxicity of **6**, IC_{50} = 0.18 μM, was attributed to the extraordinarily high uptake. The intracellular uptake of **6** was about 7.6 times higher than **1**. In contrast, thiaporphyrin **8** with only two meso aryl groups was less effective as a photosensitizer, perhaps due to poorer uptake and a lower quantum yield for the generation of singlet oxygen.

1. Introduction

Photodynamic therapy (PDT) offers a great opportunity to reduce severe side effects and enhance selectivity in cancer treatments due to its unique mechanism of action.[1,2] The expression of biological activity in PDT is derived from the combined effect of three components: a photosensitizer, light, and oxygen. Excited-state photosensitizers generate reactive oxygen species by the transfer of energy to produce singlet oxygen or by the transfer of electrons to produce superoxide, hydrogen peroxide, or hydroxyl radicals. Therefore, although photosensitizers are generally administered systemically, damage by PDT occurs only near/around the areas irradiated by the light. Although Photofrin® has been effective as a first-generation photosensitizer, it has several problems.[3,4] Physically, Photofrin® is a mixture of compounds, which makes pharmacological evaluation difficult and causes problems in terms of reproducibility in its preparation. In addition, some patients suffer long-term skin photosensitization following treatment with Photofrin®. Furthermore, Photofrin® is only weakly absorbing at its absorption maximum (~630 nm), which limits the photoefficiency of treatment and its absorption maximum limits the depth of penetration of activating light.

Core-modified porphyrins, in particular dithiaporphyrins, have several advantages: flexibility in synthesis, preparation in high purity, absorption at longer wavelengths (band I absorption maxima of ~700 nm), and high photostability.[5] In an effort to develop second-generation, dithiaporphyrin photosensitizers, we synthesized numerous core-modified porphyrins and studied the relationship between their structure and their phototoxicity.[5–11] Dithiaporphyrins with two carboxylic acid groups were found to be more efficient photosensitizers than dithiaporphyrins with one, three, and four carbox-

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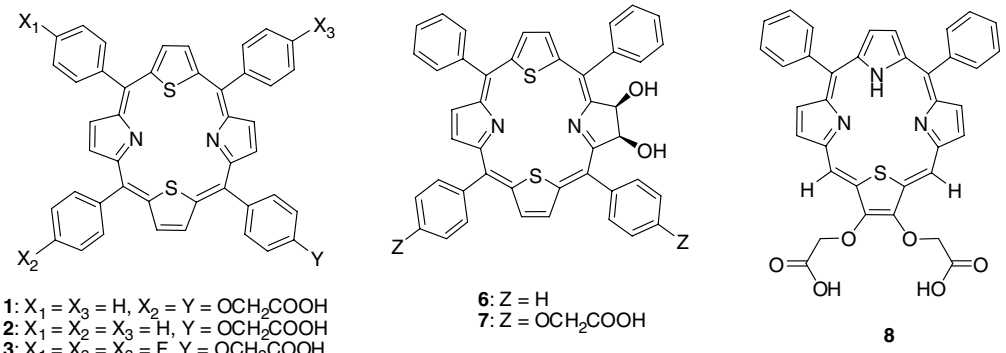


1: $X_1 = X_3 = H$, $X_2 = Y = OCH_2COOH$
2: $X_1 = X_2 = X_3 = H$, $Y = OCH_2COOH$
3: $X_1 = X_2 = X_3 = F$, $Y = OCH_2COOH$
4: $X_1 = X_2 = H$, $X_3 = C(CH_3)_3$, $Y = OCH_2COOH$
5: $X_1 = X_2 = X_3 = F$, $Y = SO_3Na$

6: Z = H
7: $Z = OCH_2COOH$

8

Figure 1. Structures of 21-mono- or 21,23-dithiaporphyrins.

ylic acid groups.[8] Among dithiaporphyrins with two carboxylic acid groups, steric effects at the meso-aryl substituents were more determinant of phototoxicity than electronic effects at the meso-aryl groups and a preference was noted for smaller substituents and two different meso substituents.[5,9]

In this report, we extend our SAR study to mono-functionalized dithiaporphyrins **2**–**5**, dihydroxydithiachlorins **6** and **7**, and monothiaporphyrin **8** bearing only two meso-aryl groups (Fig. 1). We describe the syntheses of **2**–**8** as well as the measurement of their cellular uptake and phototoxicity. In order to elucidate the determining factors for phototoxicity, the absorption spectra, relative yields for the generation of singlet oxygen, the lipophilicity ($\log D_{7.4}$), the aggregation tendency, and sites of sub-cellular localization were determined.

2. Results and discussion

2.1. Chemistry

Compounds **1** and **2** were prepared as described in our earlier work.[8] The syntheses of **3**–**8** used methods that we developed to construct asymmetric core-modified porphyrins.

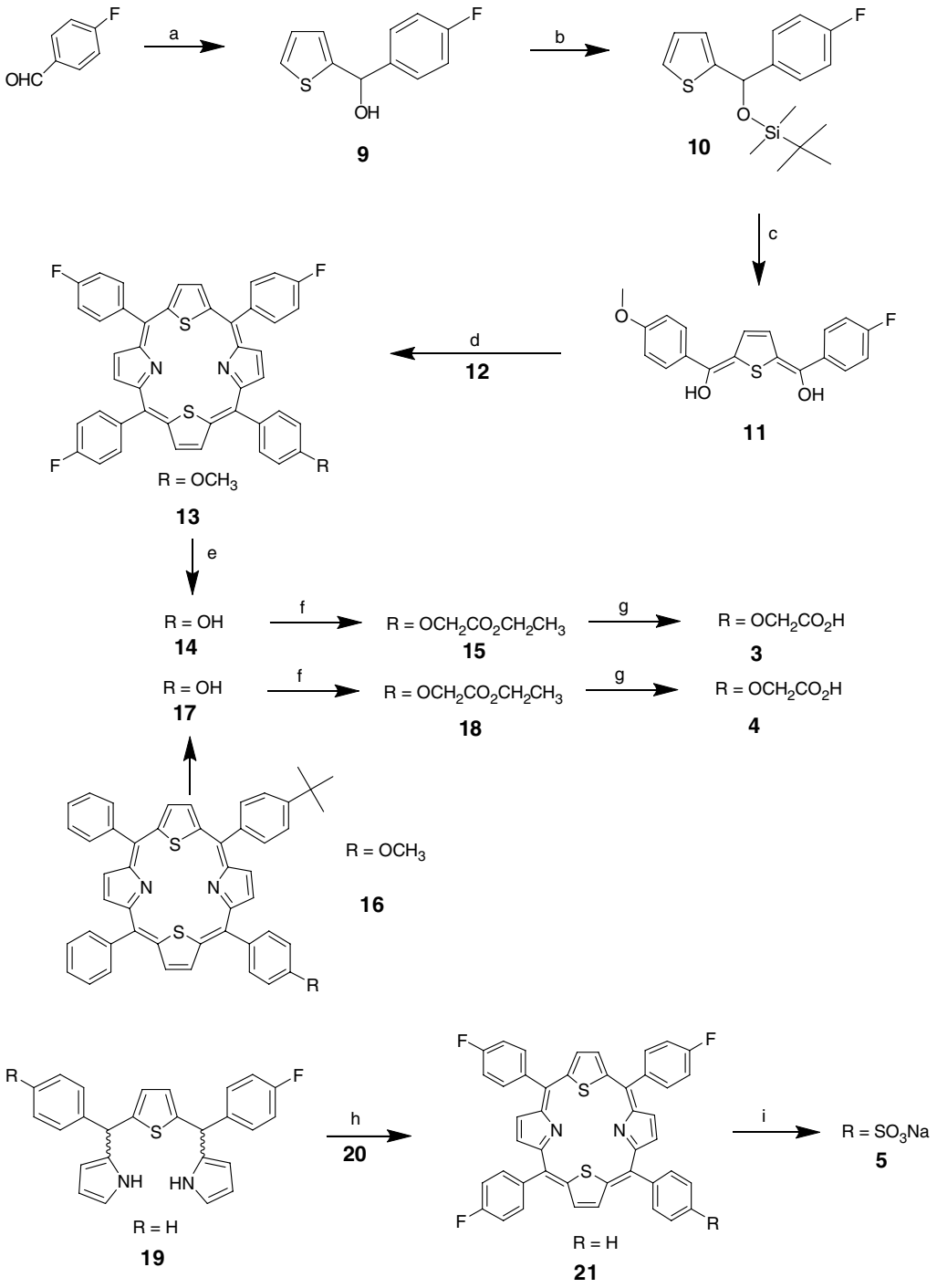
2.1.1. Synthesis of mono-functionalized core-modified porphyrins. Trifluorinated mono-carboxylic acid dithiaporphyrin, **3**, was prepared based on previous synthetic methods used for the preparation of asymmetric dithiaporphyrins.[8] Compound **9** was synthesized from thiophene and 4-fluorobenzaldehyde in 46% isolated yield. After protection of the hydroxyl group of **9** with *tert*-butyldimethylsilyl chloride (TBSCl), the dihydroxy compound **11** was prepared from **9** and *p*-anisaldehyde in 74% yield after deprotection of the TBS group with 1 M TBAF. Condensation of **11** with 2,5-bis[1-(4-fluorophenyl)-1-pyrrolomethyl]thiophene **12** afforded the dithiaporphyrin **13** in 8.5% isolated yield. Demethylation of **13** with BBr_3 gave **14** in 82% yield. The final product, **3**, was obtained after alkylation with ethyl bromoacetate to give **15** followed by saponification of the ethyl ester. Dithiaporphyrin **4** was prepared using similar chemistry to that used for the preparation of **3** starting with methoxy porphyrin **16**.[10] Compound **21** was obtained in 14% isolated yield through the condensation reaction of **19** and 2,5-bis[1-(4-fluorophenyl)-1-hydroxymethyl]-thiophene **20**. Sulfonation of the meso-phenyl group of **21** afforded **5** in 50% isolated yield (Scheme 1).

