Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2575–2577
Bioorganic & Medicinal Chemistry Letters

Peripheral substituents of di(pyridiumyl)porphyrins affected on their interactions with DNA

Song Wu,[a] Ping Wang,[a] Tian Tian,[a] Lin Wu,[a] Hanping He,[a] Xiang Zhou,[a,b,*] Xiaolian Zhang[c,*] and Xiaoping Cao[d]

[a]*College of Chemistry and Molecular Sciences, Wuhan University, Hubei, Wuhan 430072, PR China*
[b]*Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Peking University, Beijing 100871, PR China*
[c]*School of Medicine, Wuhan University, Hubei, Wuhan 430072, PR China*
[d]*National Laboratory of Applied Organic Chemistry, Lanzhou University, Gansu, Lanzhou 730000, PR China*

Received 19 November 2003; accepted 23 February 2004

Abstract—*meso*- and β-Substituted di(pyridiumyl)porphyrins **3**, **4**, and **7** have been synthesized and their interactions with DNA have been investigated. *meso*-Substituted porphyrins showed the stronger effect on DNA than that of β-substituted porphyrin. Cytoxicity of compound **3** (IC_{50}) to THP-1 tumor cell was up to 0.11 nM.

Cationic porphyrins have received a great deal of interests because of their DNA-binding properties.[1] It is known that some porphyrin derivatives are capable of intercalating into DNA, and other porphyrins are in external or groove binding modes.[1] Different DNA binding modes might count on the peripheral substituents of porphyrin.[2] In order to further investigate the structure relationship of *meso*- and β-substituted porphyrin interactions with DNA, we synthesized three di(pyridiumyl)porphyrins: **3** with weak electron withdrawing phenyl group at *meso* position, **4** with electron donating ethyl group at *meso* position and **7** with eight electron donating ethyl and methyl groups at β-positions. Herein, we shall report our preliminary results.

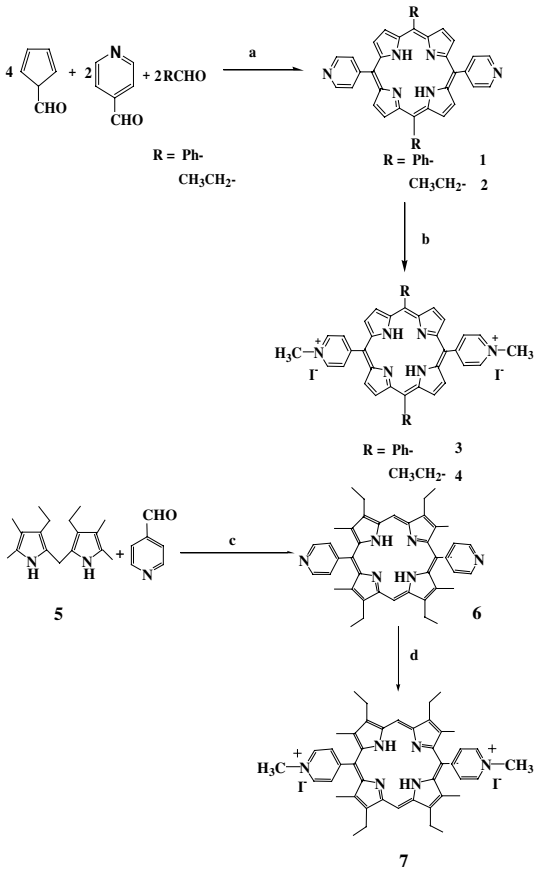
Desired compounds have been synthesized according to the procedure showed in Scheme 1. Porphyrins **1**[3] and **2** were obtained from mixing aldehyde and pyrrole in the yields of 3.4‰ and 9.5‰, respectively. Methylations of porphyrins **1** and **2** were finished by adding methyl iodide to the co-solvent of DMF and $CHCl_3$. Compounds **3**[1a] and **4** were obtained in good yields of 88.3% and 98%, respectively. Meanwhile, porphyrin **6** was obtained by mixing dipyrrolemethane **5**[4] and 4-pyridinecarboxaldehyde in a cosolvent of CH_2Cl_2 and CH_3OH (9:1) under N_2 atmosphere in the presence of trifluroacetic acid. Final methylation was carried out by mixing methyl iodide with compound **6** and di(pyridiumyl)-porphyrin **7** was obtained in a good yield of 95.3%. All the new compounds were fully characterized by NMR, UV, and HRMS.[5]

