Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3030–3033

Zinc(II) and copper(II) complexes of β-substituted hydroxylporphyrins as tumor photosensitizers

Qimao Huang,[a] Zhiquan Pan,[a,*] Ping Wang,[b] Zhangping Chen,[b] Xiaolian Zhang[c] and Hansheng Xu[b]

[a]*Hubei Key Lab of Novel Reactor & Green Chemical Technology, Wuhan 430073, PR China*
[b]*College of Chemistry and Molecular Sciences, Wuhan University, Hubei Wuhan 430072, PR China*
[c]*School of Medicine, Wuhan University, Hubei Wuhan 430072, PR China*

Received 1 February 2005; revised 4 February 2006; accepted 21 February 2006
Available online 15 March 2006

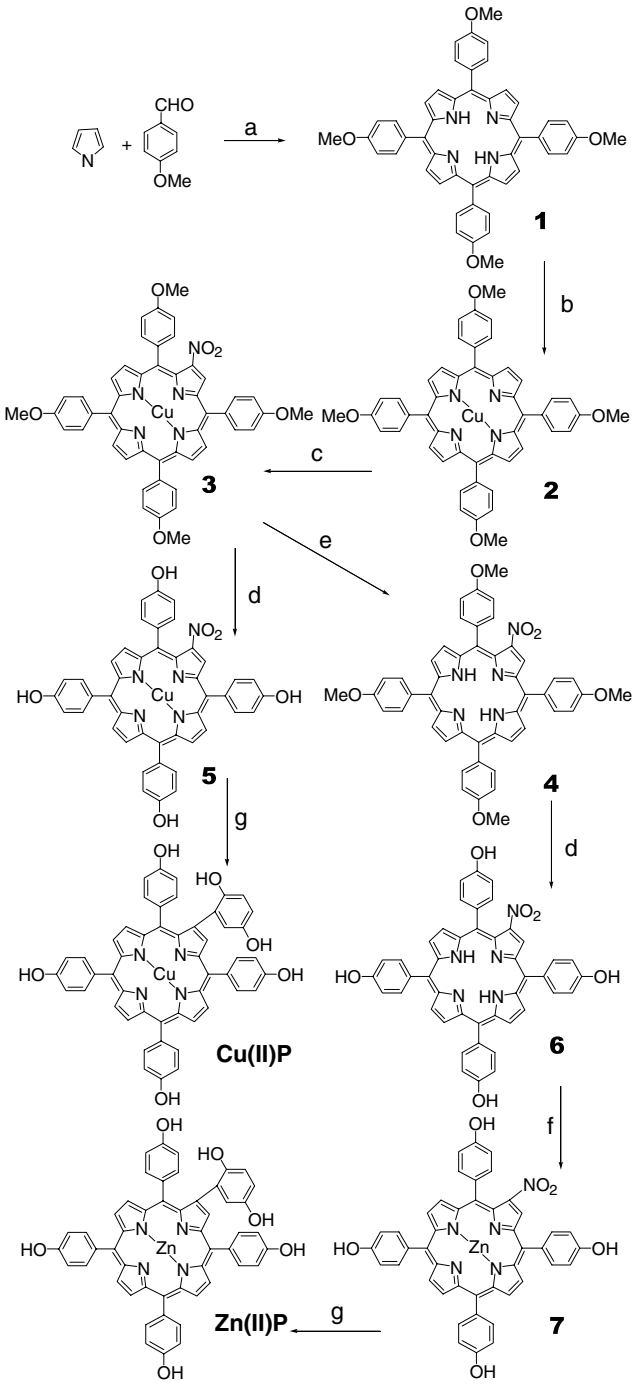
Abstract—Novel photosensitizers β-(hydroquinon-2-yl)-5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II) (Zn(II)P) and β-(hydroquinon-2-yl)-5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato copper(II) (Cu(II)P) were synthesized and characterized. Their ability of producing singlet oxygen under irradiation was detected by the measurement of decomposition of 1,3-diphenylisobenzofuran (DPBF). The preliminary biological activity studies show that the Zn(II)P has photo-toxicity on human chronic myelogenous leukemia cell (K562) and could cleave supercoiled DNA (pBR 322 DNA), while the Cu(II)P has inferior biological activity. Results showed Zn(II)P having high anti-tumor activity, which presents a promising photosensitizer for photodynamic therapy.
© 2006 Elsevier Ltd. All rights reserved.

