Monomer zinc phthalocyanine / upconversion nanoparticle coated with hyaluronic acid crosslink gel as NIR light - activated drug for in vitro photodynamic therapy

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Synthesis of ZnPc-$(COOH)_4$

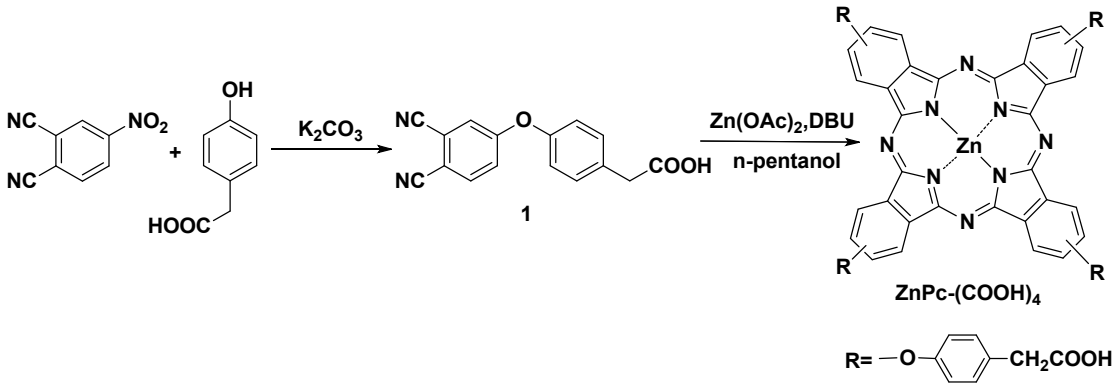

Fig S1. The synthetic route of ZnPc-$(COOH)_4$.

Compound 1

Under nitrogen atmosphere, a mixture of 4-hydroxybenzoic acid (500 mg, 3.62 mmol), *4*-nitrophthalonitrile (658.06 mg, 3.80 mmol) and potassium carbonate (1.0 g, 7.24 mmol) in 10 mL DMF was stirred at 45 °C for 6 h. After cooling to room temperature, the solution was mixed with 100 mL ice water and pH of the system was adjusted to 3-4 and get brown solid. The product was obtained by filtration and then washed with water and vacuum-dried at 40 °C for 12 h (826.7mg, 86.4%). M.p.138-140 °C; IR (KBr, ν_{max}/cm^{-1}): 3108, 2545, 2237, 1683, 1588, 1493, 1430, 1256, 1209, 1161, 852; ^{1}H-NMR (400 MHz, DMSO-d_6): δ/ppm: 13.00 (bs, 1H, COOH), 8.17 (d, 1H ,J=8.4 Hz, Ar), 8.05-8.02 (m, 2H, Ar), 7.95 (d, 1H, J=2.4 Hz, Ar), 7.56-7.53 (m, 1H, Ar), 7.28-7.25 (m, 2H, Ar).^{13}C-NMR (100 MHz, DMSO-d_6): δ /ppm: 167.0, 160.3, 158.3, 136.9, 132.4, 128.2, 124.3, 123.8, 120.0, 117.3, 116.3, 115.8, 109.7 121.3, 120.7, 115.4, 114.9, 108.7. Anal. Calcd. For $C_{15}H_8N_2O_3$: C, 68.18, H, 3.05, N, 10.60. Found: C, 67.93, H, 3.12, N, 10.48.

ZnPc-$(COOH)_4$

Under nitrogen atmosphere, a mixture of compound 2 (500 mg, 1.89 mmol) and $Zn(OAc)_2$ (216.98 mg, 1.18 mmol) in 10 mL n-pentanol was stirred at 120 °C for 1 h. Then, 1,8-diazabicyclo [5,4, 0]-undec-7-ene (DBU, 0.3 mL, 2.00 mmol) was added. The mixture was heated to 140 °C and stirred for another 12 h. After a brief cooling, the solvents were removed under reduced pressure. The residues were washed by acetone and dichloromethane for 10 times and the solvents were removed under reduced pressure. The residues were suspended in 50 mL 10% NaOH solution. The solution was heated to reflux and stirred for 12 h. Then, the pH of the water was adjusted to 2-3 by 10% HCl aqueous to obtain dark green solid. The product was obtained by filtration. The green solid was washed with acetone and vacuum-dried at 40 °C for 12 h (131.4 mg, 12.4%). M.p.>200 °C. IR (KBr, ν_{max}/cm^{-1}): 3464, 1700, 1597, 1467, 1394, 1328, 1233, 943. ^{1}H-NMR (400 MHz, DMSO-d_6): δ/ppm: 9.11 (s, 4H, Pc-H), 8.69 (s, 4H, Pc-H), 8.17 -8.06 (m, 8H, Ar), 7.85-7.78 (m, 4H, Pc-H), 7.52-7.43 (m, 8H, Ar). HRMS (MALDI-TOF) m/z: 1120.491, (Calculated. For $C_{60}H_{32}N_8O_{12}Zn$: 1120.143). Anal. Calcd for $C_{60}H_{32}N_8O_{12}Zn$: C, 64.21; H, 2.87; N, 9.98. Found: C, 64.19; H, 2.83; N, 10.02.

