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Bioorganic & Medicinal Chemistry Letters 17 (2007) 1073–1077
Bioorganic & Medicinal Chemistry Letters

Synthesis and in vitro photodynamic activity of mono-substituted amphiphilic zinc(II) phthalocyanines

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Received 7 September 2006; revised 4 November 2006; accepted 8 November 2006
Available online 10 November 2006

Abstract—A series of novel zinc(II) phthalocyanines mono-substituted with a 1,3-bis(dimethylamino)-2-propoxy group at the α- or β-position, and the corresponding di-N-methylated derivatives, have been synthesized. All these compounds can generate singlet oxygen effectively and exhibit high in vitro photodynamic activities toward HT29 human colorectal carcinoma cells with IC_{50} values down to 0.08 μM. The dicationic derivatives have a higher affinity to the cell membrane compared with the non-ionic counterparts.

Photodynamic therapy (PDT) is an innovative and promising modality to treat localized and superficial tumors.[1] The treatment utilizes the combined action of a photosensitizer, light, and molecular oxygen to cause cellular and tissue damage, in which singlet oxygen, generated through a series of photo-induced processes, is believed to be the major cytotoxic agent. Among the various considerations to improve the overall efficacy of this therapy, the behavior of the photosensitizer is of paramount importance. Over the last decade, a substantial effort has been put in the development of various classes of photosensitizers which have better absorption and photophysical properties, greater tumor specificity, and less cutaneous photosensitivity compared with the first-generation photosensitizer Photofrin.[2] We are particularly interested in phthalocyanine-based photosensitizers because of their strong absorption in the red visible region, which allows a deeper light penetration, high efficiency to generate singlet oxygen, and ease of chemical modification to facilitate the tailoring of properties such as aggregation and cellular uptake.[2,3] Recently, we have prepared a series of silicon(IV) phthalocyanines with 1,3-bis(dimethylamino)-2-propoxy group or its di-N-methylated counterpart as the axial substituent. These compounds exhibit very high photocytotoxicities toward a range of cancer cell lines with IC_{50} values (defined as the dye concentration required to kill 50% of the cells) down to 0.02 μM.[4] The most potent compound $SiPc[OC_3H_5(NMe_2)_2](OMe)$ has a high and selective affinity to the mitochondria of cells and induces apoptosis.[5] We report herein an extension of this work to zinc(II) phthalocyanines which in general have higher stability than the silicon(IV) counterparts. Four mono-substituted zinc(II) phthalocyanines with these substituents at the peripheral α or β-position have been synthesized and evaluated for their in vitro photodynamic activity against HT29 human colorectal carcinoma cells. It is expected that these mono-substituted phthalocyanines, having a high amphiphilicity, can have a high cellular uptake, which in turn may increase the photodynamic activity. Zinc(II) phthalocyanines with four of these substituents at the β-positions have been reported previously,[6] while the mono-substituted analogues reported herein have only been briefly mentioned in patents,[7] all for the study of photo-inactivation of bacteria.

The synthesis of these mono-substituted phthalocyanines is shown in Scheme 1. Treatment of 3- or

Keywords: Phthalocyanines; Photodynamic therapy; Photosensitizers; Colorectal carcinoma.
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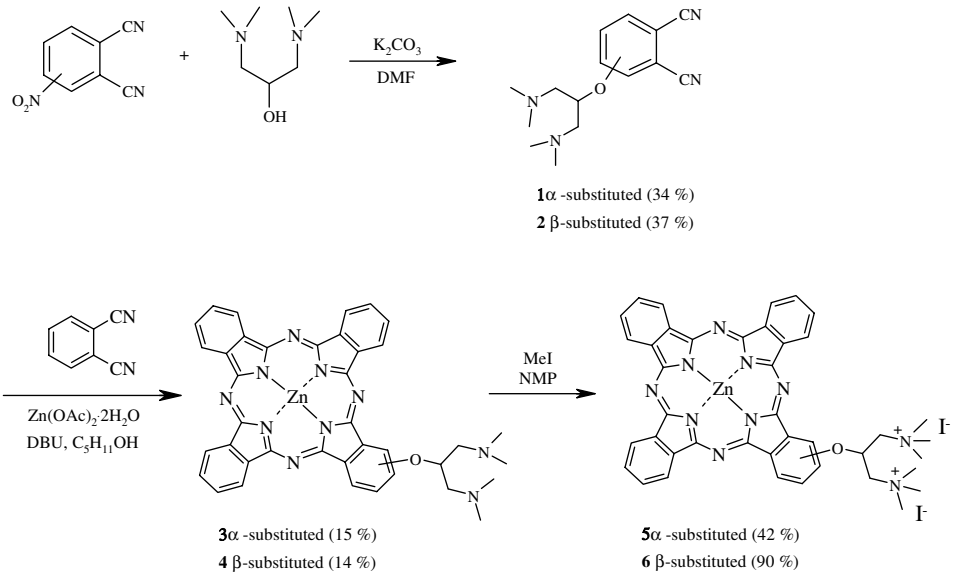
doi:10.1016/j.bmcl.2006.11.017



Scheme 1. Preparation of phthalocyanines **3**–**6**.