Photodynamic therapy (PDT) is an attractive method for treating tumors.[1] The curative activity of PDT is based on the interaction between photosensitizers and light.[2] Porphyrins have been developed as promising photosensitizers in recent years,[3] among these photosensitizers, hydroxyl-substituted porphyrins show excellent activity.[4] To our knowledge, few β-substituted porphyrins are synthesized as potential tumor photosensitizers,[5] however the sterically encumbered structure of β-substituted porphyrins is more close to that of natural porphyrin than that of meso-substituted porphyrins, and it ought to be more widely used in biological studies.[6] On the basis of these advisement by this information, we now report the synthesis and the anti-tumor activity studies of novel β-substituted hydroxylporphyrins which bear four hydroxyl group on meso position and two hydroxyl group on beta position, with the purpose of improving the hydrophilicity and anti-tumor activity. The compounds Zn(II)P and Cu(II)P were first synthesized. The ability of producing singlet oxygen was detected by the measurement of decomposition of 1,3-diphenylisobenzofuran (DPBF) for compounds Zn(II)P and Cu(II)P under irradiation. The photosensitizing activity toward supercoiled DNA (pBR 322 DNA) and K562 human chronic myelogenous leukemia cell line of these hydroxylporphyrin photosensitizers was investigated. The results show that Zn(II)P could cleave supercoiled DNA (pBR 322 DNA) and induce necrosis or apoptosis of K562 human chronic myelogenous leukemia cells under irradiation. While Cu(II)P and 5,10,15,20-tetra(4-hydroxylphenyl)porphyrin exhibit inferior photosensitizing activity and lower singlet oxygen yield. The anti-tumor activity testing results are corresponding with their ability of producing singlet oxygen under irradiation.

The synthetic route of the β-(hydroquinon-2-yl)-5,10,15,20-tetra(4-hydroxylphenyl)porphyrins is shown in Scheme 1. The condensation of pyrrole with 4-methoxylbenzaldehyde gives compound **1** (22% yield after purification by silica gel column chromatography). Metallization of **1** obtains **2** in high yield. The regioselective synthesis of **3** was realized by the reaction between **2** and copper(II) nitrate in the presence of acetic acid/acetic anhydride.[7] Demethylation of **3** offers β-NO_2-5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato copper(II) **5**. Cu(II)P was synthesized by the direct reaction of **5** with neutral hydroquinone in 93% yields.[8] Because the regioselective nitration of porphyrinato zinc(II) was not efficacious as porphyrinato copper(II), β-NO_2-5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II) **7** has to be synthesized by an indirect way:

* Corresponding author. Tel.: +86 27 87194980; fax: +86 27 87194465; e-mail: huangqim@hotmail.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.02.094



Scheme 1. Synthesis of β-hydroquinone-5,10,15,20-tetra(4-hydroxylphenyl)porphyrin and its derivatives. (a) Propanoic acid, reflux for 2.0 h (22%); (b) $Cu(OAc)_2$, $CHCl_3$, methanol, reflux for 1 h (97%); (c) $(CH_3CO)_2O/CH_3COOH$, $CHCl_3$, $Cu(NO_3)_2$, 45 °C, 5 min (97%); (d) BBr_3, CH_2Cl_2, −10 °C, 24 h (75%); (e) CF_3COOH, $CHCl_3$, rt, 30 min (95%); (f) $Zn(OAc)_2$, $CHCl_3$, methanol, reflux for 1 h (97%); (g) hydroquinone, reflux under argon protection for 1.0 h (93%).

demetallization of **3** with CF_3COOH gives porphyrin **4**, then demethylates with BBr_3 in CH_2Cl_2 at −10 °C for 20 h to give **6**, compound **6** metallized with $Zn(OAc)_2$ in methanol gaining **7**. Zn(II)P was obtained by the reaction of **7** with hydroquinone in 93% yield.[8] The new compounds were fully characterized by ^1H NMR, FAB-MS, and UV.[9]

As expected from presenting the six hydroxyl group, the β-substituted hydroxylporphyrins Cu(II)P and Zn(II)P are well soluble in methanol or component solvent of methanol and water, and insoluble in chloroform or CH_2Cl_2, while original material porphyrins(**1**–**4**) represent reverse solubility. Porphyrins(**5**–**7**) which bear a four hydroxyl group are weakly soluble in chloroform, but well soluble in acetone. These results reveal that the hydrophilic or hydrophobic property of the porphyrins achieves excellent changes, which surmount partly hindrance of water insolubility for photosensitizers.