It is known that DNA could be damaged by porphyrins after photoactivation because of their ability of producing singlet oxygen.[6] Singlet oxygen could be detected by the measurement of decomposition of 1,3-diphenylisobenzofuran (DPBF) under irradiation for compounds **3**, **4**, and **7**. The slope of the plot of bleached absorption versus illumination time is proportional to the rate of production of singlet oxygen.[7] Therefore, the rate of singlet oxygen production for these porphyrins was not significant difference (Fig. 1).

To our initial biological studies, we tested their interactions between three compounds and CTDNA (calf thymus DNA) by UV/vis.[8] When the titration of porphyrins and CTDNA (shown in Fig. 2), we observed that hypochromicities of compounds **3**, **4**, and **7** were occurred by adding CTDNA. Apparently, porphyrins **3**, **4**, and **7** have the strong abilities to interact with DNA.

* Corresponding authors. Tel.: +86-27-87645382; fax: +86-27-87336380; e-mail: zhouxiang65@hotmail.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.02.083



Scheme 1. Synthesis of di(pyridiumyl)porphyrins **3**, **4**, and **7**. Reagents and conditions: (a) Propionic acid, reflux, 1 h, 3.4‰ for 1, 9.5‰ for **2**; (b) CH_3I, DMF/CH_2Cl_2, rt, overnight, 88.3% for **3**, 98% for **4**; (c) CF_3COOH, CH_2Cl_2/CH_3OH, rt, Chloranil, 10.5% yield; (d) CH_3I, DMF/$CHCl_3$, rt, overnight, 95.3% yield.

Further study of DNA–porphyrin interactions was carried out by gel electrophoresis. Photocleavage of supercoiled pBR322 (0.15 μg) was finished with various compounds in buffer (pH=8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 3% DMF). Samples were exposed to a 50-W high-pressure mercury lamp, which placed 15 cm away

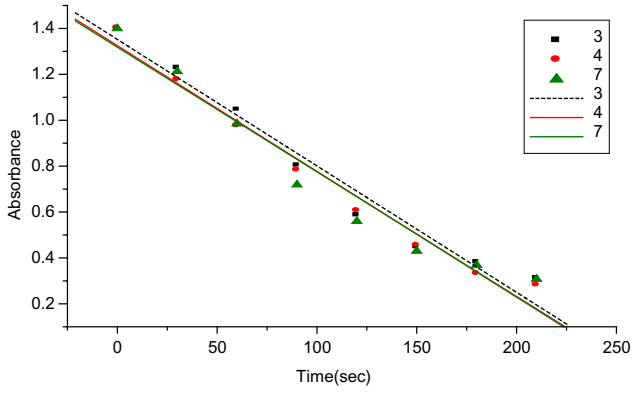


Figure 1. Decomposition of DPBF by compounds **3**, **4**, and **7**. Porphyrins (1.0×10^{-6} M) and DPBF (1.0×10^{-4} M) were irradiated in solution (pH=8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 3% DMF).

at 25 °C for 20 min. The results were analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). Results of DNA cleavage for porphyrins were illustrated in Figure 3.

No cleavage of DNA occurred without irradiation whether in control experiments or in the presence of porphyrins or DMF (see lanes 1, 3, 4, 5, 7, and 9). No cleavage was observed when DNA was only in illumination (lane 2). Apparently, compound **3** possessed the strongest ability to cleave DNA (lane 6), while compound **7** (lane 10) was the poorest cleaving agent among three compounds. As they had the closely singlet oxygen production, they might have the near abilities to cleave DNA. However, different cleavage abilities to DNA have been found. We estimated that the difference of DNA cleavage ability for porphyrins **3**, **4**, and **7** was caused by their different interaction modes with DNA. Probably, *meso*-substituted dipyridium porphyrins **3** and **4** had more favorable binding modes to DNA and then cleaved it more efficiently than that of β-substituted dipyridium porphyrin **7**. Meanwhile, octa-β-substituents (methyl and ethyl) on porphyrin might increase the porphyrin's hydrophobic environment and retard its interaction with DNA efficiently.