2.1.2. Synthesis of dihydroxydithiachlorins. Dihydroxydithiachlorins, **6** and **7**, were synthesized via oxidation of a pyrrole double bond with OsO_4 to give *syn*-dihydroxylation.[12] The precursor dithiaporphyrins, **22** and **23**, were prepared using previously reported chemistry.[6,8] 7,8-Dihydroxydithiaporphyrins **6** and **24** were prepared by oxidation with OsO_4 in a solvent mixture of chloroform and 1% pyridine, in 34% and 19% yields, respectively. Saponification of the esters in **24** gave dihydroxydithiaporphyrin **7** with two carboxylic acids in 75% yield (Scheme 2).

2.1.3. Synthesis of core-modified porphyrin 8 with two meso-aryl groups and two unsubstituted meso positions. Core-modified porphyrin **8** with two unsubstituted meso-positions was prepared in several steps from dimethoxy thiophene **25**.[13] Initial condensation of **25** with benzaldehyde and pyrrole in the presence of *p*-toluenesulfonic acid and TCBQ gave **27** in 6% isolated yield. Demethylation of **27** with BBr_3, alkylation of the resulting diol **28** with ethyl bromoacetate, and saponification of the two ester groups of **29** provided carboxylic acid **8** (Scheme 3).

2.2. Photophysical properties

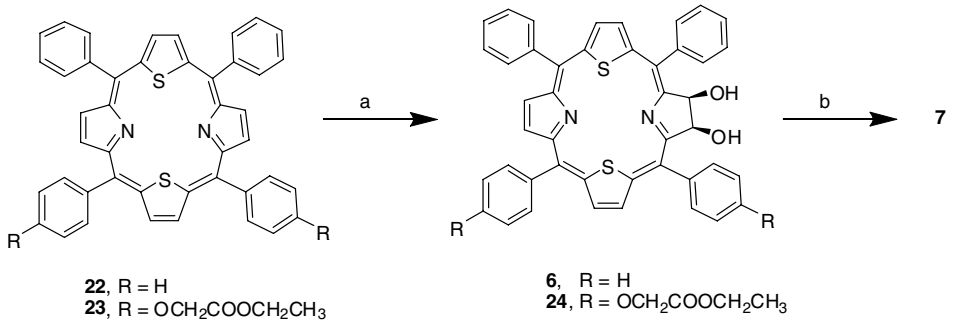
2.2.1. Absorption spectra. Mono-functionalized dithiaporphyrins **2**–**5** absorb light at wavelengths similar to those reported for other dithiaporphyrins[6–11] with band I maxima near 700 nm. Dihydroxydithiachlorins **6** and **7** have band I maxima at 698 nm consistent with reported data for other dihydroxydithiachlorins (Table 1).[12] In contrast, thiaporphyrin **8** with two unsubstituted meso positions has a band I absorption maximum at 665 nm. The shorter wavelength is presumably due to



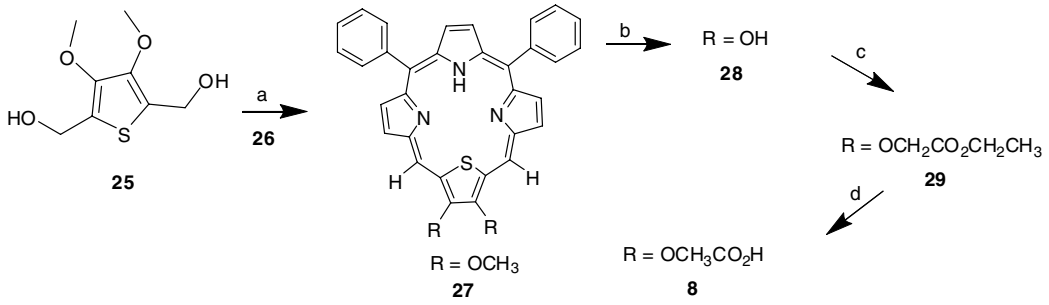
Scheme 1. Reagents: (a) i—1 equiv *n*-BuLi, ii—1 equiv thiophene; (b) TBSCl, DMAP, Et_3N; (c) i—1 equiv *n*-BuLi, ii—1 equiv 4-methoxybenzaldehyde, iii—aqueous HCl; (d) **12**, TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (e) BBr_3, CH_2Cl_2; (f) $BrCH_2CO_2Et$, K_2CO_3, acetone; (g) NaOH, aqueous THF; (h) **20**, TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (i) concd H_2SO_4.

the incorporation of only one sulfur atom in the core and the lack of two meso-aryl groups.[14]

2.2.2. Generation of singlet oxygen. To estimate the relative rates of singlet oxygen in core-modified porphyrins **1**–**8**, we measured the decrease in 1,3-diphenylisobenzofuran (DPBF) absorbance as DPBF reacted with singlet oxygen generated by irradiation of the core-modified porphyrin. A tetrahydrofuran (THF) solution of DPBF (90 μM) and core-modified porphyrin (1 μM) was irradiated with 3 mW/cm^2 of the water-filtered halogen source (400–850 nm) for 10 min (1.8 J/cm^2).[15] THF was used as a solvent to avoid potential problems from aggregation. The percentages of oxidized DPBF by each porphyrin are summarized in Table 2. Consistent with our previous studies,[5,8,9] dithiaporphyrins **1**–**5** were excellent singlet oxygen generators. Interestingly, dihydroxydithiachlorins **6** and **7** generated



Scheme 2. Reagents: (a) OsO_4, $CHCl_3$/pyridine (100:1), H_2S, NaOH; (b) NaOH, aqueous THF.



Scheme 3. Reagents: (a) 2,5-bis(2-phenyl-1-hydroxymethyl)thiophenepyrrole (**26**), TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (b) BBr_3, CH_2Cl_2; (c) $BrCH_2CO_2Et$; (d) NaOH, aqueous THF.

Table 1. UV–vis–near-IR band maxima and molar absorptivities for core-modified porphyrins in tetrahydrofuran[a]

Compound	Soret band	Band IV	Band III	Band II	Band I
1	438 (119)	516 (20.8)	547 (11.1)	633 (2.2)	698 (6.0)
2	438 (205)	516 (25.2)	547 (9.8)	633 (2.2)	698 (6.1)
3	442 (185)	520 (24.8)	548 (10.7)	635 (2.6)	699 (6.1)
4	439 (200)	513 (26.3)	548 (10.3)	634 (2.1)	698 (5.8)
5[b]	436 (132)	512 (20.3)	544 (5.6)	631 (1.5)	694 (3.2)
6	438 (154)	516 (22.8)	547 (3.3)	633 (10.9)	698 (4.9)
7	438 (108)	516 (18.7)	547 (15.0)	633 (3.6)	698 (8.6)
8	428 (70.9)	505 (21.9)	561 (3.0)	604 (5.2)	665 (2.9)

[a] λ_{max} nm ($\varepsilon \times 10^3$ M^{-1} cm^{-1}).
[b] In MeOH.

Table 2. Percentiles of oxidized DPBF by the irradiation with porphyrins and *n*-octanol/water partition coefficients in pH 7.4 phosphate buffer

Compound	Oxidized DPBF (%)	$\log D_{7.4}$
1	68	0.1
2	78	1.6
3	75	1.8
4	75	>2
5	64	0.8
6	78	>2
7	62	−0.5
8	48	0.8

singlet oxygen, 78% and 62% oxidation, respectively, as effectively as dithiaporphyrins **1**–**5**. The mono-thiaporphyrin **8**, with two unsubstituted meso-positions, was less efficient than the others for the generation of singlet oxygen with only 48% oxidized DPBF.

