Dalton Transactions

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The first silicon(IV) phthalocyanine–nucleoside conjugates with high photodynamic activity†

Cite this: *Dalton Trans.*, 2013, **42**, 10398

Received 6th April 2013,
Accepted 26th May 2013

DOI: 10.1039/c3dt50910a

www.rsc.org/dalton

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A series of novel silicon(IV) phthalocyanines conjugated axially with different nucleoside moieties (uridine, 5-methyluridine, cytidine, and 5-*N*-cytidine derivatives) have been synthesized and evaluated for their photodynamic activities. The uridine-containing compound 1 exhibits the highest photocytotoxicity against HepG2 human hepatocarcinoma cells with an IC_{50} value as low as 6 nM, which can be attributed to its high cellular uptake and non-aggregated nature in the biological media. This compound shows high affinity toward the mitochondria of HepG2 cells and causes cell death mainly through apoptosis upon illumination. The result indicates that 1 is a highly promising photosensitizer for photodynamic therapy.

Photodynamic therapy (PDT) is an attractive minimal-invasive modality for cancer treatment.[1] It typically involves the systemic administration of a non-toxic photosensitizer and the tumor-oriented illumination with light of a specific wavelength. Upon excitation by light, the tumor-localized photosensitizer generates intracellular reactive oxygen species (ROS), particularly singlet oxygen, to cause cells and tissues damage. The overall efficacy of the treatment depends greatly on the behavior of the photosensitizer.[2] Due to intense absorption in the phototherapeutic window (600–800 nm), low dark toxicity, and ease of chemical modification, phthalocyanines (Pcs) have been found to be highly promising as photosensitizers for PDT.[2,3] A substantial number of phthalocyanines with different functionalities have been prepared and examined for their photodynamic activities. Among these phthalocyanines, the analogues conjugated with biological molecules,[4] such as sugar,[5] amino acids,[6] antibodies,[7] vitamins,[8] and peptides,[9] have received considerable attention in recent years, because of their enhanced biocompatibility and cellular uptake.

As part of our ongoing interest in the development of Pc-based photosensitizers,[10] we report herein a new series of silicon(IV) phthalocyanines (SiPcs) covalently linked with two nucleoside moieties at the axial positions, including their synthesis, spectroscopic properties, and *in vitro* photodynamic activities against HepG2 human hepatocarcinoma cells. The hydrophobic and π-conjugated characterizations of the phthalocyanine skeleton favor the formation of aggregates, resulting in inefficient ROS generation and poor solubility in aqueous media.[3] By introducing two nucleoside-containing substituents at the axial positions, we aimed to improve the hydrophilicity and inhibit the self-aggregation of these compounds. More importantly, considering that nucleoside-based compounds have been widely studied for their pharmacological properties, for example, using as antiviral[11] and anticancer drugs,[12] we hope to evaluate whether this functionality has unusual effects on the photodynamic process. Indeed, the results are very interesting. One of the conjugates axially substituted with uridine moieties (compound **1**, Scheme 1) exhibits an extremely high *in vitro* photodynamic activity.

The photobiological properties of phthalocyanines functionalized with nucleoside moieties remain unexplored, although the synthesis and characterization of a few phthalocyanine–nucleoside conjugates have been reported.[13] Ng and co-workers[13a] firstly prepared adenine-containing zinc(II) phthalocyanines (ZnPcs) and examined their spectroscopic properties. Afterwards, Sessler and Torres *et al.*[13b,c] synthesized a novel zinc(II) phthalocyanine peripherally linked with cytidine derivative and investigated their interaction with guanosine and fullerene. Reddy *et al.*[13d] also reported the synthesis and spectroscopic properties of a trifuoromethylated zinc(II) phthalocyanine conjugated with deoxyribonucleosides. Nonetheless, all of them did not report the photodynamic activity of these ZnPc–nucleoside conjugates. Moreover, to the best of our knowledge, no silicon(IV) phthalocyanine containing nucleoside moieties has been reported in the literature.

