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A tumor-targeted fluorescent probe based on melatonin for HClO imaging in living cells

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1 Instrument and reagent

1.1. Materials

Chemicals and solvents purchased were of analytical grade and used directly in the experiments.

1.2. Characterizations

^{1}H and ^{13}C NMR spectroscopy was conducted on a 600 MHz spectrometer (Bruker, Germany). High-resolution mass spectrometry was performed on UHPLC-HRMS spectrometry (Thermo, American). Fluorescence spectra were recorded with a Fluoromax-4 fluorescence spectrophotometer (Horiba, Japan). All fluorescence imaging experiments were measured on V1000-IX81 confocal fluorescence microscope (Olympus, Japan).

2 Synthesis of the probe

2.1 The synthesis of **Compound 1**

4-Chloro-1,8-naphthalenedicarboxylic anhydride (1 mmol), glycine (1 mmol), and acetic acid (3 mmol) were dissolved in dimethyl sulfoxide (10 mL). The reaction was refluxed and stirred at 100 °C for 8 hours. Afterward, the reaction solution was filtered to obtain a yellow solid. The resulting residue was purified by silica gel preparative thin-layer chromatography, yielding a yellow-green powder **Compound 1** (42%).^{1}H NMR (600 MHz, DMSO-d_6) δ 13.15 (s, 1H), 8.59 (m, 2H), 8.42 (dd, J = 8.0, 1.6 Hz, 1H), 8.03 (m, 1H), 8.00 (dd, J = 8.6, 7.3 Hz, 1H), 4.73 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.65, 163.10, 162.82, 138.52, 132.48, 131.75, 131.05, 129.23, 128.98, 128.77, 128.34, 122.54, 121.24, 41.70.

2.2 The synthesis of **Compound 2**

Compound 1 (1 mmol), 5-methoxytryptamine (400 mg, 1 mmol), and 1-hydroxybenzotriazole (1 mmol) were dissolved in N, N-dimethylformamide (8 mL). The reaction was refluxed and stirred at 25 °C for 12 hours. Afterward, the solution was evaporated under reduced pressure. The resulting residue was purified by silica gel preparative thin-layer chromatography to obtain a yellow-green powder **Compound 2** (73%).^{1}H NMR (600 MHz, DMSO-d_6) δ 10.68 (d, J = 2.5 Hz, 1H), 8.63 (dd, J = 8.6, 1.1 Hz, 1H), 8.60 (dd, J = 7.3, 1.1 Hz, 1H), 8.44 (d, J = 7.8 Hz, 1H), 8.35 (t, J = 5.7 Hz,

1H), 8.06 (d, J = 7.9 Hz, 1H), 8.03 (dd, J = 8.5, 7.3 Hz, 1H), 7.22 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 8.7, 2.4 Hz, 1H), 4.66 (s, 2H), 3.75 (s, 3H), 3.33 (m, 2H), 2.79 (t, J = 7.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.80, 163.41, 163.13, 153.45, 138.15, 132.21, 131.79, 131.48, 130.74, 129.19, 129.02, 129.00, 128.27, 127.91, 123.86, 123.20, 121.90, 112.46, 111.87, 111.53, 100.45, 55.80, 43.06, 40.52, 25.60.

2.3 The synthesis of the probe

Compound 2 (1 mmol), ethyl thioglycolate (2 mmol), and potassium carbonate (3 mmol) were dissolved in N, N-dimethylformamide (8 mL). The reaction was carried out under reflux stirring at 80 °C for 4 hours. Afterward, the solution was evaporated under reduced pressure. The solid was subjected to column chromatography for purification (80%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.67 (d, J = 2.5 Hz, 1H), δ 8.66 (d, J = 8.5 Hz, 1H), 8.58 (d, J = 6.6 Hz, 1H), 8.42 (d, J = 7.9 Hz, 1H), 8.35 (t, J = 5.7 Hz, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.96 (dd, J = 8.6, 7.2 Hz, 1H), 7.22 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.01 (d, J = 2.4 Hz, 1H), 6.64 (dd, J = 17.6, 10.9 Hz, 1H), 4.66 (s, 2H), 4.37 (s, 2H), 4.15 (q, J = 7.1 Hz, 2H), 3.75 (s, 3H), 3.33 (m, 2H), 2.79 (t, J = 7.5 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.83, 169.37, 168.49, 166.95, 163.68, 163.61, 153.94, 143.04, 131.95, 131.38, 131.03, 130.46, 128.37, 127.53, 126.95, 124.73, 123.12, 122.61, 112.33, 112.20, 111.84, 100.08, 61.70, 61.52, 55.87, 41.48, 33.66, 14.15, 14.12.

