Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 8029–8032

TETRAHEDRON LETTERS

Photodynamic activities of a dicationic silicon(IV) phthalocyanine and its bovine serum albumin conjugates

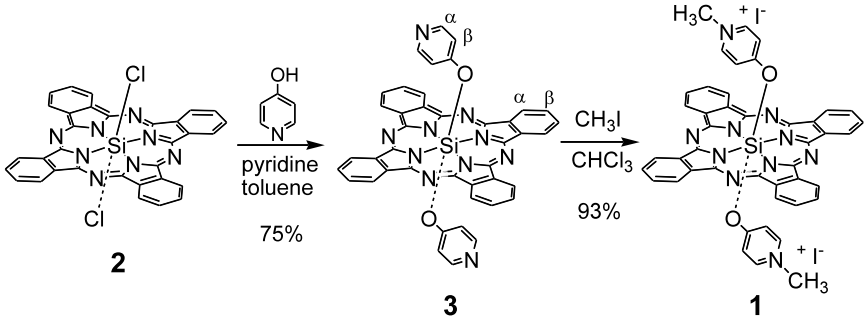
Jian-Dong Huang,[a,b] Wing-Ping Fong,[c,*] Elaine Y. M. Chan,[c] Michael T. M. Choi,[a] Wing-Kin Chan,[a] Man-Chor Chan[a] and Dennis K. P. Ng[a,*]

[a]*Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China*
[b]*Institute of Research on Functional Materials, Department of Chemistry, Fuzhou University, Fuzhou 350002, China*
[c]*Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China*

Received 9 June 2003; revised 2 July 2003; accepted 31 July 2003

Abstract—The dicationic bis(4-*N*-methylpyridiniumoxy)phthalocyaninatosilicon(IV) has been synthesised and examined for its photodynamic activities towards HepG2 and J774 cancer cell lines; the photocytotoxicity is greatly enhanced by complexation with bovine serum albumin.

Photodynamic therapy (PDT) is an innovative treatment for several types of cancer and wet age-related macular degeneration.[1] Its applications in many other areas such as removal of plaque that accumulate on arterial walls and treatment of infectious diseases, acne and pre-cancerous skin lesions are also being examined.[2] One of the critical elements determining the efficacy of this therapeutic approach rests on the photosensitising agents involved. Photofrin®, which is a complex mixture of hematoporphyrin derivatives, is still widely used clinically despite the deficiencies such as complex composition, poor absorption of tissue-penetrating red light and prolonged retention. As a result, there has been a great demand for second-generation photosensitisers which are more effective and less toxic.[3] Owing to the desirable photophysical and photochemical properties, ease of chemical modification and low toxicity in the absence of light, phthalocyanines have received much current attention.[4] While emphasis has been placed on anionic (e.g. $-SO_3^-$ and $-CO_2^-$ substituted) and non-ionic (e.g. -OH, -F substituted) phthalocyanines, photodynamic activities of cationic analogues have only been sporadically reported.[5] The very different polarity of the photosensitisers may greatly affect the cellular uptake, subcellular localisation and eventually the photosensitising efficiency. Cationic lipophilic photosensitisers appear to have selective affinity for mitochondria, which are believed to be the targets for the initiation of apoptosis by PDT.[6] Photosensitisers that localise to mitochondria seem to have higher pho-



Scheme 1.

* Corresponding authors. E-mail: wpfong@cuhk.edu.hk; dkpn@cuhk.edu.hk

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.07.007

tocytotoxic effects than those that localise at other cellular sites.[7] We describe herein the preparation and photodynamic activities of an easily accessible and structurally well-defined new dicationic phthalocyanine, namely bis(4-*N*-methylpyridiniumoxy)phthalocyaninatosilicon(IV) diiodide (**1**), together with its bovine serum albumin (BSA) conjugates towards HepG2 human hepatocarcinoma cells and J774 mouse mammary tumour cells of monocyte-macrophage origin. Serum albumins are major plasma proteins with many important functions, but their use for targeted delivery of photosensitisers remains little studied.[8]

Compound **1** was prepared from the commercially available silicon phthalocyanine dichloride (**2**) as shown in Scheme 1. Treatment of **2** with 4-hydroxypyridine in the presence of pyridine in toluene led to the substituted product **3**, which underwent methylation readily to give **1** in high yield. The new compounds **1** and **3** were fully characterised with various spectroscopic methods and elemental analysis.[9] The UV-vis spectrum of **1** in water was typical for non-aggregated phthalocyanines, showing the B band at 349 nm, a sharp Q band at 695 nm together with two vibronic bands at 624 and 662 nm. The spectrum was virtually unchanged upon standing under ambient conditions for 2 hours indicating that the compound is relatively stable in aqueous media. When excited at 615 nm in water, the compound showed a relatively strong fluorescence emission at 701 nm with a quantum yield of 0.15 [with reference to unsubstituted zinc phthalocyanine in *N*,*N*-dimethylformamide (DMF) ($\Phi_f = 0.30$)]. It is worth noting that fluorescence of phthalocyanines in aqueous media is rarely observed due to the strong hydrophobic interactions, leading to the formation of non-fluorescent aggregates.[10] The axial pyridinium groups are therefore very effective to inhibit aggregation of **1**.

