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The main subjects of year one were to prepare the diverse structures of core-modified porphyrins, and to examine their physical properties and in vitro biological activities. Twenty five dithiaporphyrin compounds were synthesized with different features in size, symmetry, and electronic property at *meso*-aromatic groups of the porphyrins. These structural analogues had similar absorption maxima (~700 nm) and quantum yields of singlet oxygen generation (~0.8). However, these structural changes showed striking difference in biological activities. Thirteen compounds expressed >50 % cell kill at 0.5μM and 5-phenyl-20-(2-thienyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin was most potent displaying 68% cell kill at 0.1 μM with 5 Jcm^{-2} of light. In a mechanistic study, the porphyrin inhibited cytochrome c oxidase although it did not initially localize in the mitochondria. This enzyme damage can be explained by re-localization of porphyrin during irradiation. Interestingly, the induction of apoptotic cell death with the porphyrin depended on the incubation time with the photosensitizer and its concentration. Longer incubation time with the sensitizer (24 hr) at an appropriate concentration (0.2 μM) gave the most apoptosis. Base on the localization pattern with 24 hr incubation and consequent apoptotic process by PDT, there might be a target site inside the cell to trigger

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Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4

Key Research Accomplishments.....5

Reportable Outcomes.....5

Conclusions.....6

References.....6

Appendices.....6

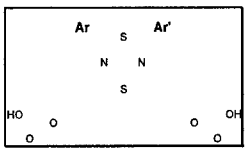
Introduction

The objectives of this project are two fold: one is to train the PI, Dr. Youngjae You, as an photodynamic cancer therapy expert in breast cancer research and the other is to perform the research to optimize the structure of 21,23-core-modified porphyrins as potential photosensitizers that are able to absorb long-wavelength light for treating breast cancer. The results of the first year term effectively address the plans in the statement of work. The goals were to obtain and determine the physical properties of diverse core-modified porphyrins and to characterize biological properties of the prepared compounds.

Body

In the course of accomplishing the first-year goals, the PI gained physical and biological (and photobiological) techniques. To determine the physical properties of the compounds, the PI learned methods to measure the quantum yields of fluorescence using a fluorimeter and singlet oxygen using a luminometer. For the biological evaluations, the PI also mastered the techniques to determine the cellular uptake of the core-modified porphyrins in cells, to investigate the sub-cellular localization using fluorescence microscopy, and to detect damage to cytochrome *c* oxidase both in isolated mitochondria and in whole cell.

Diverse arrays of 21,23-dithiaporphyrins were prepared to investigate the effects of the *meso*-aromatic rings on physical and photobiological properties of the compounds. The variation of the *meso*-aromatic rings focused on their steric and electronic properties as well as the degree of symmetry. New synthetic methods were devised to prepare the compounds having a trimethylphenyl or hydroxylphenyl group at the *meso*-position. Overall, about 25 compounds have been synthesized.



#	Ar	Ar'	#	Ar	Ar'
1	2-thienyl	2-thienyl	10	4'-chlorophenyl	4'-chlorophenyl
2	2-thienyl	phenyl	11	4'-hydroxyphenyl	4'-hydroxyphenyl
3	mesityl	mesityl	12	4'-methoxyphenyl	4'-methoxyphenyl
4	mesityl	phenyl	13	4'-dimethyl aminophenyl	4'-dimethyl aminophenyl
5	4'-tert-butylphenyl	4'-tert-butylphenyl	14	phenyl	phenyl
6	4'-tert-butylphenyl	phenyl	15	isopropyl	isopropyl
7	4'-methylphenyl	4'-methylphenyl	16	4'-trifluorophenyl	4'-trifluoromethylphenyl
8	4'-ethylphenyl	4'-ethylphenyl	17	4'-fluorophenyl	phenyl
9	4'-butylphenyl	4'-butylphenyl	18	4'-trifluoromethylphenyl	phenyl

Figure 1. The structures of 21,23-dithiaporphyrins prepared during first-year performance.

The physical properties of the compounds were determined such as the absorption maxima, extinction coefficients, quantum yields of fluorescence and singlet-oxygen generation, and log $D_{7.4}$. Most of the compounds generated singlet oxygen efficiently and absorbed the long-wavelength light (~ 700 nm) which are important factors for an ideal photosensitizer. The effects of the substituents at *meso*-aromatic ring were minimal on the physical properties except log $D_{7.4}$. The log $D_{7.4}$s, partition between *n*-octanol and phosphate buffer (pH, 7.4), ranged from -0.55 for compound **8** to 0.779 for compound **11**.

The phototoxicity was measured as a biological end point to evaluate the value of the compounds as photosensitizers. Thirteen compounds that expressed potent phototoxic activity, *i.e.*, cell kill > 50% at 0.5 μM with broad band (350-750 nm) light at 1.4mW for 1 hr, were obtained. Dark-toxicity was avoided for these compounds with concentration as high as 10 μM. Compound **2** with two small and different substituents at the *meso*-positions showed the most activity with a 68% cell kill at 0.1 μM with the same intensity of light. More interestingly, in stark contrast to the physical properties, the effects of structural modification at the *meso*-aromatic rings on biological outcome were more dramatic. From the analysis of the structure-activity relationships, the favorable structural features were deduced: 1) the size of *meso*-substituent was inversely correlated with phototoxicity, 2) breaking the symmetry of the molecule increased the activity, and 3) molecular amphiphilicity was important as seen in the case of compound **2**, which lost the activity.

To investigate the underlying mechanism of cell death by core-modified porphyrin **2**, sub-cellular localization, damage of cytochrome *c* oxidase activity, and induction of apoptosis during the cell death were investigated. Irradiation of cells in the presence of 0.2 μM compound **2** resulted in a decrease in cytochrome *c* oxidase activity while dithiaporphyrin **2** at 0.2 μM without irradiation did not affect cytochrome *c* oxidase activity in whole cells. However, compound **2** did not appear to localize in the mitochondria since images of **2**-treated cells by fluorescence microscopy show globular fluorescence inconsistent with mitochondrial localization. Photosensitizer **2** re-localized following irradiation as shown by time-dependent localization using fluorescence microscopy. Interestingly, the dynamic induction of apoptosis depends on the incubation time and concentration of the sensitizer (Figure **2**). The production of nucleosomes, which is an indicator of the apoptotic process, reached a maximum with longer incubation (24 hr) with and at appropriate concentration of compound **2** (0.2 μM). We postulate that there are target sites inside cells which can be targeted to trigger the apoptotic pathway by photodynamic treatment with compound **2**.

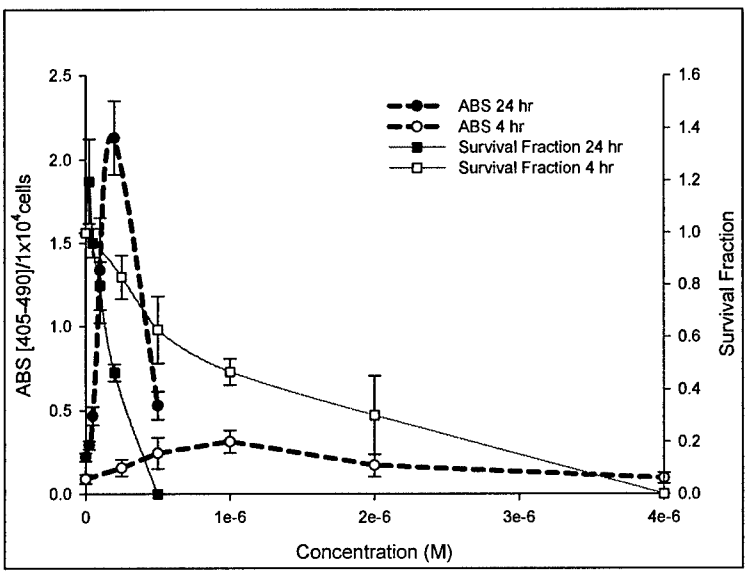


Figure 2. Cell death and detection of nucleosomes in apoptotic process

Key Research Accomplishments

Training: The PI acquired critical techniques to study the photophysical aspects of photosensitizers for photodynamic therapy as well as techniques to examine their biological effects. In addition, the PI developed new synthetic techniques for the synthesis of new core-modified porphyrins. Specifically, both hardware skills (fluorometer, luminometer, and fluorescence microscope) and biological assay skills (subcellular localization, determination of activity of cytochrome c oxidase, and detection of apoptosis and necrosis) were acquired.

Research accomplishments: 1) Synthesis of diverse sets of core-modified porphyrins, 2) determination of physical properties of prepared porphyrins, 3) evaluation of biological effects of the compounds and analysis of structure-activity relationships, and 4) elucidating of apoptotic cell death after PDT treatment.

Reportable Outcomes

Publications: 1) The part of the report was already published in peer-reviewed journal: Y. You, S.L. Gibson, R. Hilf, T.Y. Ohulchanskyy, M.R. Detty, Core-modified porphyrins. Part 4: Steric effects on photophysical and biological properties in vitro, Bioorganic & Medicinal Chemistry, 13 (6), 2235-2251, 2005.

2) The other part of the first year results is under preparation for two more publications.

3) Due to the contribution in the photodynamic cancer therapy, the PI was invited as an *ad hoc* reviewer in a high quality review journal, *Current Medicinal Chemistry-Anti-Cancer Agents*.

Poster: 1) The 229th American Chemical Society National Meeting, in San Diego, CA, March 13-17, 2005: Y. You, S.L. Gibson, R. Hilf, M.R. Detty, SAR study of new core-modified porphyrins as photosensitizers for photodynamic cancer therapy.

Conclusions

The progresses in training and research are satisfactory based on the proposed the SOW. After the first year practice, the PI is qualified to perform the photophysical, chemical, and *in vitro* biological studies necessary to perform independent research in photodynamic therapy. In addition, the PI has acquired an in-depth knowledge in PDT. The results of the first year generated a candidate for *in vivo* study of PK/PD effects and efficacy, and gained insight allowing the design of next target structures.

References

N/A

Appendices

Bioorganic & Medicinal Chemistry, 13 (6), 2235-2251, 2005

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Core-modified porphyrins. Part 4: Steric effects on photophysical and biological properties in vitro

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Abstract—21,23-Dithiaporphyrins (**2–10**) were designed and prepared as analogues of 5,20-diphenyl-10,15-bis(4-carboxylatomethoxy)phenyl-21,23-dithiaporphyrin (**1**) to examine the impact of steric bulk at the 5- and 20-*meso* positions as well as the impact of symmetry. Changes at the *meso* positions had minimal impact on the UV–vis–near-IR absorption spectra, quantum yields for the generation of singlet oxygen, and quantum yields for fluorescence and some impact on values of the octanol/water partition coefficient. Of the compounds **1–10**, 5-phenyl-20-(2-thienyl)-10,15-bis-(4-carboxylatomethoxy-phenyl)-21,23-dithiaporphyrin (**3**) showed the greatest phototoxicity toward cultured R3230AC cells, with 68% cell kill at 1×10^{-7} M and irradiation with 5 J cm^{-2} of 350–750 nm light. Results in this study suggest that smaller substituents on the *meso* ring and less symmetrical compounds are more effective as photosensitizers than compounds with two bulky substituents at adjoining *meso* sites and a higher symmetry. The mitochondria appear to be involved in the process of phototoxicity as determined by the inhibition of whole cell cytochrome *c* oxidase activity in cells treated with **3** and light. No impact upon mitochondrial cytochrome *c* oxidase activity was observed in cells treated with **3** and no light. Fluorescence microscopy studies suggest that the mitochondria are not initial sites of accumulation of **3**.

1. Introduction

Photodynamic therapy (PDT) is a promising treatment regimen for cancer that uses a dual-mode of selectivity:[1–9] a photosensitizer, which absorbs light and generates cytotoxic singlet oxygen, localizes in or around a tumor and is then irradiated in the lesion. The photosensitizer, porfimer sodium (Photofrin), has gained regulatory approval for the treatment of various cancers including lung, bladder, and oesophageal cancers in numerous countries but suffers from several drawbacks including persistent skin phototoxicity after treatment, in some cases, for up to 3 months.[10] Several second-generation compounds have been developed to overcome the deficiencies of Photofrin and other first generation photosensitizers and these are in late clinical trials for various indications.[5,11,12] Generally second-generation photosensitizers are designed to be pure, single, identifiable compounds with higher extinction coefficients at wavelengths above 600 nm.[8,13–19] They are also designed to achieve greater tumor specificity and targeted delivery to malignant lesions.[20–31] The 21,23-dithiaporphyrins are unique entities as second-generation photosensitizers. The substitution of a chalcogen atom (S, Se) for NH– at the 21- and 23-positions of the porphyrin macrocycle red shifts the absorption maxima to >690 nm.[19,32–34] The core chalcogen atoms, S or Se, exclude metal binding in the porphyrin core and 21,23-dithiaporphyrins are more efficient singlet oxygen generators than 21,23-diselenaporphyrins.[35–37]

The relationship between structural variations of the 21,23-core-modified porphyrins and the phototoxicity of the core-modified porphyrins in vitro was established previously.[32] First, dithiaporphyrins are more phototoxic toward Colo-26 cells than diselenaporphyrins or natural porphyrins containing four nitrogen atoms in

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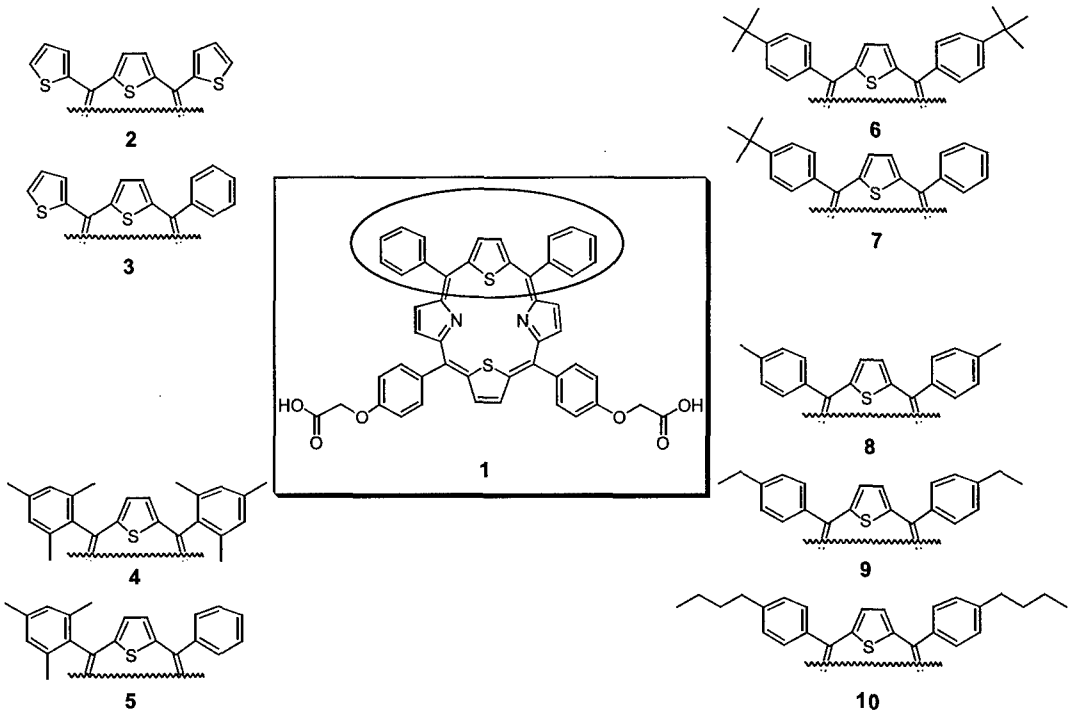


Figure 1. Structures of 21,23-dithiaporphyrins with various group substitutions at the 10,15-*meso* positions.

