Supporting Information

Dual-channel detection of viscosity and pH with near-infrared fluorescent probe for cancer visualization

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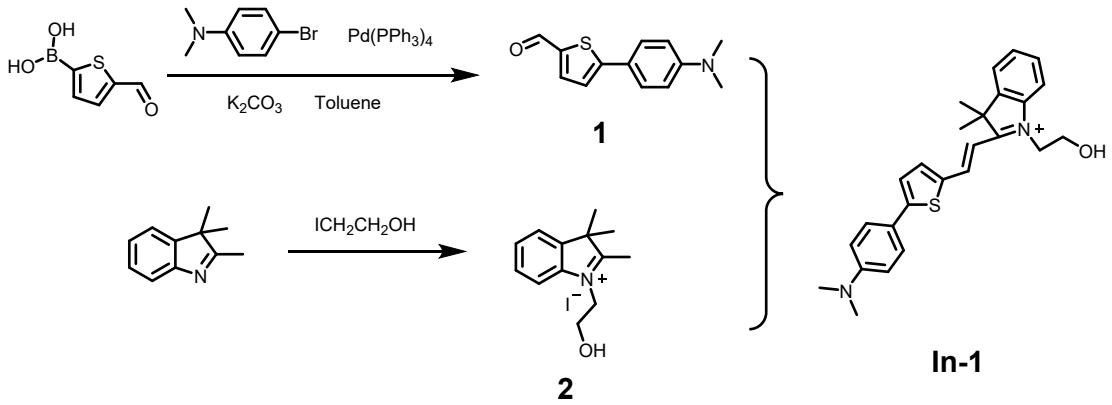
1. Experimental section

Materials and instruments

Unless specifically stated, all the chemicals were purchased form commercial suppliers and were used as received without further purification. Deionized water was used throughout all experiments. All test analytes in this experiment were papered by mixture solid in distilled water or DMSO solution.

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Reaction processes were monitored on thin layer chromatography (TLC). Ultraviolet-visible (UV-vis) spectra were recorded by Hitachi U-3900 UV-vis spectrophotometer. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer. Synthetic intermediates and probes were characterized by ^{1}H NMR and ^{13}C NMR using a Bruker AVANCE-600 MHz spectrometer and 150 MHz NMR spectrometer. HRMS were measured by the Thermo Scientific Q Exactive. The final bioimaging application were measured the Zeiss LSM880 Airyscan confocal laser scanning microscope.

Synthesis route



Synthesis of compound 1. The synthesis method of compound **1** is based on a literature report[1]. And its structure was confirmed by ^{1}H NMR and ^{13}C NMR (Fig. S4-S5). ^{1}H NMR (600 MHz,

DMSO-d_6) δ 9.82 (s, 1H), 7.95 (d, J = 4.0 Hz, 1H), 7.63 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 4.0 Hz, 1H), 6.77 (d, J = 8.9 Hz, 2H), 2.98 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 183.68, 155.11, 151.53, 140.24, 139.79, 132.79, 127.72, 122.64, 120.26, 113.47, 112.61, 40.52.

Synthesis of compound 2. 2,3,3-Trimethylindolenine (1 mL, 6.3 mmol) and 2-iodoethanol (0.49 mL, 6.3 mmol) were dissolved in 5 mL ethanol and heated at 80℃ for 12 h at reflux. After cooling to room temperature, the mixture was poured into 100 mL petroleum ether, then filtered and washed with petroleum ether for three times. The final product was black powder with a yield of 90%.

Synthesis of In-1. A mixture of **1** (46 mg, 0.2 mmol), **2** (99 mg, 0.3 mmol), CH_3COONa (16 mg, 2 mmol) in ethanol (10 mL) were stirred at rt for 8 h. After standing for 5 h, solid was precipitated and washed with n-hexane for three times to obtain the probe **In-1**(15 mg, 18%). And its structure was confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (d, J = 15.5 Hz, 1H), 8.09 (d, J = 4.0 Hz, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 7.7 Hz. 1H), 7.67 (dd, J = 15.2, 6.4 Hz, 3H), 7.56 (dt, J = 20.3, 7.4 Hz, 2H),7.15 (d, J = 15.7 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 5.22 (s, 1H), 4.63 (s, 2H), 3.89 (s, 2H), 3.44 (q, J = 7.0 Hz,1H), 3.03 (s, 6H), 1.79 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 181.59, 157.12, 151.97, 145.89, 143.58, 141.72, 141.55, 129.26, 128.73, 128.08, 124.35, 123.26, 120.20, 115.05, 112.73, 109.25, 59.06, 56.49, 52.02, 49.16, 40.60, 26.63, 19.01. HRMS: $[M- I^-]^+$ Calcd for 417.19951; Found 417.19983.

