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Porphyrin–DNA cross-linking agent hybrids: chemical synthesis and biological studies

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Abstract—Three new porphyrin–DNA cross-linking conjugates **8**, **9**, and **10** have been synthesized. Their photoinduced DNA cleavage activity have been studied. The IC_{50} values to THP-1 cells in the presence of porphyrin derivatives **8**, **9**, and **10** with photoirradiation were 5.6, 88.4, and 61.8 nM, respectively.

A great deal of attention has been directed to inducing DNA interstrand cross-link (ISC) by chemical agent or photoactivation.[1] DNA ISC plays very important roles for cancer therapy in antitumor agents by disrupting cell maintenance and replication. Some drugs (e.g. cisplatin, chlorambucil, and mitomycin C) have already been employed in clinical medicine.[2] Meanwhile, it is known that porphyrin as a photosensitizer can localize in tumor cells and be phototriggered to produce singlet oxygen to cleave DNA and damage tumor cells.[3] Previously, we have reported that a biphenol bis(quaternary ammonium) derivative (Fig. 1, **I**) could potently induce DNA cross-links by photoactivation.[4] (2-Hydroxylbenzyl) trimethyl ammonium can be photoactivated to form *o*-QMs (*o*-quinone methide) (Fig. 1, **II**),[5] a species known to alkylate DNA.[6] Considering that porphyrin has a good selectively for cancer cells and has antitumor activity by photoexcitation, and (2-hydroxylbenzyl) trimethyl ammonium could be photoactivated to form *o*-QMs, we designed novel bifunctional units as new antitumor agents by combining porphyrin (as a carrier and drug itself) and photo-inducible DNA alkylation agent **II** (as a drug 'warhead'). These new derivatives were expected to be inert prodrug bioconjugates that are nontoxic until they are activated by illumination. Herein, we report our first results from this project.

The target compounds **8**, **9**, and **10** were synthesized according to the procedures described in Scheme 1. 4-Hydroxy-3-[(dimethylamino)methyl]benzenedaldehyde **1**[7] condensed with *meso*-substituted dipyrrolemethanes **2**, **3**, and **4**, respectively, in the presence of TFA in a co-solvent of CH_2Cl_2/CH_3CH_2OH (95:5).[8] The reaction mixtures were stirred under N_2 for 24 h at room temperature.[9] After oxidation for 3–10 h, *meso*-substituted porphyrin derivatives **5**, **6**, and **7** were obtained in the yields of 6%, 16%, and 15% yields, respectively. Methylations of compounds **5**, **6**, and **7** were carried out by mixing methyl iodide in acetone. Compounds **8**, **9**, and **10** were obtained in good yields. For our mechanistic study, we also further synthesized compound **11** that could be prepared by methylation of compound **6** in the presence of K_2CO_3. All new compounds were fully characterized by ^1H NMR, UV, and HRMS.[10]

Measurement of singlet oxygen production was carried out by DPBF(1,3-diphenyl isobenzofuran) decomposition reaction.[11,12] Porphyrin (1.5 μM) and DPBF (150 μM) were dissolved in the buffer (3 mM Tris–HCl, 0.3 mM EDTA, pH = 8.0, 2.5% DMF), transferred to a