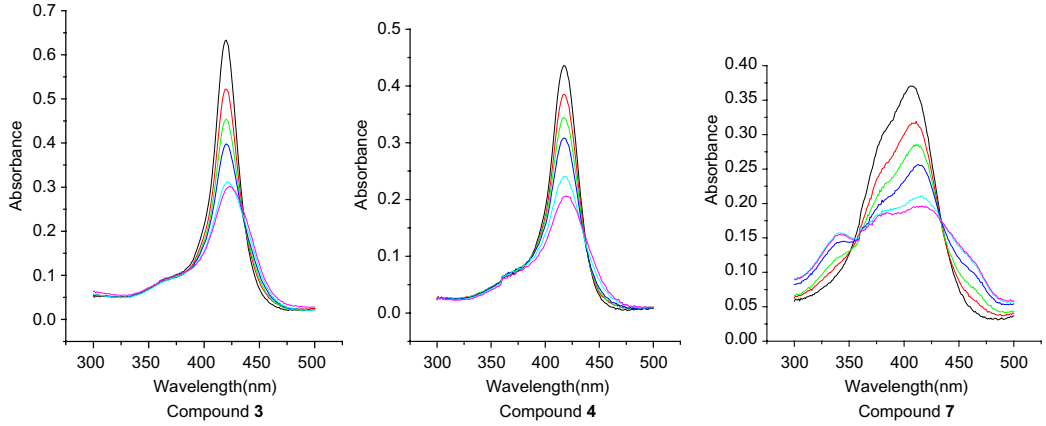


Figure 2. Titration of porphyrin **3**, **4**, and **7** by calf thymus DNA. [porphyrin **3**]=[porphyrin **4**]=[porphyrin **7**]=3.33 μM in solution (pH=8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 3% DMF); [B.P]=0, 0.34, 0.68, 1.02, 2.04, 3.06 μM from the upper to the lower curves in all porphyrin measurements.

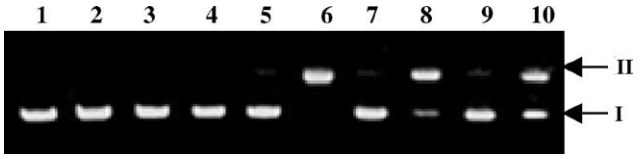


Figure 3. Cleavage of supercoiled pBR322 DNA by porphyrins **3**, **4**, and **7**. Reaction mixtures (10 μL) contained 0.15 μg of plasmid DNA. Lane 1: DNA alone; lane 2: DNA + *hv* (20 min); lane 3: DNA + 3% DMF; lane 4: DNA + 3% DMF + *hv* (20 min); lane 5: DNA + compound **3** (6 μM); lane 6: DNA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

Peripheral substituent effects of compounds **3**, **4**, and **7** were reflected by the result of cytotoxic ability to THP-1 tumor cells. A MTT assay was performed to determine THP-1 cell viability.[9] 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} value of compound **3** was 0.11 nM, compound **4** was 32 nM and compound **7** was 12 nM. It suggested that structure relationship of porphyrins could affect their interactions with DNA and finally lead the difference for cytotoxicity to tumor cells. Further peripheral substituent effects, for example, electronic effects and steric effects, on porphyrins to DNA and tumor cells are being investigated.

Acknowledgements

This work was supported by grants from the Trans-Century Training Programme Foundation for the Talents by the Ministry of Education of China and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, Ministry of Education of China. We appreciate one referee for his critical comments on this manuscript.