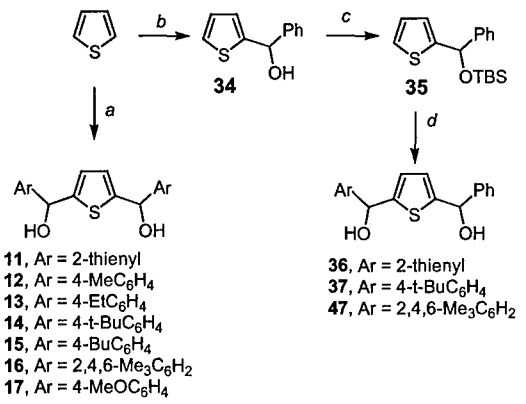
their core.[32] Second, by varying the number of sulfonate or carboxylate groups from 1 to 4 on the outer porphyrin ring, we determined that compounds with two, *cis*–*meso*, hydrophilic substituents are the most potent in vitro photosensitizers toward Colo-26 cells.[34] Third, molecules with *cis* carboxylic acid groups at the *meso* position are more potent than compounds with sulfonate groups. Specifically, studies in vitro with the dicarboxylic acid compound, 5,20-diphenyl-10,15-bis(4-carboxylatomethoxy)phenyl-21,23-dithiaporphyrin (**1**, Fig. 1), required only 0.15 μM photosensitizer to decrease the viability of R3230AC cells to 50% after irradiation with 5 J cm^{-2} of 350–750 nm light, a concentration that was 10-fold lower than that necessary for the disulfonate compound to reach the same level of cytotoxicity with the same light dose.[38]

The mitochondria are an important intracellular target for PDT due to their critical role in energy production and their involvement in apoptosis.[39] For porphyrins and porphyrin-related structures, accumulation of photosensitizer can occur in the membranes of organelles such as the mitochondria or lysosomes.[40] Earlier studies with **1** indicated that the mitochondria were targets for **1** prior to irradiation of treated cells and that irradiation lead to further mitochondrial damage.[38] Costaining studies of R3230AC cells with **1** and rhodamine 123 suggested that the mitochondria were disrupted by the presence of **1** or that the presence of **1** excluded rhodamine 123 from the mitochondria.[38]

Both the dark and phototoxicity studies and the mitochondrial studies with **1**, prompted us to ask whether further alterations in the basic structure might lead to more potent photosensitizers. Herein, we present the synthesis of a series of new 21,23-dithiaporphyrins designed to have diversity at the 5,10-*meso* aromatic rings (Fig. 1). Results demonstrate that these structural alterations did not greatly impact important photophysical properties, that is, UV–vis–near-IR absorption spectra, quantum yields for the generation of singlet oxygen, or quantum yields for fluorescence. However, biological properties including dark and phototoxicity, cellular uptake, subcellular localization, and dark and phototoxicity toward mitochondrial cytochrome *c* oxidase in isolated mitochondria or whole cells can be significantly affected by structural modifications.

2. Results and discussion

Based on our earlier studies with **1**, which showed potent phototoxicity compared to derivatives with one, three, or four carboxylic acids,[38] we prepared the dithiaporphyrins **2–10** of Figure 1 and examined the impact of alterations to the head portion of the molecule, namely the aromatic rings at *meso* positions 5 and 20, on photophysical and biological properties. A small 2-thienyl heteroaromatic ring or a larger 2,4,6-trimethylphenyl were substituted for phenyl at the 5- and/or 20-positions of the head portion of the molecule. A series of alkyl substituents (methyl, ethyl, *n*-butyl, *tert*-butyl) were also added to the 4-position of the *meso*-phenyl substituents. Combined, these substitutions created molecules with different steric demands and with different symmetries.



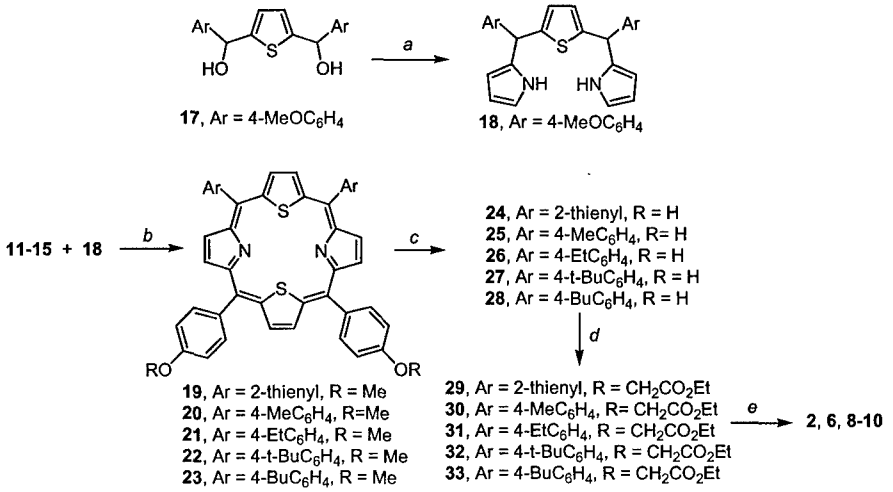
Scheme 1. Reagents and conditions: (a) i. 2.5 equiv *n*-BuLi, ii. 2 equiv aryl aldehyde; (b) i. 1 equiv *n*-BuLi, ii. 1 equiv benzaldehyde; (c) TBSCl, DMAP, Et_3N; (d) i. 1 equiv *n*-BuLi, ii. 1 equiv aryl aldehyde, iii. aqueous HCl.

2.1. Chemistry

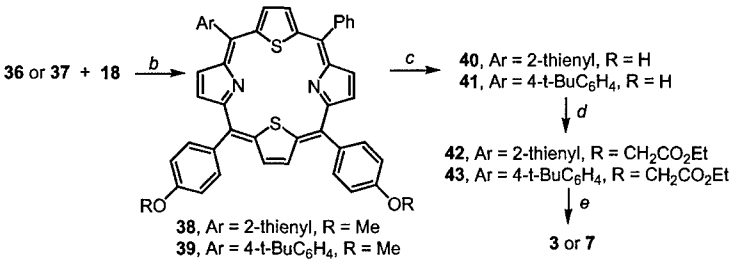
2.1.1. Synthesis of symmetrical 21,23-dithiaporphyrins 2, 6, and 8–10. The synthesis of symmetric core-modified porphyrins **2**, **6**, and **8–10** (Fig. 1) was based on our previous synthetic methods to symmetrical derivatives.[34] As shown in Scheme 1, 2,5-bis[1-(4-aryl)-1-hydroxymethyl]thiophenes **11–17** were prepared from 2,5-dilithiothiophene and various aldehydes. 2,5-Bis[1-(4-methoxyphenyl)-1-hydroxymethyl]-thiophene (**17**) was converted to 2,5-bis[1-(4-methoxyphenyl)-1-pyrrolomethyl]-thiophene (**18**) using pyrrole and boron trifluoride etherate ($BF_3{\cdot}OEt_2$) (Scheme 2). Diols **11–15** were condensed with **18** in the presence of 2,3,5,6-tetrachlorobenzoquinone (TCBQ) and *p*-toluenesulfonic acid monohydrate ($TsOH{\cdot}H_2O$) (or $BF_3{\cdot}OEt_2$) in CH_2Cl_2 to give 21,23-dithiaporphyrins **19–23** in 9–20% isolated yields (Scheme 2). Demethylation of **19–23** with BBr_3 in CH_2Cl_2 gave phenolic core-modified porphyrins **24–28**, which were alkylated with ethyl bromoacetate and K_2CO_3 in acetone. The resulting esters **29–33** were hydrolyzed with NaOH in aqueous tetrahydrofuran (THF) to give final diacid compounds **2**, **6**, and **8–10**.

2.1.2. Synthesis of unsymmetrical dicarboxylic acid derivatives 3 and 7. The synthetic strategy used for the synthesis of unsymmetrical porphyrins **3** and **7**, was elaborated in our previous report.[38] 2-(1-Hydroxy-1-phenylmethyl)thiophene (**34**) was prepared in 68% yield from thiophene using *n*-butyllithium (*n*-BuLi) as limiting reagent (Scheme 1).[38] Protection of the hydroxyl group of **34** with *tert*-butyldimethylsilyl chloride (TBSCl) gave TBS ether **35**, in 86% yield.[38] Compound **35** was then lithiated with *n*-BuLi and treated with thiophene 2-carboxaldehyde or 2,4,6-trimethylbenzaldehyde. The resulting alcohols were deprotected with tetrabutylammonium fluoride (TBAF) to afford unsymmetrical diols **36** and **37** in 43% and 68% isolated yields, respectively (Scheme 1). The unsymmetrical diols were condensed with 2,5-bis[1-(4-methoxyphenyl)-1-pyrrolomethyl]thiophene (**18**) to give the unsymmetrical 21,23-dithiaporphyrins, **38** and **39**, in 11% and 14% isolated yields, respectively (Scheme 3). Demethylation to phenols **40** and **41** with BBr_3, alkylation with ethyl bromoacetate to diesters **42** and **43**, and saponification to give 21,23-dithiaporphyrins **3** and **7** followed our standard protocols (Scheme 3).[38]

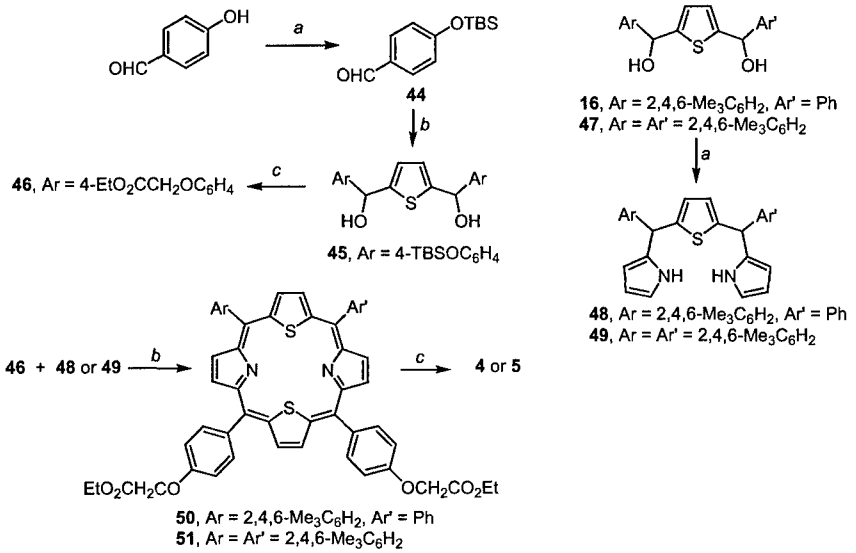
2.1.3. Synthesis of 2,4,6-trimethylphenyl-containing derivatives 4 and 5. It was necessary to prepare 21,23-dithiaporphyrins **4** and **5** bearing 2,4,6-trimethylphenyl groups via an alternative synthetic route. Attempted demethylation of the corresponding 5,20-bis(4-methoxyphenyl)-21,23-dithiaporphyrins with one or two 2,4,6-trimethylphenyl substituents with BBr_3 gave, in addition to the desired bisphenols, monobrominated products as determined by the appearance of $M^+ + Br$ peaks in the mass spectra of these molecules. The ^1H NMR spectra of these molecules also showed multiple sets of 2,4,6-trimethylphenyl peaks consistent with additional products. We were unable to purify/separate these mixtures so further characterization of these materials was not



Scheme 2. Reagents: (a) pyrrole, $BF_3{\cdot}OEt_2$; (b) TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (c) BBr_3, CH_2Cl_2; (d) $BrCH_2CO_2Et$, K_2CO_3, acetone; (e) NaOH, aqueous THF.



Scheme 3. Reagents: (a) pyrrole, $BF_3{\cdot}OEt_2$; (b) TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (c) BBr_3, CH_2Cl_2; (d) $BrCH_2CO_2Et$, K_2CO_3, acetone; (e) NaOH, aqueous THF.



Scheme 4. Reagents and conditions: (a) TBSCl, Et_3N; (b) 0.5 equiv $2,5\text{-}Li_2SC_4H_2$; (c) $BrCH_2CO_2Et$, K_2CO_3, acetone; (d) pyrrole, $BF_3{\cdot}OEt_2$; (e) TCBQ, $TsOH{\cdot}H_2O$, CH_2Cl_2; (f) NaOH, aqueous THF.

attempted. Alternative methods of demethylation were examined including BCl_3 with NaI or NaSEt in DMF. However, reaction yields were poor with these reagents.

An alternative approach to these products was devised as shown in Scheme 4. 4-Hydroxybenzaldehyde was protected with TBSCl to give **44**, which was then added to 2,5-dilithiophene to give 2,5-bis[1-(4-*tert*-butyldimethylsilyloxyphenyl)-1-hydroxymethyl]thiophene (**45**). The TBS groups of **45** were deprotected with HCl and the crude diol was alkylated with ethyl bromoacetate in acetone to give 2,5-bis[1-(4-carboxylatomethoxy)phenyl-1-hydroxymethyl]thiophene (**46**).

The addition of 2,4,6-trimethylbenzaldehyde to 2,5-dilithiothiophene gave diol **16** in 53% isolated yield and to lithiated **35** gave diol **47** in 47% isolated yield (Scheme 1). Diols **16** and **47** were treated with pyrrole and $BF_3{\cdot}OEt_2$ to give dipyrrolomethane compounds **48** and **49**—each in 78% isolated yield (Scheme 3). The condensation of diol **46** with dipyrrolomethanes **48** and **49** in the presence of TCBQ and $TsOH{\cdot}H_2O$ in CH_2Cl_2 gave diesters **50** and **51** in 13% and 22% isolated yields, respectively. Dicarboxylic acids **4** and **5** were obtained in 90% and 87% isolated yields, respectively, after the saponification of **50** and **51**.

2.2. Photophysical properties

2.2.1. Absorption maxima. Longer wavelength exciting light is advantageous in PDT because it penetrates tissue to a greater depth. The 21,23-dithiaporphyrins' band 1 absorbance maxima are all >690 nm. As shown in Table 1, changing the *meso*-aryl substituents in the dicarboxylic acid derivatives **1–10** did not significantly impact the wavelength maxima of bands.[34,38]

2.2.2. Quantum yields for the generation of singlet oxygen. Singlet oxygen ($^1O_2$) is reported to be the toxic agent responsible for the effectiveness of the majority of PDT photosensitizers.[1,3–5,41] Thus, efficient $^1O_2$ generation during irradiation of the photosensitizer is important for effective treatment. Quantum yields of singlet oxygen generation [$\phi(^1O_2)$] for dithiaporphyrins **1–7** were measured directly using the luminescence of $^1O_2$ compared to that of a rose bengal standard.[42] For this series of core-modified porphyrins, the average value of $\phi(^1O_2)$ was 0.80 with a range of 0.67 for **2** to 0.90 for **4** (Table 2).