2.2.3. *n*-Octanol/pH 7.4 buffer partition coefficients. The lipophilicity of a molecule is important not only in cellular uptake, but also in the determination of water solubility, which is closely related to the tendency to form aggregates. This is especially true for porphyrins. Partition coefficients of porphyrins **1**–**8** were determined as $\log D_{7.4}$ using *n*-octanol and pH 7.4 phosphate buffer. Experimental $\log D_{7.4}$ values reflected the effects of substituents at meso-aryl groups except **8**. Monocarboxylic acids **2**–**4** and monosulfonated porphyrin **5** had higher values of $\log D_{7.4}$ than dicarboxylic acid porphyrin, **1**. Sulfonated porphyrin **5** had a lower value of $\log D_{7.4}$ due to the higher acidity of the sulfonic acid residue relative to the carboxylic acid. Monocarboxylic acid porphyrin **4** with a *tert*-butyl group showed a higher value of $\log D_{7.4}$ than **2**. The hydrophilic effect of the two alcohol substituents of **6** was much smaller than the effect of the two carboxylic acid substituents of **1** ($\log D_{7.4}$ of >2 vs $\log D_{7.4}$ of 0.1). However, the addition of two hydro-

xyl groups in **1** to give dithiachlorin **7** significantly lowered $\log D_{7.4}$ from 0.1 for **1** to −0.5 for **7**. Interestingly, $\log D_{7.4}$ of **8** was higher than that of **1** (0.8 vs 0.1) even though both have two carboxylic acid residue and **8** lacks two aromatic meso substituents.

2.3. Biology

2.3.1. Dark and phototoxicity of core-modified porphyrins toward cultured R3230AC cells. We tested the photodynamic activity of the core-modified porphyrins against R3230AC cells. The porphyrins were added to the cells 24 h before the irradiation with water-filtered halogen light source (3 mW/cm^2 for 1 h, 10.8 J/cm^2). Cell viability was determined by MTT colorimetric assay.[16] The Hill (sigmoid Emax) equation was used to fit the data (Fig. 2) and to calculate values of IC_{50}. Values of IC_{50} ranged from 0.13 to 2.6 μM: a 20-fold difference between the best and the worst. Dithiachlorin derivative **7** and thiaporphyrin **8** with two unsubstituted meso positions showed much lower potency than the others with values of $IC_{50} > 2$ μM. Among the mono-functionalized porphyrins, **4** and **5** were less phototoxic than **2** and **3**. Interestingly, dithiachlorin **6** was more phototoxic than derivatives **2**–**5**, **7**, and **8**, and was similar to **1** in phototoxicity. In dark and light controls, no dark toxicity was observed in R3230AC cells at porphyrin concentrations up to 10 μM and no toxicity was observed in R3230AC cells treated with 10.8 J/cm^2 of water-filtered halogen light (data not shown).

2.3.2. Intracellular accumulation of core-modified porphyrins in R3230AC cells. The cellular concentration of the various porphyrins was determined by fluorescence and is expressed in fmole per cell as shown in Figure 3. Cells were treated with 10 μM core-modified porphyrin for 24 h. With the exception of the intracellular concentration of **6**, concentrations of all porphyrins were less than 1 fmole per cell (0.31–0.59 fmole/cell). In particular, the uptake of **7** and **8** was much less than the others, 0.11 and 0.16 fmole/cell, respectively. On the other hand, **6** showed much higher uptake, 4.5 fmole/cell. The uptake of the porphyrins in cells seems to correlate with phototoxicity. The potent activity of **6** is accompanied by the highest uptake (4.5 fmole/cell) among core-modified porphyrins **1**–**8**. The lower uptake of **7** and **8** also correlates with the low phototoxicity of these two compounds. The relatively low lipophilicity of **7** ($\log D_{7.4}$, −0.5) might make passage through the lipid bilayer of cell membrane difficult. This observation is consistent with our previous results where core-modified porphyrins with three or four carboxylic acids showed much lower uptake.[8] The lower efficiency of singlet oxygen generation in **8** also contributes to the lower potency of **8** (Table 2). The sulfonated porphyrin **5** showed lower potency than the carboxylated porphyrins, although the uptake (0.30 fmole/cell) was close to the uptake observed with **2** (0.31 fmole/cell) and **3** (0.33 fmole/cell) and singlet oxygen was generated nearly as effectively (64%) as the others (75–78%).

Extraordinarily high uptake of **6** (4.5 fmol/cell) was unexpected because it was highly lipophilic and formed aggregates in the media. Compound **4** also showed high lipophilicity and formed aggregates well, but uptake was much lower (0.46 fmol/cell). Compound **6** might cross cell membrane more readily due to two reasons: (1) it is smaller in size and (2) it does not have flexible hydrophilic functional group, which could interfere the porphyrin's crossing through plasma membrane as both a monomer and an aggregate. However, we do not exclude the possibility that compound **6** forms different

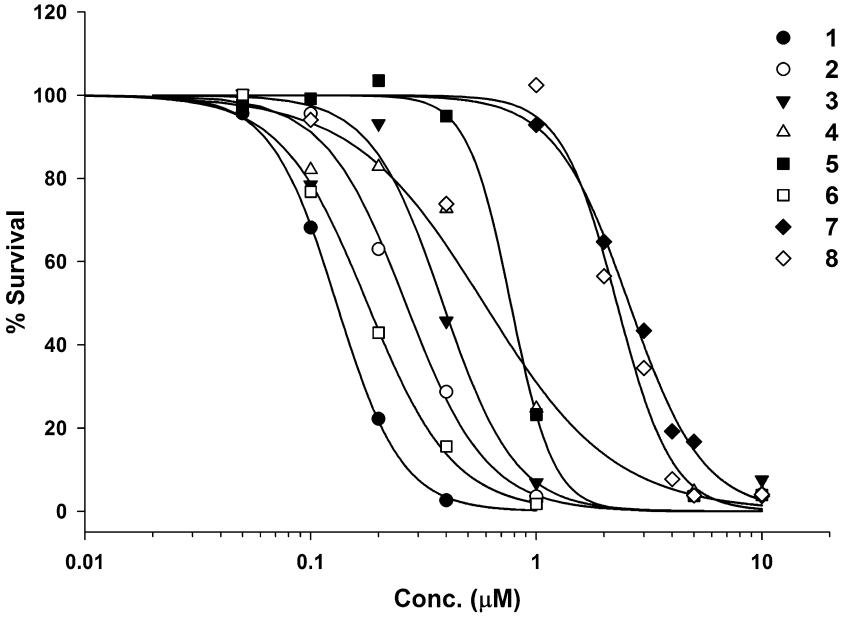


Figure 2. Cell viability of cultured R3230AC cells after photosensitization in the presence of thiaporphyrins **1**–**8**. Each data point represents the mean of at least 3 separate experiments performed in duplicate and error bars are the SEM. Data are expressed as the surviving fraction of viable cells relative to untreated controls. IC_{50} (μM): **1** (0.13), **2** (0.26), **3** (0.38), **4** (0.59), **5** (0.77), **6** (0.18), **7** (2.57), and **8** (2.28).

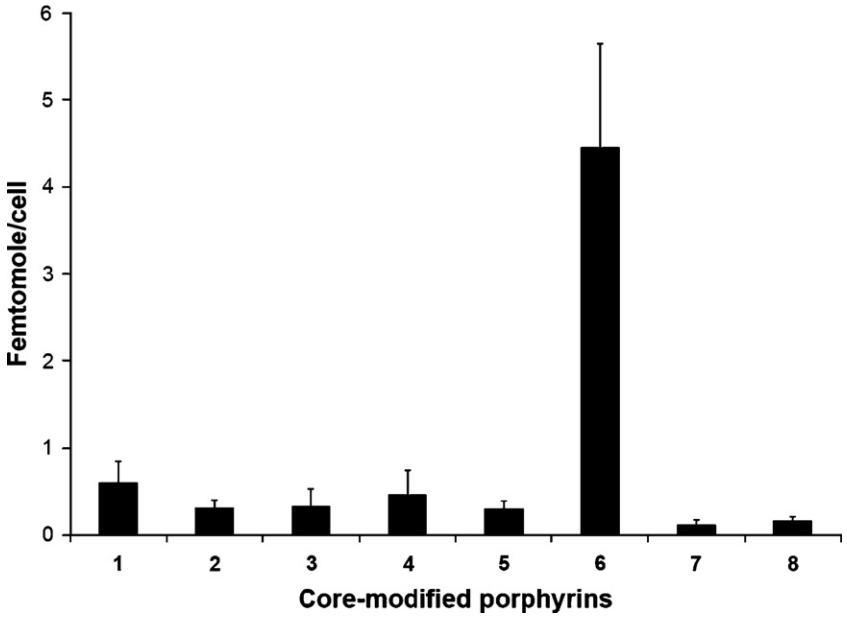


Figure 3. Cellular uptake of thiaporphyrins **1**–**8** in cultured R3230AC cells. Each bar represents the mean intracellular uptake of each compound incubated with R3230AC cells for 24 h at 1×10^{-5} M. Data are expressed as femtomole porphyrin/cell and error bars are the SEM.

type of aggregates due to the absence of the flexible hydrophilic chain.