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† Electronic supplementary information (ESI) available: Experimental details and characterization data, HPLC profiles, additional electronic absorption spectra, fluorescence spectra, and subcellular localization microscopies. See DOI: 10.1039/c3dt50910a

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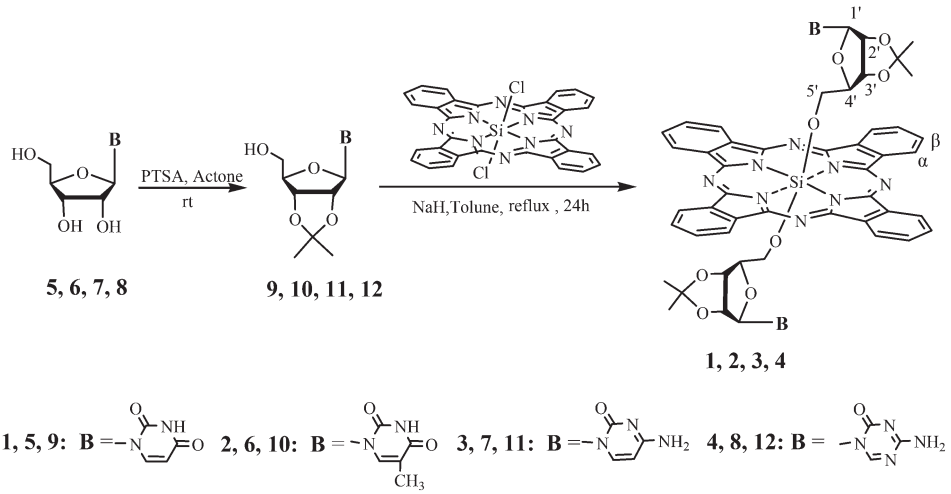

Scheme 1 Preparation of compounds **1–4**.

The preparation of the nucleoside–conjugated phthalocyanines (compounds **1–4**) is shown in Scheme 1. Treatment of uridine (**5**), 5-methyl-uridine (**6**), cytidine (**7**) or 5-*N*-cytidine (**8**) with *p*-toluenesulfonic acid (PTSA) and dry acetone gave the corresponding isopropylidene-protected products **9–12** in good yields (85–95%), respectively. These nucleoside derivatives were then treated with silicon(IV) phthalocyanine dichloride in the presence of NaH and led to axial substitution, giving compounds **1–4** in moderate yield (40–65%). Four final products are highly soluble in common organic solvents and could be readily purified by column chromatography. The purity of these new phthalocyanines was determined by high performance liquid chromatography (HPLC) and was found to be ≥98% (see Fig. S1, in the ESI†). All the prepared phthalocyanines were characterized with various spectroscopic methods including high resolution mass spectrometry (HRMS), proton nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared (FT-IR), and elemental analyses.

The spectroscopic properties of **1–4** were measured in *N*,*N*-dimethylformamide (DMF), and the data are summarized in Table 1. The electronic absorption spectra of all compounds in DMF are typical of non-aggregated phthalocyanines, exhibiting an intense and sharp Q-band at 676–678 nm. Their Q bands strictly follow the Lambert–Beer law, suggesting that these SiPcs are essentially free from aggregation in DMF (see Fig. S2 in the ESI†). The almost identical absorption positions for all these compounds indicate that the π system of the phthalocyanine ring is not perturbed by the axial substituents. Upon excitation at 610 nm, these compounds show a strong fluorescence emission at 683–687 nm with a fluorescence quantum yield (Φ_F) of 0.29–0.37 relative to unsubstituted zinc(II) phthalocyanine (ZnPc) (Φ_F = 0.28). The fluorescence spectra of these compounds are essentially a mirror image of the absorption spectra in Q-band with a small Stokes shift (*ca*. 8 nm).

To evaluate the photosensitizing activities of these SiPcs **1–4**, their singlet oxygen quantum yields (Φ_Δ) were also determined in DMF by a steady-state method using 1,3-diphenylisobenzofuran (DPBF) as the chemical quencher.[15] In this method, the concentration of DPBF was monitored spectroscopically at 413 nm with irradiation time and the singlet oxygen generation efficiency can be determined by comparing the rate of photodegradation of DPBF induced by the photosensitizers with that of unsubstituted ZnPc (Φ_Δ = 0.56). As shown in Table 1, all the prepared SiPcs are efficient single oxygen generators with the Φ_Δ value of 0.42–0.67. It seems that the photosensitizing activities of these compounds follow the trend **4** > **3** ≈ **2** ≈ **1**, and the 5-*N*-cytidine-containing compound **4** has a higher Φ_Δ than the other compounds, which may be due to the introduction of N atom into the pyrimidine ring of the nucleoside leading to the change of electronic distribution of the nucleoside moiety.