Table 1. UV–vis–near-IR band maxima and molar absorptivities for dithiaporphyrins **1–10** in methanol[a]

Compound	Soret	Band IV	Band III	Band II	Band I
1	435 (314)	513 (27.7)	549 (10.7)	632 (3.2)	698 (7.0)
2	442 (288)	520 (20.5)	558 (13.7)	640 (2.2)	707 (5.3)
3	438 (320)	516 (24.8)	553 (12.8)	636 (2.4)	702 (6.3)
4	434 (303)	512 (27.6)	546 (7.3)	633 (2.0)	697 (4.8)
5	434 (291)	513 (31.5)	547 (10.6)	633 (2.5)	697 (6.9)
6[b]	438 (216)	517 (19.3)	552 (9.3)	637 (1.4)	702 (4.8)
7[b]	438 (278)	516 (26.7)	551 (8.7)	635 (2.0)	700 (5.1)
8	436 (267)	515 (22.2)	550 (11.1)	635 (4.2)	700 (7.4)
9[b]	437 (171)	516 (16.1)	551 (7.2)	637 (1.2)	701 (4.0)
10[b]	437 (150)	516 (14.1)	552 (6.3)	636 (1.0)	700 (3.2)

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

Table 2. Quantum yields for the generation of singlet oxygen [$\phi(^1O_2)$], quantum yields for fluorescence (ϕ_F), and octanol/water partition coefficients in pH 7.4 phosphate buffer ($\log D_{7.4}$) for **1–10**[a]

Compound	$\phi(^1O_2)$	ϕ_F	$\log D_{7.4}$
1	0.80 ± 0.03	0.007 ± 0.001	0.04 ± 0.0191
2	0.67 ± 0.03	0.009 ± 0.002	−0.27 ± 0.0057
3	0.86 ± 0.03	0.009 ± 0.002	−0.28 ± 0.007
4	0.90 ± 0.03	0.007 ± 0.002	0.24 ± 0.0061
5	0.80 ± 0.03	0.006 ± 0.001	0.28 ± 0.0164
6	0.80 ± 0.03	0.006 ± 0.001	0.48 ± 0.0079
7	0.80 ± 0.03	0.005 ± 0.001	−0.14 ± 0.002
8	ND	ND	−0.21 ± 0.110
9	ND	ND	−0.55 ± 0.064
10	ND	ND	0.45 ± 0.087

[a] Detailed methods for measurements above are presented in Section 4. ND (no data) represents measurements that were not performed.

2.2.3. Quantum yields for fluorescence. The fluorescence signal emitted by photosensitizers has been used to determine intracellular localization,[43] to study pharmacokinetics in vivo,[44] and to diagnosis malignancies.[45–47] Quantum yields for fluorescence (ϕ_F) for this series of core-modified porphyrins were low (0.005–0.009, Table 2) compared to the standard rhodamine 6G fluorescence with a value of ϕ_F of 1.0. However, this weak fluorescence could still be used to observe the intracellular localization of the dithiaporphyrins (vide infra).

2.2.4. *n*-Octanol/water partition coefficients. The presence of the carboxylic acid substituents necessitated the use of buffer solutions for determining values of the *n*-octanol/water partition coefficient, *P*, for **1–10**. Values of the partition coefficient were measured at pH 7.4 in a phosphate buffer and are reported as values of $\log D_{7.4}$ in Table 2. Values of $\log D_{7.4}$ were clearly impacted by the aryl/heteroaryl substituents in the 5- and 20-positions with a range of −0.55 to 0.48. The substitution of a 2-thienyl ring for phenyl (comparison of **1** and **3**) increased the hydrophilicity, $\log D_{7.4}$ 0.04 and −0.27 for **1** and **3**, respectively. However, the addition of a second 2-thienyl substituent in **2** had no further impact on $\log D_{7.4}$ with $\log D_{7.4}$ of −0.28 for **2**. Compounds **6** and **10** with two 4-*tert*-butylphenyl or two 4-*n*-butylphenyl substituents were the most lipophilic compounds in the series and were followed closely by compounds **4** and **5** with two and one 2,4,6-trimethylphenyl substituents, respectively (Table 2). In contrast, dithiaporphyrins **7–9** were surprisingly hydrophilic with values of $\log D_{7.4}$ of −0.14 to −0.55.

2.3. Biology

2.3.1. Intracellular accumulation of core-modified porphyrins into cultured R3230AC cells. Intracellular accumulation of dithiaporphyrins was assessed in cultured R3230AC cells. Cell monolayers were incubated with dithiaporphyrins **1–10** for 24 h at concentrations of 5×10^{-6} and 1×10^{-5} M, cells were digested with 25% Scintigest, and the fluorescence in the cell digests was determined. Intracellular dithiaporphyrin content was calculated using standard fluorescence curves obtained from the individual dithiaporphyrins dissolved in 25% Scintigest. The data are expressed in Figure 2 as fmol dithiaporphyrin/cell. When 5×10^{-6} M dithiaporphyrin was incubated with cultured R3230AC cells, dithiaporphyrins accumulated intracellularly at concentrations ranging from 0.18 to 2.7×10^{-15} mol dithiaporphyrin/cell. This intracellular dye content represents a range of 2–27% of the dithiaporphyrin content in the culture medium. The uptake of dithiaporphyrins **4–7** was $<10^{-15}$ mol/cell while the uptake of **1–3** and **8–10** was in the range of 1.2×10^{-15}–2.7×10^{-15} mol/cell.

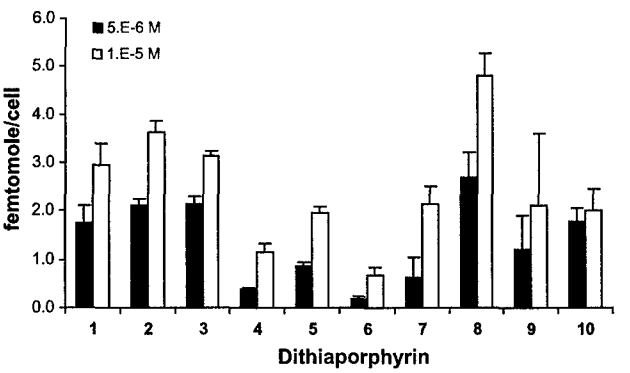


Figure 2. Cellular uptake of 21,23-dithiaporphyrins in cultured R3230AC rat mammary adenocarcinoma cells. Experimental conditions are detailed in Section 4. Each bar represents the mean intracellular uptake of each dithiaporphyrin incubated with R3230AC cells for 24 h at 5×10^{-6} or 1×10^{-5} M. The intracellular fluorescence was obtained by comparing experimental values with standard curves obtained from porphyrins dissolved in Scintigest. Data are expressed as fmol porphyrin/cell, error bars are the SEM.

Compounds **4**–**7** bear bulky 2,4,6-trimethylphenyl (**4** and **5**) and 4-*tert*-butyl (**6** and **7**) substituents, which suggests that steric factors in the *meso* substituents can impact uptake.

At 1×10^{-5} M dithiaporphyrin (Fig. 2), the least uptake was again observed with **4** and **6** bearing two bulky substituents, but the uptake of **5** with one 2,4,6-trimethylphenyl substituent and **7** with one 4-*tert*-butylphenyl were comparable to the uptake of **9** and **10** with two 4-ethylphenyl and 4-*n*-butylphenyl substituents, respectively. At 1×10^{-5} M dithiaporphyrin, the highest uptake was observed with **1**–**3** and **8**, which bear the smallest *meso* substituents. Again, uptake at 1×10^{-5} M dithiaporphyrin appears to be impacted by steric effects.

2.3.2. Dark and phototoxicity of core-modified porphyrins toward cultured R3230AC cells. The dark toxicity and phototoxicity of dithiaporphyrins **1**–**10** were determined in cultures of R3230AC cells incubated with the dyes for 24 h prior to washing or irradiation. No significant dark toxicity was observed in R3230AC cells for any of the core-modified porphyrins studied, even at the highest porphyrin concentration used, 1×10^{-5} M (cell survival $\geqslant 80\%$). At 1×10^{-7} M dithiaporphyrin with 5 J cm^{-2} of 350–750 nm light, dithiaporphyrins **1**–**3**, **5**, **7**, and **8** displayed 15–68% cell kill while dithiaporphyrins **4**, **6**, **9**, and **10** showed no phototoxicity (Fig. 3a). Increasing the dithiaporphyrin concentration to 5×10^{-7} M gave phototoxicity of >80% cell kill with 5 J cm^{-2} of 350–750 nm light with dithiaporphyrins **1**–**3**, **5**, **7**, and **8** while dithiaporphyrins **4**, **6**, **9**, and **10** displayed <35% cell kill at this concentration. Among dithiaporphyrins **1**–**10**, dithiaporphyrin **3** exhibited the greatest phototoxicity with 30% cell kill at 5×10^{-8} M, 68% cell kill at 1×10^{-7} M, and >90% cell kill at 5×10^{-7} M and the same light dose (Fig. 3b).

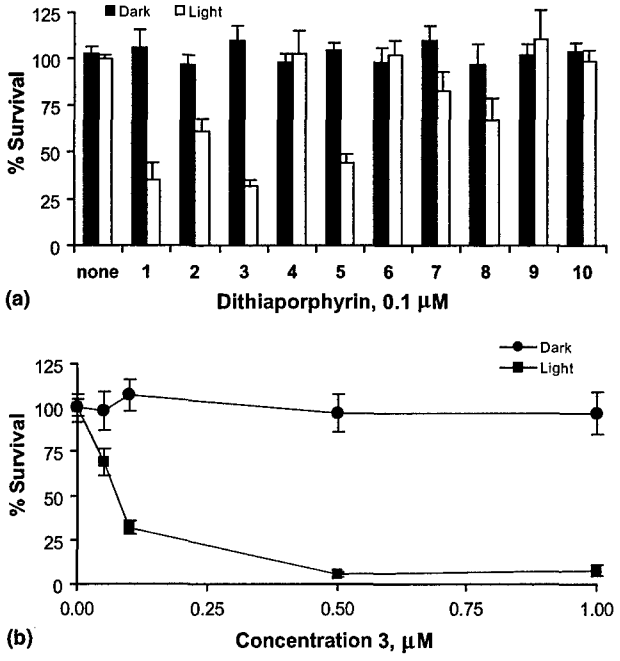


Figure 3. Cell viability of cultured R3230AC cells after photosensitization with 5 J cm^{-2} of 350–750 nm light in the presence of (a) 0.1 μM 21,23-dithiaporphyrins or (b) various concentrations of **3**. Experimental conditions are detailed in Section 4. Each data point represents the mean of at least three separate experiments performed in duplicate, error bars are the SEM. Data are expressed as the surviving fraction of viable cells relative to untreated controls.

As was observed with respect to cellular uptake of dithiaporphyrins **1**–**10**, the efficacy of the dithiaporphyrins as photosensitizers is sensitive to steric effects. Dithiaporphyrin **1** with two phenyl substituents at the 5- and 20-*meso* positions and dithiaporphyrins **2** and **3**, in which one or both phenyl substituents were replaced by the small 2-thienyl substituent, had comparable phototoxicity. Similarly, the substitution of two 4-methylphenyl substituents at the 5- and 20-*meso* positions of **8** had little impact on phototoxicity. However, a slight increase in the aryl steric bulk to 4-ethylphenyl as in compound **9** was sufficient to give a statistically significant decrease in phototoxicity for **9** relative to **1** at 5×10^{-8}, 1×10^{-7}, and 5×10^{-7} M photosensitizer ($P < 0.05$). Similarly, compound **4** with two 2,4,6-trimethylphenyl substituents and compound **6** with two 4-*tert*-butylphenyl substituents showed no significant phototoxicity at 1×10^{-7} M ($P > 0.05$). In contrast, compounds **5** and **7** with only one bulky substituent were both effective photosensitizers at 5×10^{-7} M with >80% cell kill while compound **5** was also a photosensitizer at 1×10^{-7} M with 56% cell kill. Compound **10** with two 4-*n*-butylphenyl substituents showed no significant phototoxicity ($P > 0.05$) over a concentration range of 5×10^{-8}–1×10^{-6} M relative to dark controls.

These data suggest that dithiaporphyrins bearing 4-(carboxylatomethoxy)phenyl substituents in the 10- and 15-positions can have one bulky aryl substituent in the 5- or 20-position, but not two. In this study, a bulky aryl substituent is defined as 4-ethylphenyl, 4-*n*-butylphenyl, 4-*tert*-butylphenyl, or 2,4,6-trimethylphenyl.

Phototoxicity does not appear to be solely dependent on intracellular accumulation of the dithiaporphyrins. At 5×10^{-6} M, dithiaporphyrins **9** and **10** accumulated at 1.2 and 1.8×10^{-15} mol/cell but **9** and **10** showed relatively little phototoxicity at 5×10^{-7} M relative to **5** or **7** whose intracellular concentrations were only 0.85 and 0.63×10^{-15} mol/cell, respectively. Clearly, other factors such as subcellular localization of the dithiaporphyrins or relocalization of the photosensitizers during light exposure may impact the phototoxicity of these compounds.

2.3.3. Studies on the subcellular localization of 21,23-dithiaporphyrins

2.3.3.1. Photosensitized inhibition of cytochrome *c* oxidase in isolated mitochondrial suspensions. In order to demonstrate that photodamage following irradiation of 21,23-dithiaporphyrins in the mitochondria could be responsible for cellular phototoxicity, we initially examined the inhibition of cytochrome *c* oxidase in isolated

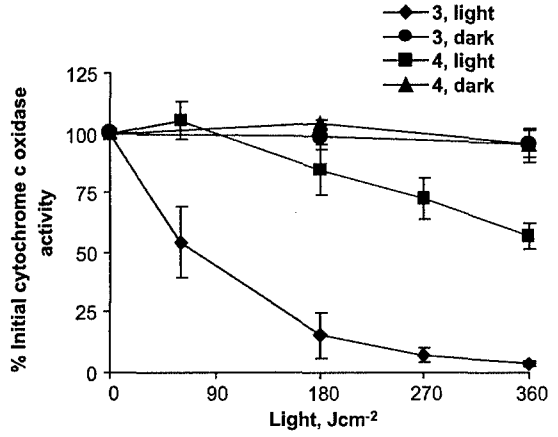


Figure 4. Effect of compounds **3** and **4** (10 μM) on mitochondrial cytochrome *c* oxidase activity in isolated mitochondrial suspensions in the dark and with photosensitization. Details of experimental conditions are described in Section 4. Data are expressed as the percent of initial preirradiation cytochrome *c* oxidase activity in mitochondrial suspensions. Each datum point represents the mean of three separate experiments; the error bars are the SEM.