2.3.3. Aggregates of core-modified porphyrins in media. The extent of core-modified porphyrin aggregation in growth medium was indirectly determined by a comparison of their fluorescence yield in medium relative to their fluorescence observed in DMSO (Fig. 4). The loss of fluorescence emission upon aggregation of porphyrins has been reported for both anionic and cationic porphyrins.[17,18] The decrease in fluorescence of lipophilic porphyrins **2**–**6** in medium compared to that in DMSO was quite apparent as shown in Figure 4. Fluorescence from the more hydrophilic porphyrins **1**, **7**, and **8** showed little difference between growth medium and DMSO. These data indicate that the lipophilic porphyrins are more prone to form aggregates than hydrophilic porphyrins in medium.

The tendency to aggregate seemed the most striking factor responsible for the lower efficiency of highly lipophilic porphyrins, for example, **4** and **6**. Although the intracellular uptake of **4** (0.46 fmole/cell) was close to that of **1** (0.59 fmole/cell), **1** was about 4.5 times more potent than **4** (IC_{50}, 0.13 μM vs 0.59 μM). The IC_{50} of **6** was slightly higher than that of **1** (0.18 vs 0.13 μM), but the cellular concentration of **6** was 7.6 times higher than that of **1** (4.5 vs 0.59 fmole/cell). On a 'molecule per cell' basis, both **4** and **6** were much less efficient as photosensitizers than **1**. In general, when dyes form aggregates, quantum yields for photophysical processes such as fluorescence emission and singlet oxygen generation are decreased. We suggest that the high tendency of **6** and **4** to form aggregates in media may be mirrored in the cytoplasm causing the lowered phototoxicity of the lipophilic photosensitizers.

2.3.4. Sub-cellular localization of core-modified porphyrin in R3230AC cells. The site of intracellular localization of the photosensitizer has been suggested to be one of the main factors in determining the efficiency of photodynamic activity.[19] This statement is supported by two observations: (1) the site of localization of the photosensitizers will be the site of cellular damage since the diffusion distance of singlet oxygen is no further than 20 nm in a biological system[20] and (2) the sensitivity of organelles to damage by singlet oxygen can be quite different. Photodynamic damage to mitochondria can lead to the induction of apoptosis,[21–23] which has made mitochondria the premier target of photosensitizers.[24,25]

In order to discern the subcellular localization of the core-modified porphyrins, R3230AC cells were treated with core-modified porphyrins **1**, **4**, or **6**, or with rhodamine-123 (**Rh-123**), a standard mitochondrial staining dye (Fig. 5).[26] R3230AC cells were incubated with 20 μM of the core-modified porphyrin for 24 h or with 13 μM **Rh-123** for 30 min and were then washed three times with media to remove residual porphyrin or **Rh-123**. The core-modified porphyrins did not provide the fine, granular fluorescence associated with mitochondrial specificity as shown with **Rh-123** in Figure 5. While all three core-modified porphyrins gave similar fluorescence images, some slight differences were observed. Staining with **4** looked more specific without more diffuse staining area. The core-modified porphyrins did not stain nuclei and, while mitochondria may be stained, the core-modified porphyrins were not specific for mitochondria. These images did not provide conclusive information to explain the differences of the efficiency between hydrophilic core-modified porphyrin, **1**, and lipophilic porphyrins, **4**, **6**.

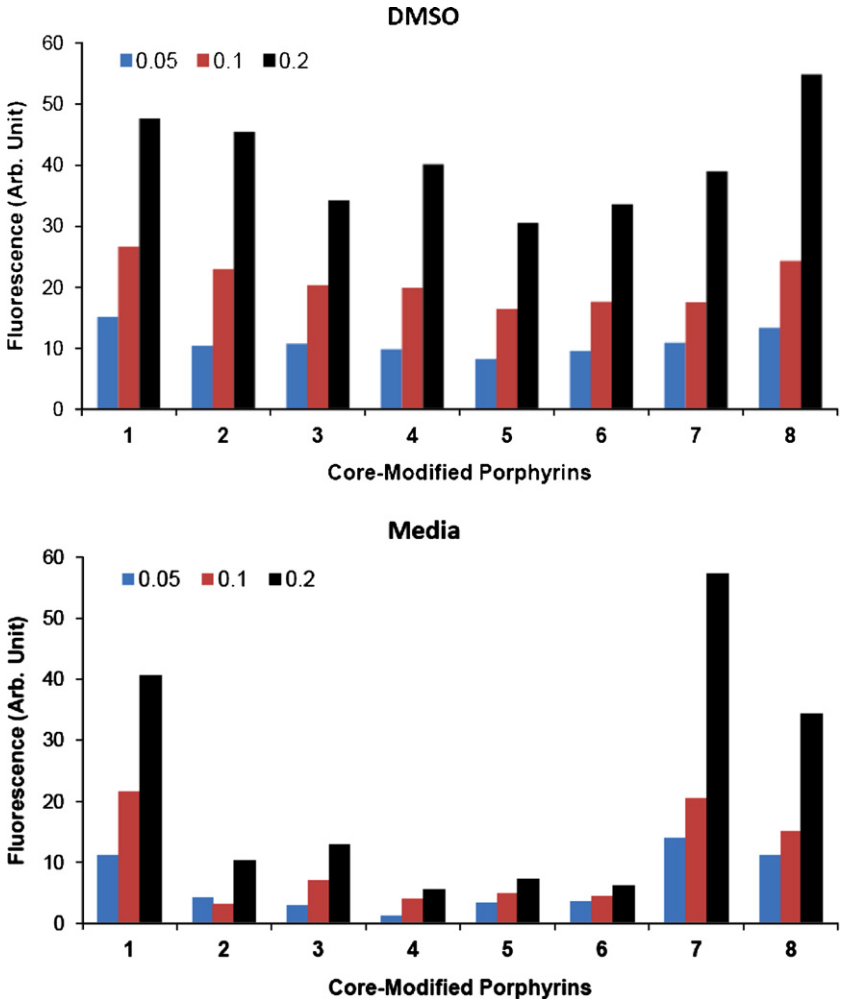


Figure 4. Fluorescence emission from core-modified porphyrins **1**–**8** in DMSO (upper) and in aqueous media (lower) at three different concentrations: 0.05, 0.1, and 0.2 μM.

3. Summary and conclusions

This study was designed to observe structure–activity relationships of core-modified porphyrins with physicochemical and biological properties as a continuation of our previous studies.[5–9] Mono-functionalized dithiaporphyrins **2**–**5**, dihydroxydithiachlorins **6** and **7**, and mono-thiaporphyrin **8** with two unsubstituted meso-positions were prepared and compared with dithiaporphyrin **1**. Compounds **2**–**8** were prepared based on our previous methods with slight modification. In the preparation of dihydroxydithiachlorins **6** and **7**, OsO_4 was used.[12] Mono-thiaporphyrin **8** was prepared as a surrogate for dithiaporphyrin with only two meso aryl groups.

The photophysical properties were similar within the series of dithiaporphyrins **1**–**7**. The band I absorption maxima of **1**–**7** were between 694 and 698 nm (665 nm for thiaporphyrin **8**). All of the dithiaporphyrins generated singlet oxygen quite effectively and oxidized 62–78% of DPBF (48% for thiaporphyrin **8**). The differences observed with **8** might be due to the core system and/or the absence of two meso aryl groups. The unusual absorption spectrum of dihydroxydithiachlorin **6** relative to tetranitrogenic chlorins was reported.[12] In the tetranitrogenic porphyrins, chlorins usually have longer-wavelength absorption maxima than the corresponding porphyrins. However, chlorins of dithiaporphyrins do not show a similar bathochromic shift. Interestingly, dihydroxydithiachlorins **6** and **7** generated singlet oxygen as effectively as dithiaporphyrin **1**. Lipophilicity was affected mainly by the functional groups.