It is well known that scientific basis for photodynamic therapy (PDT) is the photodynamic effect of certain photosensitizers: the photodynamic reactions lead to the production of many reactive oxygen species (ROS) that induced the tumor necrosis or apoptosis.[10] In order to determine the photosensitive activity of the novel β-substituted porphyrins Zn(II)P and Cu(II)P, we investigated their cleaving of DNA (supercoiled pBR322) by using agarose gel electrophoresis. All experiments were performed in buffer solutions (pH 8.0, 10 mM Tris–HCl, 1 mM EDTA, and 4% DMF). High-pressure mercury lamp (50 W) was used when irradiation was needed. The distance from the sample to the lamp was ca. 25 cm. A control containing photosensitizers kept in darkness was analyzed at the same time (Fig. 1, lanes 2, 4, and 6). The photo-assisted DNA-cleaving activity of 5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II) was also performed as a control compound (Fig. 2, lane 8). The results reveal that the novel hydroxylporphyrin showed cleaving ability only when they irradiated with light (Fig. 1, lanes 3, 5, and 7; Fig. 2, lane 6), while there was almost no observed cleavage of DNA when Cu(II)P and 5,10,15,20-tetra(4-hydroxylphe-

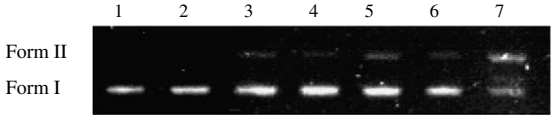


Figure 1. Cleavage of supercoiled pBR322 DNA by compound Zn(II)P. [Lane 1, 0.1 μg pBR322 + 2 mL DMF, hυ (2.5 h); lane 2, 0.1 μg pBR322 + Zn(II)P (50 μM); lane 3, 0.1 μg pBR322 + Zn(II)P (50 μM), hυ (2.5 h); lane 4, 0.1 μg pBR322 + Zn(II)P (100 μM); lane 5, 0.1 μg pBR322 + Zn(II)P (100 μM), hυ (2.5 h); lane 6, 0.1 μg pBR322 + Zn(II)P (200 μM); lane 7, 0.1 μg pBR322 + Zn(II)P (200 μM), hυ (2.5 h).]

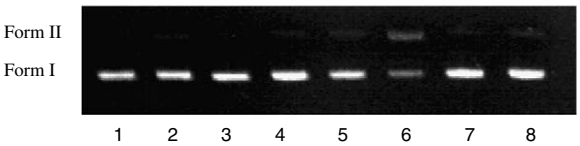


Figure 2. Cleavage of supercoiled pBR322 DNA by compound Zn(II)P and 5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II) ($Zn(II)P_4$ [lane 1, 0.1 μg pBR322; lane 2, 0.1 μg pBR322, hυ (2.5 h); lane 3, 0.1 μg pBR322 + 1 mL DMF; lane 4, 0.1 μg pBR322 + 1 mL DMF, hυ (2.5 h); lane 5, 0.1 μg pBR322 + Zn(II)P (200 μM); lane 6, 0.1 μg pBR322 + Zn(II)P (200 μM), hυ (2.5 h); lane 7, 0.1 μg pBR322 + Zn(II)P4 (200 μM); lane 8, 0.1 μg pBR322 + Zn(II)P4 (200 μM), hυ (2.5 h).]

nyl)porphyrinato zinc(II) replaced Zn(II)P even with irradiation (Fig. 2, lane 8). It means that the porphyrins did not chemically modify the target DNA, but photodynamically. As increasing the concentration of the Zn(II)P, the cleavage efficiency to DNA increased (Fig. 1, lanes 3, 5, and 7). In respect to that with increasing the concentration of porphyrin under irradiation, the concentration of reactive oxygen increased. Their ability of producing singlet oxygen under irradiation was detected by the measurement of decomposition of 1,3-diphenylisobenzofuran (DPBF) (Fig. 5). DPBF is an excellent quencher of singlet oxygen. Relative reduction percentage of DPBF corresponds to the ability of photosensitizers to produce singlet oxygen. The results reveal that singlet oxygen producing ability of β-substituted hydroxylporphyrins Zn(II)P is superior to that of 5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II). Cu(II)P has inferior ability to produce singlet oxygen, which may be owing to that Cu(II) as a quencher could inhibit the production of singlet oxygen.[11] Antibacterial activity of Cu(II)P and Zn(II)P exhibits similar tendency to be able to produce singlet oxygen: the compound Zn(II)P possesses a higher anti-microbial activity under illumination for *Staphylococcus aureus ATCC 25923* than that of Cu(II)P. It is believed that major reactive oxygen species originated from this photodynamic course is singlet oxygen.