3 Determination of fluorescence spectroscopy

The probe was dissolved in EtOH at a concentration of 250 μM as the initial solution. In the experiment, the concentration of the probe was selected as 5 μM. For all spectral test systems, a mixed buffer solution of ethanol and PBS (1 mM, 2%, v/v, pH = 7.4) was selected. The excitation and emission spectra were 425 nm and 502 nm, respectively, and the slit was 2 nm.

4 Cell imaging research

4.1 Cytotoxicity test

Five kinds of cells (HUVEC, MCF-7, RAW 264.7, HeLa, Hep G2) were cultured in DMEM medium (10% fetal bovine serum, 1% penicill-streptomycin, 37 °C, 5%

CO_2). The Cell cytotoxicity test was measured by CCK-8 assay. After cells were incubated with different concentrations of probe (0-50 μM) for 12 h, 10 μL CCK-8 was added and incubated for 1 h. Finally, the cell viability was calculated by measuring the absorbance at 450 nm using a microplate reader (SpectraMax iD).

4.2 Fluorescence imaging experiment in living cell

(1) Detection of exogenous HClO

HeLa cells were selected as experimental objects, and the cells were cultured in DMEM medium (10% fetal bovine serum, 1% penicill-streptomycin, 37 ℃ and 5% CO_2). First, the probe (10 μM) was added to the cells and cultured for 1 h. And then HClO (20 μM) were added to the cells and cultured for 1 h. Finally, the cells were washed three times with PBS for cell imaging research.

(2) Detection of endogenous HClO

Hela cells were selected and divided into five groups. The first group served as the control group. The remaining four were incubated with probe (10 μM) for 1 h, and for the last three experimental groups, cells were supplemented with N-acetylcysteine (NAC, 1 μg/mL), phorbol 12-myristate 13-acetate (PMA, 1 μg/mL), and the co-treatment of PMA and NAC, respectively, with each group subjected to a 1-hour incubation period. Then the cells were washed with PBS for 3 times for fluorescence imaging.

(3) Tumor cell screening detection

Five kinds of cells (HUVEC, RAW 264.7, HeLa, MCF-7, Hep G2) were selected for fluorescence imaging. First, NAC was applied to remove HClO. Then, the probe (10 μM) was added to the cells and cultured for 1 h. Finally, the cells were washed with PBS for 3 times for fluorescence imaging.

5 Figure legend

Fig.S1 CKK-8 experiment of the probes in HUVEC cells.

Fig. S2 CKK-8 experiment of the probes in RAW 264.7 cells.

Fig. S3 CKK-8 experiment of the probes in HeLa cells.

Fig. S4 CKK-8 experiment of the probes in MCF-7 cells.

Fig. S5 CKK-8 experiment of the probes in Hep G2 cells.