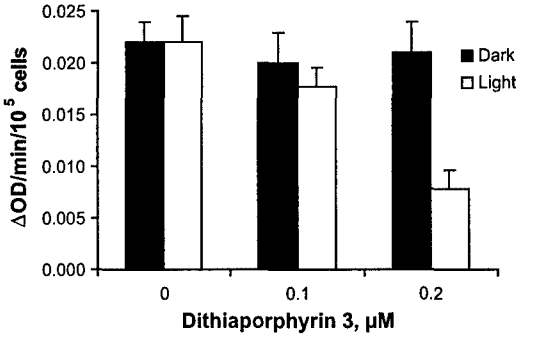


Figure 5. Effect of **3** on mitochondrial cytochrome *c* oxidase activity in cultured whole R3230AC cells following irradiation. Cell culture and light exposure conditions are detailed in Section 4. The activity values represent the change in optical density (OD) units per minute for 1×10^5 cells that were exposed to **3** at indicated concentrations 24 h after washing (dark) or 24 h after light exposure (light). Each data point represents the mean of at least three separate experiments; the error bars are the SEM.

mitochondrial suspensions. Mitochondrial suspensions were prepared from rat liver using a previously published method.[48] Mitochondrial suspensions were treated with 5×10^{-6} M solutions of **3** or **4** for 5 min. The suspensions were centrifuged, the pellets resuspended and irradiated with filtered 530–750 nm light delivered at 100 mW cm^{-2}. Aliquots were removed at various times and cytochrome *c* oxidase activity was compared to that of mitochondrial suspensions maintained in the dark (Fig. 4). No significant reduction in enzyme activity was observed for mitochondrial suspensions kept in the dark while exposure of suspensions to **3** or **4** and light resulted in a light-dose-dependent inhibition of cytochrome *c* oxidase. At 360 J cm^{-2}, dithiaporphyrins **3** and **4** inhibited cytochrome *c* oxidase activity by 97% and 43%, respectively. These results suggest that the dithiaporphyrin photosensitizers—even those bearing a bulky substituent—can inhibit mitochondrial function if the dithiaporphyrins reach the mitochondria.

2.3.3.2. Photosensitized inhibition of cytochrome *c* oxidase in cultured whole R3230AC cells. We next examined the inhibition of mitochondrial cytochrome *c* oxidase in cultured whole R3230AC cells using **3** and 5 J cm^{-2} of 350–750 nm light—the same light conditions used in the phototoxicity studies. Thirty minutes after the irradiation, at a time when there was no significant cytotoxicity, cells were harvested and cytochrome *c* oxidase activity was assessed (Fig. 5). At concentrations of $\leqslant 1 \times 10^{-7}$ M, no significant inhibition of cytochrome *c* oxidase was observed ($P > 0.05$). However, at 2×10^{-7} M **3**, 60% of the enzyme activity was inhibited after light exposure, which was significantly different from dark and untreated controls ($P < 0.05$).

2.3.3.3. Intracellular localization of 3 via fluorescence microscopy. The fluorescence of 21,23-dithiaporphyrin **3** was sufficient to make an image of subcellular localization via fluorescence microscopy. Cellular staining with **3** at 5×10^{-6} M resulted in globular cytosolic fluorescence as shown in Figure 6. Under these conditions, fluorescence was not detected in the nucleus, the nuclear membrane or the plasma membrane. Furthermore, the fluorescence pattern was not consistent with mitochondrial localization in R3230AC cells treated with the mitochondrial stain rhodamine 123.[38] Other studies have demonstrated that staining of lipid droplets with the fatty acid fluorescence probe, C_1-BODIPY 500/510 C_{12} (Molecular Probes, Inc., Eugene, OR), showed similar globular fluorescence patterns in the cytosol.[49,50]

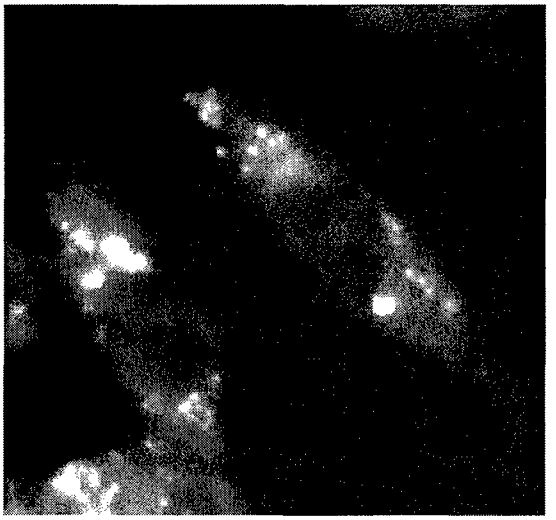


Figure 6. Fluorescence image of R3230AC cells treated with 5×10^{-6} M of **3** for 24 h. Cell culture conditions and experimental details are detailed in Section 4.

3. Summary and conclusions

We have further developed synthetic routes to 21, 23-dithiaporphyrins with different 5- and 20-*meso*

substituents with 4-caboxylatomethoxyphenyl substituents as the 10- and 15-*meso* substituents. We prepared nine new 21,23-dithiaporphyrins and compared their physical and photophysical properties to those of 5,20-diphenyl-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (**1**).[38] Changes in the *meso* substituents had little effect on the absorption spectra, quantum yields for the generation of singlet oxygen, and quantum yields for fluorescence. The substituent changes did have some impact on the *n*-octanol/water partition coefficient, but these changes showed no correlation with either cellular uptake or cellular dark or phototoxicity.

None of the 21,23-dithiaporphyrins **1–10** showed any significant dark toxicity following a 24 h incubation with $\leqslant 1 \times 10^{-5}$ M **1–10** (>80% cell viability). However, phototoxicity was structure dependent. The presence of two bulky substituents, that is, substituents more bulky than 4-methylphenyl at the 5- and 20-positions (dithiaporphyrins **4**, **6**, **9**, and **10**), gave greatly decreased phototoxicity relative to dithiaporphyrins **1–3** and **8** with two smaller substituents (phenyl, 2-thienyl, or 4-methylphenyl) or dithiaporphyrins **5** and **7** with one phenyl substituent and one bulky substituent (4-*tert*-butyl or 2,4,6-trimethylphenyl) at the 5- and 20-positions. Among the photosensitizers **1–3**, **5**, **7**, and **8**, dithiaporphyrin **3** with 5-phenyl-20-2-thienyl substituents displayed the greatest phototoxicity with 68% cell kill at a concentration of 1×10^{-7} M with 5 J cm^{-2} of 350–750 nm light.

Irradiation of cells in the presence of compound **3** at 2×10^{-7} M gives a decrease in cytochrome *c* oxidase activity while dithiaporphyrin **3** at 2×10^{-7} M without irradiation does not affect cytochrome *c* oxidase activity in whole cells. However, compound **3** does not appear to localize in the mitochondria since images of **3**-treated cells by fluorescence microscopy show globular fluorescence inconsistent with mitochondrial localization. Photosensitizer **3** may relocalize following irradiation. We are now trying to uncover the detailed biological responses after the irradiation with core-modified porphyrin **3** as well as in vivo antitumor efficacy.

4. Experimental

4.1. General methods

Solvents and reagents were used as received from Sigma–Aldrich Chemical Co. (St. Louis, MO) unless otherwise noted. Cell culture medium was purchased from GIBCO (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Atlanta, GA). Concentration in vacuo was performed on a Buchi rotary evaporator. NMR spectra were recorded at 23 °C on a Varian Gemini-300, Inova 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). Infrared spectra were recorded on a Perkin–Elmer FT-IR instrument. UV–vis–near-IR spectra were recorded on a Perkin–Elmer Lambda 12 spectrophotometer equipped with a circulating constant temperature bath for the sample chambers. Elemental analyses were conducted by Atlantic Microlabs, Inc. Q-TOF 2 electrospray and ESI mass spectrometry and were conducted by the Campus Chemical Instrumentation Center of The Ohio State University (Columbus, OH) and the Instrument Center of the Department of Chemistry at the University at Buffalo. The purity of compounds **1–10** was accessed by elemental analysis and the results were within ±0.4% of the theoretical values. 2,5-Bis[1-(4-methoxyphenyl)-1-hydroxymethyl]thiophene (**17**),[34] 2,5-bis[1-(4-methoxy-phenyl)-1-pyrrolomethyl]thiophene (**18**),[32] **34**,[34] **35**,[38] and **44**[51] were prepared as previously described. For biological studies, core-modified porphyrins **1–10** were dissolved in DMSO to make a stock solution at 2×10^{-3} M. The stock solutions were used after appropriate dilutions with sterilized doubly distilled water.

4.2. Chemistry

4.2.1. 2,5-Bis(2-thienyl-1-hydroxymethyl)thiophene (11). Thiophene (4.4 g, 52 mmol) was added to a solution of *n*-BuLi (80 mL of a 1.6 M solution in hexanes, 128 mmol) and TMEDA (20 mL, 133 mmol) in 200 mL of hexanes under an Ar atmosphere. The reaction mixture was heated at reflux for 1 h, cooled to ambient temperature, and transferred via a cannula to a pressure-equalizing addition funnel. This dilithiothiophene suspension was then added dropwise to a solution of 2-thiophenecarboxaldehyde (14.4 g, 128 mmol) in 200 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, 300 mL of NH_4Cl (aqueous 1 M solution) was added, and the organic phase was separated. The aqueous phase was extracted with ether (3 × 300 mL). The combined organic extracts were washed with water (3 × 300 mL) and brine (300 mL), dried over $MgSO_4$, and concentrated to give yellow oil. The crude product was precipitated by the slow addition of hexanes to an ether solution of **11** to give 13.0 g (80%) of predominantly one diastereomer of **11** as white amorphous powder. IR (KBr): 3390, 3104, 2869, 2247, 1604 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (2H, br s), 6.31 (2H, s), 6.95 (2H, s), 7.05 (2H, s), 7.10 (2H, s), 7.36 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 68.9, 124.3, 125.0, 125.0, 125.6, 125.6, 127.1. High-resolution EI MS: *m/z* 308.0000 (calcd for $C_{14}H_{12}O_2S_3$, 307.9999).

4.2.2. 2,5-Bis[1-(4-methyl)phenyl-1-hydroxymethyl]thiophene (12). Diol **12** was prepared as described for the preparation of **11** using thiophene (2.0 g, 25 mmol), *n*-BuLi (34 mL of 1.6 M in hexanes, 55 mmol), TMEDA (8.7 mL, 57 mmol), and 4-methylbenzaldehyde (5.7 g, 47 mmol). Product yield was 2.0 g (25%) of **12** as white amorphous powder. IR (KBr): 3377, 2955, 2922, 2868, 1513 cm^{-1}. ^{1}H NMR (400 MHz, 1:1 $CDCl_3$/CD_3OD): δ 2.19 (6H, s), 5.74 (2H, s), 6.52 (2H, s), 7.00 (4H, d, J = 7.6 Hz), 7.16 (4H, d, J = 7.6 Hz). ^{13}C NMR (75 MHz, 1:1 $CDCl_3$/CD_3OD): δ 21.3, 81.8, 125.1, 127.2, 127.5, 127.8, 129.5, 138.1, 138.2, 146.6. High-resolution ESI MS: *m/z* 347.1057 (calcd for $C_{20}H_{20}O_2S$ + Na, 347.1082).

4.2.3. 2,5-Bis[1-(4-ethyl)phenyl-1-hydroxymethyl]thiophene (13). Diol **13** was prepared as described for the preparation of **11** using thiophene (1.5 g, 19 mmol), *n*-BuLi (26 mL of 1.6 M in hexanes, 41 mmol), TMEDA (6.5 mL, 43 mmol), and 4-ethylbenzaldehyde (4.9 g, 37 mmol). Product yield was 5.0 g (76%) of **13** as white amorphous powder. IR (KBr): 3410, 2962, 2928, 2872, 1514 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.14 (6H, t, J = 7.5 Hz), 2.55 (4H, q, J = 7.5 Hz), 5.79 (2H, s), 6.03 (2H, s), 6.65 (2H, s), 7.15 (4H, d, J = 8.0 Hz), 7.28 (4H, d, J = 8.0 Hz). ^{13}C NMR (75 MHz, 1:1 $CDCl_3/CD_3OD$): δ 16.4, 28.5, 71.3, 123.4, 126.7, 128.1, 143.0, 143.2, 150.1. High-resolution ESI MS: *m/z* 352.1492 (calcd for $C_{22}H_{24}O_2S$, 352.1491).

4.2.4. 2,5-Bis[1-(4-*tert*-butyl)phenyl-1-hydroxymethyl]thiophene (14). Diol **14** was prepared as described for the preparation of **11** using thiophene (1.4 g, 16 mmol), *n*-BuLi (22 mL of 1.6 M in hexanes, 36 mmol), TMEDA (5.6 mL, 37 mmol), and 4-*tert*-butylbenzaldehyde (5.1 g, 32 mmol). Product yield was 4.0 g (60%) of **14** as white amorphous powder. IR (KBr): 3366, 2960, 2904, 2868, 1511 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.26 (18H, s), 5.79 (2H, m), 6.03 (2H, m), 6.66 (2H, m), 7.30 (4H, d, J = 6.5 Hz), 7.34 (4H, d, J = 8 Hz). ^{13}C NMR (126 MHz, DMSO-d_6): δ 31.2, 34.2, 70.6, 122.7, 124.8, 125.7, 125.8, 142.0, 149.1, 149.4. High-resolution EI MS: *m/z* 408.2114 (calcd for $C_{26}H_{32}O_2S$, 408.2118).

4.2.5. 2,5-Bis[1-(4-butyl)phenyl-1-hydroxymethyl]thiophene (15). Diol **15** was prepared as described for the preparation of **11** using thiophene (1.3 g, 16 mmol), *n*-BuLi (21 mL of 1.6 M in hexanes, 34 mmol), TMEDA (5.4 mL, 36 mmol), and 4-butylbenzaldehyde (4.9 g, 30 mmol). Product yield was 4.0 g (63%) of **15** as colorless oil as a mixture of *meso*- and *d,l*-diastereomers and used for next reaction without further purification. IR (film): 3364, 2955, 2928, 2857, 1512 cm^{-1}. ^1H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 0.75–0.93 (6H, m), 1.30–1.40 (4H, m), 1.55–1.62 (4H, m), 2.54–2.60 (4H, m), 5.36/5.87 (2H, s), 6.62–6.65 (2H, m), 7.10–7.17 (4H, m), 7.24–7.32 (4H, m). ^{13}C NMR (75 MHz, 1:1 $CDCl_3/CD_3OD$): δ 13.8, 22.4, 33.7, 35.4, 81.6, 125.0, 127.0, 128.6, 138.1, 142.9, 146.4. High-resolution ESI MS: *m/z* 408.2118 (calcd for $C_{26}H_{32}O_2S$, 408.2111).

4.2.6. 2,5-Bis[1-hydroxy-1-(2,4,6-trimethylphenyl)methyl]-thiophene (16). Using thiophene (2.1 g, 25 mmol), *n*-BuLi (34.3 mL of 1.6 M in hexanes, 55 mmol), TMEDA (8.7 mL, 57 mmol), and mesitylaldehyde (7.0 g, 47 mmol), **16** was prepared as described in the method for **11** to afford 5.0 g (53%) of **16** as colorless oil. IR (KBr): 3408, 2921, 1611 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.19 (6H, s), 2.21 (12H, s), 5.90 (2H, d, J = 4.4 Hz), 6.13 (2H, d, J = 4.4 Hz), 6.27 (2H, d, J = 7.6 Hz), 6.79 (4H, s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.1, 20.4, 67.2, 121.9, 129.4, 135.8, 136.1, 137.3, 147.5. High-resolution Q-TOF MS: *m/z* 403.1736 (calcd for $C_{24}H_{28}O_2S$ + Na, 403.1708).

