Eur. J. Org. Chem. **2008** · © WILEY-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2008 · ISSN 1434–193X

SUPPORTING INFORMATION

Title: A Model for Light-Triggered Porphyrin Anticancer Prodrugs Based on an *o*-Nitrobenzyl Photolabile Group
Author(s): Weiying Lin,* Daiwei Peng, Bin Wang, Lingliang Long, Cancheng Guo, Jinbin Yuan
Ref. No.: O200700972

Experimental Section

General Procedures

^{1}H NMR spectra were recorded with an INOVA-400 spectrometer, using TMS as an internal standard. ESI/APCI-MS analyses were performed using a Waters Micromass ZQ-4000 spectrometer. Electronic absorption spectra were recorded with a labtech UV-visible spectrometer. Elementary analyses data were obtained by a Vario El III Elemental Analyzer. Melting point of the intermediates was measured with a Beijing taike XT-4 microscopy melting point apparatus, but the melting point was uncorrected. TLC analyses were performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals. All photochemical reactions were conducted in a Rayonet RPR-600 Reactor using 350nm mercury lamps. Compounds **1** and **2** were purified by Recycling Preparative HPLC (LC-9201, Japan Analytical Industry Co., Ltd.). HPLC analyses were conducted using an Agilent 1100 Series HPLC. Pyrrole and benzaldehyde were redistilled before use. Unless otherwise noted, the reagents were commercially available and used as received.

Synthesis

4-bromomethyl-benzaldehyde (3) [1-3]**:** To a solution of 4-bromomethyl-benzonitrile (6.00 g, 30.6 mmol) in 120 mL anhydrous toluene under nitrogen pressure at 0 °C , a solution of DIBAL-H in *n*-hexane (43.2 mL, 1 M, 43.2 mmol) was added in a dropwise manner over a period of 20 min. After stirring for 2.5 h at 0 °C, 150 mL of chloroform was then added followed by 200 mL 10% aqueous HCl, and the mixture was then stirred at room temperature for 1 h. The organic layer was separated and the aqueous layer was extracted twice with chloroform. The organic layers were then combined, washed with distilled water, dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure and the residue was recrystallized from *n*-hexane. The product appeared as a white crystal. Yield: 5.12 g (84.1%). M.p.: 97-99 °C (lit.[2] 97-100°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.52 (s, 2H, CH_2-Br), 7.56 (d, J = 8.0 Hz, 2H), 7.87 (d, J = 8.0 Hz, 2H), 10.02 (s, 1H, CHO) ppm. ESI-MS m/z: 200.0 $[M+H]^+$.

4-bromomethyl-3-nitrobenzaldehyde (4) [1]**:** To a solution of 7.5 mL 98% conc. H_2SO_4 and KNO_3 (757.5 mg, 7.5 mmol) at 0 °C, a solution of 4-bromomethyl-benzaldehyde (1250.0 mg, 6.25 mmol) in 5 mL of chloroform was added slowly in a dropwise manner over 15 min. The mixture was stirred for 2.5 h at 0 °C and then poured into 500 mL of ice water. The precipitate was separated by filtration, dried under vacuum and recrystallized from Et_2O. The product appeared as a pale yellow solid. Yield: 1.2 g (78.6%). M.p.: 74.5-75.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.87 (s, 2H, CH_2-Br), 7.79 (d, J = 8.0 Hz, 1H), 8.13 (dd, J = 8.0/1.2 Hz, 1H), 8.53 (d, J = 1.2 Hz, 1H), 10.09 (s, 1H, CHO) ppm. ESI-MS m/z: 245.2 $[M+H]^+$.

5-(4-bromomethyl-3-nitro-phenyl)-10, 15, 20-triphenylporphyrin (5): To a solution of 4-bromomethyl-3-nitrobenzaldehyde (268.4mg, 1.1 mmol), benzaldehyde (466.9mg, 4.4 mmol) and pyrrole (368.5mg, 5.5 mmol) in 550 mL chloroform, $BF_3 \cdot Et_2O$ (149 μL, 0.55 mmol) was added, and the reaction mixture was stirred for 5 h at room temperature in the dark under nitrogen. Then *p*-chloranil (811.4mg, 3.3 mmol) was added and heated under reflux for 2 h. After removal of the solvent under reduced pressure, the resulting residue was purified by flash chromatography over silica gel eluting with dichloromethane/petroleum ether (1:3) twice to afford compound **5** as a shiny purple fine crystal. Yield: 86.9 mg (10.5%). ^{1}H NMR (400MHz, $CDCl_3$): δ -2.78 (s, 2H, NH), 5.10 (s, 2H, CH_2-Br), 7.73 (m, 9H), 7.83 (d, 1H, J = 8.0 Hz), 8.20 (d, 6H, J = 6.8 Hz), 8.36 (dd, 1H, J = 8.0 Hz/1.6 Hz), 8.73 (d, 2H, J = 4.8 Hz), 8.89 (m, 7H) ppm. ESI-MS m/z: 752.8 $[M+H]^+$.UV/Vis λ_{max} nm (logε, $CHCl_3$): 277 (4.45), 419 (5.50), 515 (4.24), 550 (3.88), 589 (3.72), 645 (3.51) nm. Anal. Calcd for $C_{45}H_{30}BrN_5O_2 \cdot 1.5H_2O$: C, 69.32; H, 4.27; N, 8.98. Found: C, 69.09; H, 4.58; N, 8.78.