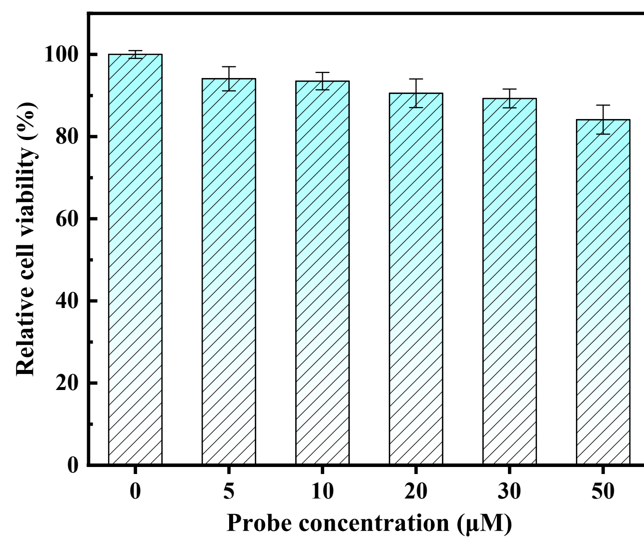


Fig. S1

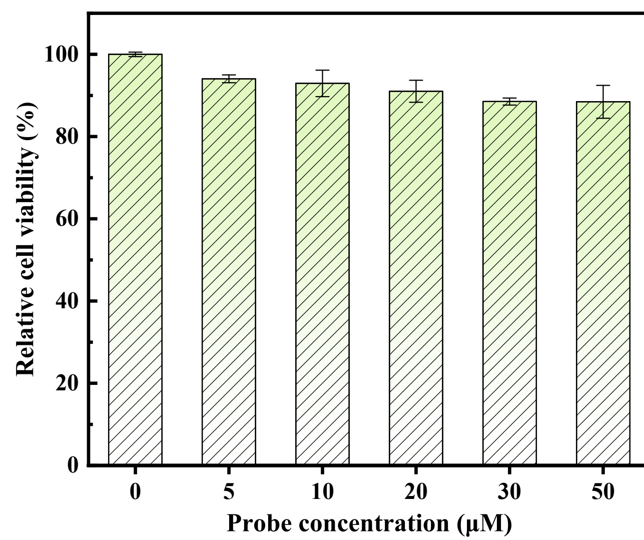
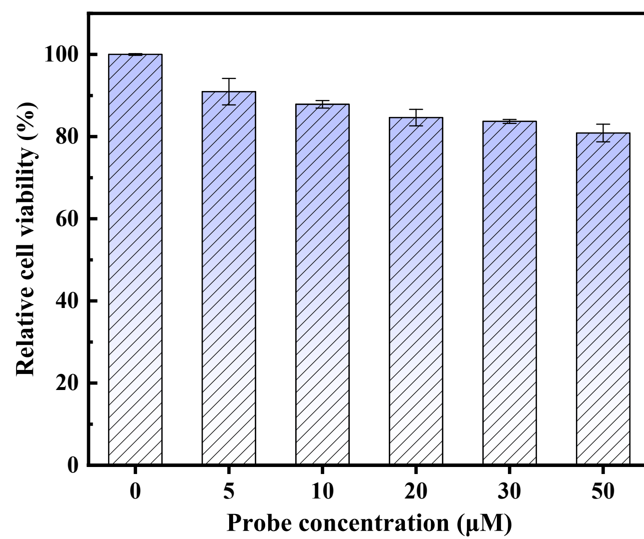


Fig. S2



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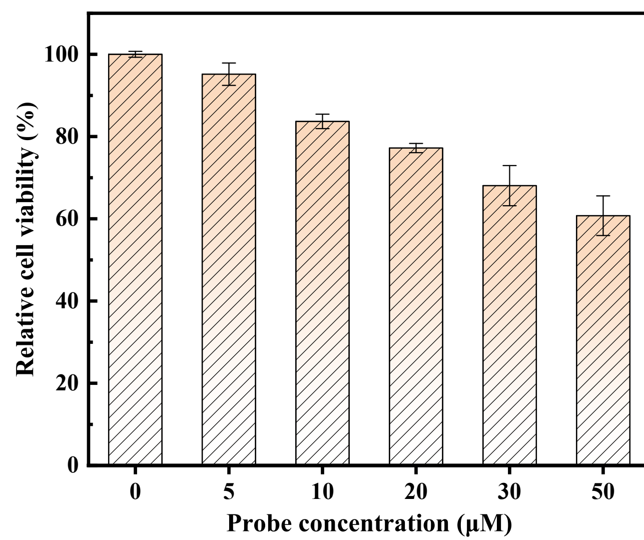

