Folate Receptor Mediated Targeted Delivery of Porphyrin Photosensitizer

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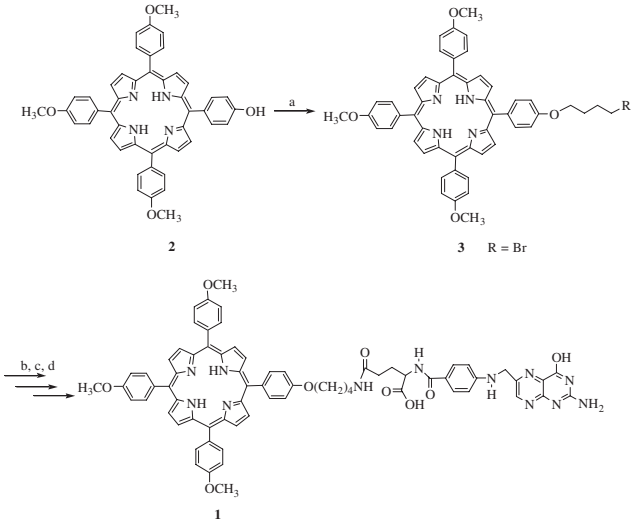
A folate–porphyrin conjugate **1** has been synthesized and characterized. The cellular uptake of conjugate **1** by Hela cells was about 35 times higher than that of precursor porphyrin **3** after 24 h of incubation and can be inhibited competitively by free folic acid. Moreover, conjugate **1** exhibited much lower dark cytotoxicity against Hela cells in vitro but significant photocytotoxicity with 86.4% cell growth inhibition ratio after irradiation.

As an alternative, noninvasive modality for cancer therapy, photodynamic therapy (PDT) has attracted wide attention.[1–3] Despite promising results, current PDT leaves much to be desired. A main limitation is the lack of selectivity of the first and second generation photosensitizers, which can result in severe side effects, such as prolonged cutaneous phototoxicity.[4–7] Targeted PDT offers the opportunity of enhancing photodynamic efficiency by directly targeting diseased cells and tissues. Since the folate receptor is overexpressed in human epithelial cancer cells but is absent from most normal cells and folic acid can specifically bind to folate receptor with high affinity ($K_d \approx 10^{-9}$ M),[8–10] there has been much interest in exploiting this natural cellular uptake mechanism for the targeted delivery of chemotherapeutic compounds.[11–13]

Despite the many anticancer drugs that have been conjugated with folic acid either directly or by the use of a carrier over the past decade, very little effort has been devoted to the synthesis of a targeted photosensitizer mediated by folate–receptor, so we designed and synthesized a folate–porphyrin photosensitizer following a four-step procedure shown in Scheme 1. First, the hydroxy group of porphyrin **2** was reacted with stable 1,4-dibromobutane in DMF to generate bromoporphyrin **3** in 81.5% yield, then porphyrin **3** was reacted with potassium phthalimide in DMF to give amide porphyrin **4** in 89% yield, which was followed by the hydrolyzation in THF/hydrazine hydrate to offer amido-terminated porphyrin **5** in 92% yield. Finally, the amine group of porphyrin **5** was reacted with the γ-carboxylic group of folic acid, which was activated in advance with DCC and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONb) at room temperature in the dark, to offer the conjugate **1** in 54.2% yield. Due to much higher reactivity, the coupling reaction would occur mainly at the γ-carboxylic group rather than the α-carboxylic group of folic acid.[14,15] In comparison with similar compounds reported in the literature,[16,17] the porphyrin block in conjugate **1** conjugated with folic acid through an ether bond, which can efficiently avoid being hydrolyzed by enzyme in the body, so conjugate **1** is more stable. And due to the application of Gabriel reaction in the synthesis of conjugate **1**, the procedures were easier and the total yield was increased to 36%. The structure of conjugate **1** was verified by UV–vis, IR, ^{1}H NMR, and Ms spectra.[19]



Scheme 1. Reagents and conditions: (a) 1,4-dibromobutane, DMF, K_2CO_3, KI, 80 °C; (b) potassium phthalimide, DMF, 100 °C; (c) THF, NH_2NH_2, reflux; (d) DCC, HONb, DMSO, folic acid, pyridine, rt, in dark.