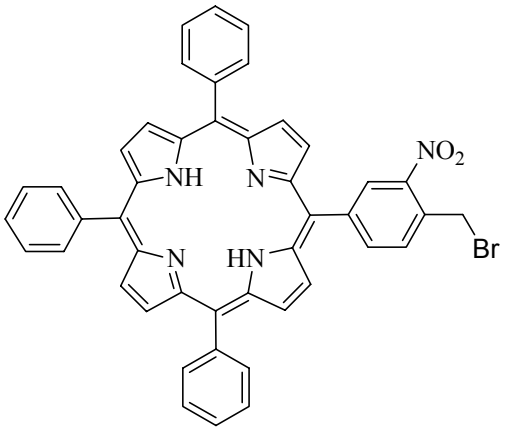

Figure S1. The structure of compound **5**

5-(α-Tegafur-*m*-nitro-*p*-tolyl)-10, 15, 20-triphenylporphyrin (1):5-(4-bromomethyl -3-nitro-phenyl)-10, 15, 20-triphenylporphyrin **5** (60.0 mg, 0.08 mmol), tegafur (63.8 mg, 0.32 mmol) and anhydrous potassium carbonate (22.0 mg, 0.16 mmol) were dissolved in anhydrous DMF (2.0 mL). The resulting solution was then heated under nitrogen at 70°C for 10 h. After removal of DMF under reduced pressure, the residue was dissolved in $CHCl_3$ and washed with water three times. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in a vacuum. The residue was then purified by flash silica gel column (dichloromethane/petroleum ether, 1/3). The product appeared as a shiny purple fine crystal. Yield: 63.8 mg (92.6%). ^{1}H NMR (400MHz, $CDCl_3$): δ -2.80 (s, 2H, NH), 1.97(m, 1H), 2.13 (m, 2H), 2.50 (m, 1H), 4.03 (q, 1H), 4.26 (m, 1H), 5.88(q, 1H), 6.10 (d, 1H, *J* = 3.2Hz), 7.56 (t, 2H), 7.76 (s, 9H), 8.21 (d, 6H, *J* = 6.4Hz), 8.40 (dd, 1H, *J* = 8.0/1.2Hz), 8.77 (s, 2H), 8.89 (m, 6H), 8.94 (d, 1H, *J* = 1.6Hz) ppm. ESI-MS m/z: 872.9 $[M+H]^+$. UV/Vis λ_{max} nm (logε, THF): 270 (4.44), 416 (5.53), 513 (4.31), 547 (3.92), 589.5 (3.76), 646 (3.56) nm. Anal. Calcd for $C_{53}H_{38}FN_7O_5 \cdot 3H_2O$: C, 68.75; H, 4.79; N, 10.59. Found: C, 68.77; H, 4.52; N, 10.42.