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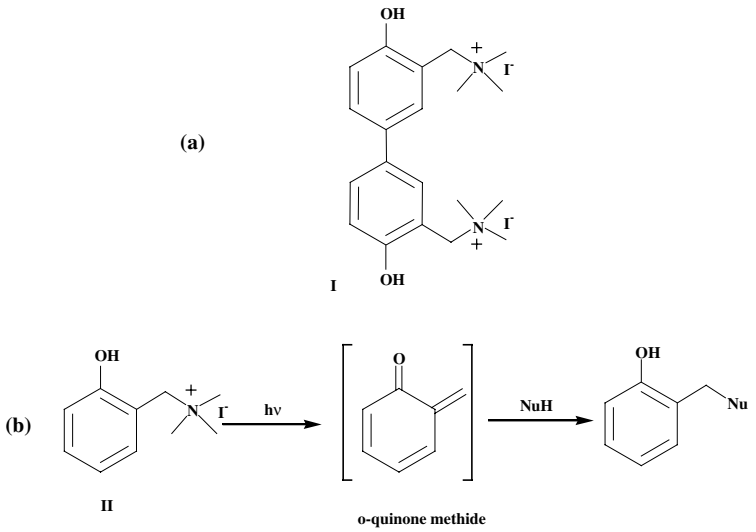
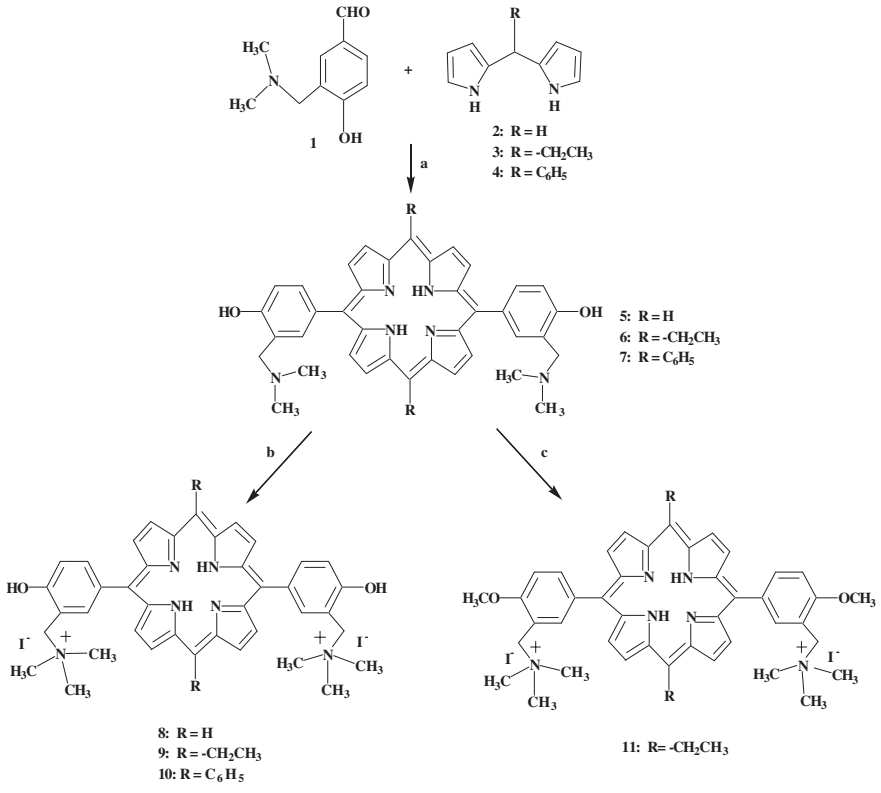


Figure 1. *o*-Quinone methide formation by photoactivation.



Scheme 1. Synthesis of phenol quaternary ammonium porphyrins. (a) CH_2Cl_2/CH_3OH (95:5), N_2, 20 h for **5** or 24 h for **6** and **7**, then added 2,3,5,6-tetrachloro-1,4-benzoquinone, stirring for 3 h (6.2% yield of **5**) or 10 h (15.8% yield of **6**) or 10 h (15% yield of **7**); (b) CH_3I, acetone, rt, 1 h; (c) CH_3I, K_2CO_3, N_2, DMF, 24 h, yield: 62%.

glass tube, then irradiated under a high-pressure lamp (50 W). A decrease of DPBF concentration was measured by an absorbance at 415 nm. Results were shown in Figure 2. The slopes of the plots of bleached absorption of DPBF versus illumination time were −0.00461 for **8**, −0.0045 for **9**, −0.00423 for **10**, 0.00509 for **11**, respectively. And the slope is proportional to the rate of production of singlet oxygen. Therefore, the rates of singlet oxygen production for these porphyrins were not significant different.