Although none of compounds **2**–**8** was more potent than **1**, we were able to observe relations between structure and activity. The phototoxicity of the core-modified porphyrins was dependent on multiple factors such as intracellular uptake, lipophilicity, the tendency to form aggregates, and singlet-oxygen quantum yield. The higher potency of **6** is attributed to extraordinarily high uptake compared to the other core-modified porphyrin derivatives. The lower efficiency of highly lipophilic porphyrins, for example, **4** and **6**, might be, in part, due to the formation of inactive aggregates in the cytoplasm. Water-soluble derivative **7** had the lowest uptake in the series and poor phototoxicity. On the other hand, the poor phototoxicity of **8** might be due to the com-

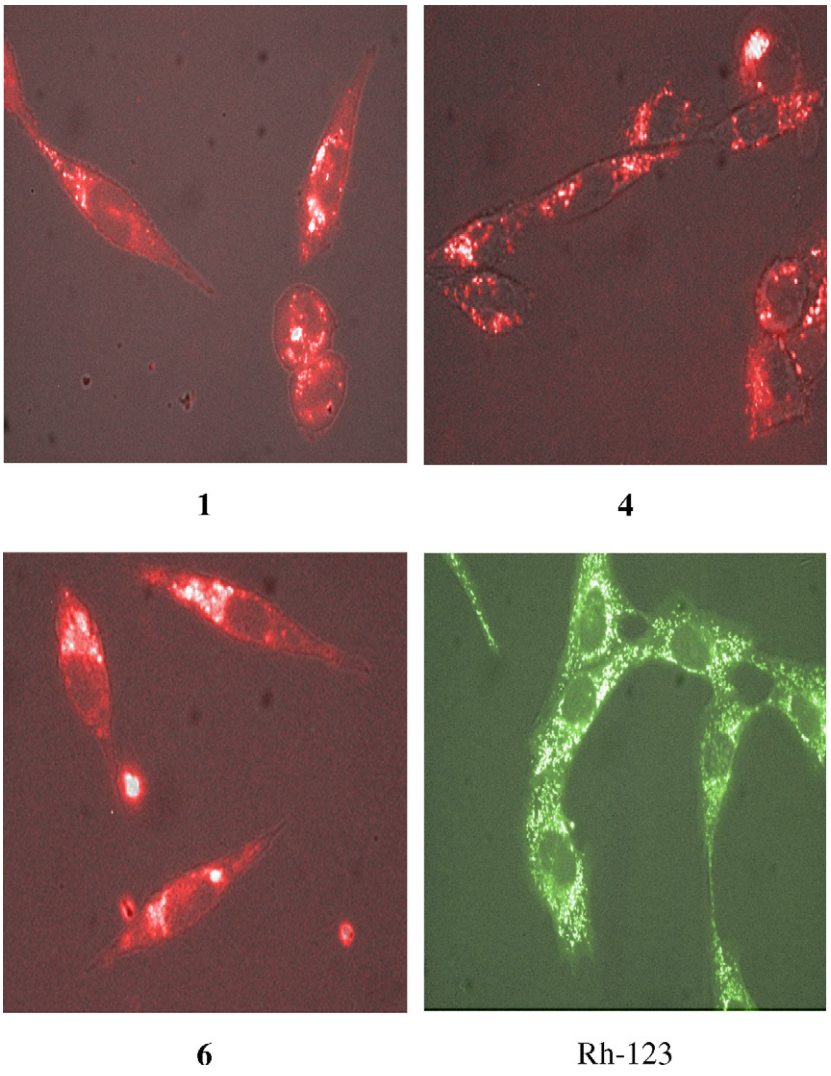


Figure 5. Fluorescence images of R3230AC cells treated with 20 μM of **1**, **4**, **6**, and 13 μM of Rh-123. Cell culture conditions and experimental details are described in Section 4.

bined effects of lower uptake and less effective generation of singlet oxygen.

4. Experimental

4.1. General methods

Solvents and reagents were used as received from PHARMCO-AAPER, Sigma-Aldrich Chemical Co. or Thermo Fisher Scientific, Inc. unless otherwise noted. Chemicals for tissue culture were purchased from Sigma-Aldrich Chemical Co. or Thermo Fisher Scientific, Inc. NMR spectra were recorded at 23 °C on a Varian Gemini-300, Inova 400 (Bruker AVANCE 400), or Inova 500 instrument with residual solvent signal as the internal standard: $CDCl_3$ (δ 7.26 for proton, δ 77.16 for carbon). UV–visible near-IR spectra were recorded on a Perkin-Elmer Lambda 12 or CHEM4-UV-FIBER spectrophotometer (Ocean Optics Inc.). Elemental analyses were conducted by Atlantic Microlabs, Inc. Q-TOF 2 electrospray and ESI mass spectrometry were conducted by the Campus Chemical Instrumentation Center of the Ohio State University (Columbus, OH), the Instrument Center of the Department of Chemistry at the University at Buffalo, or the Campus Mass Spectrometry Facility of at South Dakota State University.

4.2. Synthesis

Compounds **1**, **2**, **12**, **16**, **20**, **22**, **23**, and **26** were prepared as previously described in our earlier works.[5–8,10]

4.2.1. Synthesis of 2-[1-(4-fluorophenyl)-1-hydroxymethyl]-thiophene (9). Thiophene (12.0 mL, 150 mmol) was added to a solution of *n*-butyllithium (93.8 mL of a 1.6 M solution in hexanes, 150 mmol) and TMEDA (22.7 mL, 150 mmol) in 400 mL of hexanes under an Ar atmosphere. The reaction mixture was heated at reflux for 1 h, cooled to ambient temperature to make 2-lithiophene. This 2-lithiothiophene suspension was transferred via a cannula to a solution of 4-fluorobenzaldehyde (15.0 mL, 143 mmol) in 400 mL of anhydrous THF cooled to 0 °C, which had been degassed with Ar

for 15 min. After the addition was complete, the mixture was warmed to ambient temperature, 500 mL of aqueous 1 M NH_4Cl was added, and the organic phase was separated. The aqueous phase was extracted with ether (3×400 mL). The combined organic extracts were washed with water (3×400 mL) and brine (400 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel column chromatography with the mixture of EtOAc and hexanes giving 13 g (46%) of **9** as a light yellow oil. The structure was confirmed by the comparison with the reference data.[27]

4.2.2. 2-[1-(4-Fluorophenyl)-1-(*tert*-butyldimethylsilyloxy)-methyl]thiophene (10). Compound **10** was prepared following literature procedures.[8] Briefly, a solution of compound 9, TBSCl (28.0 g, 186 mmol), DMAP (7.5 g, 61 mmol), and Et_3N (11.0 mL, 188 mmol) in 300 mL of dry CH_2Cl_2 was stirred at 0 °C for 2 h and was then warmed to ambient temperature for 24 h. The reaction mixture was partitioned between 400 mL of ether and 400 mL of saturated aqueous $NaHCO_3$. The organic layer was washed with water (3×400 mL) and brine (400 mL), dried over $MgSO_4$, and concentrated to give a yellow oil. Chromatography on SiO_2 eluted with 25% EtOAc/hexanes gave 14.7 g (74%) of **10** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (3H, s), 0.11 (3H, s), 0.99 (9H, s), 6.01 (1H, s), 7.25 (2H, t, $J = 8.7$ Hz), 7.44–7.65 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.89, −4.70, 18.42, 25.91, 72.72, 115.10, 115.38, 123.75, 124.86, 126.52, 127.91, 128.02, 140.42, 140.46, 150.33, 160.65, 163.90; HR Q-TOF MS: *m*/*z* 345.1105 (calcd for $C_{17}H_{23}FOSSi + Na$, 345.1121).

4.2.3. 2-[1-(4-Fluorophenyl)-1-hydroxymethyl]-5-[1(4-methoxyphenyl)-1- hydroxymethyl]thiophene (11). Compound **10** (7.0 g, 22 mmol) was added to a solution of *n*-butyllithium (14.9 mL, 1.6 M in hexanes, 24 mmol) and TMEDA (3.6 mL, 24 mmol) in 50 mL of hexanes under an Ar atmosphere. The reaction mixture was stirred at ambient temperature for 30 min. The suspension of lithio-**10** was transferred dropwise via cannula to a 0 °C solution of 4-methoxybenzaldehyde (2.5 mL, 21 mmol) in anhydrous THF (50 mL), which had been degassed with Ar for 15 min. After addition was complete, the mixture was allowed to warm to ambient temperature, 100 mL of a 1 M solution of NH_4Cl was added, and the organic phase was separated. The aqueous phase was extracted with ether (3×150 mL). The combined organic extracts were washed with water (3×150 mL) and brine (150 mL), dried over $MgSO_4$, and concentrated to give a yellow oil. The oil was dissolved in a 1 M solution of Bu_4NF in THF (50 mL, 50 mmol) and stirred at ambient temperature for 1 h at which point 50 mL of saturated aqueous NH_4Cl was added. The resulting mixture was extracted with ether (4×70 mL). The combined organic extracts were washed with water (3×150 mL) and brine (150 mL), dried over $MgSO_4$, and concentrated. The crude diol was purified by column chromatography on SiO_2 eluted with 25% EtOAc/hexanes to give 5.5 g (74%) of **11** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (3H, s), 5.78 (1H, m), 5.85 (1H, m), 6.02 (1H, d, $J = 3.4$ Hz), 6.17 (1H, d, $J = 3.4$ Hz), 6.65 (2H, m), 6.88 (2H, d, $J = 6.5$ Hz), 7.15 (2H, t, $J = 6.9$ Hz), 7.29 (2H, m), 7.42 (2H, t, $J = 5.2$ Hz); HR Q-TOF MS: *m*/*z* 344.0882 (calcd for $C_{19}H_{17}FO_3S$, 344.0885).