Since the *in vitro* photodynamic assay was determined in RPMI 1640 cell culture medium, the absorption spectra of compounds **1–4** (formulated with Cremophor EL[16]) were also recorded in this culture medium. As shown in Fig. 1, these compounds exhibit very similar absorption spectra with a sharp and intense Q-band peaking at 681 nm, which also obeys the Lambert–Beer law (see the inset of Fig. 1, using **1** as an example). Moreover, these compounds in the culture medium show a strong fluorescence emission peaking at

Table 1 Photophysical and photochemical data for **1–4** in DMF

Compound	λ_{max} (nm)	λ_{em} [a] (nm)	Stokes shift (nm)	$\varepsilon \times 10^5$ (M^{-1} cm^{-1})	Φ_F [b]	Φ_Δ [c]
1	678	687	9	2.00	0.37	0.44
2	676	684	8	1.76	0.29	0.46
3	677	685	8	2.13	0.36	0.42
4	676	683	7	1.80	0.30	0.67

[a] Excited at 610 nm. [b] Using unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF as the reference (Φ_F = 0.28).[14] [c] Determined using 1,3-diphenylisobenzofuran (DPBF) as the chemical quencher, and using ZnPc in DMF as the reference (Φ_Δ = 0.56).[15]

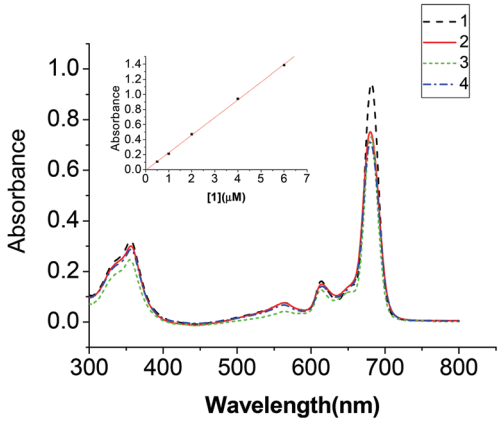

Fig. 1 Electronic absorption spectra of **1–4** (4 μM), formulated with Cremophor EL, in cellular culture medium. The inset plots the Q-band absorbance at 681 nm *versus* the concentration of **1**.

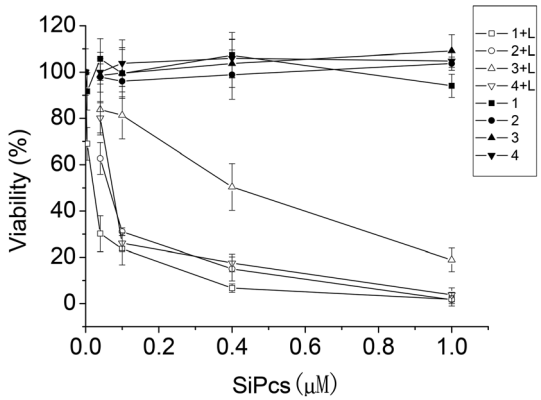

Fig. 2 Cytotoxic effects of SiPcs **1–4** on HepG2 cells in the absence (closed symbols) and presence (open symbols, +L) of light. For the latter, the cells were irradiated with red light ($\lambda > 610$ nm, 15 mW cm^{-2}, 27 J cm^{-2}). Values represent mean ± standard deviation of three separate experiments, each performed in quadruplicate.