To enhance the biocompatibility and selectivity, compound **1** was complexed with BSA, which is a common protein carrier for anticancer drugs to improve their passive targeting properties.[11] The conjugates were prepared by stirring mixtures containing different molar ratios of **1** (dissolved in <5% DMF) and BSA (20:1, 10:1 and 2.5:1) in 50 mM tris(hydroxymethyl)aminomethane–HCl buffer of pH 7.4 with 0.1 M NaCl at ambient temperature for 2 h, followed by chromatography on a G-100 Sephadex column (dry bead diameter = 40–120 μm) using a 20 mM aqueous NH_4HCO_3 (pH 8.3) eluent. The **1**-BSA conjugates collected as the first blue fraction were then lyophilised to remove NH_4HCO_3 and water. The protein contents were determined with the Bio-Rad protein assay kit using BSA as standard,[12] while the phthalocyanine concentrations were measured spectroscopically in DMF ($\varepsilon_{670} = 2.2\times10^5$ mol^{-1} dm^3 cm^{-1}). The molar ratios of **1** to BSA were found to be 8:1, 5:1 and 1:1 in the three conjugates. Interestingly, the UV-vis spectra of these conjugates are very different from that of **1**. As shown in Figure 1, the spectrum of **1**-BSA (1:1) conjugate in phosphate buffered saline (PBS) shows three absorptions in the visible and near-IR region, which is remarkably different from the typical non-aggregated

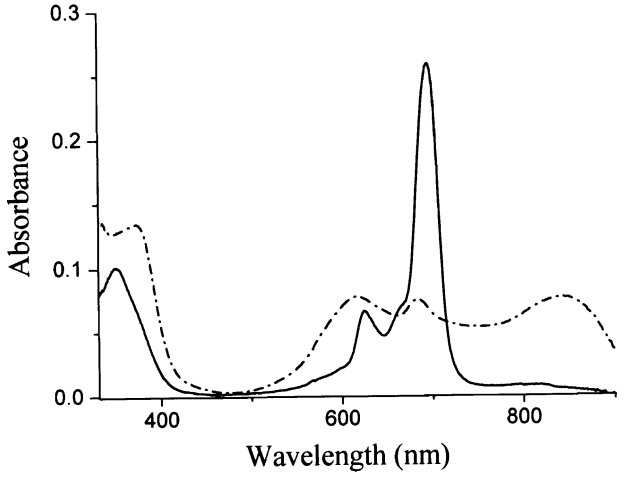


Figure 1. Electronic absorption spectra of **1** (—) and **1**-BSA (1:1) conjugate (---) in PBS (pH 7.4).

phthalocyanine spectrum of **1**. The exact nature of these peculiar bands, in particular the near-IR band at ca. 840 nm, remains elusive at this stage, but similar spectral features were observed in the solid-state spectra of distorted oxo(phthalocyaninato)titanium(IV).[13] Molecular distortion due to π–π atomic contacts along the molecular stack lifts the doubly degenerate excited state of this compound, giving two absorption bands at 660 and 840 nm.

The photodynamic activities of **1** and its BSA conjugates, under illumination with a red light (690 nm diverged from a Ti:sapphire laser) with a fluence rate of ca. 1 mW cm^{-2}, towards HepG2 and J774 cell lines were examined.[14] The cell survival was determined by means of the colourimetric MTT assay.[15] Figure 2 shows the survival curve for HepG2 using **1** as the photosensitiser. While **1** is relatively non-toxic in the

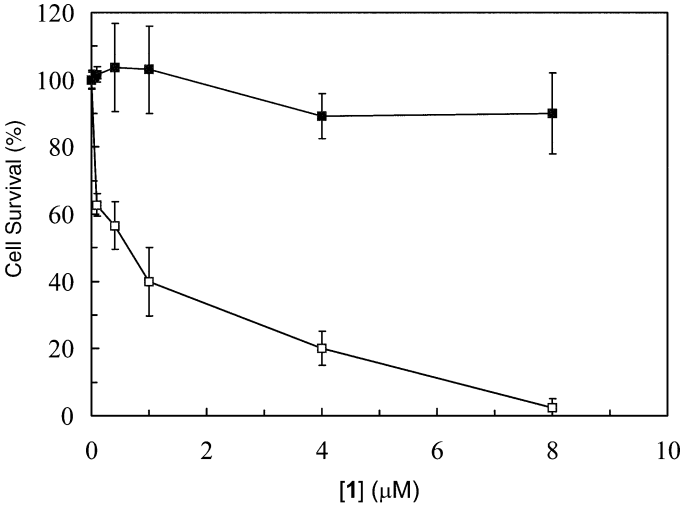


Figure 2. Dark- (■) and photo- (□) cytotoxicities of **1** towards HepG2. For the latter, the cells were illuminated with a red laser light (690 nm, 1.5 mW cm^{-2}, 5.5 J cm^{-2}). Values are expressed as mean±SD ($n = 3$).