Photocleavage of supercoiled pBR322 (0.15 μg) was finished with various porphyrins in buffer (pH = 8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 2.5% DMF). Samples were exposed to a 50 W high-pressure mercury lamp, which placed 15 cm away at 25 °C for 20 min. The results were

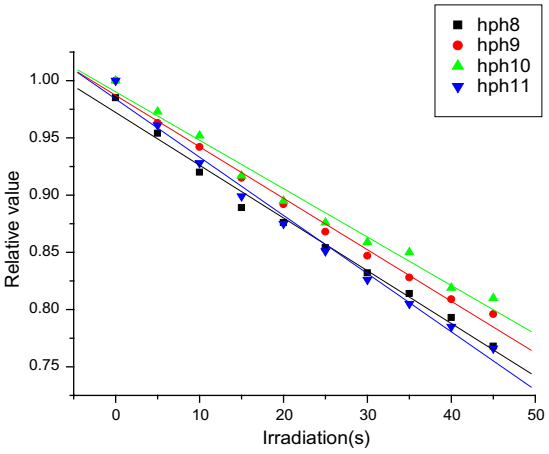


Figure 2. Decomposition of DPBF by singlet oxygen generated by compound porphyrin **8**, **9**, **10**, **11** (1.5 μM), and DPBF (150 μM) were irradiated in the solution (3 mM Tris–HCl, 0.3 mM EDTA, pH = 8.0, 2.5% DMF).

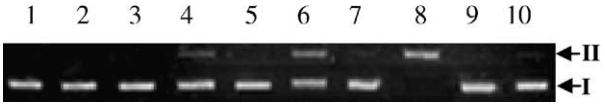


Figure 3. Cleavage of supercoiled pBR322 DNA by porphyrins **8**, **9**, **10**, and **11**. Reaction mixtures (10 μL) contained 0.15 μg of plasmid DNA. Lane 1: DNA + 2.5% DMF; lane 2: DNA + 2.5% DMF + hν (20 min); lane 3: DNA + porphyrin **8** (2 μM) + 2.5% DMF; lane 4: DNA + porphyrin **8** (2 μM) + 2.5% DMF + hν (20 min); lane 5: DNA + porphyrin **9** (2 μM) + 2.5% DMF; lane 6: DNA + porphyrin **9** (2 μM) + 2.5% DMF + hν (20 min); lane 7: DNA + porphyrin **10** (2 μM) + 2.5% DMF; lane 8: DNA + porphyrin **10** (2 μM) + 2.5% DMF + hν (20 min); lane 9: DNA + porphyrin **11** (2 μM) + 2.5% DMF; lane 10: DNA + porphyrin **11** (2 μM) + 2.5% DMF + hν (20 min).

Figure 4. Cleavage of supercoiled pBR322 DNA by porphyrins **11**. Lane1: DNA + 2.5% DMF; lane 2: DNA + 2.5% DMF + hν (20 min); lane 3: DNA + porphyrin **11** (2 μM) + 2.5% DMF; lane 4: DNA + porphyrin **11** (2 μM) + 2.5% DMF + hν (20 min); lane 5: DNA + porphyrin **11** (5 μM) + 2.5% DMF + hν (20 min); lane 6: DNA + porphyrin **11** (20 μM) + 2.5% DMF + hν (20 min).

analyzed by gel electrophoresis (0.9%). Results of DNA cleavage by porphyrins were illustrated in Figure 3.

Further photocleavage titration of compound **11** with pBR322 was finished under the same conditions. The result was shown in Figure 4. Apparently, the ability of photocleavage of DNA by compound **9** was twice times stronger than that of compound **11**.

A MTT assay was performed to determine THP-1 cell viability.[13] Cytotoxic data were expressed as IC_{50} values (the concentration of the test agent inducing 50% reduction in cell numbers compared with control cultures). The IC_{50} values of **8**, **9**, **10**, and **11** were 5.6, 88.4, 61.8, and 1.4×10^{3} nM, respectively.

These results indicated that the cytotoxic activities of porphyrin **8**, **9**, and **10** were much higher than that of porphyrin **11** (nearly 100 times). Peripheral substituents of porphyrins **8**, **9**, **10**, and **11** might affect porphyrin's interactions with DNA because of their binding modes.[14] Our previous and current findings suggested that abilities of photocleavage of DNA by porphyrins had not big difference (1–5 times) if peripheral substituents on the porphyrin were introduced. Therefore, the

