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Asymmetric ZnPc–TEG photosensitizers: the effect of Pc substitution on phototoxicity

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ABSTRACT

Asymmetric zinc phthalocyanine (ZnPc) isomers with triethylene glycol (TEG) moieties in the peripheral (**1a**) and non-peripheral (**1b**) substitution were designed, synthesized, characterized, and their phototoxicity on colon-26 cells evaluated. TEG moieties as water solubilizing groups were incorporated at peripheral or non-peripheral positions to investigate the effect of their position on the Pc on the photodynamic activity. UV/vis analyses indicated a small red-shifting of the Q band of the non-peripheral ZnPc–TEG **1b** (ca. 4 nm) relative to **1a** in dichloromethane and THF. *n*-Octanol/pH 7.4 buffer partition coefficients revealed that **1b** was marginally more hydrophilic than **1a** with log $D_{7.4}$ values of 2.44 and 2.46, respectively. Fluorescence emission aggregation studies indicate that **1b** is ca. 61% (DMSO) and 32% (phosphate buffer) less aggregated than the peripherally substituted ZnPc **1a** suggesting that non-peripheral substitution decreased overall aggregation of the photosensitizers. Singlet oxygen generation studies are reported. Both ZnPc–TEGs showed negligible dark toxicity on colon 26 cells with 10 μM **1a** and **1b**. Irradiation (690 nm, 45 J/cm^2) of the cells produced an IC_{50} value >25 μM for **1a** and an IC_{50} value between 1 and 5 μM for **1b**. Overall, the non-peripheral isomer **1b** was found to be more photodynamically active.

Introduction

Several reports have emerged depicting amphiphilic photosensitizers to be generally more photodynamically active than symmetrically hydrophobic or hydrophilic counterparts.[1,2] This has been attributed to improved cellular uptake of the amphiphilic photosensitizers with localization at the hydrophobic-hydrophilic membrane interfaces and protein surfaces.[1–4] Amphiphilic molecules are those that possess partial hydrophobic and hydrophilic parts at different positions allowing each region of the compound to interact independently with the solvent.[5] Amphiphilicity can also affect the degree of aggregation of a photosensitizer (PS), consequently influencing its photoactivity. Hydrophilic photosensitizers are reported to be transported by human albumin and other serum proteins but the sensitizers do not diffuse across cellular bi-layer lipid membranes due to their low lipophilicities.[6] Intravenously introduced hydrophobic sensitizers are thought to bind to low density lipoproteins (LDLs) and are retained at the lipophilic regions of the cells often leading to mutagenicity and skin hypersensitivity due to slow clearance.[6,7] On the other hand, amphiphilic compounds are believed to bind to hydrophobic-hydrophilic interfacial proteins increasing their transport in and out of cells.[5]

Asymmetric phthalocyanines (Pcs) have been reported to be more photodynamically active than their symmetric analogues. Di-sulfonated AlPcs[8] and ZnPcs[1,9] were observed to be more phototoxic toward EMT-6 and Chinese hamster cells than the corresponding symmetric counterparts. Similarly, mono-substituted cationic,[10] mono-glycosylated ZnPcs,[11] and di-α-substituted ZnPcs[12] have shown lower IC_{50} values than their symmetric analogues. Structurally, the macrocyclic *Pc* core is largely hydrophobic in nature. Substitutions should be aimed at introducing hydrophilicity. Mono-substitutions are made on one of the four quadrants, leaving the other three same (Fig. 1).

Recently, Ng and co-workers indicated there was increased photodynamic activity in Pcs with peripheral substitution compared to non-peripheral. In two separate reports, ZnPc-β-Gal and di-cationic β-ZnPc had significantly lower IC_{50} values toward HT 29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells compared to their corresponding α-substituted ZnPcs.[10,11] However, they also observed that the α-mono-glycosylated ZnPc was more photodynamically active than the β-ZnPc-Glu.[11] Herein, we revisit this study on the effect of site of Pc substitution. ZnPc isomers with peripheral and non-peripheral

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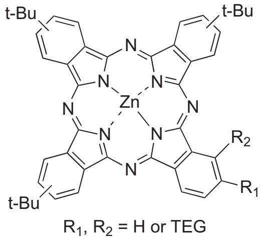
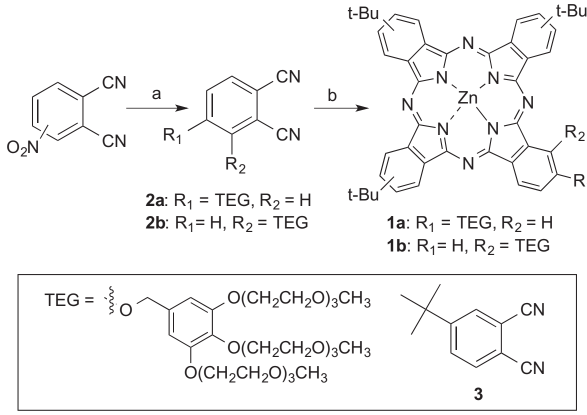


Figure 1. Asymmetric ZnPc isomers with peripheral (R_1) and non-peripheral (R_2) substitution of TEG group.