absence of light, it has a high photocytotoxicity which increases with the concentration. The activity of **1** is significantly enhanced by conjugation with BSA. As shown in Figure 3, the photodynamic efficiency is higher for conjugate with a higher BSA content; the 1:1 complex is the most efficient system in this study. Reducing the light dose also reduces the photoactivity. When 4 μM of **1** was used, the cell viability increases from 20 to 81% as the light dose decreases from 5.5 to 1.8 J cm^{-2} (Figures 2 and 3). The enhancement in photoactivity by conjugation with BSA is even greater for J774. With 4 μM of **1** and under a total fluence of 1.8 J cm^{-2}, the cell viability greatly decreases from 74 (for **1**) to 2% [for **1**-BSA (1:1)] (data not shown), probably due to the macrophage origin of J774 which has a high affinity for BSA.[8b]

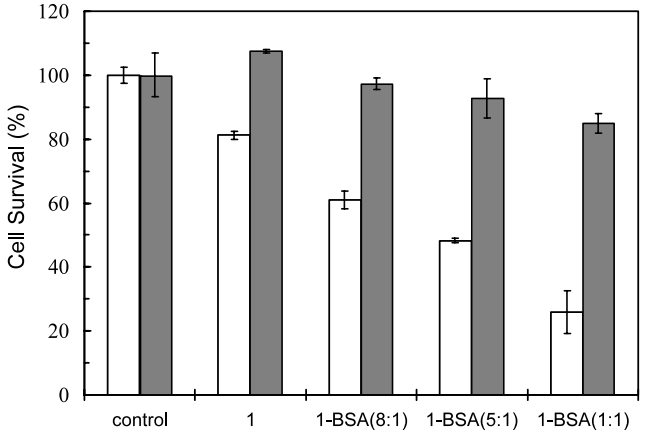


Figure 3. Comparison of the photocytotoxicities (white columns) of **1** and its BSA conjugates towards HepG2. The cells were incubated with **1** or **1**-BSA (4 μM based on **1**) for 2 h and then illuminated with a red laser light (690 nm, 0.6 mW cm^{-2}, 1.8 J cm^{-2}). Grey columns represent data in the absence of light. Values are expressed as mean±SD ($n=3$).

In summary, we report herein the dicationic phthalocyanine **1**, which apparently fulfils most of the criteria for second-generation photosensitisers including ease of preparation, unique composition, high stability and being non-aggregated in aqueous media, strong absorption in the red visible region, low dark-toxicity and high photoactivity. The photodynamic activities of this compound are greatly enhanced by conjugation with BSA protein, which serves as a promising vehicle for targeted delivery of phthalocyanine photosensitisers. Further studies are in progress to optimise the efficiency of these systems.

Acknowledgements

We thank the Croucher Foundation for a Chinese Visitorship to J.-D.H. This work was supported by The Chinese University of Hong Kong (Direct Grant 2001-02, Project Code 2060214), Natural Science Foundation of China (Grant No. 20201005) and Foundation for University Key Teachers by the Ministry of Education, China. The laser facilities used in this work were supported by the Special Equipment Grant of the Science Faculty, The Chinese University of Hong Kong.