692–694 nm when excited at 610 nm (see Fig. S3 in the ESI†). These observations indicate that **1–4** mainly exist as a monomeric form in the RPMI 1640 medium with 1% Cremophor EL. Therefore, these axial nucleoside moieties are effective in decreasing aggregation of the phthalocyanine ring as we expected due to their steric effect and hydrophilicity. The non-aggregated characterization of these compounds is extremely important for PDT application, since aggregation provides an efficient non-radiative energy relaxation pathway, thereby greatly shortening the triplet lifetime of the phthalocyanine molecule and then drastically reducing the overall photosensitizing efficiency.[17]

The photodynamic activities of **1–4** in Cremophor EL emulsions were evaluated against HepG2 human hepatocarcinoma cells. As shown in Fig. 2, all these compounds are essentially non-cytotoxic in the absence of light, but become cytotoxic upon illumination with red light. The IC_{50} values, defined as the photosensitizer concentration demanded to kill 50% of the cells, can be obtained from the dose-dependent survival curves

Table 2 Comparison of the IC_{50} values of SiPcs **1–4** against HepG2 cells

Compd	IC_{50} [a] (μM)
1	0.006
2	0.051
3	0.400
4	0.080

[a] Defined as the photosensitizer concentration required to kill 50% of the cells.

in the presence of light. These data are summarized in Table 2. It can be seen that the photodynamic activity of these compounds greatly depends on the axial substituents. The photocytotoxicity towards HepG2 cells follows the trend **1** > **2** ≈ **4** > **3** ($P < 0.01$ for **1** *vs.* **2** or **3** or **4**; $P > 0.05$ for **2** *vs.***4**; and $P < 0.05$ for **3** *vs.* **2** or **4**). The compound **1** conjugated with uridines exhibits the highest photoactivity with an IC_{50} value as low as 6 nM, while the analogue **3** conjugated with cytidines shows the lowest photoactivity with an IC_{50} value of 400 nM. Compounds **2** and **4** present a comparable photocytotoxicity against HepG2 cells (IC_{50} = 51 and 80 nM, respectively).

It is worth noting that compound **1** is highly promising as an efficient photosensitizer. Recently, Ng's group has made significant progress in the rational modification of SiPc-based photosensitizers.[5b,18] They obtained a highly photocytotoxic glucosylated SiPc with an IC_{50} down to 6 nM toward HepG2 cells.[5b] The result was found to be much higher than that of the classical photosensitizers such as porfimer sodium and pheophorbide a. The photoactivity of compound **1** against HepG2 cancer cells is comparable with that of the glucoconjugated SiPc as well as other highly potent Pc-based photosensitizers.[19]

To further account for the photocytotoxicity results, the cellular uptake of these compounds was examined by fluorescence microscopy. Fig. 3 shows the bright-field and fluorescence microscopic images of HepG2 cells after incubation with **1–4** (2 μM) for 2 h. The average relative fluorescence intensity per cell of these compounds was also measured and described in Fig. 4. It can be seen that the intracellular fluorescence intensity follows the trend **1** > **2** > **4** > **3**. As discussed above, these compounds are essentially free from aggregation in biological environment and show similar fluorescence emission properties. Hence, the trend in intracellular fluorescence intensity of these compounds can reflect their trend in cellular uptake.

The observed trend of photocytotoxicity toward HepG2 cells, **1** > **2** ≈ **4** > **3**, may be a result of two effects of cellular uptake (**1** > **2** > **4** > **3**) and singlet oxygen yield (**4** > **3** ≈ **2** ≈ **1**). Compound **1** shows much stronger intracellular fluorescence in HepG2 cells than the other compounds, suggesting **1** having the highest cellular uptake and then giving the highest photocytotoxicity toward HepG2 cells. Compound **4** shows a comparable photoactivity relative to **2** due to its higher singlet oxygen yield, although the cellular uptake of **4** is slightly lower than that of **2**.