4-nitrophthalonitrile with 1,3-bis(dimethylamino)-2-propanol in the presence of K_2CO_3 in DMF gave the corresponding substituted products **1** and **2**. These precursors were then treated with the unsubstituted phthalonitrile in the presence of $Zn(OAc)_2{\cdot}2H_2O$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *n*-pentanol to give the desired '3+1' products **3** and **4**, which could be isolated and purified by column chromatography in 14–15% yield. To enhance the amphiphilicity, compounds **3** and **4** were methylated using methyl iodide in *N*-methyl-2-pyrrolidinone (NMP). The resulting dicationic compounds **5** and **6** were isolated by precipitation with diethyl ether followed by extensive washing with diethyl ether, hexane, and dichloromethane.[8]

The electronic absorption and photophysical data of **3**–**6** measured in DMF are summarized in Table 1. The absorption spectra of **3**–**6** in DMF are typical for non-aggregated phthalocyanines, showing a B (or Soret) band at 334–346 nm, an intense and sharp Q band at 672–679 nm, together with a vibronic band at 606–612 nm. The Q band straightly follows the Lambert–Beer law up to 10 μM. Upon excitation at 610 nm, these compounds show a fluorescence emission at 676–682 nm with a fluorescence quantum yield (Φ_F) of 0.22–0.31. Both the absorption position of the Q-band and the fluorescence emission are slightly red-shifted for the α-substituted analogues (**3** and **5**) compared with the β-substituted counterparts (**4** and **6**). The dicationic analogues **5** and **6** are readily soluble in water, but they are highly aggregated in aqueous media as shown by absorption spectroscopy. As shown in Figure 1, the absorption spectrum of **5** in water shows a broad signal peaking at ca. 620 and 670 nm in the Q band region. The former band is characteristic for face-to-face or H-aggregates of phthalocyanines.[9] This spectral feature is very different from that recorded in DMF, in which the phthalocyanine exists mainly in monomeric state (Fig. 1).

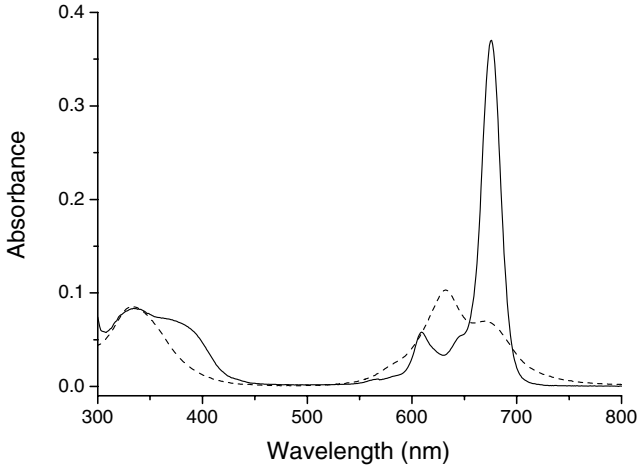


Figure 1. Electronic absorption spectra of **5** in water (---) and in DMF (—) (both at 2 μM).

Table 1. Electronic absorption and photophysical data for **3**–**6** in DMF

Compound	λ_{max} (nm) (log ε)	λ_{em}[a] (nm)	Φ_F[b]	Φ_Δ[c]
3	339 (4.69), 612 (4.53), 679 (5.31)	682	0.22	0.50
4	346 (4.74), 606 (4.52), 673 (5.29)	677	0.31	0.52
5	334 (4.63), 609 (4.48), 676 (5.28)	682	0.24	0.57
6	344 (4.67), 606 (4.46), 672 (5.25)	676	0.26	0.53

[a] Excited at 610 nm.
[b] Using unsubstituted zinc(II) phthalocyanine (ZnPc) in 1-chloronaphthalene as the reference [fluorescence quantum yield (Φ_F) = 0.30].
[c] Using ZnPc as the reference [singlet oxygen quantum yield (Φ_Δ) = 0.56 in DMF].