The compounds Zn(II)P and Cu(II)P were used to test in vitro photosensitizing activity on human chronic myelogenous leukemia cell (K562). Trypan blue exclusion assay was employed to evaluate the effect of the concentration on the phototoxic potential of Zn(II)P and Cu(II)P. The cells were treated with photosensitizers (16 nmol/L, 80 nmol/L, 160 nmol/L, and 320 nnmol/L) for 24 h at 37 °C. Irradiation experiments (high-pressure mercury lamp) and a control containing photosensitizers kept in darkness were analyzed at the same time. Cell cultures not being treated with Zn(II)P or Cu(II)P were irradiated under similar conditions. Survival rate was 100% for cells under irradiation in the absence of photosensitizers (Fig. 3a). Result reveals that cytotoxicity which is induced by Zn(II)P with illumination is significantly stronger than that of Cu(II)P. The cytotoxicity of photosensitizers with irradiation is also stronger than that of those maintained in dark. The Zn(II)P induces

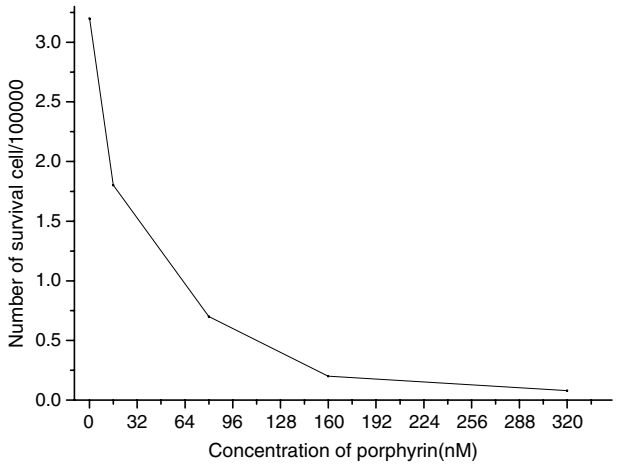


Figure 4. Inactivation of the human chronic myelogenous leukemia cell (K562) incubated with Zn(II)P in different concentrations irradiated with high-pressure mercury lamp.

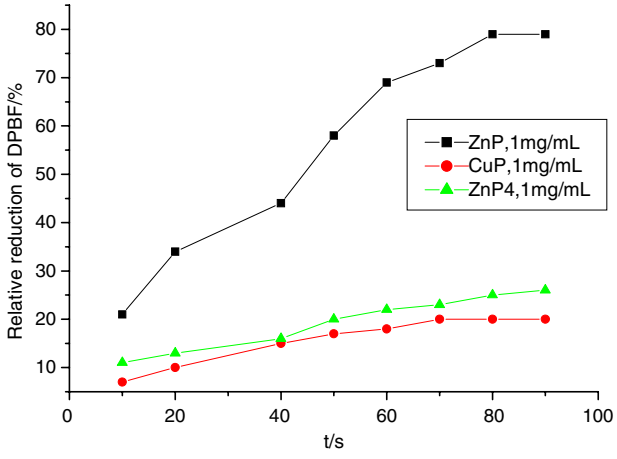


Figure 5. Decomposition of DPBF by compounds Zn(II)P, Cu(II)P, and 5,10,15,20-tetra(4-hydroxylphenyl)porphyrinato zinc(II) ($Zn(II)P_4$); solvent: ethanol/water, 1:1 (v/v); DPBF (1.0×10^{-4} M), porphyrin: 1 mg/mL.

more than 95% cell death even if the concentration of Zn(II)P were 0.32×10^{-6}mol/L (Fig. 3b). Figure 4 shows the survival rate of the human chronic myelogenous leukemia cell (K562) that was incubated with irradiated