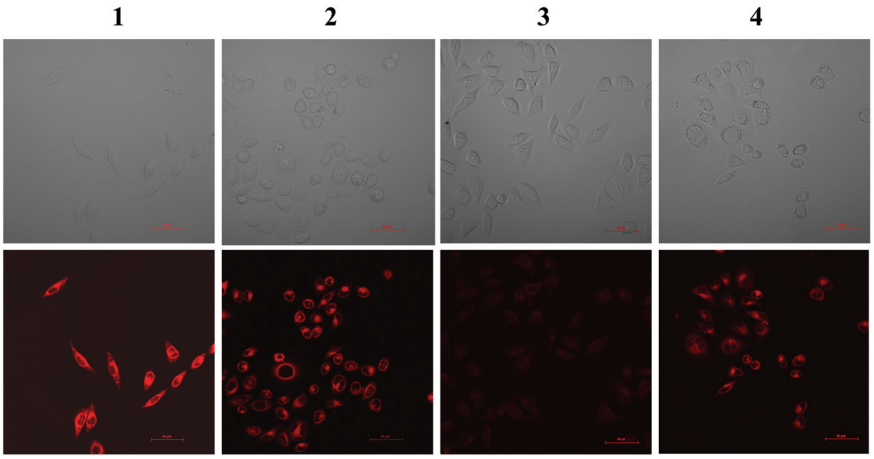

Fig. 3 Bright-field (row 1) and intracellular fluorescence (row 2) images of HepG2 cells after incubation with **1** (column 1), **2** (column 2), **3** (column 3), and **4** (column 4) (all at 2 μM) for 2 h, respectively.

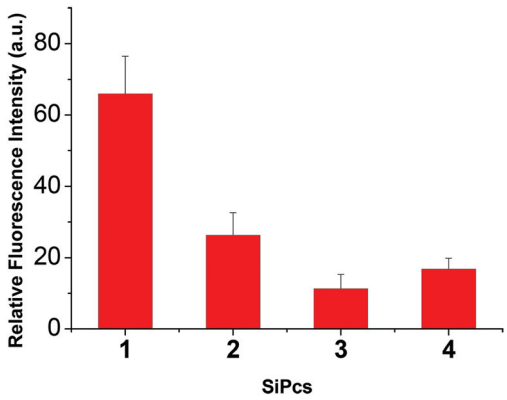

Fig. 4 Comparison of the intracellular fluorescence intensities of SiPcs **1–4** in HepG2 cells. Data are expressed as mean ± standard deviation (the number of cells = 20).

The subcellular localization of compounds **1–4** in HepG2 cells was investigated by confocal microscopy. Since mitochondria are an important target of the initiation of apoptosis induced by PDT,[20] it would be interesting to reveal whether the photosensitizer has an affinity to the subcellular component. We stained the cells with MitoTracker Green, which is a specific fluorescence dye for mitochondria, together with **1–4**. As shown in Fig. S4,† in HepG2 cells, the fluorescence caused by MitoTracker Green (excited at 488 nm, monitored at 499–529 nm) is mainly superimposed with the fluorescence caused by **1** (excited at 637 nm, monitored at 640–700 nm). Similarly, the fluorescence images of **2–4** and MitoTracker can also be superimposed (Fig. S5–7 in the ESI†). These indicate that compounds **1–4** can target the mitochondria of HepG2, which is similar to Pc4, a known axially substituted SiPc.[21]

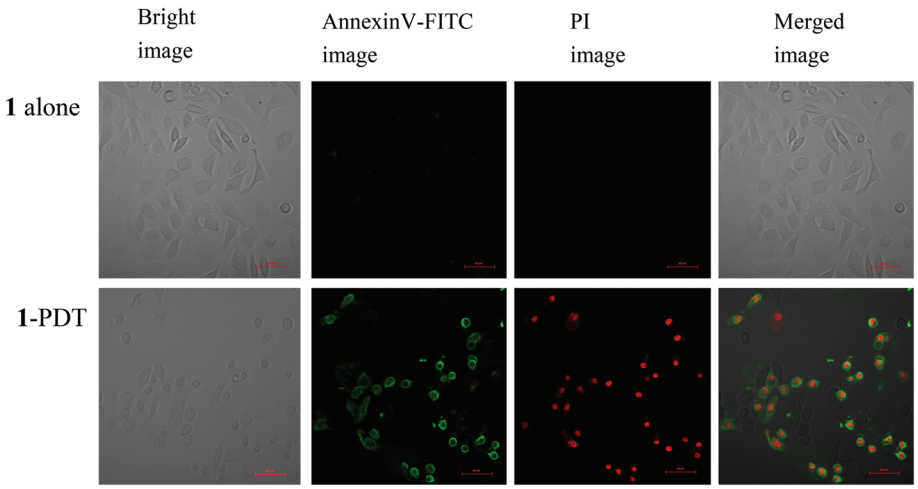

Fig. 5 The intracellular fluorescence images of Annexin V-FITC (in green) and PI (in red) in HepG2 cells after treatment with **1** (8 nM) in combination with light (**1**-PDT) or without light (**1** alone). The bright field image is given in column 1. Scale bar: 50 μm.