Cell culture

HL-7702 and HepG-2 cells were obtained from Procell Life Science & Technology Co., Ltd. HepG-2 and HL-7702 cells were cultured in a Dulbecco's modified Eagle's medium supplemented with 1% antibiotics and 12% Fetal Bovine Serum. The cells are cultured at 37 °C in a 5% CO_2

atmosphere cell incubator. Cells were plated on a cell culture dish, cultured for 24 hours and adhered to the wall.

Colocalization

First, the cells were incubated with **In-1** (10 μM) for 10 min. Then, the HL-7702 cells were incubated with Mito-Tracker Red (500 nM) and Lyso-Tracker Red (500 nM) at 37 ℃ for 20 min, respectively. And then washed with PBS (10 mM, pH 7.4) for three times. The cells were collected CLSM images directly.

Cell imaging pH

The cells were first incubated with the probe (10 μM) for 10min at 37℃, then washed with PBS for three times, and incubated with chloroquine with different concentrations (10 μM, 30 μM, 50 μM, 100 μM) for 30min, respectively. And the cells were imaging and photographed by a Zeiss LSM710 Airyscan confocal laser scanning microscope.

Cell imaging viscosity

HL-7702 cells were incubated with **In-1** (10 μM) for 10 min, then washed with PBS for three times, and then treated with 50 μM dexamethasone, and recorded its red channel fluorescence intensity over time by a Zeiss LSM710 Airyscan confocal laser scanning microscope.