4.2.4. 5,15,20-Tri(4-fluorophenyl)-10-(4-methoxyphenyl)-21,23-dithiaporphyrin (13). Diol **11** (4.0 g, 12 mmol), 2,5-bis[1-(4-fluorophenyl)-1-pyrrolomethyl]thiophene (**12**, 5.0 g, 12 mmol), and 2,3,5,6-tetrachloro-1,4-benzoquinone (TCBQ, 8.6 g, 35 mmol) were dissolved in 500 mL CH_2Cl_2. *p*-Toluenesulfonic acid monohydrate (2.2 g, 12 mmol) was added and the reaction mixture was stirred for 0.5 h in the dark. The reaction mixture was concentrated and the residue was redissolved in minimal CH_2Cl_2. The crude product was purified via chromatography on basic alumina eluted with CH_2Cl_2. A red band containing dithiaporphyrin **13** was isolated. The crude product was washed with acetone to give 0.72 g (9%) of **13** as a purple solid. Mp: >300 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.13 (3H, s), 7.40 (2H, d, $J = 8.6$ Hz), 7.54 (6H, t, $J = 8.6$ Hz), 8.22 (8H, m), 8.68 (3H, m), 8.75 (1H, d, $J = 4.5$ Hz), 9.68 (3H, m) 9.77 (1H, d, $J = 5.0$ Hz); HR ESI MS: *m*/*z* 733.1602 (calcd for $C_{45}H_{27}F_3N_2OS_2 + H$, 733.1595).

4.2.5. 5,15,20-Tri(4-fluorophenyl)-10-(4-hydroxyphenyl)-21,23-dithiaporphyrin (14). Dithiaporphyrin **13** (0.47 g, 0.64 mmol) was dissolved in 50 mL of CH_2Cl_2 and BBr_3 (0.2 mL, 1.9 mmol) was added at 0 °C. The resulting solution was stirred overnight at ambient temperature. The reaction mixture was added to 150 mL of EtOAc and 150 mL of saturated $NaHCO_3$. The organic layer was separated and washed three times with 150 mL of brine, dried over $MgSO_4$, and concentrated. The crude solid was washed with 25% EtOAc/hexanes several times to give 0.38 g (82%) of **14** as a dark blue solid. Mp: >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.45 (2H, d, $J = 8.5$ Hz), 6.89 (6H, m), 7.23 (2H, d, $J = 8.4$ Hz), 7.45 (6H, m), 7.78 (3H, m), 7.86 (1H, d, $J = 4.5$ Hz), 8.87 (3H, m), 8.97 (1H, d, $J = 5.1$ Hz), 9.26 (1H, s); HR Q-TOF MS: *m*/*z* 719.1433 (calcd for $C_{44}H_{25}F_3N_2OS_2 + H$, 719.1442).

4.2.6. 5,15,20-Tri(4-fluorophenyl)-10-[4-(ethoxycarbonylmethyleneoxy)-phenyl]-21,23-dithiaporphyrin (15). Dithiaporphyrin **14** (0.33 g, 0.46 mmol), K_2CO_3 (0.63 g, 4.6 mmol), and ethyl bromoacetate (0.5 mL, 4.6 mmol) in 200 mL acetone were heated at reflux for 10 h. The reaction mixture was cooled to ambient temperature and the K_2CO_3 was removed by filtration. The filter cake was washed with acetone until the filtrate became colorless. The combined filtrates were concentrated. The crude product was washed with MeOH to give 0.32 g (87%) of **15** as a purple solid. Mp: 168–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (3H, t, $J = 7.1$ Hz), 4.44 (2H, q, $J = 7.1$ Hz), 4.94 (2H, s), 7.40 (2H, d, $J = 8.6$ Hz), 7.54 (6H, t, $J = 8.6$ Hz), 8.22 (8H, m), 8.68 (3H, m), 8.73 (1H, d, $J = 4.5$ Hz), 9.67 (3H, m), 9.74 (1H, d, $J = 5.1$ Hz); HR Q-TOF MS: *m*/*z* 805.1801 (calcd for $C_{48}H_{31}F_3N_2O_3S_2 + H$, 805.1812).

4.2.7. 5,15,20-Tri(4-fluorophenyl)-10-[4-(carboxymethyleneoxy)-phenyl]-21,23-dithiaporphyrin (3). Core-modified porphyrin **15** (0.30 g, 0.37 mmol) was dissolved in

40 mL THF and 40 mL of 1 M aqueous NaOH was added. The resulting solution was stirred at ambient temperature for 15 h. The solution was acidified by the addition of 4.1 mL of 10 N HCl. The reaction mixture was diluted with 100 mL H_2O and the products were extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated. The crude product was washed with several portions of hexanes/MeOH to give 0.19 g (66%) of **3** as a purple solid. Mp: >300 °C: ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.15 (2H, s), 6.59 (2H, d, J = 8.6 Hz), 6.88 (6H, t, J = 8.7 Hz), 7.34 (2H, d, J = 8.6 Hz), 7.44 (6H, m), 7.78 (3H, m), 7.82 (1H, d, J = 4.5 Hz), 8.88 (3H, m), 8.93 (1H, d, J = 5.1 Hz); HR Q-TOF MS: *m*/*z* 777.1509 (calcd for $C_{46}H_{27}F_3N_2O_3S_2$ + H, 777.1488).

4.2.8. 5-(4-*tert*-Butylphenyl)-10-(4-methoxyphenyl)-15,20-bis-phenyl-21,23-dithiaporphyrin (16). Dithiaporphyrin **16** was prepared as previously described.[10]

4.2.9. 5-(4-*tert*-Butylphenyl)-10-(4-hydroxyphenyl)-15,20-diphenyl-21,23-dithiaporphyrin (17). Dithiaporphyrin **16** (0.16 g, 0.21 mmol) was treated with BBr_3 (0.25 mL, 2.7 mmol) as described for the preparation of **14** to give 0.14 g (92%) of **17** as a metallic purple solid. Mp: >300 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.71 (4H, dd, J = 4.9, 8.8 Hz), 8.71 (4H, dd, J = 4.3, 6.1 Hz), 8.25 (4H, dd, J = 1.6 Hz, 5.5 Hz), 8.19 (2H, d, J = 7.9 Hz), 8.11 (2H, d, J = 8.2 Hz), 7.82 (8H, dd, J = 7.9 Hz, 8.2 Hz), 7.23 (2H, d, J = 8.5 Hz), 5.09 (1H, br s), 1.62 (9H, s); ^{13}C NMR (300 MHz, $CDCl_3$): δ 157.2, 156.6, 151.5, 148.5, 148.3, 141.8, 138.7, 136.1, 135.8, 135.2, 134.9, 134.9, 134.8, 134.7, 134.7, 134.4, 134.3, 128.5, 127.9, 125.0, 115.0, 35.5, 32.2; HR ESI MS: *m*/*z* 721.2693 (calcd for C48H36ON2S2 + H, 721.2710).

4.2.10. 5-(4-*tert*-Butylphenyl)-10-[4-(ethoxycarbonylmethyleneoxy)-phenyl]-15,20-bis-phenyl-21,23-dithiaporphyrin (18). Dithiaporphyrin **17** (0.14 g, 0.19 mmol) was reacted with ethyl bromoacetate (0.67 mL, 6.0 mmol) and K_2CO_3 (0.82 g, 5.8 mmol) in 50 mL of acetone as described for the preparation of **15** to give 0.15 g (95%) of **18** as a dark purple solid. Mp: 185–187 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.70 (4H, dd, J = 4.9, 8.8 Hz), 8.70 (4H, dd, J = 4.0, 6.1 Hz), 8.22 (8H, dd, J = 5.8, 8.2 Hz), 7.82 (8H, dd, J = 7.3, 8.8 Hz), 7.36 (2H, d, J = 7.3 Hz), 4.92 (2H, s), 4.42 (2H, q, J = 7.0 Hz), 1.61 (9H, s), 1.42 (3H, t, J = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.5, 158.6, 157.2, 156.9, 151.5, 148.4, 148.4, 141.9, 138.8, 136.2, 135.9, 135.3, 135.0, 134.9, 134.8, 134.5, 134.4, 134.1, 134.0, 128.5, 128.0, 125.0, 114.3, 66.3, 62.1, 35.5, 32.2, 14.8; HR ESI MS: *m*/*z* 807.2693 (calcd for $C_{52}H_{43}O_3N_2S_2$ + H, 807.2710).