4.2.7. 5,20-Bis(2-thienyl)-10,15-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (19). Diol **11** (3.1 g, 10 mmol), 2,5-bis[1-(4-methoxyphenyl)-1-pyrrolomethyl]thiophene (**18**, 4.6 g, 10 mmol), and TCBQ (12.6 g, 51 mmol) were dissolved in 600 mL of CH_2Cl_2. $BF_3{\cdot}OEt_2$ (0.57 mL, 0.45 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. Porphyrin **19** was isolated as the first red band. The crude product was washed with acetone and was then recrystallized from CH_2Cl_2/MeOH to give 0.96 g (13%) of **19** as a purple solid; mp > 300 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 4.09 (6H, s), 7.35 (4H, d, J = 8.1 Hz), 7.57 (2H, d, J = 4.2 Hz), 7.93 (2H, d, J = 5.4 Hz), 7.96 (2H, d, J = 3.3 Hz), 8.17 (4H, d, J = 8.1 Hz), 8.70 (2H, d, J = 4.5 Hz), 8.87 (2H, d, J = 4.2 Hz), 9.69 (2H, s), 9.92 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 77.2, 113.2, 125.6, 127.1, 128.9, 133.4, 133.7, 134.3, 134.8, 135.0, 135.6, 135.8, 142.0, 148.6, 148.9, 156.9, 157.4, 160.0. High-resolution Q-TOF MS: *m/z* 721.1144 (calcd for $C_{42}H_{28}N_2O_2S_4$ + H, 721.1112).

4.2.8. 5,20-Bis(4-methylphenyl)-10,15-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (20). Diol **12** (2.2 g, 6.8 mmol), thiophene **18** (3.1 g, 6.8 mmol), $TsOH{\cdot}H_2O$ (1.3 g, 6.8 mmol), and TCBQ (5.0 g, 20 mmol) were treated as described for the preparation of **19** to give 0.31 g (6%) of **20** as a purple solid; mp > 300 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 4.09 (6H, s), 7.35 (4H, d, J = 8.1 Hz), 7.57 (2H, d, J = 4.2 Hz), 7.93 (2H, d, J = 5.4 Hz), 7.96 (2H, d, J = 3.3 Hz), 8.17 (4H, d, J = 8.1 Hz), 8.70 (2H, d, J = 4.5 Hz), 8.87 (2H, d, J = 4.2 Hz), 9.69 (2H, s), 9.92 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 77.2, 113.2, 125.6, 127.1, 128.9, 133.4, 133.7, 134.3, 134.8, 135.0, 135.6, 135.8, 142.0, 148.6, 148.9, 156.9, 157.4, 160.0. High-resolution ESI MS: *m/z* 737.2308 (calcd for $C_{48}H_{36}N_2O_2S_2$ + H, 737.2296).

4.2.9. 5,20-Bis(4-ethylphenyl)-10,15-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (21). Diol **13** (2.8 g, 8.0 mmol), thiophene **18** (3.6 g, 7.9 mmol), $TsOH{\cdot}H_2O$ (1.5 g, 7.9 mmol), and TCBQ (5.8 g, 24 mmol), were treated as described for the preparation of **19** to give 1.2 g (20%) of **21** as a purple solid; mp > 300 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 1.54 (6H, t, J = 7.5 Hz), 3.02 (4H, q, J = 7.5 Hz), 4.12 (6H, s), 7.38 (4H, d, J = 8.0 Hz), 7.67 (4H, d, J = 7.5 Hz), 8.19 (4H, d, J = 7.0 Hz), 8.20 (4H, d, J = 6.5 Hz), 8.70–8.74 (4H, m), 9.73 (4H, s). High-resolution ESI MS: *m/z* 765.2575 (calcd for $C_{50}H_{40}N_2O_2S_2$ + H, 765.2604).

4.2.10. 5,20-Bis(4-*tert*-butylphenyl)-10,15-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (22). Thiophene **18** (3.0 g, 6.6 mmol), diol **14** (2.7 g, 6.6 mmol), and TCBQ (4.9 g, 20 mmol) were dissolved in 500 mL of CH_2Cl_2. $TsOH{\cdot}H_2O$ (1.3 g, 6.6 mmol) was added and the reaction mixture was stirred for 0.5 h at ambient temperature and was then heated at reflux for 1 h in the dark. Porphyrin **22** was isolated as described for **19** to give 0.90 g (17%) of **22** as a purple solid; mp > 300 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.82 (18H, s), 4.29 (6H, s), 7.56 (4H, s), 8.04 (4H, s), 8.39 (8H, br s), 8.94 (4H, s), 9.93

(4H, s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 31.8, 35.1, 55.7, 113.2, 124.5, 133.8, 134.0, 134.2, 134.3, 134.5, 134.7, 135.4, 135.6, 138.5, 148.1, 148.1, 151.0, 156.6, 156.7, 159.9. High-resolution ESI MS: *m/z* 821.3218 (calcd for $C_{54}H_{48}N_2O_2S_2$ + H, 821.3230).

4.2.11. 5,20-Bis(4-butylphenyl)-10,15-bis(methoxyphenyl)-21,23-dithiaporphyrin (23). Diol **15** (3.4 g, 8.2 mmol), thiophene **18** (3.5 g, 8.1 mmol), $TsOH{\cdot}H_2O$ (1.6 g, 8.1 mmol), and TCBQ (6.0 g, 24 mmol), were treated as described for the preparation of **19** to give 0.6 g (9%) of **23** as a purple solid; mp 248–250 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10 (6H, t, J = 7.0 Hz), 1.58–1.65 (4H, m), 1.90–1.97 (4H, m), 2.97 (6H, t, J = 8.0 Hz), 4.11 (6H, s), 7.39 (4H, d, J = 10.5 Hz), 7.65 (4H, d, J = 7.0 Hz), 8.18 (4H, d, J = 8.0 Hz), 8.21 (4H, d, J = 8.0 Hz), 8.72 (4H, m), 9.73 (4H, s). High-resolution ESI MS: *m/z* 821.3235 (calcd for $C_{54}H_{48}N_2O_2S_2$ + H, 821.3230).

4.2.12. 5,20-Bis(2-thienyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (24). Dithiaporphyrin **19** (0.5 g, 0.69 mmol) was dissolved in 100 mL of CH_2Cl_2 and BBr_3 (1.3 mL, 14 mmol) was added at 0 °C. The resulting solution was stirred for 5 h at ambient temperature. The reaction mixture was added to 200 mL of EtOAc and 200 mL of saturated $NaHCO_3$. The organic layer was separated and washed three times with brine, dried over $MgSO_4$, and concentrated. The crude solid was washed with 25% EtOAc/hexanes several times to give 0.42 g (87%) of **24** as a dark blue solid; mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.26 (4H, d, J = 8.4 Hz), 7.62 (2H, d, J = 4.2 Hz), 8.01 (2H, br s), 8.01 (4H, d, J = 7.6 Hz), 8.18 (2H, d, J = 5.2 Hz), 8.62 (2H, d, J = 4.8 Hz), 8.75 (2H, d, J = 4.4 Hz), 9.72 (2H, s), 9.88 (2H, s), 10.28 (2H, br s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 114.9, 125.1, 127.6, 130.3, 130.8, 133.7, 134.0, 135.1, 135.6, 135.8, 136.4, 140.5, 147.5, 147.7, 156.0, 156.2, 158.0. High-resolution Q-TOF MS: *m/z* 693.0754 (calcd for $C_{40}H_{24}N_2O_2S_4$ + H, 693.0799).

4.2.13. 5,20-Bis(4-methylphenyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (25). Dithiaporphyrin **20** (240 mg, 0.33 mmol) was treated with BBr_3 (0.31 mL, 3.2 mmol) as described for the preparation of **24** to give 0.20 g (87%) of **25** as a dark blue solid; mp > 300 °C. ^{1}H NMR (400 MHz, 1:1 $CDCl_3/CD_3OD$): δ 2.60 (6H, s), 7.17 (4H, d, J = 8.4 Hz), 7.51 (4H, d, J = 7.6 Hz), 7.97 (4H, d, J = 8.4 Hz), 8.00 (4H, d, J = 8.0 Hz), 8.56 (2H, d, J = 4.4 Hz), 8.60 (2H, d, J = 4.4 Hz), 9.57 (2H, s), 9.63 (2H, s). ^{13}C NMR (75 MHz, 1:1:1 $CDCl_3$/CD_3OD/DMSO-d_6): δ 26.6, 120.1, 133.6, 137.1, 139.1, 139.3, 139.5, 139.8, 140.8, 152.6, 153.1, 161.4, 161.6, 163.0. High-resolution ESI MS: *m/z* 709.1992 (calcd for $C_{46}H_{32}N_2O_2S_2$ + H, 709.1983).

4.2.14. 5,20-Bis(4-ethylphenyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (26). Dithiaporphyrin **21** (400 mg, 0.52 mmol) was treated with BBr_3 (0.50 mL, 5.2 mmol) as described for the preparation of **24** to give 0.35 g (91%) of **26** as a dark blue solid; mp > 300 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.52 (6H, t, J = 8.0 Hz), 3.01 (4H, q, J = 7.5 Hz), 7.33 (4H, d, J = 8.0 Hz), 7.69 (4H, d, J = 7.5 Hz), 8.06 (4H, d, J = 8.0 Hz), 8.08 (4H, d, J = 7.5 Hz), 8.59 (2H, d, J = 4.5 Hz), 8.64 (2H, d, J = 4.5 Hz), 9.68 (2H, m), 9.72 (2H, s). High-resolution ESI MS: *m/z* 737.2285 (calcd for $C_{48}H_{36}N_2O_2S_2$ + H, 737.2291).

4.2.15. 5,20-Bis(4-*tert*-butylphenyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (27). Dithiaporphyrin **22** (500 mg, 0.61 mmol) was treated with BBr_3 (0.60 mL, 6.1 mmol) as described for the preparation of **24** to give 0.43 g (89%) of **27** as a dark blue solid; mp > 300 °C. ^{1}H NMR (400 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.60 (18H, s), 7.29 (4H, d, J = 7.6 Hz), 7.83 (4H, d, J = 7.6 Hz), 8.08 (4H, d, J = 7.6 Hz), 8.15 (4H, d, J = 7.6 Hz), 8.69 (2H, d, J = 3.6 Hz), 8.71 (2H, d, J = 4.4 Hz), 9.72 (2H, s), 9.76 (2H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 35.7, 39.1, 119.0, 128.8, 136.8, 138.4, 138.5, 138.6, 138.7, 138.8, 139.9, 140.0, 142.5, 152.0, 152.4, 155.5, 160.8, 161.0, 161.9. High-resolution ESI MS: *m/z* 793.2932 (calcd for $C_{52}H_{44}N_2O_2S_2$ + H, 793.2917).

4.2.16. 5,20-Bis(4-butylphenyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (28). Dithiaporphyrin **23** (430 mg, 0.52 mmol) was treated with BBr_3 (0.50 mL, 5.2 mmol) as described for the preparation of **24** to give 0.38 g (92%) of **28** as dark blue solid; mp > 300 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.06 (6H, t, J = 7.0 Hz), 1.52–1.62 (4H, m), 1.86–1.93 (4H, m), 2.95 (6H, t, J = 8.0 Hz), 4.11 (6H, s), 7.35 (4H, d, J = 6.5 Hz), 7.61–7.70 (4H, m), 8.12 (4H, d, J = 8.0 Hz), 8.15 (4H, d, J = 7.5 Hz), 8.69 (2H, s), 8.73 (4H, d, J = 4.0 Hz), 9.70–9.80 (4H, m). High-resolution ESI MS: *m/z* 793.2908 (calcd for $C_{52}H_{44}N_2O_2S_2$ + H, 793.2917).

4.2.17. Diethyl 5,20-bis(2-thienyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (29). Dithiaporphyrin **24** (0.29 g, 0.42 mmol), K_2CO_3 (2.9 g, 21 mmol), ethyl bromoacetate (4.64 mL, 42 mmol) in 60 mL of acetone were heated at reflux for 15 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 was colorless. The combined filtrates were concentrated. The crude product was washed with MeOH to give 0.27 g (75%) of **29** as a purple solid; mp 186–188 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (6H, t, J = 7.2 Hz), 4.43 (4H, q, J = 6.8 Hz), 4.93 (4H, s), 7.36 (4H, d, J = 8.4 Hz), 7.57 (2H, dd, J = 5.2, 3.2 Hz), 7.93 (2H, d, J = 5.2 Hz), 7.96 (2H, d, J = 3.2 Hz), 8.17 (4H, d, J = 3.2 Hz), 8.67 (2H, d, J = 4.4 Hz), 8.87 (2H, d, J = 4.8 Hz), 9.66 (2H, s), 9.92 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 61.8, 65.9, 114.0, 125.7, 127.1, 129.0, 129.2, 133.5, 133.6, 134.4, 134.5, 134.7, 134.8, 134.9, 135.6, 135.7, 135.9, 141.9, 148.5, 149.0, 156.9, 157.4, 158.3, 169.1. High-resolution Q-TOF MS: *m/z* 865.1432 (calcd for $C_{48}H_{36}N_2O_6S_4$ + H, 865.1456).

4.2.18. Diethyl 5,20-bis(4-methylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (30). Dithiaporphyrin **25** (0.22 g, 0.31 mmol), K_2CO_3 (1.07 g, 7.8 mmol), and ethyl bromoacetate (0.86 mL, 7.8 mmol) in 60 mL of acetone were treated as described

for the preparation of **29** to give 0.24 g (88%) of **30** as a purple solid; mp 134–136 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (6H, t, J = 7.0 Hz), 2.71 (6H, s), 4.42 (4H, q, J = 7.0 Hz), 4.92 (4H, s), 7.36 (4H, d, J = 8.5 Hz), 7.62 (4H, d, J = 7.5 Hz), 8.13 (4H, d, J = 8.0 Hz), 8.17 (4H, d, J = 8.5 Hz), 8.67 (2H, d, J = 4.5 Hz), 8.69 (2H, d, J = 4.0 Hz), 9.68 (2H, s), 9.69 (2H, s). ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.5, 21.8, 61.8, 66.0, 114.0, 128.4, 133.6, 134.4, 134.4, 134.6, 134.8, 135.0, 135.5, 135.6, 135.6, 138.0, 138.6, 148.1, 156.7, 156.8, 158.2, 169.2. High-resolution ESI MS: *m/z* 881.2709 (calcd for $C_{54}H_{44}N_2O_6S_2$ + H, 881.2719).

4.2.19. Diethyl 5,20-bis(4-ethylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (31). Dithiaporphyrin **26** (0.35 g, 0.47 mmol), K_2CO_3 (0.66 g, 4.8 mmol), and ethyl bromoacetate (0.50 mL, 4.8 mmol) in 50 mL of acetone were treated as described for the preparation of **29** to afford 0.36 g (83%) of **31** as a purple solid; mp 238–240 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (6H, t, J = 7.0 Hz), 1.54 (6H, t, J = 7.5 Hz), 3.02 (4H, q, J = 8.0 Hz), 4.42 (4H, q, J = 7.0 Hz), 4.93 (4H, s), 7.39 (4H, d, J = 8.0 Hz), 7.67 (4H, d, J = 7.5 Hz), 8.19 (4H, d, J = 8.0 Hz), 8.21 (4H, d, J = 8.0 Hz), 8.70 (2H, d, J = 4.5 Hz), 8.73 (2H, d, J = 4.0 Hz), 9.71 (2H, m), 9.73 (2H, s). High-resolution ESI MS: *m/z* 909.3015 (calcd for $C_{56}H_{48}N_2O_6S_2$ + H, 909.3027).