To evaluate the photosensitizing efficiency of these compounds, their singlet oxygen quantum yields (Φ_Δ) were determined by a steady-state method using 1,3-diphenylisobenzofuran as the scavenger. The concentration of the quencher was monitored spectroscopically at 411 nm along with time, from which the values of Φ_Δ could be determined.[10] As shown in Table 1, all the phthalocyanines have similar Φ_Δ values (0.50–0.57) and are as efficient as the unsubstituted zinc(II) phthalocyanine (ZnPc) ($\Phi_\Delta = 0.56$) in sensitizing the formation of singlet oxygen in DMF.

The photodynamic activities of compounds **3**–**6** in Cremophor EL emulsions were investigated against HT29 human colorectal carcinoma cells. Figure 2, which shows the effects of **4** on HT29, is a typical dose-dependent survival curve for all these phthalocyanines. While all these compounds are essentially non-cytotoxic in the absence of light, they exhibit a high photocytotoxicity. As shown in Table 2, which summarizes the IC_{50} and IC_{90} values of these compounds, the β-substituted phthalocyanines (**4** and **6**) are generally more photocytotoxic than the α-substituted counterparts (**3** and **5**), and

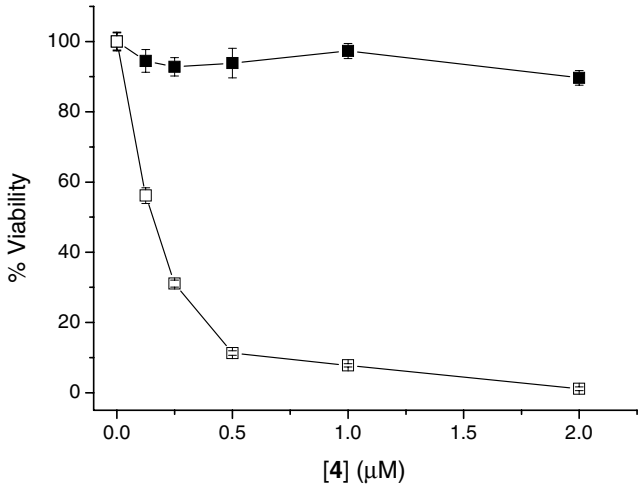


Figure 2. Effects of **4** on HT29 in the absence (■) and presence (□) of light. For the latter, the cells were illuminated with a red light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± S.E.M. of three independent experiments, each performed in triplicate.

Table 2. Comparison of the IC_{50} and IC_{90} values of **3**-**6** against HT29

Compound	IC_{50} (μM)	IC_{90} (μM)
3	0.48	1.67
4	0.15	0.67
5	0.64	1.04
6	0.08	0.12

the β-di-N-methylated phthalocyanine **6** is particularly potent with an IC_{50} value of 0.08 μM. Apparently, its photocytotoxicity is much higher than that of the classical photosensitizer Photofrin ($IC_{50} = 7.5$ μgmL^{-1} vs 0.08 μgmL^{-1} for **6**),[11] pheophorbide *a* ($IC_{50} = 0.5$ μM),[11] and several other chlorin-based photosensitizers.[12]

To have a better understanding of the in vitro photodynamic action of these compounds, we examined their absorption and photophysical properties in the culture medium.[13] While a strong and sharp Q band was retained for **4** and **6** in the medium, only a weak Q band was observed for **3** and **5**. The fluorescence emission for the former two compounds was also substantially higher (see Figures S1 and S2 in Supporting information). These observations seemed to suggest that the β-substituted analogues are less aggregated than the α-substituted counterparts in the culture medium. However, their singlet-oxygen generation efficiency did not seem to have a great difference. We employed imidazole/4-nitrosodimethylaniline (RNO)[14] as a probe to monitor the singlet oxygen formation. It was found that the rates of decay of RNO (as monitored by the decrease in absorbance at 440 nm) induced by **4** and **6** were only marginally faster than those by using **3** and **5** as the photosensitizers (see Figure S3 in Supporting information).

We also employed fluorescence microscopy to study the cellular uptake and subcellular localization. After incubation with compounds **3**–**6** for 2 h and upon excitation at 630 nm, the HT29 cells showed a strong intracellular fluorescence (see Figure S4 in Supporting information), indicating that there were substantial uptakes of the dyes. As mitochondria are believed to be the targets for the initiation of apoptosis by PDT,[15] it would be important to reveal whether the dyes have a selective affinity to these subcellular components. We stained the HT29 cells with MitoTracker Green FM, which is