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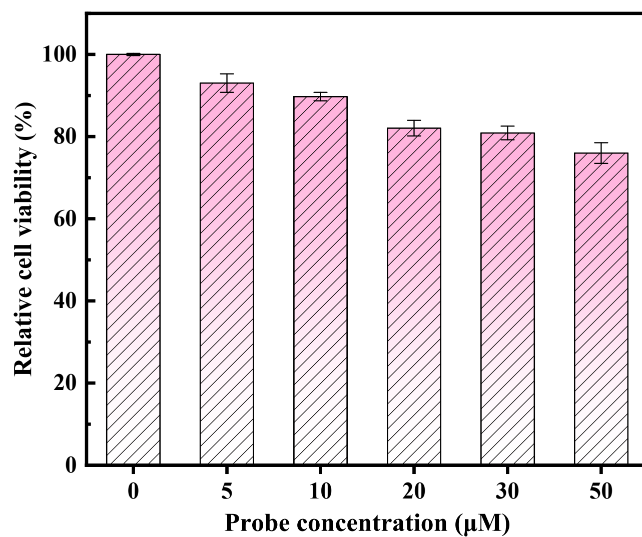


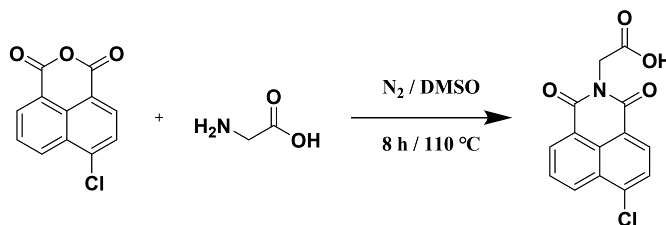
Fig. S5

6 Scheme legend

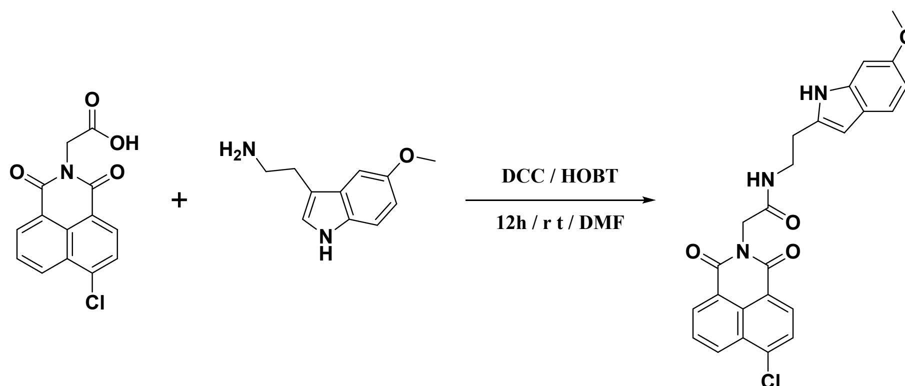
Scheme S1 The synthesis of **Compound 1**.

Scheme S2 The synthesis of **Compound 2**.

Scheme S3 The synthesis of the probe.



Scheme 1



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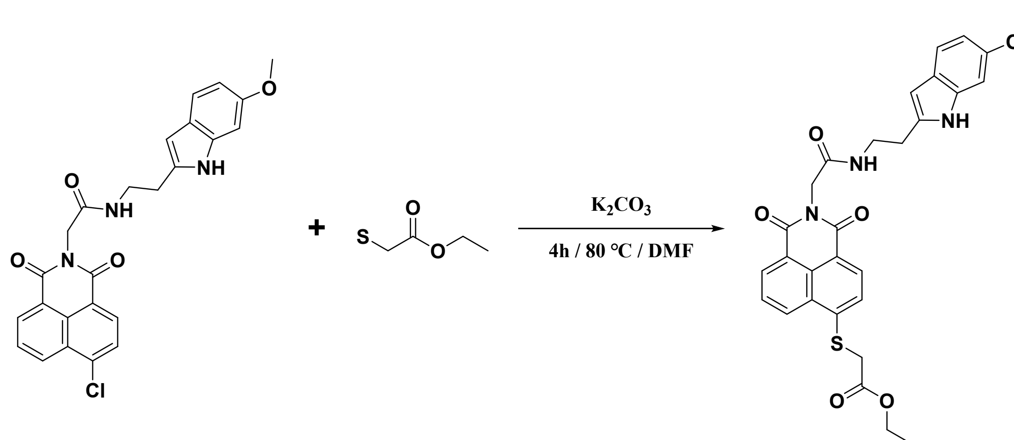

Scheme 3