4.2.20. Diethyl 5,20-bis(4-*tert*-butylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (32). Dithiaporphyrin **27** (0.40 g, 0.50 mmol), K_2CO_3 (0.70 g, 5.0 mmol), and ethyl bromoacetate (0.6 mL, 5.0 mmol) in 50 mL of acetone were treated as described for the preparation of **29** to give 0.37 g (76%) of **32** as a purple solid; mp 170–172 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (6H, t, J = 7.6 Hz), 1.62 (18H, s), 4.42 (4H, q, J = 7.2 Hz), 4.93 (4H, s), 7.36 (4H, d, J = 8.4 Hz), 7.83 (4H, d, J = 8.0 Hz), 8.18 (8H, d, J = 8.0 Hz), 8.68 (2H, d, J = 4.4 Hz), 8.73 (2H, d, J = 4.4 Hz), 9.68 (2H, s), 9.72 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 31.8, 35.1, 61.7, 65.9, 113.9, 124.5, 133.4, 134.3, 134.4, 134.8, 135.0, 135.3, 135.5, 135.7, 138.4, 148.0, 148.1, 151.0, 156.6, 158.2, 169.1. High-resolution Q-TOF MS: *m/z* 793.2932 (calcd for $C_{52}H_{44}N_2O_2S_2$ + H, 793.2917).

4.2.21. Diethyl 5,20-bis(4-butylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (33). Using core-modified porphyrin **28** (0.35 g, 0.44 mmol), K_2CO_3 (0.61 g, 4.4 mmol), and ethyl bromoacetate (0.49 mL, 4.4 mmol) in 50 mL of acetone, **33** was prepared as described for the preparation of **29** to give 0.32 g (75%) of **33** as a purple solid; mp 120–122 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10 (6H, t, J = 7.0 Hz), 1.42 (6H, t, J = 7.0 Hz), 1.58–1.66 (4H, m), 1.90–2.00 (4H, m), 2.97 (6H, t, J = 7.5 Hz), 4.42 (4H, q, J = 7.0 Hz), 4.93 (4H, s), 7.29 (2H, d, J = 7.5 Hz), 7.39 (2H, d, J = 8.5 Hz), 7.65 (4H, d, J = 7.5 Hz), 8.16–8.22 (8H, m), 8.69 (2H, d, J = 4.5 Hz), 8.73 (2H, d, J = 4.5 Hz), 9.70–9.74 (4H, m). High-resolution ESI MS: *m/z* 965.3658 (calcd for $C_{60}H_{56}N_2O_6S_2$ + H, 965.3653).

4.2.22. 2-(1-Hydroxy-1-phenylmethyl)-5-[1-hydroxy-1-(2-thienyl)methyl]thiophene (36). 2-[1-(*tert*-Butyldimethylsilyloxy)-1-phenyl methyl]thiophene[40] (**35**, 5.0 g, 16 mmol) was added to a solution of *n*-BuLi (11 mL of 1.6 M in hexanes, 18 mmol) and TMEDA (2.5 mL, 16 mmol) in 150 mL of hexanes under an Ar atmosphere. The reaction mixture was stirred at ambient temperature for 30 min, and was transferred via cannula to a pressure-equalizing addition funnel. The suspension of 2-lithio **35** was then added dropwise to a solution of 2-thiophenecarboxaldehyde (1.38 mL, 15 mmol) in 150 mL of anhydrous THF at 0 °C, which was degassed with Ar for 15 min. After addition was complete, the mixture was warmed to ambient temperature and 300 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 × 300 mL). The combined organic extracts were washed with water (3 × 300 mL) and brine (300 mL), dried over $MgSO_4$, and concentrated to give yellow oil. The oil was dissolved in a 1 M solution of Bu_4NF in THF (95 mL, 95 mmol) and stirred at ambient temperature for 1 h at which point 100 mL of saturated aqueous NH_4Cl was added. The resulting mixture was extracted with ether (4 × 100 mL). The combined organic extracts were washed with water (3 × 400 mL) and brine (400 mL), dried over $MgSO_4$, and concentrated to brown oil. The crude diol was purified by column chromatography on SiO_2 eluted with 25% EtOAc/hexanes to give 3.6 g (73%) of predominantly one diastereomer of **36** as yellow amorphous powder. IR (KBr): 3468, 3085, 1455, 1391 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, 2H, J = 8.0 Hz), 7.30–7.36 (m, 2H), 7.25–7.30 (m, 1H), 7.21–7.30 (m, 1H), 6.97 (br s, 1H), 6.93 (dd, 1H, J = 5.4, 2.3 Hz), 6.80 (d, 1H, J = 3.3 Hz), 6.69 (d, 1H, J = 3.2 Hz), 6.17 (s, 1H), 5.95 (s, 1H), 2.54 (s, 1H), 2.41 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.5, 147.3, 147.0, 143.0, 128.7, 128.2, 126.9, 126.4, 125.7, 125.2, 124.8, 124.6, 72.7, 68.8. High-resolution Q-TOF MS: *m/z* 325.0314 (calcd for $C_{16}H_{14}O_2S_2$ + Na, 325.0333).

4.2.23. 2-[1-(4-*tert*-Butylphenyl)-1-hydroxymethyl]-5-(1-hydroxy-1-phenylmethyl)-thiophene (37). Thiophene **35** (6.0 g, 19.7 mmol), *n*-BuLi (13.5 mL of 1 M in hexanes, 22 mmol), TMEDA (3.6 mL, 24 mmol), and 4-*tert*-butylbenzaldehyde (4.0 mL, 24 mmol) were treated as described for the preparation of **36** to give 3.0 g (43%) of **37** as colorless oil. IR (film): 3367, 2962, 2869, 1711 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (9H, s), 5.94 (1H, s), 5.97 (1H, s), 6.70 (2H, s), 7.32–7.50 (9H, m). ^{13}C NMR (126 MHz, $CDCl_3$): δ 31.4, 34.6, 72.3, 72.4, 124.3, 124.3, 124.5, 124.5, 125.4, 126.1, 126.2, 126.4, 126.4, 127.9, 128.5, 140.0, 143.0, 147.9, 148.2, 150.9. High-resolution ESI MS: *m/z* 375.1387 (calcd for $C_{22}H_{24}O_2S$ + Na, 375.1389).

4.2.24. 5-Phenyl-20-(2-thienyl)-10,15-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (38). Diol **36** (3.1 g, 10 mmol) and thiophene **18** (4.7 g, 10 mmol) in 600 mL of CH_2Cl_2 were treated with $BF_3{\cdot}OEt_2$ (0.57 mL, 4.5 mmol) and TCBQ (13 g, 52 mmol) as described for the preparation of **19** to give 0.84 g (11%) of **38** as a purple solid;

mp > 300 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.11 (6H, s), 7.36 (4H, d, J = 8.1 Hz), 7.57 (1H, br s), 7.81 (3H, br s), 7.92 (1H, d, J = 4.8 Hz), 7.96 (1H, br s), 8.17 (4H, d, J = 8.4 Hz), 8.23 (2H, br s), 8.61 (1H, br s), 8.69 (2H, br s), 8.88 (1H, d, J = 4.5 Hz), 9.67 (1H, d, J = 5.1 Hz), 9.70 (2H, br s), 9.91 (1H, d, J = 5.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 113.2, 127.0, 127.6, 128.2, 128.9, 133.3, 133.8, 134.3, 134.8, 135.4, 135.6, 135.7, 141.4, 160.0, 165.2. High-resolution Q-TOF MS: *m/z* 715.1615 (calcd for $C_{44}H_{30}N_2O_2S_3$ + H, 715.1547).

4.2.25. 20-(4-*tert*-Butylphenyl)-5-phenyl-15,20-bis(4-methoxyphenyl)-21,23-dithiaporphyrin (39). Diol **37** (3.9 g, 11 mmol) and thiophene **18** (5.0 g, 11 mmol) in 600 mL of CH_2Cl_2 were treated with $TsOH{\cdot}H_2O$ (3.1 g, 11 mmol) and TCBQ (8.1 g, 33 mmol) as described for the preparation of **19** to give 1.2 g (14%) of **39** as a purple solid; mp 210–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.6 (9H, s), 4.0 (6H, s), 7.4 (3H, m), 7.62–7.98 (9H, m), 8.07–8.32 (5H, m), 8.60–8.80 (4H, m), 9.64–9.78 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.8, 35.1, 55.7, 113.2, 114.0, 120.1, 124.6, 127.3, 127.6, 128.1, 128.2, 128.4, 133.7, 133.8, 133.9, 134.2, 134.3, 134.4, 134.5, 134.6, 134.7, 134.7, 134.9, 135.5, 135.5, 135.8, 138.4, 141.4, 142.7, 142.8, 147.8, 147.9, 148.0, 148.2, 151.1, 151.1, 156.4, 156.5, 156.7, 156.8, 158.8, 159.9. High-resolution ESI MS: *m/z* 765.2604 (calcd for $C_{50}H_{40}N_2O_2S_2$ + H, 765.2631).

4.2.26. 5-Phenyl-20-(2-thienyl)-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (40). Dithiaporphyrin **38** (300 mg, 0.42 mmol) was treated with BBr_3 (0.79 mL, 8.3 mmol) as described for the preparation of **24** to give 0.27 g (94%) of **40** as dark purple solid; mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.26 (2H, d, J = 8.4 Hz), 7.26 (2H, d, J = 8.4 Hz), 7.61 (1H, dd, J = 5.0, 4.0 Hz), 7.83 (3H, m), 8.00 (1H, d, J = 3.6 Hz), 8.02 (2H, d, J = 8.8 Hz), 8.02 (2H, d, J = 8.4 Hz), 8.17 (3H, m), 8.53 (1H, d, J = 4.4 Hz), 8.63 (2H, dd, J = 4.4, 2.4 Hz), 8.77 (1H, d, J = 4.8 Hz), 9.64 (1H, d, J = 5.2 Hz), 9.75 (2H, s), 9.88 (1H, d, J = 5.2 Hz), 10.26 (2H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 115.3, 125.1, 128.2, 128.8, 130.7, 131.2, 134.0, 134.2, 135.3, 136.0, 136.3, 140.5, 140.8, 156.0, 156.2, 158.2. High-resolution Q-TOF MS: *m/z* 687.1235 (calcd for $C_{42}H_{26}N_2O_2S_3$ + H, 687.1234).

4.2.27. 20-(4-*tert*-Butylphenyl)-5-phenyl-10,15-bis(4-hydroxyphenyl)-21,23-dithiaporphyrin (41). Dithiaporphyrin **39** (620 mg, 0.81 mmol) was treated with BBr_3 (0.80 mL, 8.1 mmol) as described for the preparation of **24** to give 0.57 g (95%) of **41** as dark purple; mp > 300 °C. ^{1}H NMR (400 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.59 (9H, s), 7.28 (3H, t, J = 8.4 Hz), 7.62 (1H, t, J = 8.4 Hz), 7.68–7.74 (3H, m), 7.77–7.87 (5H, m), 8.08 (1H, d, J = 8.0 Hz), 8.14 (2H, d, J = 7.2 Hz), 8.20–8.25 (2H, m), 8.63–8.75 (4H, m), 9.67 (4H, q, J = 5.2 Hz), 9.73 (1H, q, J = 4.8 Hz), 9.78 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.6, 35.1, 114.9, 115.5, 121.8, 124.8, 126.5, 127.8, 128.4, 128.7, 132.7, 134.4, 134.6, 134.7, 134.8, 135.7, 136.0, 138.4, 141.4, 142.7, 148.0, 148.0, 148.1, 151.5, 156.4, 156.7, 156.8, 157.0, 157.9. High-resolution ESI MS: *m/z* 737.2291 (calcd for $C_{48}H_{36}N_2O_2S_2$ + H, 737.2297).

4.2.28. Diethyl 5-phenyl-20-(2-thienyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (42). Dithiaporphyrin **40** (0.32 g, 0.47 mmol), K_2CO_3 (0.64 g, 4.7 mmol), and 0.52 mL of ethyl bromoacetate in 50 mL of acetone were treated as described for the preparation of **29** to afford 0.36 g (90%) of **42** as a purple solid; mp 198–200 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (6H, t, J = 7.2 Hz), 4.43 (4H, q, J = 6.8 Hz), 4.93 (4H, s), 7.37 (4H, d, J = 8.4 Hz), 7.81 (3H, m), 7.92 (1H, d, J = 4.8 Hz), 7.96 (1H, d, J = 3.2 Hz), 8.17 (4H, d, J = 8.4 Hz), 8.24 (2H, m), 8.66 (1H, d, J = 4.4 Hz), 8.68 (2H, m), 8.89 (1H, d, J = 4.4 Hz), 9.68 (1H, d, J = 4.8 Hz), 9.69 (2H, s), 9.92 (1H, d, J = 4.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 114.0, 125.4, 127.0, 127.6, 128.2, 128.9, 133.4, 134.0, 134.2, 134.3, 134.3, 134.4, 134.5, 134.6, 134.8, 135.4, 135.6, 135.7, 135.8, 141.3, 142.0, 147.8, 148.2, 148.5, 149.1, 156.7, 156.9, 157.3, 158.3, 169.1. High-resolution Q-TOF MS: *m/z* 859.1964 (calcd for $C_{50}H_{38}N_2O_6S_3$ + H, 859.1970).

4.2.29. Diethyl 20-(4-*tert*-butylphenyl)-5-phenyl-10,15-bis(4-carboxylatomethoxy-phenyl)-21,23-dithiaporphyrin (43). Dithiaporphyrin **41** (0.55 g, 0.75 mmol), K_2CO_3 (1.0 g, 7.5 mmol), and ethyl bromoacetate (0.8 mL, 7.5 mmol) were treated as described for the preparation of **29** to give 0.58 g (86%) of **43** as a purple solid; mp 136–138 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (6H, t, J = 7.2 Hz), 1.62 (9H, s), 4.30 (4H, q, J = 7.2 Hz), 4.84 (4H, s), 7.38 (3H, br s), 7.69–7.93 (8H, m), 8.19 (3H, d, J = 8.0 Hz), 8.25 (2H, m), 8.67–8.79 (4H, m), 9.65–9.79 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 31.8, 35.1, 61.6, 65.7, 113.9, 114.8, 120.6, 124.6, 127.6, 128.2, 128.6, 133.3, 133.4, 134.3, 134.3, 134.4, 134.4, 134.5, 134.8, 134.8, 135.0, 135.5, 135.9, 138.3, 141.3, 142.7, 147.7, 147.7, 148.0, 148.2, 151.1, 156.3, 156.4, 156.8, 157.0, 158.1, 169.0. High-resolution ESI MS: *m/z* 909.3027 (calcd for $C_{56}H_{48}N_2O_6S_2$ + H, 909.3022).