4.2.11. 5-(4-*tert*-Butylphenyl)-10-[4-(carboxymethyleneoxy)-phenyl]-15,20-bis-phenyl-21,23-dithiaporphyrin (4). Dithiaporphyrin **18** (0.15 g, 0.18 mmol) was hydrolyzed by 1 M NaOH (16 mL) as described for the preparation of **3** to give 0.095 g (68%) of **4** as a purple solid; mp: >300 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.69 (4H, dd, J = 4.9, 8.8 Hz), 8.67 (4H, dd, J = 4.3, 7.9 Hz), 8.17 (8H, m), 7.81 (8H, dd, J = 5.2, 8.2 Hz), 7.35 (2H, dd, J = 7.6 Hz), 4.94 (2H, s), 1.62 (9H, s); ^{13}C NMR (300 MHz, $CDCl_3$): δ 158.2, 157.1, 156.8, 151.5, 148.5, 148.3, 141.7, 138.7, 136.1, 135.9, 135.5, 135.2, 134.9, 134.9, 134.7, 134.5, 134.4, 133.7, 128.6, 127.9, 125.0, 114.3, 65.7, 35.5, 32.2; HR ESI MS: *m*/*z* 779.2421 (calcd for $C_{50}H_{39}O_3N_2S_2$ + H, 779.2397).

4.2.12. 2-[1-(4-Fluorophenyl)-1-pyrrolomethyl]-5-[1-phenyl-1-pyrrolomethyl]-thiophene (19). 2-[1-(4-Fluorophenyl)-1-hydroxymethyl]-5-[1-phenyl-1-hydroxylmethyl]-thiophene[5] (6.0 g, 18 mmol) was dissolved in excess pyrrole (25 mL). Boron trifluoride etherate was added (0.40 mL, 3.6 mmol) and the resulting mixture was stirred for 1 h at ambient temperature. The reaction was stopped by the addition of CH_2Cl_2 (150 mL), followed by 40% NaOH (80 mL). The organic layer was separated, washed with water (3 × 200 mL) and brine (200 mL), dried over $MgSO_4$, and concentrated. The excess pyrrole was removed at reduced pressure at ambient temperature. The residual oil was purified via chromatography on SiO_2 eluted with 25% EtOAc/hexanes to give 5.0 g (68%) of **19** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.61 (1H, s), 5.63 (1H, s), 5.96 (1H, s), 6.00 (1H, s), 6.22 (2H, s), 6.67 (1H, s), 6.69 (1H, s), 6.76 (2H, s), 7.06 (2H, dd, J = 8.1, 7.9 Hz), 7.23–7.36 (5H, m), 7.38 (2H, d, J = 6.8 Hz), 7.95 (2H, br s); HR EI MS: *m*/*z* 412.1404 (calcd for $C_{26}H_{21}FN_2S$, 412.1404).

4.2.13. 5,15,20-Tri-(4-fluorophenyl)-10-phenyl-21,23-dithiaporphyrin (21). Cyclization of 2,5-bis[(4-fluorophenyl)-hydroxymethyl]-thiophene[7] (**20**, 2.9 g, 7.0 mmol) and **19** (2.3 g, 6.9 mmol) with $TsOH{\cdot}H_2O$ (1.3 g, 6.9 mmol) and TCBQ (5.1 g, 21 mmol) was performed as described for the preparation of **13** to give 0.67 g (14%) of **21** as a purple solid; mp: >300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.55 (6H, m), 7.85 (3H, m), 8.23 (8H, m), 8.69 (4H, m), 9.68 (4H, m); HR EI MS: *m*/*z* 702.1406 (calcd for $C_{44}H_{25}F_3N_2S_2$, 702.1407).

4.2.14. 5,15,20-Tri-(4-fluorophenyl)-10-(4-sulfonatophenyl)-21,23-dithiaporphyrin sodium salt (5). Dithiaporphyrin **21** (0.30 g, 0.43 mmol) was dissolved in excess concentrated H_2SO_4 (40 mL) and allowed to stir at 100 °C overnight. The acid was slowly neutralized with concentrated NaOH until the solution was slightly basic. An equal volume of MeOH was added, and the solid Na_2SO_4 was removed by filtration. The filtrate was concentrated, and the residue was dissolved in acetone. The resulting solution was chilled precipitating more Na_2SO_4, which was removed via filtration. The acetone solution was concentrated, and the residue was recrystallized from 10% aqueous MeOH to give 0.17 g (50%) of **5**as a purple solid. Mp: >300 °C; ^{1}H NMR (400 MHz, CD_3OD): δ7.78 (6H, t, J = 8.4 Hz), 8.16 (2H, d, J = 7.3 Hz), 8.26 (2H, d, J = 7.4 Hz), 8.35 (6H, t, J = 5.8 Hz), 8.67–8.73 (4H, s), 9.76–9.83 (4H, s); HR ESI MS: *m*/*z* 783.1052 (calcd for $C_{44}H_{24}F_3N_2O_3S_3$ + H, 783.1069).

4.2.15. 5,20-Bis-phenyl-10,15-bis[4-(ethoxycarbonylmethyleneoxy)-phenyl]-21,23-dithia-7,8-dihydroxychlorin (24). Dihydroxylation of dithiaporphyrin **23** followed litera-

ture procedures.[12] Briefly, dithiaporphyrin **23** (0.60 g 0.70 mmol) was dissolved in a 100:1 mixture of $CHCl_3$:pyridine (140 mL). To the solution, OsO_4 (0.27 g, 1.1 mmol) was added and the flask was closed with stoppers, covered with aluminum foil, and stirred at ambient temperature for 3 d. The reaction was then quenched by purging with H_2S for 5 min and the excess H_2S was trapped by 6 M NaOH aqueous solution. The solution was filtered through a plug of Celite and the filtrate was evaporated. The resulting residue was purified on a silica gel column eluted with a mixture of CH_2Cl_2 and MeOH to give 0.15 g (24%) of **24** as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ 1.39 (3H, t, J = 4.4 Hz), 1.42 (3H, t, J = 4.7 Hz), 3.19 (1H, d, J = 4.2 Hz), 3.25 (1H, d, J = 4.3 Hz), 4.35–4.44 (4H, s), 4.87 (2H, s), 4.90 (2H, s), 6.38–6.47 (2H, m), 7.19 (4H, s), 7.28–7.35 (2H, m), 7.70–7.83 (5H, m), 7.80 (1H, d, J = 7.9 Hz), 7.92–7.98 (1H, m), 8.04–8.24 (5H, m), 8.46–8.54 (2H, m), 9.12 (2H, t, J = 7.1 Hz), 9.45 (1H, d, J = 5.0 Hz), 9.48 (1H, d, J = 4.9 Hz); HR ESI MS: m/z 887.2974 (calcd for $C_{52}H_{42}N_2O_8S_2$ + H, 887.2461).

4.2.16. 5,20-Diphenyl-10,15-bis[4-(carboxymethyleneoxy)-phenyl]-21,23-dithia-7,8-dihydroxychlorin (7). Dithiaporphyrin **24** (0.14 g, 0.16 mmol) was hydrolyzed by 1 M NaOH (40 mL) as described for the preparation of **3** to give 0.10 g (76%) of **7** as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ 4.87 (2H, s), 4.90 (2H, s), 6.36 (2H, s), 7.29 (1H, dd, J = 6.1, 1.8 Hz), 7.30–7.40 (3H, m), 7.59 (2H, s), 7.65–7.93 (7H, m), 8.03–8.24 (5H, m), 8.39 (1H, d, J = 4.4 Hz), 8.42 (1H, d, J = 4.4 Hz), 9.13 (1H, d, J = 5.0 Hz), 9.17 (1H, d, J = 5.0 Hz), 9.47 (1H, d, J = 5.0 Hz), 9.51 (1H, d, J = 5.0 Hz); HR ESI MS: m/z 831.2313 (calcd for $C_{48}H_{34}N_2O_8S_2$ + H, 831.1835).

4.2.17. 5,20-Bis-phenyl-12,13-dimethoxy-21-thiaporphyrin (27). Cyclization of 2,5-bis(hydroxymethyl)-3,4-dimethoxy-thiophene[28] **25**(0.50 g, 2.5 mmol), pyrrole (0.52 mL, 7.5 mmol), and benzaldehyde (0.42 mL, 4.2 mmol) with BF_3·etherate (0.052 mL, 0.41 mmol) and TCBQ (1.7 g, 7.4 mmol) was performed as described for the preparation of **13** to give 0.08 g (6%) of **27** as a purple solid. Mp: 159–161 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.73 (6H, s), 7.60–7.72 (6H, m), 8.06 (4H, d, J = 5.4 Hz), 8.56 (2H, d, J = 3.5 Hz), 8.71 (2H, s), 8.89 (2H, d, J = 3.5 Hz), 10.42 (2H, s); HR ESI MS: m/z 540.2460 (calcd for $C_{34}H_{25}N_3O_2S$ + H, 540.1746).