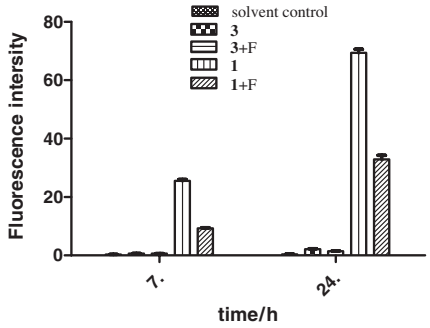


Figure 1. Cellular uptake efficiency of porphyrin **3** and conjugate **1** by Hela cells in or without present of free folic acid (F).

The cellular uptake of conjugate **1** by human cervix carcinoma Hela cells, which are one of the many tumor cell types that over-express folate receptors,[18] was investigated first by fluorescence spectroscopy (HITACHI F-4500, Japan) with porphyrin **3** as control. As shown in Figure 1, the cellular uptake of conjugate **1** increased obviously with the increase of the incubation time, after 24 h the accumulation of conjugate **1** in cells was almost 35-fold higher than that of porphyrin **3**. Moreover, the presence of excess of free folic acid inhibited conspicuously the cellular uptake of conjugate **1**. However, both the increase of porphyrin **3** in cells and the inhibition of folic acid for porphyrin **3** were not significant. These findings suggested that free folic acid mole-

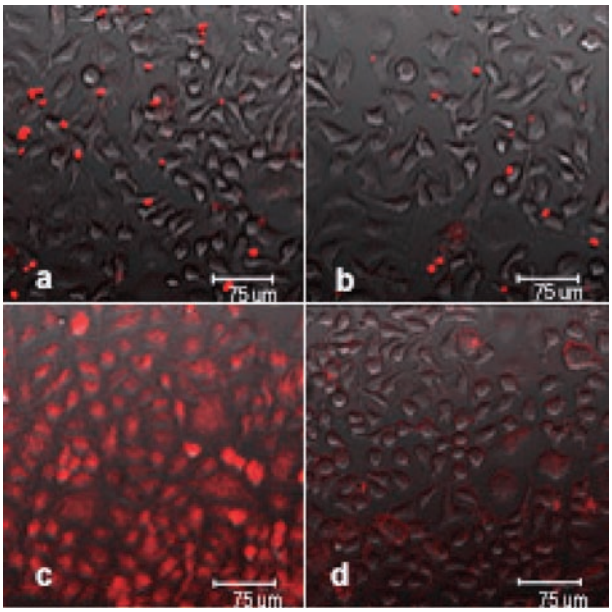


Figure 2. Confocal laser scanning microscopy of Hela cells incubated with 5×10^{-5} M porphyrin **3** (a), 5×10^{-5} M porphyrin **3** + 5×10^{-3} M folic acid (b), 5×10^{-5} M conjugate **1** (c), 5×10^{-5} M conjugate **1** + 5×10^{-3} M folic acid (d) for 24 h at 37 °C.

cules prevented the cellular uptake of conjugate **1** though competitively binding to the folate–receptors on the cell surface; namely, the enhanced cellular uptake of conjugate **1** compared with that of porphyrin **3** should be attributed to the endocytosis mediated by folate–receptor.

The results of laser scanning confocal microscopy (Leica, TCS SP2, Germany) are shown in Figure 2. When cells were incubated with porphyrin **3** (Figure 2a), porphyrin molecules simply aggregated on the cell surface through a passive diffusion pathway possibly and no significant difference between Figures 2a and 2b was observed. However, strong red fluorescence of porphyrin was observed in the cytoplasm and the nucleus when the cells were incubated with the conjugate **1** (Figure 2c), and the fluorescence intensity weakened evidently with the addition of free folic acid (Figure 2d). These results were similar to that of fluorescence microscopy. The strong signals of porphyrin appeared in the nucleus and cytoplasm indicated that the conjugate **1** was internalized by the cells through an endocytic process mediated by folate receptor rather than nonspecific cell absorption.