Due to the high potency of **1**, the mode of cell death induced by **1** was also investigated using double staining with Annexin V-FITC and propidium iodide (PI) by fluorescence confocal microscopy.[22] As shown in Fig. 5, most of the HepG2 cells are negative for Annexin V-FITC and PI after treatment with **1** (8 nM) in the absence of light, indicating that **1** is essentially non-cytotoxic toward HepG2 cells in darkness. However, upon illumination, cells stained with Annexin V-FITC are obviously observed, and cells stained with PI can also be seen. This suggests that apoptotic changes of these cells have occurred after PDT treatment. More than 50% of **1**-PDT-treated HepG2 cells were stained with both Annexin V-FITC and PI, implying that the pathway of cell death was mainly apoptosis.

In conclusion, we have reported the first synthesis of silicon phthalocyanines bearing nucleosides at the axial positions. The axial nucleoside moieties can effectively reduce the self-aggregation of these phthalocyanines not only in organic solvent, but also in cell culture medium. The photodynamic activities of these compounds depend on the kind of nucleosides as the result of different cellular uptake properties toward cancer cells. The uridine-containing derivative (compound **1**) is the most potent one. The IC_{50} value of **1** is as low as 6 nM toward HepG2 cells. As revealed by confocal microscopy, compound **1** shows a high localization in the mitochondria of HepG2 cells and causes predominately apoptosis upon illumination. These results indicate that **1** is a highly promising photosensitizer for photodynamic therapy. The actual role of the nucleoside moieties and *in vivo* PDT efficacies of these compounds are under investigation.

We thank Prof. Huang-Hao Yang for providing the confocal microscopic facility. This work was supported by the Natural Science Foundation of China (grant no. 20872016 and 21172037), the Natural Science Foundation of Fujian, China (grant no. 2011J01040), and the Specialized Research Fund for the Doctoral Program of Higher Education (grant no. 201135141001).

Notes and references

1 (*a*) D. E. J. G. J. Dolmans, D. Fukumura and R. K. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380–387; (*b*) S. B. Brown, E. A. Brown and I. Walker, *Lancet Oncol.*, 2004, **5**, 497–508; (*c*) M. Ethirajan, Y. Chen, P. Joshi and R. K. Pandey, *Chem. Soc. Rev.*, 2011, **40**, 340–362.

2 (*a*) M. R. Detty, S. L. Gibson and S. J. Wagner, *J. Med. Chem.*, 2004, **47**, 3897–3915; (*b*) K. Plaetzer, B. Krammer, J. Berlanda, F. Berr and T. Kiesslich, *Lasers Med. Sci.*, 2009, **24**, 259–268; (*c*) C. A. Robertson, D. H. Evans and H. Abrahamse, *J. Photochem. Photobiol., B.*, 2009, **96**, 1–8; (*d*) R. R. Allison and C. H. Sibata, *Photodiagn. Photodyn. Ther.*, 2010, **7**, 61–75.

3 (*a*) T. Nyokong, *Coord. Chem. Rev.*, 2007, **251**, 1707–1722; (*b*) C. M. Allen, W. M. Sharman and J. E. van Lier, *J. Porphyrins Phthalocyanines*, 2001, **5**, 161–169; (*c*) J.-Y. Liu, P.-C. Lo, X.-J. Jiang, W.-P. Fong and D. K. P. Ng, *Dalton Trans.*, 2009, 4129–4135; (*d*) F. Dumoulin, M. Durmuş, V. Ahsen and T. Nyokong, *Coord. Chem. Rev.*, 2010, **254**, 2792–2847; (*e*) S.-I. Ogura, K. Tabata, K. Fukushima, T. Kamachi and I. Okura, *J. Porphyrins Phthalocyanines*, 2006, **10**, 1116–1124.