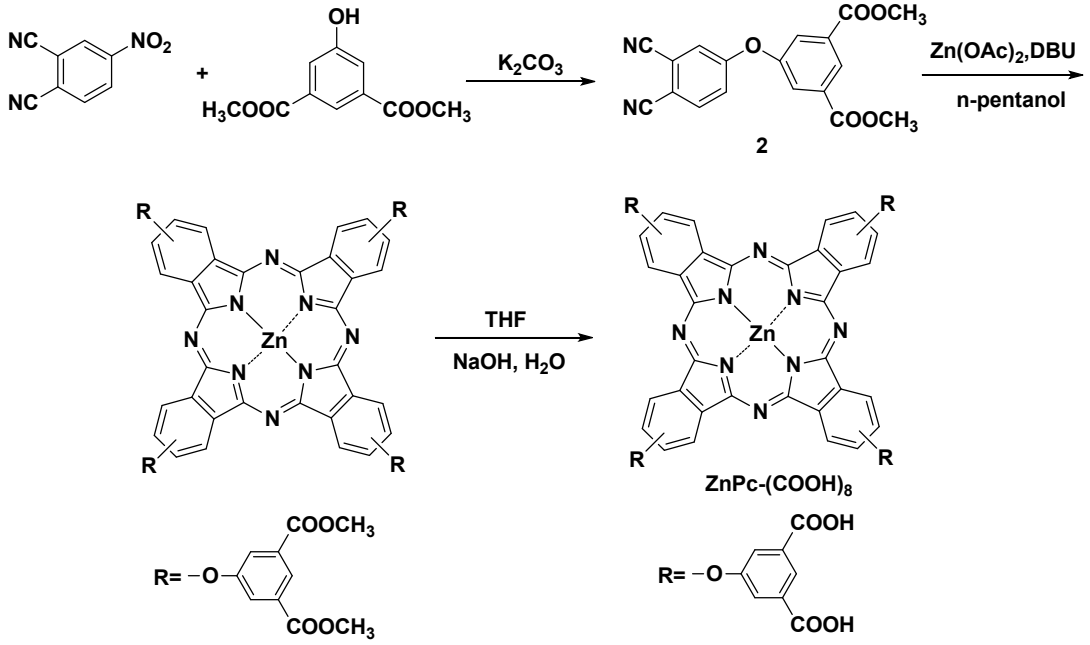

Fig S2. The synthetic route of ZnP-$(COOH)_8$.

Compound 2

Under nitrogen atmosphere, a mixture of 4-Nitrophthalonitrile (500 mg, 2.89 mmol), dimethyl 5-hydroxyisophthalate (607.2 mg, 2.89 mmol) and potassium carbonate (798.28 g, 5.78 mmol) in 8 mL DMF was stirred at 75 °C for 8 h. After cooling to room temperature, the solution was mixed with 50 mL water and extracted by ethyl acetate. The products in ethyl acetate were washed by water and saturated sodium chloride for 3 times and the solvents were removed under reduced pressure. The combined organic extracts were dried over Na_2SO_4 and further purification with silica gel column chromatography using ethyl acetate / petroleum ether (1:2, v/v) with as the eluent to give compound 2 as white solid (899.4 mg, 92.6%). M.p.123-128 °C; IR (KBr, ν_{max}/cm^{-1}): 3425, 3084, 2233, 1735, 1584, 1493, 1316, 1264, 1008, 837, 765. ^{1}H-NMR (400 MHz, DMSO-d_6): δ/ppm: 8.32 (t, 1H, J=1.6 Hz, Ar), 8.13 (d, 1H, J=8.8 Hz, Ar), 7.90 (d, 2H, J=1.6 Hz, Ar), 7.86 (d, 1H, J=2.8 Hz, Ar), 7.54 -7.51 (m, 1H, Ar). ^{13}C-NMR (100 MHz, DMSO-d_6): δ /ppm: 165, 160.7, 154.9, 136.8, 133.0, 126.9, 125.7, 123.6, 123.3, 117.3, 116.2, 115.7, 109.7. Anal. Calcd for $C_{18}H_{12}N_2O_5$: C, 64.29; H, 3.60; N, 8.33. Found: C, 64.31; H, 3.57; N, 8.29.