2. Characterization of In-1

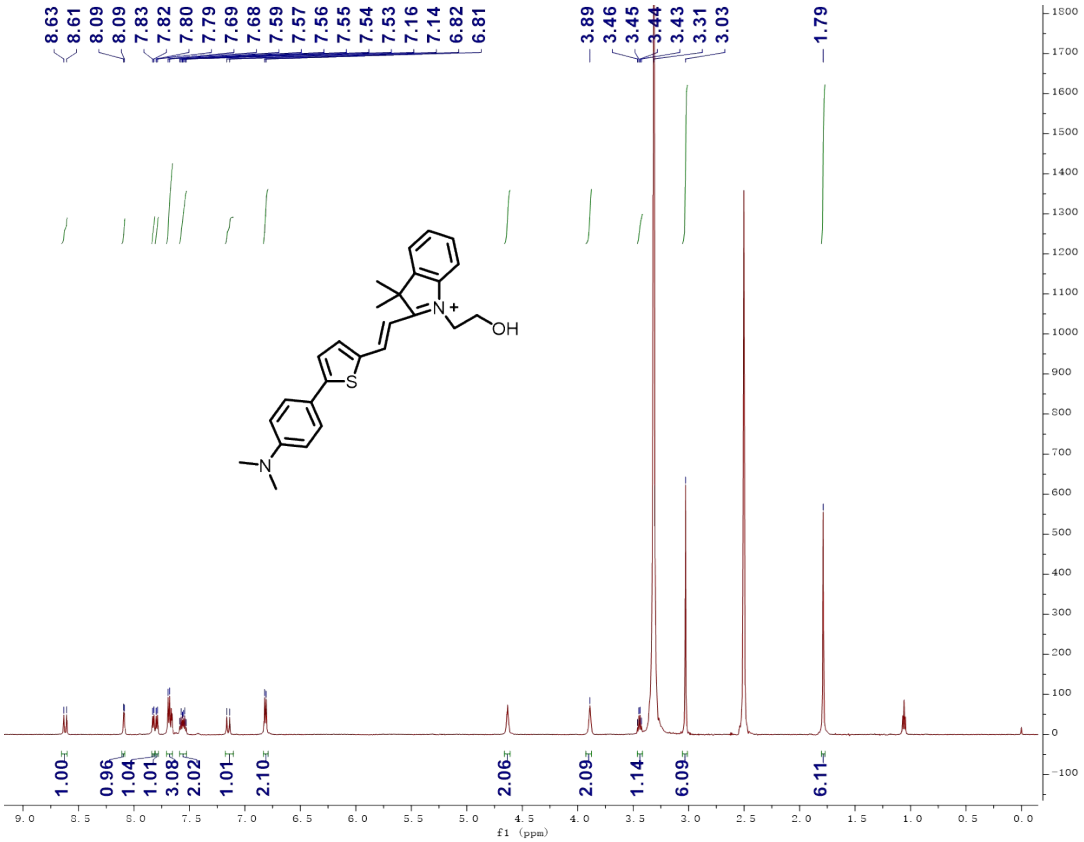


Fig. S1: ^{1}H NMR spectrum of **In-1** in DMSO-d_6

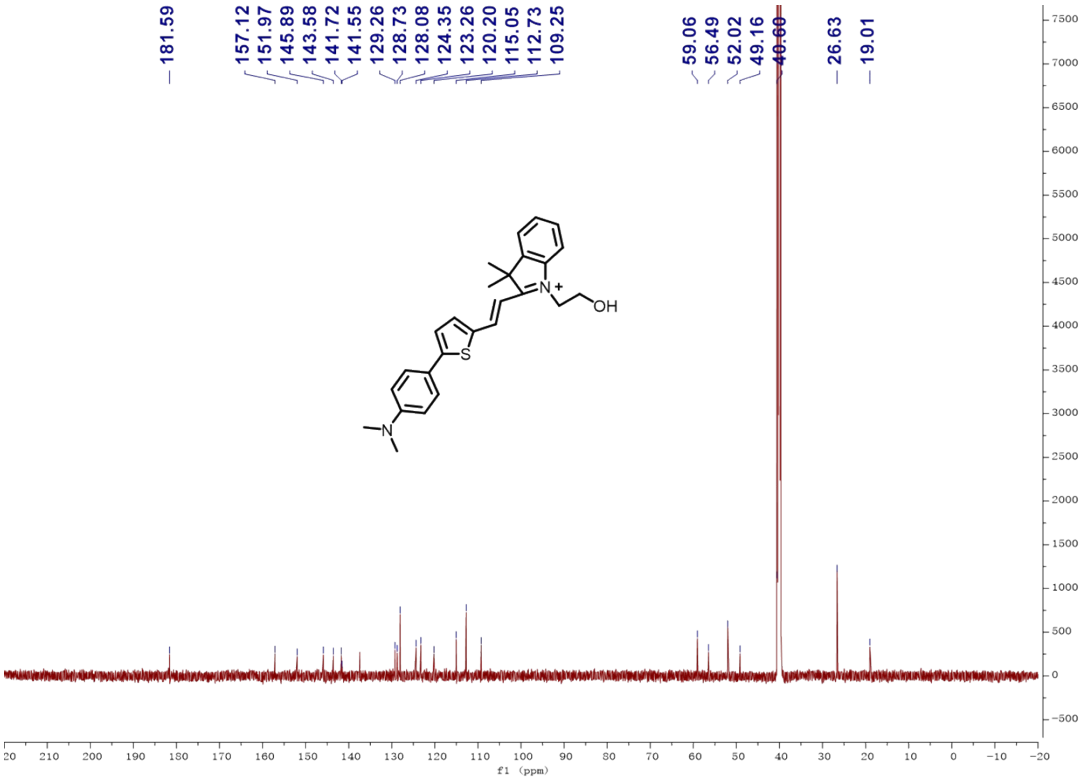


Fig. S2: ^{13}C NMR spectrum of **In-1** in DMSO-d_6.