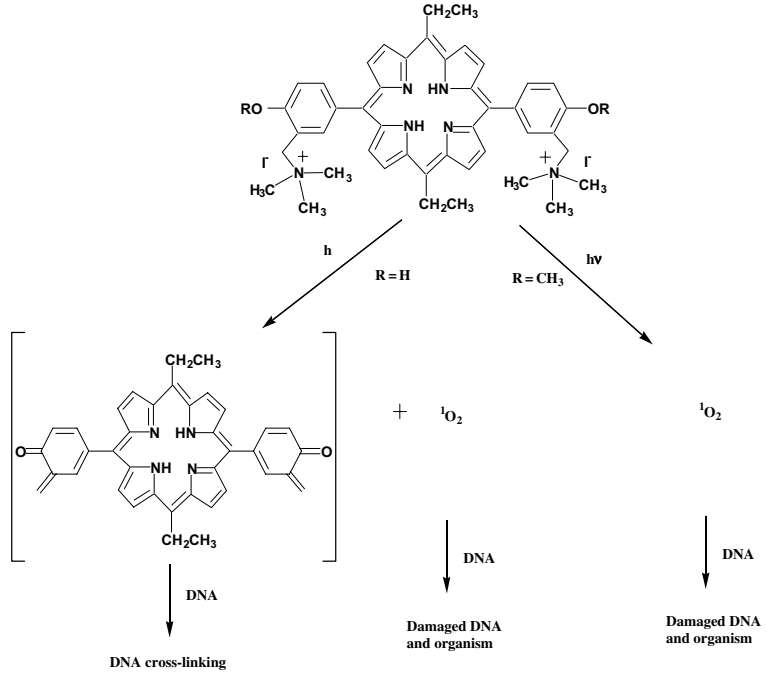


Figure 5. Possible mechanism for these porphyrin's action.

difference of cytotoxic to tumor cell among these porphyrins **8**, **9**, **10**, and **11** did not depend on the content of their interactions with DNA and photocleavage of DNA. Therefore, we suggested that compounds **8**, **9**, and **10** could produce both singlet oxygen and *o*-QMs under photoactivation[11] Singlet oxygen and *o*-QMs could damage tumor cells efficiently. However, hybrid porphyrin **11** could only produce singlet oxygen.[11] As their production of singlet oxygen were nearly equal for compounds **8**, **9**, **10**, and **11** (Fig. 2), porphyrin hybrid **11** without *o*-QMs formation under illumination could damage tumor cells much less than that of compounds **8**, **9**, and **10** (Fig. 5).

In conclusion, we have synthesized three porphyrin–DNA cross-linking agent hybrids **8**, **9**, and **10**. They showed the photocleavage to DNA. The IC_{50} values to THP-1 cells in the presence of porphyrin derivatives **8**, **9**, and **10** with photoirradiation were at nM level. The cytotoxicities of compounds **8**, **9**, and **10** might be involved in both singlet oxygen and *o*-QMs mechanism. under photoactivation.