References

1. (a) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer Inst*. **1998**, *90*, 889–905; (b) Sharman, W. M.; Allen, C. M.; van Lier, J. E. *Drug Discovery Today* **1999**, *4*, 507–517; (c) Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach: Amsterdam, 2000.
2. (a) Rouhi, A. M. *Chem. Eng. News* **1998**, *Nov. 2*, 22–27; (b) Lane, N. *Sci. Amer*. **2003**, *Jan*., 26–33.
3. (a) Ali, H.; van Lier, J. E. *Chem. Rev*. **1999**, *99*, 2379–2450; (b) Bonnett, R. *J. Heterocyclic Chem*. **2002**, *39*, 455–470.
4. (a) Lukyanets, E. A. *J. Porphyrins Phthalocyanines* **1999**, *3*, 424–432; (b) Allen, C. M.; Sharman, W. M.; van Lier, J. E. *J. Porphyrins Phthalocyanines* **2001**, *5*, 161–169; (c) Tedesco, A. C.; Rotta, J. C. G.; Lunardi, C. N. *Curr. Org. Chem*. **2003**, *7*, 187–196.
5. (a) Leznoff, C. C.; Vigh, S.; Svirskaya, P. I.; Greenberg, S.; Drew, D. M.; Ben-Hur, E.; Rosenthal, I. *Photochem. Photobiol*. **1989**, *49*, 279–284; (b) Zaidi, S. I. A.; Agarwal, R.; Eichler, G.; Rihter, B. D.; Kenney, M. E.; Mukhtar, H. *Photochem. Photobiol*. **1993**, *58*, 204–210; (c) Peeva, M.; Shopova, M.; Michelsen, U.; Wöhrle, D.; Petrov, G.; Diddens, H. *J. Porphyrins Phthalocyanines* **2001**, *5*, 645–651; (d) Soncin, M.; Fabris, C.; Busetti, A.; Dei, D.; Nistri, D.; Roncucci, G.; Jori, G. *Photochem. Photobiol. Sci*. **2002**, *1*, 815–819.
6. Oleinick, N. L.; Morris, R. L.; Belichenko, I. *Photochem. Photobiol. Sci*. **2002**, *1*, 1–21.
7. (a) Dummin, H.; Cernay, T.; Zimmermann, H. W. *J. Photochem. Photobiol. B: Biol*. **1997**, *37*, 219–229; (b) Ball, D. J.; Mayhew, S.; Wood, S. R.; Griffiths, J.; Vernon, D. I.; Brown, S. B. *Photochem. Photobiol*. **1999**, *69*, 390–396.
8. (a) Larroque, C.; Pelegrin, A.; van Lier, J. E. *Brit. J. Cancer* **1996**, *74*, 1886–1890; (b) Brasseur, N.; Langlois, R.; La Madeleine, C.; Ouellet, R.; van Lier, J. E. *Photochem. Photobiol*. **1999**, *69*, 345–352.
9. Characterising data for **1**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.75–9.78 (m, 8H, Pc-H_α), 8.63–8.66 (m, 8H, Pc-H_β), 7.19 (d, $J=6.9$ Hz, 4H, Py-H_α), 3.24 (s, 6H, CH_3), 3.10 (d, $J=6.9$ Hz, 4H, Py-H_β); $^{13}C\{^1H\}$ NMR (DMSO-d_6, 75.4 MHz) δ 172.7, 162.1, 149.6, 145.2, 133.4, 124.6, 115.4, 44.9; HRMS (FAB) m/z 379.1167 [calc. for $(M-2I)^{2+}$ 379.1161]; UV-Vis (H_2O) [λ_{max}/nm (log ε)] 349 (4.88), 624 (4.59), 662 (sh), 695 (5.35). Anal. Calc. for $C_{44}H_{32}I_2N_{10}O_3Si$ (**1** H_2O): C, 51.27; H, 3.13; N, 13.59; I, 24.62. Found: C, 50.83; H, 3.24; N, 13.17; I, 24.21%. **3**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.64–9.67 (m, 8H, Pc-H_α), 8.40–8.43 (m, 8H, Pc-H_β), 6.76 (d, $J=6.6$ Hz, 4H, Py-H_α), 2.44 (d, $J=6.6$ Hz, 4H, Py-H_β); UV-vis ($CHCl_3$) [λ_{max}/nm (log ε)] 357 (4.86), 614 (4.55), 654 (4.48), 684 (5.32). Anal. calcd for $C_{43}H_{28}N_{10}O_3Si$ (**3** CH_3OH): C, 67.88; H, 3.71; N, 18.41. Found: C, 68.05; H, 3.32; N, 18.47%.

10. Ng, A. C. H.; Li, X.-y.; Ng, D. K. P. *Macromolecules* **1999**, *32*, 5292–5298 and references cited therein.
11. Stehle, G.; Wunder, A.; Schrenk, H. H.; Hartung, G.; Heene, D. L.; Sinn, H. *Anti-cancer Drugs* **1999**, *10*, 785–790.
12. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
13. Mizuguchi, J.; Rihs, G.; Karfunkel, H. R. *J. Phys. Chem.* **1995**, *99*, 16217–16227.
14. About 2×10^4 HepG2 cells and 3×10^4 J774 cells per well in 100 μL RPMI medium 1640 (Life Technologies) supplemented with 10% fetal calf serum were inoculated in 96-multiwell plates and incubated overnight at 37°C under 5% CO_2. The cells were rinsed with PBS and incubated with 100 μL of different concentrations of **1** or its conjugates in the same medium for 2 h under the same conditions. The cells were then rinsed again with PBS and re-fed with 100 μL of the growth medium before being illuminated with a red light at ambient temperature.
15. MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide. Tada, H.; Shiho, O.; Kuroshima, K.; Koyama, M.; Tsukamoto, K. *J. Immunol. Methods* **1986**, *93*, 157-165.