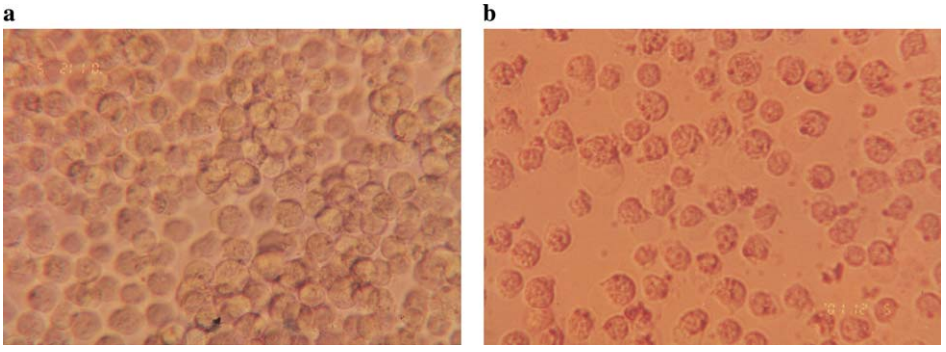


Figure 3. Trypan blue staining of human chronic myelogenous leukemia cell (K562). (a) Control cultures, cell incubated for 16 h and illuminated for 30 min with 50 W high-pressure mercury lamp (wavelength >400 nm); (b) Cell incubated with Zn(II)P 320 nmol/L for 16 h and illuminated for 30 min with 50 W high-pressure mercury lamp (wavelength >400 nm).

Zn(II)P in different concentrations. Further studies are in progress using other β-substituted hydroxyporphyrins and various metalloporphyrins, and extensive biological studies are ongoing.

Acknowledgments

The authors are grateful to National Nature Science Foundation of China for financial support (Nos. 20471045, 29872033).

References and notes

1. (a) Rosenthal, D. I.; Glatstein, E. *Ann. Med.* **1994**, *26*, 405; (b) Schuitmaker, J.; Baas, P.; van Leengoed, H. L. L. M.; van der Meulen, F. W.; Star, W. M.; van Zandwijk, N. *J. Photochem. Photobiol. B: Biol.* **1996**, *34*, 3; (c) Hsi, R. A.; Rosenthal, D. I.; Glatstein, E. *Drugs* **1999**, *57*, 725.
2. (a) Dougherty, T. J. *Photochem. Photobiol.* **1993**, *58*, 895; (b) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W. *J. Natl. Cancer Inst.* **1998**, *90*, 889.
3. (a) Bonnett, R. *Chem. Soc. Rev.* **1995**, *24*, 19; (b) Sternberg, E. D.; Dolphin, D.; Brucker, C. *Tetrahedron* **1998**, *54*, 4151.
4. (a) Bonnett, R.; White, D. R.; Winfield, U. J.; Berenbaum, M. C. *Biochem. J.* **1989**, *261*, 277; (b) Ferrand, Y.; Bourre, L.; Simonneaux, G.; Thibaut, S.; Odobel, F.; Lajat, Y.; Patrice, T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 833; (c) Maestrin, A. P. J.; Tedesco, A. C.; Neri, C. R.; Gandini, M. E. F.; Serra, O. A.; Iamamoto, Y. *J. Braz. Chem. Soc.* **2004**, *15*, 708.
5. Chen, B.; Qin, W.; Wang, P.; Tian, T.; Ma, H.; Cao, X.; Wu, X.; Zhou, X. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3731.
6. Zhou, X.; Tse, M. K.; Wan, T. S. M.; Chan, K. S. *J. Org. Chem.* **1996**, *61*, 3590.
7. Huang, Q.; Chen, Z.; Xu, H.; Zhao, Y. *Chin. J. Org. Chem.* **2001**, *21*, 746.
8. Huang, Q.; Chen, Z.; Xu, H.; Zhou, X. *Acta Chim. Sinica* **2004**, *62*, 2182.
9. Selected data: Cu(II)P UV–vis (CH_3OH) λ_{max}: 417, 517, 541 nm; FAB-MS: Anal. Calcd for $C_{50}H_{32}N_4O_6Cu$: 848. Found: 848 (M+) Zn(II)P 1H NMR (300 MHz, CD_3COCD_3, ppm) δ: 8.9 (2H, s, 10,15-meso-Ar-OH), 8.6–8.4 (6H, m, β-pyrrole-H), 8.1–8.0 (8H, m, 10,15-meso-Ar-H), 7.9–7.8 (9H, m, 5,20-meso-Ar-H, 3-pyrrole-H), 7.3 (1H, s, 2-6′-H), 7.2–7.1 (2H, m, 2-3′-H,2-4′-H), 3.6–3.5 (4H, m, 2-2′-OH,2-5′-OH, 5, 20-meso-Ar-OH); UV–vis (CH_3OH) λ_{max}: 426, 548, 589 nm; FAB-MS: Anal. Calcd for $C_{50}H_{32}N_4O_6Zn$: 850. Found: 850 (M+).
10. Henderson, R. W.; Dougherty, T. J. *Photochem. Photobiol.* **1992**, *55*, 145.
11. Ochsner, M. *J. Photochem. Photobiol. B: Biol.* **1997**, *39*, 1.