4 (*a*) J.-P. Taquet, C. Frochot, V. Manneville and M. Barberi-Heyob, *Curr. Med. Chem.*, 2007, **14**, 1673–1687; (*b*) A. M. Bugaj, *Photochem. Photobiol. Sci.*, 2011, **10**, 1097–1109; (*c*) F. Schmitt and L. Juillerat-Jeanneret, *Anticancer Agents Med. Chem.*, 2012, **12**, 500–525; (*d*) P.-C. Lo, J. T. F. Lau and D. K. P. Ng, in *Handbook of Porphyrin Science*, ed. K. M. Kadish, K. M. Smith and R. Guilard, World Scientific, Singapore, 2012, vol. 18, pp. 239–301.

5 (*a*) Y. Zorlu, M. A. Ermeydan, F. Dumoulin, V. Ahsen, H. Savoie and R. W. Boyle, *Photochem. Photobiol. Sci.*, 2009, **8**, 312–318; (*b*) P.-C. Lo, C. M. H. Chan, J.-Y. Liu, W.-P. Fong and D. K. P. Ng, *J. Med. Chem.*, 2007, **50**, 2100–2107; (*c*) A. R. Soares, J. P. Tomé, M. G. Neves, A. C. Tomé, J. A. Cavaleiro and T. Torres, *Carbohydr. Res.*, 2009, **344**, 507–510.

6 S. L. Haywood-Small, D. I. Vernon, J. Griffiths, J. Schofield and S. B. Brown, *Biochem. Biophys. Res. Commun.*, 2006, **339**, 569–576.

7 (*a*) C. J. Gomer, A. Ferrario, M. Luna, N. Rucker and S. Wong, *Lasers Surg. Med.*, 2006, **38**, 516–521; (*b*) M. B. Vrouenraets, G. W. Visser, C. Loup, B. Meunier, M. Stigter, H. Oppelaar, F. A. Stewart, G. B. Snow and G. A. Dongen, *Int. J. Cancer*, 2000, **88**, 108–114; (*c*) M. Mitsunaga, M. Ogawa, N. Kosaka, L. T. Rosenblum, P. L. Choyke and H. Kobayashi, *Nat. Med.*, 2011, **17**, 1685–1691.

8 I. G. Meerovich, V. V. Jerdeva, V. M. Derkacheva, G. A. Meerovich, E. A. Lukyanets, E. A. Kogan and A. P. Savitsky, *J. Photochem. Photobiol. B: Biol.*, 2005, **80**, 57–64.

9 (*a*) E. Ranyuk, N. Cauchon, K. Klarskov, B. Guérin and J. E. Lier, *J. Med. Chem.*, 2013, **56**, 1520–1534; (*b*) M.-R. Ke, S.-L. Yeung, W.-P. Fong, D. K. P. Ng and P.-C. Lo, *Chem.–Eur. J.*, 2012, **18**, 4225–4233; (*c*) B. G. Ongarora, K. R. Fontenot, X. Hu, I. Sehgal, S. D. Satyanarayana-Jois and M. G. Vicente, *J. Med Chem.*, 2012, **55**, 3725–3738.

10 (*a*) Y. J. Zhu, J.-D. Huang, X. J. Jiang and J. C. Sun, *Inorg. Chem. Commun.*, 2006, **9**, 473–477; (*b*) X. J. Jiang, J.-D. Huang, Y. J. Zhu, F. X. Tang, D. K. P. Ng and J. C. Sun, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2450–2453; (*c*) M.-R. Ke, J.-D. Huang and S. M. Weng, *J. Photochem. Photobiol. A: Chem.*, 2009, **201**, 23–31; (*d*) X. M. Shen, X. J. Jiang, C. C. Huang, H. H. Zhang and J.-D. Huang, *Tetrahedron*, 2010, **66**, 9041–9048; (*e*) B. Y. Zheng, T. Lin, H. H. Yang and J.-D. Huang, *Dyes Pigm.*, 2013, **96**, 547–553.

11 (*a*) D. Komiotis, S. Manta, E. Tsoukala and N. Tzioumaki, *Curr. Med. Chem.: Anti-Infect. Agents*, 2008, **7**, 219–244; (*b*) C. E. De and A. Holý, *Nat. Rev. Drug Discovery*, 2005, **4**, 928–940.

12 (*a*) W. D. Meng and F. L. Qing, *Curr. Top. Med. Chem.*, 2006, **6**, 1499–1528; (*b*) A. Matsuda and T. Sasaki, *Cancer Sci.*, 2004, **95**, 105–111.