ZnPc-$(COOH)_8$

Under nitrogen atmosphere, a mixture of compound 2 (500 mg, 1.49 mmol) and $Zn(OAc)_2$ (170.48 mg, 0.93 mmol) were added to 8 mL dried n-pentanol and stirred at 90 °C for 1 h. Then, DBU (0.2 mL, 1.33 mmol) was added. The mixture was heated to 130 °C and stirred for another 12 h. After a brief cooling, the solvents were removed under reduced pressure. The product was purified with silica gel column chromatography using ethyl acetate / petroleum ether (1:2, v/v) and methanol/ dichloromethane (1:5, v/v); the result product was vacuum-dried at 40 °C for 12 h. The dried dark green solid was solved by THF and the solution was dropped to the complex of NaOH and methanol (1:4, v/v, 50 mL) at 60 °C for 7 h. The impurities were separated by centrifugation. The pH value of the supernatant was adjusted to 2-3 by 10% HCl until many dark green solid precipitates. The precipitates were separated by centrifugation. Then, the green precipitates were dissolved in 10 mL water (pH=9-10), and precipitated by adjust pH to 2-3 by 10% HCl again. Above procedure was carried out for 5 times to purify ZnPc. Then, the crude product was washed with distilled water and acetone for 3 times. The final product was obtained as dark green solid after vacuum-dried at 40 °C for 12 h (125.6 mg, 24%). M.p.>200 °C, IR (KBr, ν_{max}/cm^{-1}): 3436, 3083, 2922, 1710, 1593, 1463, 1265, 1227, 1116, 974, 838, 758.^{1}H-NMR (400 MHz, DMSO-d_6): δ/ppm: 13.37 (br, 8H, COOH), 8.93-8.54 (m, 4H, Pc-H), 8.34-7.76 (m, 12H, Ar), 7.47-7.24(m, 8H, Pc-H). HRMS (MALDI-TOF) m/z: 1296.682, (Calculated. For $C_{64}H_{32}N_8O_{20}Zn$: 1296.102). Anal. Calcd for $C_{64}H_{32}N_8O_{20}Zn$: C, 59.20; H, 2.48; N, 8.63. Found: C, 59.19; H, 2.47; N, 8.65.

Dark toxicity of drugs to non-tumoral cell line

Photodynamic therapy (PDT) is based on selective illumination at tumor position, which can avoid the side-effect of PDT to healthy surrounding tissues. So, for non-tumoral cell line, dark toxicity is meaningful for safety analysis of PDT. So, the in vitro dark toxicity to non-tumoral cell line (human embryonic kidney 293 cells) was carried out at the same experiment condition with the SGC-7901 cells one. As showed in Fig. S3, low dark toxicity of all drugs to human embryonic kidney 293 cells were detected, which indicated that they are safe without irradiation.

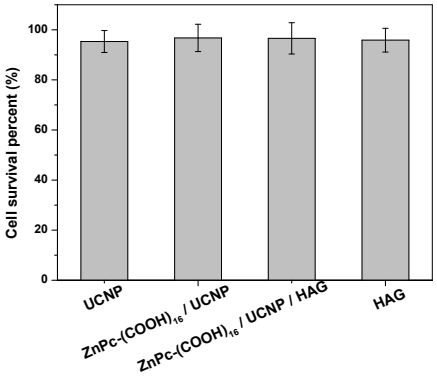

Fig S3. Dark toxicity of ZnPc-$(COOH)_{16}$, UCNP, ZnPc-$(COOH)_{16}$/UCNP and ZnPc-$(COOH)_{16}$/UCNP/HAG to non-tumoral cells (human embryonic kidney 293 cells);