Scheme 1. Synthesis of ZnPc–TEGs **1a** and **1b**. Reagents and conditions: (a) TEG-OH, K_2CO_3, DMSO, 50 °C, 16 h (**2a**, 54%; **2b**, 35%); (b) compound **3**, $Zn(OAc)_2$, DBU, 1-pentanol, 160 °C, 16 h (**1a**, 31%; **1b**, 23%).

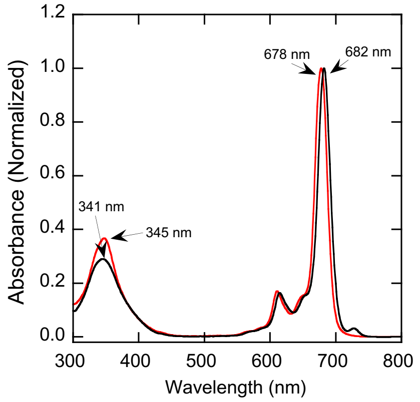


Figure 2. UV/vis absorption spectra (normalized at Q-band) of ZnPc–TEGs **1a** (red) and **1b** (black) in DCM.

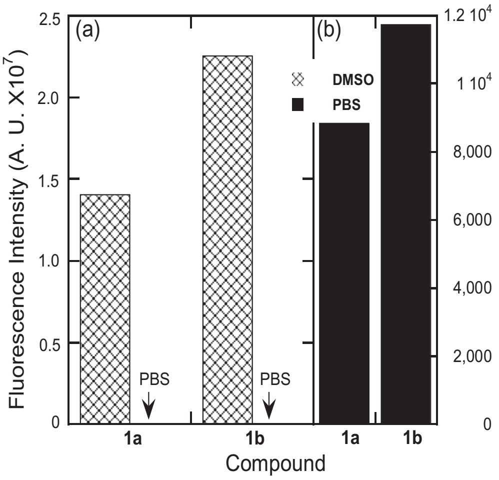


Figure 3. Aggregation of ZnPc–TEGs **1a** and **1b** as measured by fluorescence emission.

mono-substitution were designed, synthesized, characterized, and their phototoxicity on colon-26 cells studied. Triethylene glycol (TEG) groups were used to impart hydrophilicity on the ZnPcs since they have been used in the literature to confer water solubility.[13–15]

Results and Discussion

Treatment of commercially available nitro-phthalonitriles with TEG-OH[16] under basic conditions gave tegylated phthalonitriles (Pns) **2a** and **2b** in moderate yields. These yields were expected because nitro-phthalonitriles have been observed to react with K_2CO_3 in DMSO at elevated temperatures forming competing products.[17] Addition of excess of nitro-phthalonitriles to the reaction did not improve the yields of the desired products. ZnPc–TEGs **1a** and **1b** were obtained from statistical condensation of the commercially available *t*-butylphthalonitrile and Pns **2a** and **2b** under basic conditions (Scheme 1).

ZnPc–TEGs **1a** and **1b** showed the expected characteristic absorption peaks with Q-band maxima at 678 nm and 682 nm in DCM, respectively (Fig. 2). The slight bathochromic shift in non-peripheral substituted ZnPc **1b** is expected because there exists more HOMO contribution from the ortho position relative to the peripheral one as reported.[18] *n*-Octanol/pH$_{7.4}$ buffer partition coefficients revealed that **1b** was marginally more hydrophilic than **1a** (Table 1).

Pcs are inherently planar and aggregate via π–π co-facial packing to form dimers, and other higher order aggregates. For PDT purposes, aggregation is undesired for it leads to decreased singlet oxygen quantum yields consequently shutting down PS

Table 1
UV/vis absorption band maxima and extinction coefficients in DMSO and *n*-octanol/pH 7.4 buffer partition coefficients of **1a** and **1b**

Compounds	B band		Q band		% Q-band degradation over 10 min		Log$D_{7.4}$
	DCM[a] (ε)	THF[a] (ε)	DCM[a] (ε)	THF[a] (ε)	DCM	THF	
1a	350 (0.89)	348 (0.80)	678 (2.31)	673 (2.29)	0.66[b]	1.50	2.66
1b	347 (0.80)	346 (0.82)	682 (2.55)	678 (2.84)	2.52[b]	0.40	2.44

[a] Molar extinction coefficients (ε × 10^5 M^{-1} cm^{-1}) were obtained from serial dilutions in μM range. Log$D_{7.4}$ values were obtained as log[Abs$_{n\text{-octanol}}$/Abs$_{PBS}$].
[b] % degradation measured for 6 min.