13 (*a*) X. Y. Li and D. K. P. Ng, *Tetrahedron Lett.*, 2001, **42**, 305–309; (*b*) J. L. Sessler, J. Jayawickramarajah, A. Gouloumis, G. D. Pantos, T. Torres and D. M. Guldi, *Tetrahedron*, 2006, **62**, 2123–2131; (*c*) T. Torres, A. Gouloumis, S. G. David, J. Jayawickramarajah, W. Seitz, D. M. Guldi and J. L. Sessler, *Chem. Commun.*, 2007, **3**, 292–294; (*d*) M. R. Reddy, N. Shibata, Y. Kondo, S. Nakamura and T. Toru, *Angew. Chem., Int. Ed.*, 2006, **45**, 8163–8166.

14 I. Scalise and E. N. Durantini, *Bioorg. Med. Chem.*, 2005, **13**, 3037–3045.

15 (*a*) M. D. Maree, N. Kuznetsova and T. Nyokong, *J. Photochem. Photobiol., A*, 2001, **140**, 117–125; (*b*) W. Spiller, H. Kliesch, D. Wohrle, S. Hackbarth, B. Roder and G. Schnurpfeil, *J. Porphyrins Phthalocyanines*, 1998, **2**, 145–148.

16 Compounds **1–4** were first dissolved in DMF (1.0 mM) and the solutions were diluted to 80 μM with 1% (wt) aqueous solution of Cremophor EL (Sigma, 1 g in 100 mL of water). The solutions were then diluted with the cellular culture medium to appropriate concentrations.

17 (*a*) L. Howe and J. Z. J. Zhang, *Phys. Chem. A*, 1997, **101**, 3207–3213; (*b*) X.-Y. Li, X. He, A. C. H. Ng, C. Wu and D. K. P. Ng, *Macromolecules*, 2000, **33**, 2119–2123.

18 (*a*) X.-J. Jiang, S. L. Yeung, P.-C. Lo, W.-P. Fong and D. K. P. Ng, *J. Med. Chem.*, 2011, **54**, 320–330; (*b*) X.-J. Jiang, P.-C. Lo, Y.-M. Tsang, S.-L. Yeung, W.-P. Fong and D. K. P. Ng, *Chem.–Eur. J.*, 2010, **16**, 4777–4783; (*c*) X.-J. Jiang, P.-C. Lo, S.-L. Yeung, W.-P. Fong and D. K. P. Ng, *Chem. Commun.*, 2010, **46**, 3188–3190; (*d*) P.-C. Lo, J.-D. Huang, D. Y. Y. Cheng, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Chem.–Eur. J.*, 2004, **10**, 4831–4838.

19 (*a*) J. W. Hofman, F. Zeeland, S. Turker, H. Talsma, S. A. G. Lambrechts, D. V. Sakharov, W. E. Hennink and C. F. Nostrum, *J. Med. Chem.*, 2007, **50**, 1485–1494; (*b*) H. Li, T. J. Jensen, F. R. Fronczek and M. G. H. Vicente, *J. Med. Chem.*, 2008, **51**, 502–511.

20 (*a*) J. Morgan and A. R. Oseroff, *Adv. Drug Delivery Rev.*, 2001, **49**, 71–86; (*b*) P. Mroz, A. Yaroslavsky, G. B. Kharkwal and M. R. Hamblin, *Cancers*, 2011, **3**, 2516–2539.

21 (*a*) R. Agarwal, N. J. Korman, R. R. Mohan, D. K. Feyes, S. Jawed, M. Zaim and H. Mukhtar, *Photochem. Photobiol.*, 2008, **63**, 547–552; (*b*) M. Lam, A. H. Hsia, Y. Liu, M. Guo, A. R. Swick, J. C. Berlin, T. S. McCormick, M. E. Kenney, N. L. Oleinick, K. D. Cooper and E. D. Baron, *Clin. Exp. Dermatol.*, 2011, **36**, 645–651.

22 I. Vermes, C. Haanena, H. Steffens-Nakkena and C. Reutellingsperger, *J. Immunol. Methods*, 1995, **184**, 39–51.