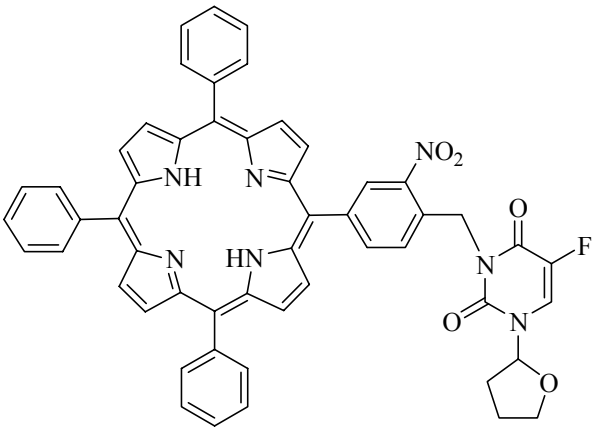

Figure S2. The structure of compound **1**

5-(α-^{1}*N*-uracil-*m*-nitro-*p*-tolyl)-10,15,20-triphenylporphyrin (2):5-(4-bromomethyl -3-nitro-phenyl)-10,15,20-triphenylporphyrin **5** (20.0 mg, 0.027 mmol), uracil (35.8 mg, 0.319 mmol) and anhydrous potassium carbonate (18.4 mg, 0.133 mmol) were dissolved in anhydrous DMF (2.0 mL). The resulting solution was then heated under nitrogen at 50°C for 8 h. After removal of DMF under reduced pressure, the residue was dissolved in $CHCl_3$ and washed with water three times. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in a vacuum. The residue was then purified by flash silica gel column (dichloromethane/acetone, 100/1). The product appeared as a shiny purple fine crystal. Yield: 17.2 mg (82.5 %). ^{1}H NMR (400MHz, [D_6]DMSO): δ -2.94 (s, 2H, NH), 5.60 (s, 2H), 5.83 (d, H, *J* = 7.6 Hz), 7.71 (d, H, *J* = 8.0 Hz), 7.84 (m, 9H), 7.98 (d, H, *J* = 8.0 Hz), 8.23 (d, 6H, *J* = 5.2 Hz), 8.61 (dd, H, *J* = 8.0 Hz/2.0Hz), 8.88 (m, 9H), 11.61 (s, H, NH) ppm. APCI-MS m/z: 784.2 $[M+H]^+$. UV/Vis λ_{max} nm (logε, CH_2Cl_2): 262 (4.43), 418 (5.52), 514 (4.30), 549 (3.90), 590 (3.74), 645 (3.53) nm. Anal. Calcd for $C_{49}H_{33}N_7O_4 \cdot 2H_2O$: C, 71.78; H, 4.55; N, 11.96. Found: C, 71.87; H, 4.62; N, 11.82.

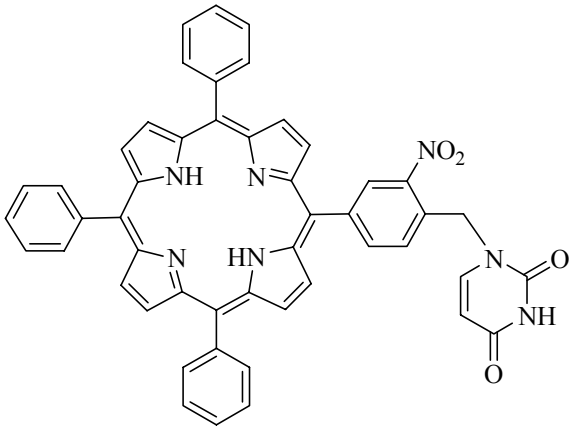

Figure S3. The structure of compound **2**

Photolysis and Quantum Yield

A solution of 50 μM 5-(α-Tegafur-*m*-nitro-*p*-tolyl)-10, 15, 20-triphenylporphyrin **1** in 2% THF /98% Water was placed in a quartz curvette. Photolysis was performed using a photochemical reactor (Rayonet Model RMR-600, the light fluence rate was calculated as 16mW/cm^2) with 350 nm UV lamps. Aliquots of 20 μL were removed at different time intervals of photolysis and analyzed by analytical HPLC (monitored at 270 nm) using a Eclipse XDB-C18 (4.6×250mm, 5μM) column and eluting with a mixture of 98%MeOH and 2%Water at a flow rate of 1 mL/min. The photochemical quantum yield was determined using the ferrioxalate actinometry method according to the literature.[4] Control **2** was photolyzed by using the same procedure as above.

In vitro cytotoxicity assay

The cytotoxic effects of tegafur, 5-(α-Tegafur-*m*-nitro-*p*-tolyl)-10, 15, 20-triphenylporphyrin **1**, and control **2** in the absence or presence of UV light was determined using the standard MTT assay. Briefly, MCF-7 mammary cancer cells at a density of 1×10^4 cell/well in RPMI medium were plated in 96-well plates and then were incubated for 24 h (37°C, 5% CO2, humidity). Tegafur, prodrug **1** and control **2** were predissolved in THF and then added to culture media to furnish a final THF concentration of 2%. The control indicates that THF, at this concentration, does not appear to induce any untoward effects on cell viability. After incubating tegafur, prodrug **1** (pre-irradiated or not pre-irradiated with UV light) or control **2** with cells for 10 h, the cell culture was illuminated with 350 nm lamps (the light fluence rate was calculated as $16mW/cm^2$) for 25 min or not illuminated as control. The cells were then incubated for additional 48 h and the cell quantities were determined by the standard MTT protocol.[5-7]

References

[1] C. Weßler, A. Homann, U. Fricke, J. Lehmann, *Eur. J. Med. Chem*. **2003**, *38*, 581–586.

[2] L. Wen, M. Li, J. B. Schlenoff, *J. Am. Chem. Soc.* **1997,** *119,* 7726–7733.

[3] H. Fukushima, D. M. Taylor, *Langmuir* **1995***, 11,* 3523–3528.

[4] H. C. G.atchard, C. A. Parker, *Proc. R. Soc. London* **1956**, *A220*, 518–536.

[5] C. C. Guo, R. B. Tong, K. L. Li, *Bioorg. Med. Chem.* **2004**, *12*, 2469–2475.

[6] H. Azuma, R. Takao, K. Shikata, H. Niiro, T. Tachibana, K. Ogino, *J. Med. Chem.* **2003**, *46*, 3445–3447.

[7] M. Hadjeri, E. –L. Peiller, C. Beney, N. Deka, M. A. Lawson, C. Dumontet, A. Boumendjel, *J. Med. Chem.* **2004**, *47*, 4964–4970.