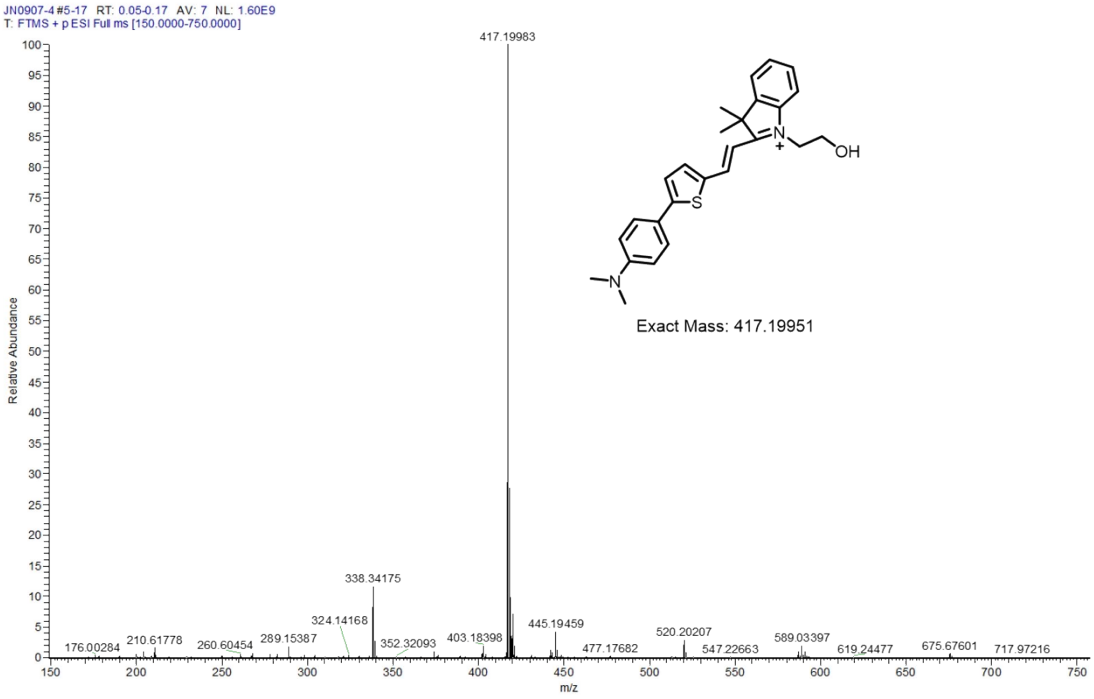


Fig. S3: HRMS spectra of **In-1.**

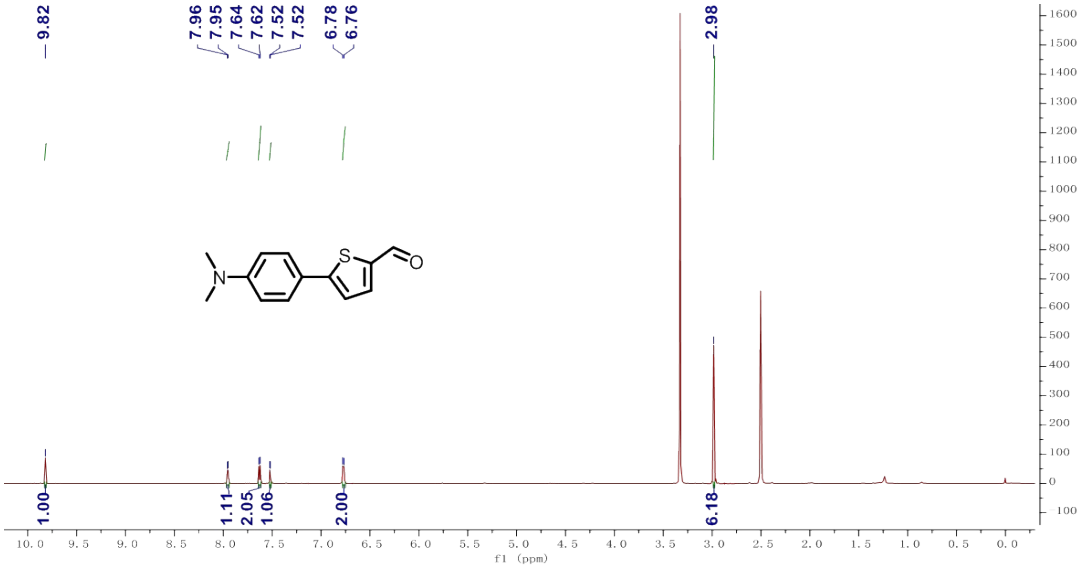


Fig. S4: ^{1}H NMR spectrum of compound **1** in DMSO-d_6

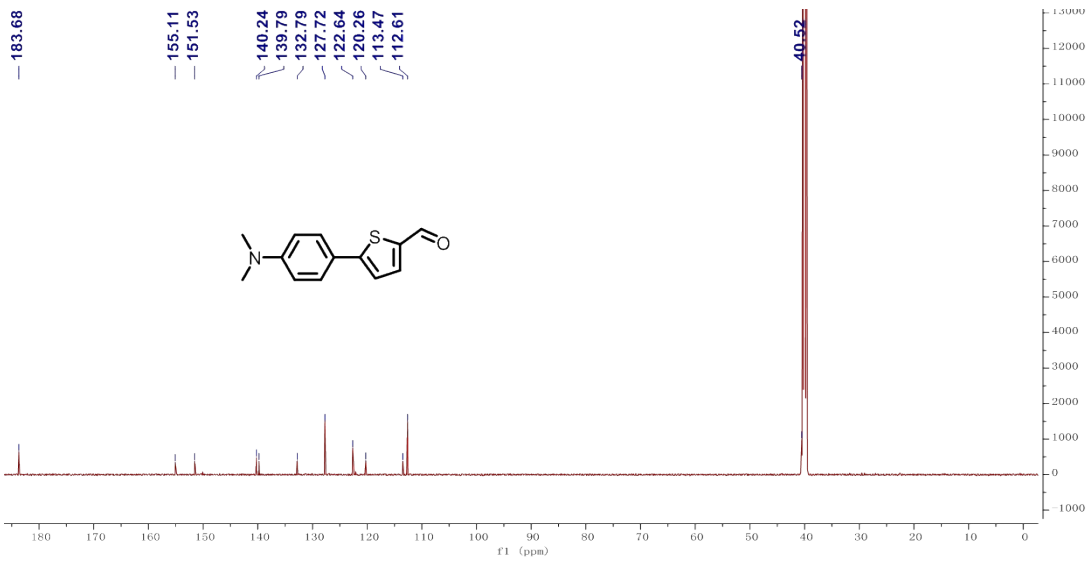


Fig. S5: ^{13}C NMR spectrum of compound **1** in DMSO-d_6

3. Spectrometric Studies of In-1