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References and notes

1. (a) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723; (b) Hartley, J. A.; Hazrati, A.; Hokdgkinson, T. J.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F. *Chem. Commun.* **2000**, 2325; (c) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.
2. (a) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153; (b) Li, V.-S.; Choi, D.; Tang, M.-S.; Kohn, H. *J. Am. Chem. Soc.* **1996**, *118*, 3765.
3. (a) Sternberg, E. D.; Dolphin, D. *Tetrahedron* **1998**, *54*, 4151; (b) Dougherty, T. J. *J. Natl. Cancer Inst.* **1998**, *90*, 889; (c) Sessler, J. L.; Weghorn, S. J. *Expanded, Contracted and Isomeric Porphyrins*; Elsevier: Oxford, 1997; (d) Bonnett, R.; Martinez, G. *Tetrahedron* **2001**, *57*, 9513; (e) Kim, Y.-S.; Song, R.; Kim, D. H.; Jun, M. J.; Sohn, Y. S. *Bioorg. Med. Chem.* **2003**, *11*, 1753.
4. Wang, P.; Liu, R.-P.; Wu, X.-J.; Ma, H.-J.; Cao, X.-P.; Zhou, P.; Zhang, J.-Y.; Weng, X.-C.; Zhang, X.-L.; Qi, J.; Zhou, X.; Weng, L.-H. *J. Am. Chem. Soc.* **2003**, *125*, 1116.
5. Modica, E.; Zanaletti, R.; Freccero, M.; Mella, M. *J. Org. Chem.* **2001**, *66*, 41.
6. Pande, P.; Shearer, J.; Yang, J.; Greenberg, W. A.; Rokita, S. E. *J. Am. Chem. Soc.* **1999**, *121*, 6773.
7. O'Brien, P. M.; Slikovic, D. R.; Blankley, C. J.; Roth, B. D.; Willson, M. W.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. *J. Med. Chem.* **1994**, *37*, 1810.
8. (a) Wang, Q. M.; Bruce, D. W. *Synlett* **1995**, 1267; (b) Lee, C. H.; Lindsey, J. S. *Tetrahedron* **1994**, *50*, 11436.
9. (a) Lindsey, J. S.; Wagner, R. W. *J. Org. Chem.* **1989**, *54*, 828; (b) Little, B. J.; Ciringh, Y.; Lindsey, J. S. *J. Org. Chem.* **1999**, *64*, 2864.
10. Selected data **8**: ^{1}H NMR (δ DMSO, 300 MHz), 10.61 (s, 2H), 9.65 (s, 4H), 9.15 (m, 4H), 8.26 (m, 4H), 7.50 (d, 2H, $J = 8.10$ Hz), 4.77 (s, 4H), 3.28 (s, 18H), −3.23 (br s, 2H); UV–vis (CH_3OH): λ_{max} (nm, $\log\varepsilon$) = 408.0 (5.07), 508 (4.06), 544.5 (3.87), 576.5 (3.78), 635.5 (3.58); ESI HRMS for $C_{40}N_6O_2H_{42}$ ([$(M^+ - 2I)/2$]) calcd 319.1679, found 319.1687. **9**: ^{1}H NMR (δ DMSO, 300 MHz), 9.68 (m, 4H), 8.92 (m, 4H), 8.16 (m, 4H), 7.42 (d, 2H, $J = 8.10$ Hz), 5.03 (m, 4H), 4.74 (s, 4H), 3.26 (s, 18H), 2.08 (t, 6H, $J_1 = 6.60$ Hz, $J_2 = 7.50$ Hz), −2.85 (br s, 2H); UV–vis (CH_3OH) λ_{max} (nm, $\log\varepsilon$): 421.0 (5.02), 517.5 (3.90), 553.0 (3.81), 596.0 (3.49); ESI HRMS for $C_{44}N_6O_2H_{50}$ ([$(M^+ - 2I)/2$]) calcd 347.1997, found 347.2002. **10**: ^{1}H NMR (δ DMSO, 300 MHz), 9.01 (d, 4H), 8.85 (s, 4H), 8.24 (m, 8H), 7.87 (m, 6H), 7.47 (m, 2H), 4.76 (s, 4H), 3.26 (s, 18H), −2.90 (br s, 2H); UV–vis (CH_3OH) λ_{max} (nm, $\log\varepsilon$): 421.0 (5.55), 516.5 (4.39), 552.0 (4.25), 648.5 (3.96); ESI HRMS for $C_{52}N_6O_2H_{50}$ ([$(M^+ - 2I)/2$]) calcd 395.1992, found 395.1998.
11. Zhang, J.-Y.; Wu, X.-J.; Cao, X.-P.; Yang, F.; Wang, J.-F.; Zhou, X.; Zhang, X.-L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1097.
12. (a) Michelsen, D.; Kliesch, H.; Wohrle, D. *Photochem. Photobiol.* **1996**, *64*, 694; (b) Oda, K.; Ogura, S.; Osekura, I. *J. Photochem. Photobiol. B* **2000**, *59*, 20.
13. Alley, M. C.; Scudiero, D.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1998**, *48*, 589.
14. (a) Chen, B.; Qin, W.; Wang, P.; Tian, T.; Ma, H.-J.; Cao, X.-P.; Wu, X.-J.; Zhou, X.; Zhang, X.-L.; Liu, F.; Zheng, F.; Li, X. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3731; (b) Wu, S.; Wang, P.; Tian, T.; Wu, L.; He, H.-P.; Zhou, X.; Zhang, X.-L.; Cao, X.-P. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 2575.