4.2.30. 2,5-Bis[1-(4-*tert*-butyldimethylsilyloxyphenyl)-1-hydroxymethyl]thiophene (45). Thiophene (3.3 g, 41 mmol) was treated with *n*-BuLi (51.5 mL of 1.6 M in hexanes, 82 mmol), TMEDA (13.1 mL, 86 mmol), and **44** (19.0 g, 2.0 mmol) as described for the preparation of **11** to give 6.5 g (28%) of **45** as colorless oil. IR (film): 3410 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.21 (12H, s), 1.00 (18H, s), 5.94 (2H, br s), 6.70 (2H, br s), 6.84 (4H, d, J = 8.4 Hz), 7.30 (4H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.3, 18.3, 25.8, 72.4, 120.2, 124.4, 127.8, 135.9, 148.4, 155.6. High-resolution ESI MS: *m/z* 579.2389 (calcd for $C_{30}H_{44}O_4S_1Si_2$ + Na, 579.2391).

4.2.31. Diethyl 2,5-bis[1-(4-carboxylatomethoxy)phenyl-1-hydroxymethyl]thiophene (46). Diol **45** (6.5 g, 11.7 mmol) and TBAF (13 mL of 1 M in THF, 13 mmol) were dissolved in 15 mL of THF, then the solution was stirred for 1 h with several sonications. The solid product was collected by filtration and washed with acetone to give 2.5 g (65%) of 2,5-bis-[1-(4-hydroxyphenyl)-

1-hydroxymethyl]thiophene as a white amorphous powder, which was used for further reaction without further purification. Using 2,5-bis-[1-(4-hydroxyphenyl)-1-hydroxymethyl]-thiophene(2.5 g, 7.6 mmol), K_2CO_3 (4.2 g, 30 mmol), and ethyl bromoacetate (3.4 mL, 30 mmol) in 50 mL of acetone were treated as described for the preparation of **29** to give 0.37 g (76%) of **46** as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (6H, t, J = 7.6 Hz), 4.27 (4H, q, J = 7.2 Hz), 4.61 (4H, s), 5.93 (2H, s), 6.68 (2H, s), 6.88 (4H, d, J = 8.8 Hz), 7.34 (4H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 61.5, 65.6, 72.2, 114.8, 124.4, 127.8, 136.5, 148.3, 157.8, 169.0. High-resolution ESI MS: *m/z* 523.1397 (calcd for $C_{26}H_{28}O_8S_1$ + Na, 523.1397).

4.2.32. 2-(1-Hydroxy-1-phenylmethyl)-5-[1-hydroxy-1-(2,4,6-trimethylphenyl)methyl]-thiophene (47). Thiophene **35** (8.0 g, 26 mmol) was treated with *n*-BuLi (20 mL of 1 M in hexanes, 32 mmol), TMEDA (5.0 mL, 33 mmol), and mesitaldehyde (4.6 mL, 32 mmol) as described for the preparation of **36** to give 6.0 g (68%) of **47** as colorless oil. IR (film): 3402, 3029, 2920, 1708, 1610 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.19 (3H, s), 2.21 (6H, s), 5.82 (1H, d, J = 3.5 Hz), 5.93 (1H, t, J = 4.0 Hz), 6.09 (1H, q, J = 4.5 Hz), 6.13 (1H, s), 6.32 (1H, d, J = 2.5 Hz), 6.61 (1H, dd, J = 16.5, 3.0 Hz), 6.78 (2H, s), 7.23 (1H, t, J = 7.0 Hz), 7.31 (2H, t, J = 7.5 Hz), 7.38 (2H, d, J = 5.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.2, 20.4, 67.3, 70.7, 121.7, 121.8, 123.2, 126.1, 127.0, 128.0, 129.4, 135.9, 136.1, 137.1, 145.0, 148.2, 148.3, 148.4, 148.5. High-resolution EI MS: *m/z* 338.1332 (calcd for $C_{21}H_{22}O_2S_1$, 338.1335).

4.2.33. 2-(1-Phenyl-1-pyrrolomethyl)-5-[1-pyrrolo-1-(2,4,6-trimethylphenyl)methyl]-thiophene (48). Diol **47** (3.5 g, 10.2 mmol) was dissolved in excess pyrrole (28 mL), and the resulting solution was degassed with Ar. $BF_3{\cdot}OEt_2$ was added (0.3 mL, 2.0 mmol), and the resulting mixture was stirred for 1 h at ambient temperature. The reaction was stopped by addition of CH_2Cl_2 (200 mL) followed by 40% NaOH (50 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 3.5 g (78%) of **48** as yellow oil. IR (film): 3410, 2968, 2919, 1727 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (6H, s), 2.51 (3H, s), 5.80 (1H, s), 6.15 (1H, s), 6.23 (1H, s), 6.28 (1H, s), 6.39 (2H, br s), 6.85 (2H, M), 6.90 (2H, d, J = 3.0 Hz), 7.09 (2H, s), 7.44–7.64 (5H, m), 8.07 (1H, br s), 8.12 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9, 20.9, 29.4, 40.7, 46.1, 103.8, 107.0, 107.6, 107.9, 108.4, 108.5, 116.4, 117.3, 125.1, 125.3, 127.1, 128.5, 128.7, 130.4, 132.1, 133.2, 135.7, 136.7, 137.5, 142.9, 144.8, 144.9, 145.1, 145.2. High-resolution ESI MS: *m/z* 437.2055 (calcd for $C_{29}H_{28}N_2S$ + H, 437.2046).

4.2.34. 2,5-Bis[1-pyrrolo-1-(2,4,6-trimethylphenyl)methyl]-thiophene (49). Diol **16** (5.1 g, 13 mmol) in 35 mL of pyrrole was treated with 0.3 mL of $BF_3{\cdot}OEt_2$ (2.7 mmol) as for the preparation of **48** to give 5.0 g (78%) of **49** as yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (12H, s), 2.19 (6H, s), 5.66 (2H, s), 5.85 (2H, s), 5.90 (2H, s), 6.47 (2H, s), 6.66 (2H, s), 6.80 (4H, s), 10.52 (2H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9, 40.7, 107.0, 107.3, 108.5, 116.4, 125.0, 132.2, 135.8, 136.6, 137.4, 144.0. High-resolution ESI MS: *m/z* 479.2515 (calcd for $C_{32}H_{34}N_2S$ + H, 479.2512).

4.2.35. Diethyl 5-phenyl-20-(2,4,6-trimethylphenyl)-10,15-bis(4-carboxylatomethoxy-phenyl)-21,23-dithiaporphyrin (50). Diol **46** (4.7 g, 9.3 mmol) and thiophene **48** (4.1 g, 9.3 mmol) in 250 mL of CH_2Cl_2 were treated with $TsOH{\cdot}H_2O$ (1.8 g, 9.3 mmol) and TCBQ (6.9 g, 28 mmol) as described for the preparation of **19** except SiO_2 was used as a stationary phase instead of basic alumina to give 1.1 g (13%) of **50** as a purple solid; mp 152–154 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (6H, t, J = 7.6 Hz), 1.87 (6H, s), 2.62 (3H, s), 4.41 (4H, q, J = 6.8 Hz), 4.92 (4H, s), 7.29 (2H, s), 7.35 (4H, d, J = 8.4 Hz), 7.75–7.81 (3H, m), 8.16 (2H, d, J = 8.4 Hz), 8.18 (2H, d, J = 8.8 Hz), 8.22 (1H, s), 8.24 (1H, d, J = 2.8 Hz), 8.48 (1H, d, J = 4.4 Hz), 8.62 (1H, d, J = 4.4 Hz), 8.66 (2H, q, J = 4.4 Hz), 9.41 (1H, d, J = 5.2 Hz), 9.61 (1H, d, J = 5.2 Hz), 9.67 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 21.6, 61.7, 65.9, 113.9, 127.5, 128.1, 133.0, 133.4, 133.6, 133.7, 133.8, 134.2, 134.5, 134.6, 134.8, 135.0, 135.0, 135.5, 135.5, 135.7, 137.7, 138.1, 138.9, 141.4, 147.2, 148.0, 148.1, 148.1, 156.6, 156.7, 156.8, 158.2, 169.1. High-resolution ESI MS: *m/z* 895.2903 (calcd for $C_{55}H_{46}N_2O_6S_2$ + H, 895.2887).

4.2.36. Diethyl 5,20-bis(2,4,6-trimethylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (51). Compounds **46** (2.8 g, 5.6 mmol) and **49** (2.7 g, 5.6 mmol) in 500 mL of CH_2Cl_2 were treated with $TsOH{\cdot}H_2O$ (1.1 g, 5.6 mmol) and TCBQ (4.1 g, 17 mmol) as described for the preparation of **19** to give 1.1 g (22%) of **51** as a purple solid; mp 160–162 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (6H, t, J = 7.0 Hz), 1.90 (12H, s), 2.63 (6H, s), 4.42 (4H, q, J = 7.0 Hz), 4.93 (4H, s), 7.30 (4H, s), 7.36 (4H, d, J = 8.0 Hz), 8.16 (4H, d, J = 8.5 Hz), 8.48 (2H, d, J = 4.0 Hz), 8.62 (2H, d, J = 4.0 Hz), 9.35 (2H, s), 9.66 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 21.6, 21.7, 61.7, 65.9, 113.9, 128.1, 132.8, 133.3, 133.5, 134.6, 134.9, 135.3, 135.4, 137.6, 138.1, 138.9, 147.3, 148.0, 156.6, 156.8, 158.2, 169.1. High-resolution Q-TOF MS: *m/z* 937.3315 (calcd for $C_{58}H_{52}N_2O_6S_2$ + H, 937.3345).

4.2.37. 5,20-Bis(2-thienyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (2). Core-modified porphyrin **29** (0.22 g, 0.25 mmol) was dissolved in 50 mL of THF and 20 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 8.6 mL of acetic acid. The reaction mixture was diluted with 100 mL of 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 portion of hexanes/MeOH to give 0.18 g (87%) of dithiaporphyrin **2** as a purple solid; mp 236–238 °C. ^{1}H NMR

(400 MHz, $CDCl_3$): δ 4.93 (4H, s), 7.43 (4H, d, J = 8.4 Hz), 7.61 (2H, dd, J = 4.8, 3.6 Hz), 8.00 (2H, d, J = 2.8 Hz), 8.09 (2H, d, J = 5.6 Hz), 8.19 (4H, d, J = 8.4 Hz), 8.58 (2H, d, J = 4.4 Hz), 8.76 (2H, d, J = 4.4 Hz), 9.68 (2H, s), 9.90 (2H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 65.5, 114.6, 126.1, 128.4, 131.1, 133.4, 134.5, 135.0, 135.3, 135.7, 136.0, 136.6, 137.0, 141.0, 148.2, 148.3, 156.6, 156.9, 158.8, 171.0. High-resolution Q-TOF MS: *m/z* 809.0901 (calcd for $C_{44}H_{28}N_2O_6S_4$ + H, 809.0908). Anal. C, H, N.

4.2.38. 5-Phenyl-20-(2-thienyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (3). Dithiaporphyrin **42** (0.18 g, 0.21 mmol) in 40 mL of THF was treated with 20 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.15 g (89%) of dithiaporphyrin **3** as a purple solid; mp 226–228 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 4.90 (4H, s), 7.37 (4H, d, J = 8.5 Hz), 7.55 (2H, dd, J = 5.0, 4.0 Hz), 7.79 (3H, m), 7.93 (2H, d, J = 3.5 Hz), 8.14 (4H, d, J = 8.5 Hz), 8.20 (2H, m), 8.63 (1H, d, J = 4.5 Hz), 8.66 (2H, d, J = 4.5 Hz), 8.85 (1H, d, J = 5.0 Hz), 9.67 (1H, d, J = 5.0 Hz), 9.69 (2H, s), 9.91 (1H, d, J = 5.5 Hz). ^{13}C NMR (75 MHz, 1:1 $CDCl_3/CD_3OD$): δ 65.6, 114.1, 125.7, 127.3, 127.8, 128.5, 129.3, 133.6, 134.3, 134.4, 134.5, 134.7, 134.9, 135.7, 135.9, 136.0, 141.3, 141.9, 147.9, 148.3, 148.6, 149.1, 156.9, 157.0, 157.5, 158.5, 171.5. High-resolution Q-TOF MS: *m/z* 803.1379 (calcd for $C_{46}H_{30}N_2O_6S_3$ + H, 803.1344). Anal. C, H, N.

4.2.39. 5,20-Bis(2,4,6-trimethylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (4). Core-modified porphyrin **51** (0.41 g, 0.46 mmol) in 80 mL of THF was treated with 40 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.35 g (87%) of dithiaporphyrin **4** as a purple solid; mp > 300 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3$/DMSO-d_6): δ 1.83 (12H, s), 2.57 (6H, s), 4.91 (4H, s), 7.28 (4H, s), 7.38 (4H, d, J = 8.5 Hz), 8.14 (4H, d, J = 8.5 Hz), 8.39 (2H, d, J = 4.0 Hz), 8.59 (2H, d, J = 4.0 Hz), 9.31 (2H, s), 9.69 (2H, s). ^{13}C NMR (126 MHz, 1:1 $CDCl_3$/DMSO-d_6): δ 21.7, 21.8, 65.6, 114.4, 128.6, 132.8, 133.6, 133.9, 134.0, 135.0, 135.5, 135.6, 135.9, 137.4, 138.3, 138.4, 147.2, 147.9, 156.4, 156.5, 158.7, 170.8. High-resolution Q-TOF MS: *m/z* 881.2747 (calcd for $C_{54}H_{44}N_2O_6S_2$ + H, 881.2719). Anal. C, H, N.

4.2.40. 5-Phenyl-20-(2,4,6-trimethylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (5). Core-modified porphyrin **50** (0.50 g, 0.56 mmol) in 100 mL of THF was treated with 50 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.42 g (90%) of dithiaporphyrin **5** as a purple solid; mp > 300 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.84 (6H, s), 2.58 (3H, s), 4.89 (4H, s), 7.27 (2H, s), 7.37 (4H, d, J = 6.5 Hz), 7.78 (3H, s), 8.11–8.17 (4H, m), 8.19 (2H, br s), 8.47 (1H, d, J = 4.5 Hz), 8.62 (2H, s), 8.64 (1H, s), 9.42 (1H, d, J = 2.5 Hz), 9.62 (1H, d, J = 2.5 Hz), 9.70 (2H, s). ^{13}C NMR (126 MHz, 1:1 $CDCl_3/CD_3OD$): δ 21.2, 65.4, 113.9, 127.6, 128.2, 128.3, 133.3, 134.0, 134.1, 134.2, 134.5, 135.1, 135.5, 135.5, 135.7, 135.8, 136.0, 138.4, 138.7, 147.2, 148.0, 148.1, 148.2, 156.8, 158.4, 171.5. High-resolution ESI MS: *m/z* 839.2250 (calcd for $C_{51}H_{38}N_2O_6S_2$ + H, 839.2244). Anal. C, H, N.