4.2.18. 5,20-Bis-phenyl-12,13-dihydroxy-21-thiaporphyrin (28). Mono-thiaporphyrin **27** (0.18 g, 0.33 mmol) was demethylated with BBr_3 (0.16 mL, 1.67 mmol) in CH_2Cl_2 as described for the preparation of **14** to give 0.15 g (88%). The crude product was used without further purification.

4.2.19. 5,20-Bis-phenyl-12,13-bis(ethoxycarboxylmethyleneoxy)-21-thiaporphyrin (29). Mono-thiaporphyrin **28** (0.30 g, 0.59 mmol) was treated with ethyl bromoacetate (0.70 mL, 5.9 mmol) and K_2CO_3 (0.81 g, 5.9 mmol) in 150 mL of acetone as described for the preparation of **15** to give 0.20 g (51%) of **29** as a dark purple solid. Mp: 119–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (6H, t, J = 7.1 Hz), 4.44 (4H, q, J = 7.1 Hz), 5.76 (4H, s), 7.75 (9H, m), 8.18 (4H, d, J = 7.9 Hz), 8.67 (2H, d, J = 4.4 Hz), 8.91 (1H, d, J = 1.9 Hz), 9.01 (2H, d, J = 4.4 Hz), 10.71 (1H, s); HR ESI MS: m/z 684.2468 (calcd for $C_{40}H_{33}N_3O_6S$ + H, 684.2168).

4.2.20. 5,20-Bis-phenyl-12,13-carboxylatomethoxy-21-thiaporphyrin (8). Mono-thiaporphyrin **30** (0.20 g, 0.29 mmol) was hydrolyzed by 1 M NaOH (4 mL) as described for the preparation of **3** to give 0.10 g (55%) of **8** as a purple solid. Mp: 199–201 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ −1.61 (1H, br s), 7.86 (4H, s), 8.22 (6H, s), 8.22 (4H, s), 8.56 (2H, s), 8.92 (2H, s), 8.92 (2H, s), 10.82 (2H, s), 12.12 (2H, s); HR ESI MS: m/z 628.1807 (calcd for $C_{36}H_{25}N_3O_6S$ + H, 628.1542).

4.3. Photophysical properties

4.3.1. Relative quantum yields for singlet oxygen generation. A stock solution of 1,3-diphenylisobenzofuran (DPBF) (A, 180 μM) was prepared by dissolving 4.9 mg of DPBF in 100 mL of THF. Stock solutions of the porphyrins (B, 2 mM) were prepared by dissolving the desired amount of porphyrin in THF. Ten microliters of (B) was added to 10 mL of THF to get a 2 μM stock solution of the porphyrins (C). The reaction mixture was prepared by mixing 2 mL of (A) and 2 mL of (C), so that the final concentration of DPBF is 90 μM and that of porphyrin is 1 μM.

The UV absorbance of the reaction mixture was obtained before irradiation, using THF as a blank solution. The irradiation was carried out using a halogen lamp with a water filter (400–850 nm). The light intensity was 3 mW/cm^2 and the reaction mixture was continuously stirred during the irradiation. The progress of reaction was monitored after every 2 min of irradiation up to 10 min, using UV absorbance. 300 μL of the sample was used each time for UV measurement.

4.3.2. Determination of *n*-octanol/water partition coefficients at pH 7.4 phosphate buffer. The *n*-octanol/water partition coefficients were determined at pH 7.4 using the absorbance of the core-modified porphyrins. A 'shake flask' direct measurement[29] with minor modification was used. Individual porphyrins were dissolved in a mixture of equal volumes of *n*-octanol and a pH 7.4 phosphate buffer, then placed in an ultrasound bath for 30 min. The mixture was then left to settle for 4 h and the partition coefficients determined by measuring the absorbance of the core-modified porphyrins, using an Ocean Optics USB4000 UV–vis spectrometer. Results were reported as $\log D_{7.4}$ values.

4.4. Biology

4.4.1. Cells and culture conditions. Cells cultured from the rodent mammary adenocarcinoma cell line (R3230AC) were used. The cells were maintained on 60 mm diameter polystyrene dishes (Becton Dickinson Labware, Franklin lakes, NJ) in 7 mL minimum essential medium (α-MEM) supplemented with 10% bovine

growth serum (HyClone, No.: SH3054103), 50 units/mL penicillin G, 50 μg/mL streptomycin, and 1.0 g/mL fungizone (complete medium). Cells were incubated at 37 °C in 5% CO_2 using an incubator (Sanyo MCO-18AIC-UV). Passage was accomplished by aspirating the culture medium, then adding a 1.0 mL solution containing 0.25% trypsin and waiting for 4–5 min to remove the cells from the dish's surface. New culture dishes were then seeded with the appropriate number of cells in 7.0 mL of complete medium. Cell counts were done using a hematocytometer. The cell doubling time was approximately 20 h.

4.4.2. Incubation of cell cultures with dithiaporphyrins. In experiments to determine the porphyrin intracellular accumulation, R3230AC cells were seeded on 96-well plates at cell densities between 2.0 and 3.0×10^4 cells/well in the complete medium and incubated at 37 °C in 5% CO_2 for 24 h. The porphyrins were dissolved in DMSO at 2 mM. The stock solutions were diluted to the appropriate concentrations with complete medium immediately before the addition to cells. The porphyrin samples were then added to the wells and incubated for 24 h. After incubation, the medium was removed and the cell monolayer rinsed twice with a 0.9% NaCl solution. 190 μL of DMSO was then added to solubilize the cells and the fluorescence from the porphyrins read using a fluorescence multi-well plate reader (Molecular Devices, SpectraMax M2 model) set at the appropriate excitation and emission wavelengths. The intracellular porphyrin concentrations were then determined from a standard fluorescence curve obtained by dissolving porphyrin standards in DMSO. Results were expressed in fmol/cell.

In experiments to determine the cytotoxicity of the porphyrins in either the dark (dark toxicity) or after light exposure (phototoxicity), R3230AC cells were seeded on 96-well plates at cell densities between 1.0 and 1.5×10^4 cells/well in the complete medium. Cultures were then incubated for 24 h at 37 °C in 5% CO_2, and then the porphyrins, dissolved in the complete medium to the appropriate concentrations, were added to the wells and again incubated for 24 h. The medium was then removed and the cell monolayer rinsed twice with 190 μL of a 0.9% NaCl solution. Clear medium without phenol red and bovine growth serum, was then added to the wells and the well plates either kept in the dark (dark toxicity) or irradiated (phototoxicity) for an hour. After this the clear medium was removed and 190 μL of complete medium added. The cultures were then incubated at 37 °C in 5% CO_2, for 24 h, after which the cytotoxicity was determined by MTT assay and expressed as a percent of the controls. The controls used in the dark toxicity tests were cells kept in the dark and in the absence of porphyrins, while those used in the phototoxicity tests were cells exposed to light in the absence of porphyrins.

4.4.3. Irradiation of cultured cells. After 24 h of incubation with the porphyrins, the medium was removed, and the cell monolayer was rinsed twice with 190 μL of a 0.9% NaCl solution. Clear medium, was then added to the wells and the well plate placed on an orbital shaker (Lab-line, Barnstead International, IA). The well plate's lid was then removed and the wells exposed for an hour to broadband visible light delivered at 3 mW cm^{-2} from a 60 W halogen light source, through a 3.5 cm water filter (400–850 nm). Uniform illumination of the entire well plate was achieved by gently orbiting the well plate on the shaker. After an hour of irradiation, the clear medium was removed, and 190 μL of complete medium added to the wells. The cultures were incubated at 37 °C in 5% CO_2, and in the dark for 24 h, after which the cytotoxicity was determined by MTT assay and expressed as a percent of the controls, cells exposed to light in the absence of porphyrins.

4.4.4. Fluorescence microscopy with thiaporphyrins. R3230AC cells were seeded at cell densities between 2.0 and 3.0×10^4 cells/well in a 24-well plate containing 12 mm diameter coverslips in 1 mL of complete medium and then incubated at 37 °C in 5% $CO_2$for 24 h. The porphyrin samples, dissolved in medium, were then added to the well plate at 2.0×10^{-5} M and incubated again for 24 h. The medium was then removed and the cell monolayer rinsed twice with 3 mL of complete medium. After this the cover slide was immediately removed and mounted on a slide and the images taken. The images were captured using a Leica DMI4000B fluorescence microscope fitted with a QImaging Fast 1394 camera and Qcapture processing software. The images were modified for better visualization with Adobe Photoshop Element 5.0.

4.4.5. Statistical analyses. Statistical analyses were performed using the Student's *t*-test for pairwise comparisons. A P value of <0.05 was considered significant. The Hill (sigmoid Emax) equation was fitted to the data to obtain IC_{50} values.

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