Tumor-Targeting and Enzyme-Activated Nanoparticles for Simultaneous Cancer Diagnose and Photodynamic Therapy

Huaxia Shi, a Wucheng Sun, a Changbing Liu, a Guiying Gu, c Bo Ma, c Weili Si, b Nina Fu, a Qi Zhang, * c Wei Huang, * a,b and Xiaochen Dong * b

aKey Laboratory for Organic Electronics & Information Displays (KLOEID), Nanjing University of Posts and Telecommunications, Nanjing 210023, China.

bKey Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing 211816, China.

E-mail: iamxcdong@njtech.edu.cn, iamwhuang@njtech.edu.cn

cSchool of Pharmaceutical Science, Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing 211816, China. Email: zhangqi@njtech.edu.cn
Synthesis of Compound 1

4-Hydroxybenzaldehyde (0.1 mol, 12.2 g), 3-Bromo-1-propanol (0.11 mol, 15.2 g) and K₂CO₃ (0.3 mol, 40 g) were added into 200 mL acetone. The mixture was stirred for 24 h at 60 °C. The desired residue was purified with chromatography (petroleum ether/ethyl acetate=1, v/v) to yield yellow oil (10.3 g, 61.8%) (compound 1). ¹HNMR (400 MHz, DMSO-d6) δ 9.75 (s, 1H), 7.73-7.70 (m, 2H), 6.93-6.89 (m, 2H), 4.13-4.09 (m, 2H), 3.80-3.76 (m, 2H), 2.01-1.97 (m, 2H); ¹³CNMR (400 MHz, DMSO-d6) δ 199.9, 161.5, 144.4, 130.2, 127.1, 124.2, 114.9, 64.8, 58.1, 25.7; MS (EI) calcd for [M]+: 167.07, found 166.45.

Synthesis of Compound 2

Benzoyl chloride (1 g, 7.8 mmol) and 2, 4-dimethylpyrrole (2.0 mL, 19.6 mmol) were added into 100 mL CH₂Cl₂ under Ar atmosphere. 10 mL riethylmine and 10 mL BF₃·OEt₂ were drop added in an ice-water bath, and the mixture was stirred until the completed reaction, monitored by thin-layer chromatography (TLC). The solvent was removed under reduced pressure to obtain a residue, which was dissolved in CH₂Cl₂ and washed with saturated Na₂CO₃ solution and water. The organic layers were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, PE/ EA=50:1, v/v) to obtain an orange powder (Compound 2). Yield: 42.4% (1.08 g). ¹HNMR (400 MHz, CDCl₃) δ 7.50-7.45 (m, 3H), 7.29-7.26 (m, 2H), 5.98 (s, 2H), 2.56 (s, 6H), 1.37 (s, 6H); ¹³CNMR (400 MHz, CDCl₃) δ 155.4, 143.2, 141.7, 135.0, 129.2, 129.0, 127.9, 77.2, 14.6, 14.4; MS (EI) calcd for [M]+: 324.16, found 323.74.

Synthesis of Compound 3

Compound 2 (0.32 g, 1 mmol) in 20 mL CH₂Cl₂ was added into N-iodosuccinimide (NIS) (2.25 g, 10 mmol), and the mixture was stirred. After that, the solvent was evaporated under reduced pressure. The residual was purified by column chromatography (silica gel, pure PE) to give a red solid (Compound 3). Yield: 87.0% (0.50 g). ¹HNMR (400 MHz, CDCl₃) δ
7.54-7.50 (m, 3H), 7.27-7.23 (m, 2H), 2.65 (s, 6H), 1.38 (s, 6H); $^{13}$CNMR (400 MHz, CDCl$_3$) δ 156.8, 145.4, 141.4, 134.8, 129.6, 129.5, 127.8, 85.7, 17.0, 16.1; MS (EI) calcd for [M]$^+$: 575.95, found 574.50.

**Synthesis of Compound 4**

**Compound 3** (0.52 mmol, 0.3 g) was dissolved into 15 mL dry DMF. **Compound 1** (1.15 mmol, 0.21 g) was added, followed by acetic acid (30 drops) and piperidine (30 drops). The mixture was put under Ar atmosphere before it was subjected to microwave irradiation (5 min, 150 °C, 1 min pre-stirring). After removing the solvent under reduced pressure, the mixture was purified by column chromatography (silica gel. Petroleum ether/ethyl acetate=1:1, v/v) to get dark green powder (Compound 4). Yield: 43.6% (0.20 g). $^1$HNMR (400 MHz, DMSO-d6) δ 8.02-7.87 (m, 2H), 7.73-7.59 (m, 2H), 7.58-7.54 (m, 4H), 7.49-7.28 (m, 4H), 4.12-4.05 (m, 4H), 3.60-3.53(m,4H), 1.91-1.83(m,4H), 1.59-1.11(m,6H); $^{13}$CNMR (400 MHz, DMSO-d6) δ 192.4, 166.7, 166.3, 165.7, 147.1, 141.1, 135.3, 134.7, 134.2, 134.0, 133.3, 128.9, 124.3, 122.5, 121.8, 120.3, 120.1, 70.4, 63.4, 43.6, 38.0, 21.9, 19.5, 5.9; MS (EI) calcd for [M]$^+$: 900.09, found 899.62.

**Synthesis of DBHA**

Under N$_2$ atmosphere, **Compound 4** (0.04 mmol, 36 mg), Hyaluronan acid (0.02 mmol, 8.2 mg), EDC (0.5 mmol, 95.9 mg), DMAP (0.5 mmol, 61 mg) were added into 30 mL anhydrous THF. The mixture was stirred at 40 °C for 72 h. After the reaction, the solvent was removed under reduced pressure, followed by adding 50 mL deionized (DI) water and then filtering to get the filtrate. The filtrate was further purified by dialyzing with dialysis membrane (molecular weight cut-off: 3.5 kDa) for 48 h. And the final product, **DBHA**, was obtained by freezing-dry.

**Preparation of DBHA-NPs**
Typically, 20 mg **Compound 4** was dissolved into 1 mL DI water, further added into 100 mL DI water under ultrasound, and freezing-dried to obtain **DBHA-NPs**.

![Fluorescence spectrum of DBHA dissolved in PBS (5 mg/ml, excited at 600 nm) and diiodo styryl bodipy dissolved in THF (5 mg/ml, excited at 610 nm).](image)

Fig. S1 Fluorescence spectrum of DBHA dissolved in PBS (5 mg/ml, excited at 600 nm) and diiodo styryl bodipy dissolved in THF (5 mg/ml, excited at 610 nm).
Fig. S2 Visualization of the intracellular fluorescence of HCT-116 cells using filter sets specific for DBHA-NPs (in red, column 1), Mito tracker and ER tracker (in green, column 2). The corresponding merged images in bright field were given in column 3.

Fig. S3 Flow cytometric analysis of the cell death mechanism induced by DBHA-NPs upon PDT treatment (λ > 600 nm) on HCT-116 cells, with different concentration: control, 0.25, 0.5, 0.75, and 1 mg/mL without light. Horizontal ordinate, propidium iodide (PI); and vertical coordinates, annexine V (GFP). All data were expressed as the mean ± standard deviation of three independent experiments.
Fig. S4 The relationship between the body weight and treated time for the mice.