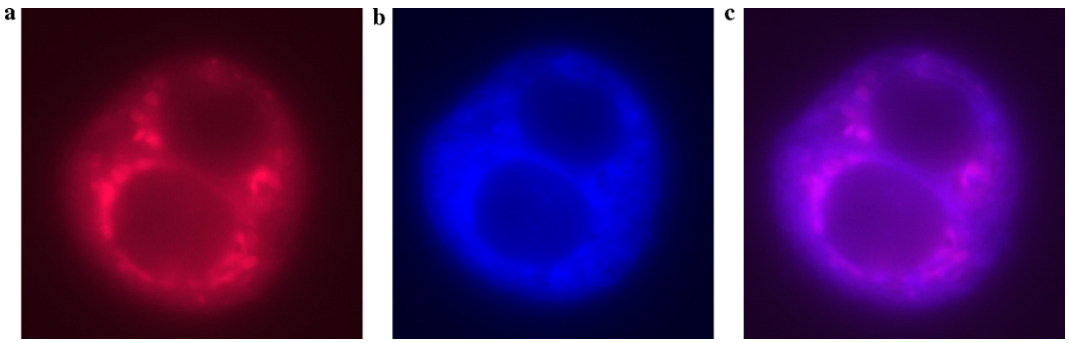


Figure 3. Visualization of intracellular fluorescence of HT29 using filter sets specific for (a) the MitoTracker (in red) and (b) phthalocyanine **4** (in blue). (c) The corresponding superimposed image.

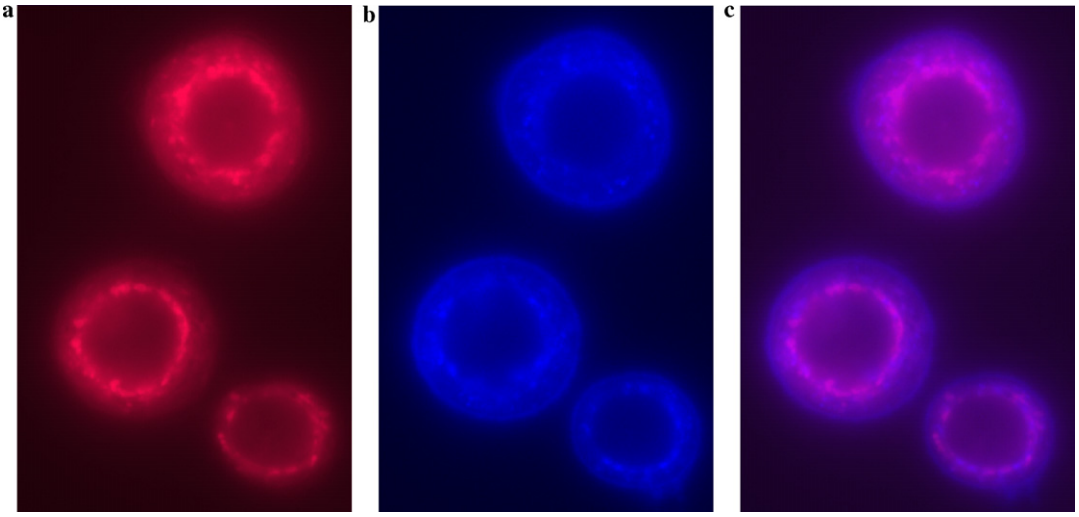


Figure 4. Visualization of intracellular fluorescence of HT29 using filter sets specific for (a) the MitoTracker (in red) and (b) phthalocyanine **6** (in blue). (c) The corresponding superimposed image.

a specific fluorescence dye for mitochondria, prior to the treatment with **3**–**6**. Figures 3 and 4 show the images for compounds **4** and **6**, which are similar to those for the α-substituted analogues **3** and **5**, respectively (see Figures S5 and S6 in Supporting information). It can be seen that the fluorescence caused by the phthalocyanines (excited at 630 nm, monitored at >660 nm) is diffused throughout the cytoplasm and cannot be perfectly superimposed with the fluorescence caused by the MitoTracker (excited at 490 nm, monitored at 500–575 nm). This observation indicates that these compounds are not exclusively localized in the mitochondria. A close examination of these figures reveals that compound **6**, as well as **5**, gives a brighter emission in the cell membrane region compared with the non-ionic counterparts. It is likely that due to the dicationic nature of these compounds, they are preferentially retained in the membrane's lipid bilayer.

In summary, we have prepared four mono-substituted zinc(II) phthalocyanines with an amphiphilic character. These compounds are highly photodynamically active toward HT29 cells. The dicationic phthalocyanines **5** and **6** have a higher affinity to the cell membrane compared to their neutral counterparts.

Acknowledgments

We thank Wallace C. Y. Yip for technical support in the imaging study. This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. 402103), and a strategic investments scheme administered by The Chinese University of Hong Kong.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.017.