References and notes

1. (a) Uno, T.; Aoki, K.; Shikimi, T.; Hiranuma, Y.; Tomisugi, Y.; Ishikawa, Y. *Biochemistry* **2002**, *41*, 13059; (b) Lauceri, R.; Purrello, R.; Shetty, S. J.; Vicente, M. G. H. *J. Am. Chem. Soc.* **2001**, *123*, 5935; (c) Pasternack, R. F.; Ewen, S.; Rao, A.; Meyer, A. S.; Freedman, M. A.; Collings, P. J.; Frey, S. L.; Rannen, M. C.; de Paula, J. C. *Inorg. Chim. Acta* **2001**, *317*, 59; (d) Beune, S. A.; Dube, D.; McMillin, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 11480; (e) Lugo-Ponce, P.; McMillin, D. R. *Coord. Chem. Rev.* **2000**, *208*, 169; (f) Bennett, M.; Krah, A.; Wien, F.; Garman, E.; Mckenna, R.; Sanderson, M.; Neidle, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9476; (g) Marzilli, L. G. *New. J. Chem.* **1990**, *14*, 409; (h) Fiel, R. J.; Howard, J. C.; Mark, E. H.; Datta-Gupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093.
2. Pasternack, R. F.; Gibbs, E. J.; Bruzewicz, D.; Engstrom, K. S. *J. Am. Chem. Soc.* **2002**, *124*, 3533.
3. Fleischer, E. B.; Shachter, M. *Inorg. Chem.* **1991**, *30*, 3763.
4. (a) Young, R.; Chang, C. K. *J. Am. Chem. Soc.* **1985**, *107*, 898; (b) Clezy, P. S.; Liepa, A. J. *Aust. J. Chem.* **1970**, *23*, 2443.
5. Selected data: compound **4** ^{1}H NMR δ_H([$^2H_6$]-DMSO, 300 MHz): 9.91 (s, 4H), 9.45 (m, 4H), 9.00 (m, 8H), 5.12 (m, 4H), 4.73 (m, 6H), 2.05 (m, 6H), −2.95 (s, 2H); δ_C([$^2H_6$]-DMSO, 75 MHz): 158.3, 144.6, 132.8, 132.3, 130.8, 124.1, 113.9, 28.5, 26.9, 24.1; UV–vis λ_{max} (DMF, nm, log ε): 422.0 (5.11), 518.0 (3.90), 556.0 (3.69), 596.0 (3.24), 654.0 (3.80); HRMS (ESI, *m*/*z*): calcd for $C_{36}H_{34}N_6$[(M^+−2I)/2]: 275.1417 found: 275.1417.
Compound **7** ^{1}H NMR δ_H([$^2H_6$]-DMSO, 300 MHz): 10.39 (s, 2H), 9.48 (m, 4H), 9.06 (m, 4H), 4.79 (s, 6H), 4.10 (m, 8H), 2.54 (s, 12H), 1.76 (t, $J_1 = J_2 = 3.3$ Hz, 12H), −2.50 (s, 2H); δ_C([$^2H_6$]-DMSO, 75 MHz): 157.6, 146.2, 144.8, 142.8, 141.0, 140.9, 134.8, 131.5, 112.4, 97.7, 19.0, 17.6, 17.5, 15.4; UV–vis λ_{max} (DMF, nm, log ε): 410.5 (5.08), 508.0 (4.12), 543.5 (3.79), 577 (3.75), 628.5 (3.46); HRMS (ESI, *m*/*z*): calcd for $C_{44}H_{50}N_6$[(M^+−2I)/2]: 331.2043, found: 331.2044.
6. (a) Dougherty, T. J. *J. Natl. Cancer Inst.* **1998**, *90*, 889; (b) Sternberg, E. D.; Dolphin, D.; Bruckner, C. *Tetrahedron* **1998**, *54*, 4151; (c) Bonnett, R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science: Amsterdam, 2000.
7. (a) Ishikawa, Y.; Yamakawa, N.; Uno, T. *Bioorg. Med. Chem.* **2002**, *10*, 1953; (b) Oda, K.; Ogura, S.; Okura, I. *J. Photochem. Photobiol. B* **2000**, *59*, 20; (c) Kochevar, I. E.; Redmond, R. W. *Methods Enzymol.* **2000**, *319*, 20; (d) Michelsen, M.; Kliesch, H.; Wohrle, D. *Photochem. Photobiol.* **1996**, *64*, 694.
8. (a) Fiel, R. J. *J. Biomol. Struct. Dyn.* **1989**, *6*, 1259; (b) Kubat, P.; Lang, K.; Anzenbacher, P., Jr.; Jursikova, K.; Kral, V.; Ehrenberg, B. *J. Chem. Soc., Perkin. 1* **2000**, *6*, 933.
9. 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.