4.2.41. 5,20-Bis(4-*tert*-butylphenyl)-15,20-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (6). Dithiaporphyrin **32** (0.35 g, 0.42 mmol) in 40 mL of THF was treated with 20 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.29 g (88%) of dithiaporphyrin **6**; mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.57 (18H, s), 5.00 (4H, s), 7.45 (4H, d, J = 8.4 Hz), 7.92 (4H, d, J = 7.6 Hz), 8.19 (4H, s), 8.21 (4H, s), 8.61 (4H, d, J = 4.4 Hz), 8.65 (4H, d, J = 4.4 Hz), 9.75 (4H, d, J = 6.8 Hz), 13.25 (2H, br s). ^{13}C NMR (75 MHz, DMSO-d_6): δ 32.0, 35.3, 65.6, 114.5, 125.1, 133.8, 134.2, 134.5, 135.2, 135.8, 136.3, 138.1, 147.6, 147.9, 151.3, 156.3, 156.5, 158.8, 170.8. High-resolution ESI MS: *m/z* 909.3027 (calcd for $C_{56}H_{48}N_2O_6S_2$ + H, 909.3029). Anal. C, H, N.

4.2.42. 20-(4-*tert*-Butylphenyl)-5-phenyl-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (7). Dithiaporphyrin **43** (0.50 g, 0.55 mmol) in 100 mL of THF was treated with 50 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.42 g (90%) of dithiaporphyrin **7**; mp 227–230 °C. ^{1}H NMR (500 MHz, 1:1 $CDCl_3$/DMSO-d_6): δ 1.57 (9H, s), 4.85 (4H, s), 7.39 (3H, m), 7.71–7.87 (9H, m), 8.11–8.17 (4H, m), 8.21–8.27 (2H, br s), 8.61–8.71 (4H, m), 9.63–9.77 (4H, m), 12.75–13.33 (2H, br s). ^{13}C NMR (75 MHz, 1:1 $CDCl_3$/DMSO-d_6): δ 31.7, 35.2, 65.6, 114.2, 114.9, 121.0, 124.8, 124.8, 127.8, 127.8, 128.3, 128.5, 128.8, 133.7, 133.8, 134.5, 134.6, 134.6, 134.7, 134.8, 134.9, 135.1, 135.8, 135.9, 136.2, 138.4, 138.4, 141.4, 141.4, 142.8, 147.9, 148.0, 148.2, 148.3, 151.5, 156.7, 156.8, 156.9, 157.0, 157.0, 157.4, 158.6, 171.6. High-resolution ESI MS: *m/z* 853.2401 (calcd for $C_{52}H_{40}N_2O_6S_2$ + H, 853.2403). Anal. C, H, N.

4.2.43. 5,20-Bis(4-methylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (8). Core-modified porphyrin **30** (0.18 g, 0.20 mmol) in 40 mL of THF was treated with 16 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.16 g (95%) of dithiaporphyrin **8**; mp > 300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.65 (6H, s), 4.89 (4H, s), 7.37 (4H, d, J = 7.0 Hz), 7.61 (4H, d, J = 7.0 Hz), 8.06 (4H, d, J = 7.0 Hz), 8.12 (4H, d, J = 7.5 Hz), 8.61 (4H, s), 9.66 (2H, s), 9.70 (2H, s), 13.07 (2H, s). ^{13}C NMR (126 MHz, 1:1 $CDCl_3$/DMSO-d_6): δ 14.5, 21.8, 61.8, 66.0, 114.0, 128.4, 133.6, 134.4, 134.4, 134.6, 134.8, 135.0, 135.5, 135.6, 135.6, 138.0, 138.6, 148.1, 156.7, 156.8, 158.2, 169.2. High-resolution ESI MS: *m/z* 825.2052 (calcd for $C_{50}H_{36}N_2O_6S_2$ + H, 825.2098). Anal. C, H, N.

4.2.44. 5,20-Bis(4-ethylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (9). Core-modified porphyrin **31** (0.35 g, 0.38 mmol) in 40 mL of THF was treated with 40 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.25 g (76%) of dithiaporphyrin **9**; mp 245–247 °C. ^{1}H NMR

(500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.51 (6H, t, $J = 7.5$ Hz), 2.99 (4H, q, $J = 8.0$ Hz), 4.91 (4H, s), 7.41 (4H, d, $J = 8.0$ Hz), 7.66 (4H, d, $J = 8.0$ Hz), 8.11 (4H, d, $J = 7.5$ Hz), 8.14 (4H, d, $J = 8.5$ Hz), 8.64 (2H, d, $J = 4.5$ Hz), 8.66 (2H, d, $J = 4.5$ Hz), 9.70 (2H, m), 9.71 (2H, s). High-resolution ESI MS: *m/z* 853.2401 (calcd for $C_{52}H_{40}N_2O_6S_2 + H$, 853.2394). Anal. C, H, N.

4.2.45. 5,20-Bis(4-butylphenyl)-10,15-bis(4-carboxylatomethoxyphenyl)-21,23-dithiaporphyrin (10). Core-modified porphyrin **33** (0.35 g, 0.36 mmol) in 40 mL of THF was treated with 40 mL of 1 M aqueous NaOH as described for the preparation of **2** to give 0.27 g (82%) of dithiaporphyrin **10**; mp 250–252 °C. ^1H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 1.07 (6H, t, $J = 7.0$ Hz), 1.56–1.62 (4H, m), 1.87–1.94 (4H, m), 2.95 (6H, t, $J = 7.5$ Hz), 4.91 (4H, s), 7.43 (4H, d, $J = 8.0$ Hz), 7.66 (4H, d, $J = 7.5$ Hz), 8.14 (4H, d, $J = 7.5$ Hz), 8.19 (4H, d, $J = 8.0$ Hz), 8.68 (2H, d, $J = 4.5$ Hz), 8.70 (2H, d, $J = 4.5$ Hz), 9.75 (2H, s), 9.76 (2H, s). High-resolution ESI MS: *m/z* 909.3027 (calcd for $C_{56}H_{48}N_2O_6S_2 + H$, 909.3037). Anal. C, H, N.

4.3. Photophysical properties

4.3.1. Determination of quantum yields for the generation of singlet oxygen. The quantum yields for singlet oxygen generation [$\phi(^1O_2)$] of 21,23-dithiaporphyrins, **2–10**, were measured by direct methods in MeOH in a manner identical to the determination of $\phi(^1O_2)$ for **1**.[38,42] A SPEX 270M spectrometer (Jobin Yvon) equipped with InGaAs photodetector (Electrooptical Systems Inc., USA) was used for recording singlet oxygen emission spectra. A diode-pumped solid state laser (Millenia X, Spectra-Physics) at 532 nm was the excitation source. The sample solution, in a quartz cuvette, was placed directly in front of the entrance slit of the spectrometer, and the emission signal was collected at 90° relative to the exciting laser beam. An additional long-pass filter (850LP) was used to attenuate the excitation laser and the fluorescence from the photosensitizer.

4.3.2. Determination of quantum yields for fluorescence. The quantum yields for fluorescence (ϕ_F) of the core-modified porphyrins were measured in MeOH as described previously [38] and were compared to the standard rhodamine 6G ($\phi_F = 1.0$).

4.3.3. Determination of octanol/water partition coefficients at pH 7.4. The octanol/water partition coefficients were determined at pH 7.4 using the absorbance of the core-modified porphyrins. A 'shake flask' direct measurement[51] with 3–5 min mixing followed by 4 h settling period was used. Equilibration and measurements were made at 23 °C using a Perkin–Elmer Lambda 12 spectrophotometer. Values are reported as $\log D_{7.4}$.

4.4. Photophysical properties

4.4.1. Cells and culture conditions. Cells cultured from the rodent mammary adenocarcinoma (R3230AC) were used for these studies. The R3230AC tumors were maintained by transplantation in the abdominal region of 100–120 g Fischer female rats, using the sterile trochar technique described earlier by Hilf et al.[51] R3230AC cells were cultured from tumor homogenates using the method described earlier.[52] All cell lines were maintained in passage culture on 35 mm diameter polystyrene dishes (Becton Dickinson, Franklin Lakes, NJ) in 3.0 mL of minimum essential medium (α-MEM) supplemented with 10% FBS, 50 units/mL penicillin G, 50 μg/mL streptomycin, and 1.0 μg/mL Fungizone (complete medium). Only cells from passages 1–10 were used for experiments and cells from passages 1–4, stored at −86 °C, were used to initiate cultures. Cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere (Forma Scientific, Marietta, OH). Passage was accomplished by removing the culture medium, adding a 1.0 mL solution containing 0.25% trypsin, incubating at 37 °C for 2–5 min to remove the cells from the surface followed by seeding new culture dishes with an appropriate number of cells in 3.0 mL of α-MEM. Cell counts were performed using a particle counter (model ZM, Coulter Electronics, Hialeah, FL).

4.4.2. Incubation of cell cultures with dithiaporphyrins. For experiments designed to determine the amount of intracellular porphyrin after incubation with core-modified porphyrins, R3230AC cells were seeded on 96 well plates as above. Compounds **1–10** were added at the appropriate concentrations in the complete medium 24 h after cell seeding. Cells were incubated at 37 °C in the dark for various periods, the medium removed, monolayers washed once with 0.9% NaCl, and 200 μL of a 25% solution of Scintigest were added to solubilize the cells. The intracellular porphyrin content was determined using a fluorescence multi-well plate reader (Molecular Devices, Sunnyvale, CA) set at the appropriate excitation and emission wavelengths. Intracellular dye concentration was determined by comparing fluorescence values obtained from solubilized cells with dye standards dissolved in 25% Scintigest. Data are expressed as fmol porphyrin/cell.

For experiments designed to determine cell viability in the presence of individual core-modified porphyrins (**1–10**) in the dark or after light exposure, R3230AC cells were seeded on 96 well plates at $1–1.5 \times 10^4$ cells/well in complete medium. Cultures were then incubated for 24 h after which the appropriate concentrations of **1–10** were added directly to the wells in the complete medium.

4.4.3. Irradiation of cultured cells. Following incubation of R3230AC cells with porphyrins, the medium was removed, cells were washed once with 0.2 mL of 0.9% NaCl and 0.2 mL of medium minus FBS, and phenol red (clear medium) was added. Plates, with lids removed, were positioned on a orbital shaker (LabLine, Melrose Park, IL) and exposed for various times to broadband visible light (350–750 nm) delivered at 1.4 mW cm^{-2} from a filtered 750 W halogen source defocused to encompass the whole 96 well plate. The culture plates were gently orbited on the shaker in order to ensure uniform illumination of all of the wells

on the plate. The clear medium was then removed, 0.2 mL of fresh complete medium was added and cultures were incubated at 37 °C for 24 h in the dark. Cell monolayers were also maintained in the dark undergoing the same medium changes and addition of dyes as those that were irradiated. Cell counts, as above, were performed on irradiated cells, cells maintained in the dark or cells exposed to neither porphyrins nor light (control cells). Cell viability, obtained for experimental samples is expressed as the percent of control cell counts.

4.4.4. Fluorescence microscopy with dithiaporphyrin 3. R3230AC cells were seeded at $3–5 \times 10^5$ cells/dish in 35 mm diameter culture dishes containing sterilized 12 mm diameter cover slips in 3 mL complete medium for 24 h at 37 °C as described above. After the 24 h incubation the medium was removed and fresh complete medium containing dye **3** at 5×10^6 M was added for 24 h. Following the incubation period the medium was removed, the dish was washed once with 0.9% NaCl and 3 mL clear medium was added. The cover slips with the cells attached were removed immediately and mounted in a holder and images were captured using a Nikon inverted fluorescence microscope fitted with a Dage CCD 72 camera (Dage MTI, Michigan City, IN), and a Genisys image intensifier and Image I/AT processing software (Universal Imaging, Media, PA).

4.4.5. Preparation of mitochondrial suspensions and measurement of cytochrome *c* oxidase activity. Preparation of isolated mitochondrial suspensions from homogenized rat liver followed a method described earlier.[48] Mitochondrial suspensions were divided into 0.5 mL aliquots (6–10 mg of mitochondrial protein/mL) and stored at −86 °C until used for experiments.

Mitochondrial suspensions were removed from storage and thawed on ice. Stock solutions, 2×10^{-3} M, of **3** or **4** were prepared in DMSO and diluted with phosphate buffer (pH 7.4) to give 1×10^{-3} M. Dyes **3** or **4** were added to 1 mL of mitochondrial suspension at a final concentration of 5×10^{-6} M and allowed to incubate in the dark on ice for 5 min. Dye containing suspensions were then centrifuged at 8000*g* for 3 min using an Eppendorf microcentrifuge (Model 3200, Brinkmann Ind., Westbuty, NY), the supernatant was discarded and the pellet was resuspended in 1.0 mL of mitochondrial preparation buffer containing 0.33 M sucrose, 1.0 mM dithiothreitol, 1.0 mM EDTA, 0.3% BSA, and 100 mM KCl (pH 7.1). The suspension was then transferred to a 3.0 mL quartz cuvette, which was positioned in a focused, 1.0 cm diameter, filtered (530–750 nm) light beam emitted from a 750 W tungsten source. The intensity of the beam was uniform over the wavelength band used and was delivered at a fluence rate of 100 mW cm^{-2}. Beam intensity was measured using a radiometer (Model 210, Coherent Inc., Palo Alto, CA). The light was cooled by passing it through a water filter, eliminating thermal effects as the sample temperature did not rise above 25 °C. The mitochondrial suspensions were magnetically stirred continuously during the 1.0 h irradiation period. Aliquots (10 μL) were removed at various times during irradiation for determination of cytochrome *c* oxidase activity. A portion of the mitochondria/dye suspension was maintained in the dark, and determinations of cytochrome *c* oxidase activity were performed on 10 μL aliquots from these samples as dark controls. Measurement of cytochrome *c* oxidase activity was performed according to a method described earlier.[32,48] Initial enzyme activity was adjusted to obtain a decrease in the reduced cytochrome *c* oxidase absorbance at 550 nm of 0.4–0.6 OD units/min. Data are expressed as the % of initial, preirradiation cytochrome *c* oxidase activity.

4.4.6. Measurement of cytochrome *c* oxidase activity in cultured, whole cells. To determine whether dithiaporphyrin **3** affected mitochondrial cytochrome *c* oxidase activity in whole cells, thus taking into account cellular uptake and distribution of the dyes, R3230AC cells were plated on 12 well culture plates as described above until they reached $4–7 \times 10^5$ cells/well, a number when the cells were still in log phase growth. Stock solutions of core-modified porphyrin **3**, were diluted with sterilized doubly distilled water to give appropriate concentrations, and 50 μL of the diluted solutions was added to the each well for final concentrations ranging from 0 to 1.0×10^{-7} M. Cells were incubated for 24 h in the dark at 37 °C, the medium was removed, and the cells were washed with 0.9% NaCl, and 1.0 mL of clear medium was added. Monolayers were then exposed to filtered, 350–750 nm light from a 750 W tungsten source (1.4 mW cm^{-2}) for a total fluence of 5 J cm^{-2}. Immediately following irradiation, the cell number was determined by counting, cells were harvested and the activity of cytochrome *c* oxidase was determined according to the method described earlier.[38,48,53] Data are expressed as ΔOD/min/10^5 cells at 550 nm and compared to that determined from cells not exposed to dye or light.

4.4.7. Statistical analyses. All statistical analyses were performed using the Student's *t*-test for pairwise comparisons. A P value of <0.05 was considered significant.

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