Cytotoxicity against Hela cells in vitro was measured by MTT assay. The results demonstrated that no cytotoxicity was observed when Hela cells were incubated with 5×10^{-5} M of conjugate **1** for 24 h in dark, but with the same concentration, conjugate **1** can inhibit the Hela cell proliferation with growth inhibition ratio of 86.4% after irradiation with a semiconductor laser therapy instrument at a power density of 12 J cm^{-2} (66 mW cm^{-2} for 3 min) and a wavelength of 630 nm. However, at the same concentration, porphyrin **3** showed no dark cytotoxicity and photocytotoxicity against Hela cells. These results suggested that as a photosensitizer, conjugate **1** possessed some desirable properties of higher photocytotoxicity but minimal or no dark toxicity.

In summary, a photosensitizer was designed and synthesized for the purpose of improving the tumor targeting of photosensitizer via the interaction between folate and folate receptor and the endocytosis mediated by the receptor. It was found that the photosensitizer exhibited significant targeting effects and higher photocytotoxicity to Hela cells. More extensive and deeper biological studies are ongoing. It is anticipated that this folate–porphyrin-like targeting photosensitizer will provide a promising platform for targeted photodynamic therapy in the near future.

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References and Notes

1 R. Ackroyd, C. Kelty, N. Brown, M. Reed, *Photochem. Photobiol.* **2001**, *74*, 656.
2 T. J. Dougherty, *J. Clin. Laser Med. Surg.* **2002**, *20*, 3.
3 D. K. Chatterjee, L. S. Fong, Y. Zhang, *Adv. Drug Delivery Rev.* **2008**, *60*, 1627.
4 Y. N. Konan, M. Berton, R. Gurny, E. Allémann, *Eur. J. Pharm. Sci.* **2003**, *18*, 241.
5 U. O. Nseyo, B. Shumaker, E. A. Klein, K. Sutherland, *J. Urol.* **1998**, *160*, 39.
6 M. R. Detty, S. L. Gibson, S. J. Wagner, *J. Med. Chem.* **2004**, *47*, 3897.
7 E. D. Sternberg, D. Dolphin, C. Bruchner, *Tetrahedron* **1998**, *54*, 4151.
8 A. J. Clifford, A. Arjomand, S. R. Dueker, P. D. Schneider, B. A. Buchholz, J. S. Vogel, *Adv. Exp. Med. Biol.* **1998**, *445*, 239.
9 M. D. A. Salazar, M. Ratnam, *Cancer Metastasis Rev.* **2007**, *26*, 141.
10 C. P. Leamon, *Curr. Opin. Invest. Drugs* **2008**, *9*, 1277.
11 I. R. Vlahov, H. K. R. Santhapuram, P. J. Kleindl, S. J. Howard, K. M. Stanford, C. P. Leamon, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5093.
12 J. Sudimack, R. Lee, *Adv. Drug Delivery Rev.* **2000**, *41*, 147.
13 C. P. Leamon, *Curr. Opin. Invest. Drugs* **2008**, *9*, 1277.
14 S. Wang, R. J. Lee, C. J. Mathias, M. A. Green, P. S. Low, *Bioconjugate Chem.* **1996**, *7*, 56.
15 S. Wang, J. Luo, D. A. Lantrip, D. J. Waters, C. J. Mathias, M. A. Green, P. L. Fuchs, P. S. Low, *Bioconjugate Chem.* **1997**, *8*, 673.
16 R. Schneider, F. Schmitt, C. Frochot, Y. Fort, N. Lourette, F. Guillemin, J. F. Muller, M. Barberi-Heyob, *Bioorg. Med. Chem.* **2005**, *13*, 2799.
17 J. Gravier, R. Schneider, C. Frochot, T. Bastogne, F. Schmitt, J. Didelon, F. Guillemin, M. Barberi-Heyob, *J. Med. Chem.* **2008**, *51*, 3867.
18 N. Parker, M. J. Turk, E. Westrick, J. D. Lewis, P. S. Low, C. P. Leamon, *Anal. Biochem.* **2005**, *338*, 284.
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