References and notes

1. (a) Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nat. Rev. Cancer* **2003**, *3*, 380; (b) Brown, S. B.; Brown, E. A.; Walker, I. *Lancet Oncol.* **2004**, *5*, 497.
2. (a) Ali, H.; van Lier, J. E. *Chem. Rev.* **1999**, *99*, 2379; (b) Nyman, E. S.; Hynninen, P. H. *J. Photochem. Photobiol., B* **2004**, *73*, 1; (c) Detty, M. R.; Gibson, S. L.; Wagner, S. J. *J. Med. Chem.* **2004**, *47*, 3897.
3. (a) Lukyanets, E. A. *J. Porphyrins Phthalocyanines* **1999**, *3*, 424; (b) Allen, C. M.; Sharman, W. M.; van Lier, J. E. *J. Porphyrins Phthalocyanines* **2001**, *5*, 161.
4. (a) Lo, P.-C.; Huang, J.-D.; Cheng, D. Y. Y.; Chan, E. Y. M.; Fong, W.-P.; Ko, W.-H.; Ng, D. K. P. *Chem. Eur. J.* **2004**, *10*, 4831; (b) Huang, J.-D.; Lo, P.-C.; Chen, Y.-M.; Lai, J. C.; Fong, W.-P.; Ng, D. K. P. *J. Inorg. Biochem.* **2006**, *100*, 946; (c) Lo, P.-C.; Leung, C.-H.; Chan, E. Y. M.; Fong, W.-P.; Ko, W.-H.; Ng, D. K. P. *Photodiag. Photodyn. Ther.* in press.
5. Lai, J. C.; Lo, P.-C.; Ng, D. K. P.; Ko, W.-H.; Leung, S. C. H.; Fung, K.-P.; Fong, W.-P. *Cancer Biol. Ther.* **2006**, *5*, 413.
6. (a) De Filippis, M. P.; Dei, D.; Fantetti, L.; Roncucci, G. *Tetrahedron Lett.* **2000**, *41*, 9143; (b) Segalla, A.; Borsarelli, C. D.; Braslavsky, S. E.; Spikes, J. D.; Roncucci, G.; Dei, D.; Chiti, G.; Jori, G.; Reddi, E. *Photochem. Photobiol. Sci.* **2002**, *1*, 641.
7. (a) Roncucci, G.; Fantetti, L.; De Filippis, M. P.; Dei, D.; Jori, G. Eur. Pat. Appl. EP 1 164 135 A1, 2001; (b) Roncucci, G.; Fantetti, L.; De Filippis, M. P.; Dei, D.; Jori, G. US Pat. Appl. US 0170178 A1, 2003.
8. The experimental procedure and full characterization data for these compounds are given in Supporting information.
9. Tai, S.; Hayashi, N. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1275.
10. (a) Spiller, W.; Kliesch, H.; Wöhrle, D.; Hackbarth, S.; Röder, B.; Schnurpfeil, G. *J. Porphyrins Phthalocyanines* **1998**, *2*, 145; (b) Maree, M. D.; Kuznetsova, N.; Nyokong, T. *J. Photochem. Photobiol., A* **2001**, *140*, 117.
11. Hajri, A.; Wack, S.; Meyer, C.; Smith, M. K.; Leberquier, C.; Kedinger, M.; Aprahamian, M. *Photochem. Photobiol.* **2002**, *75*, 140, In this work, the light (640 nm for Photofrin and 670 nm for pheophorbide *a*) was delivered by a halogen lamp fitted with specific 10 nm-width band-pass filters with a total fluence of 5 J cm^{-2}.

12. Laville, I.; Figueiredo, T.; Loock, B.; Pigaglio, S.; Maillard, Ph.; Grierson, D. S.; Carrez, D.; Croisy, A.; Blais, J. *Bioorg. Med. Chem.* **2003**, *11*, 1643, In this work, the cells were irradiated with an Ar^+ laser at 514 nm with a light fluence of 5–25 J cm^{-2}. By using 1 μM of the photosensitizers, the cell survival fraction was found to be ca. 5–30%.
13. Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, cat no. 10313-021) supplemented with fetal calf serum (10%), penicillin–streptomycin (100 U mL^{-1} and 100 μg mL^{-1}, respectively), L-glutamine (2 mM), and transferrin (10 μg mL^{-1}).
14. Kraljic, I.; El Mohsni, S. *Photochem. Photobiol.* **1978**, *28*, 577.
15. Oleinick, N. L.; Morris, R. L.; Belichenko, I. *Photochem. Photobiol. Sci.* **2002**, *1*, 21.