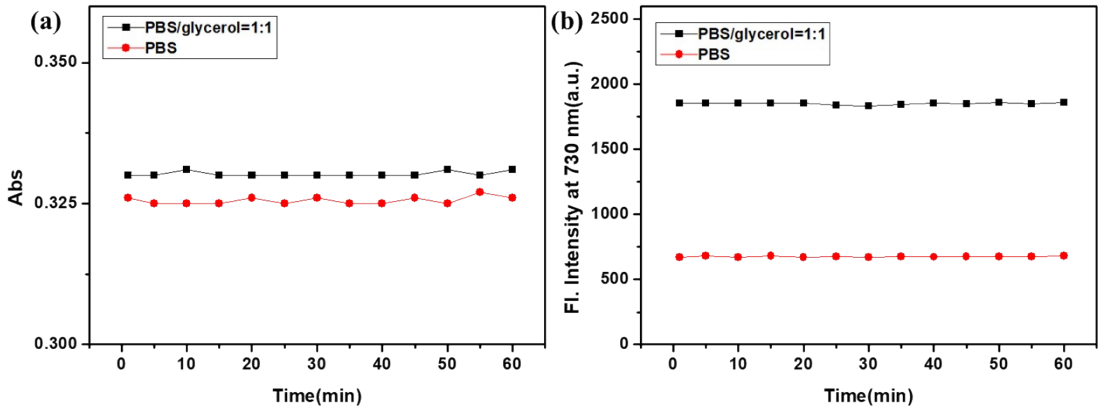


Fig. S6: (a) The changes of the absorption of probe at 570 nm within 1 hour in PBS and PBS/glycerol = 1:1 (pH = 5.0). (b) The changes of the fluorescence intensity of probe 730 nm within 1 hour in PBS and PBS/glycerol = 1:1(pH = 5.0).

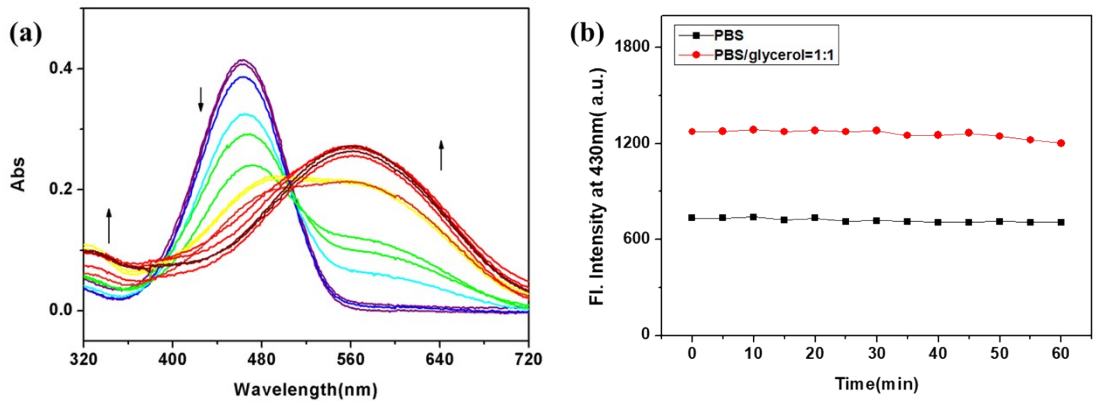


Fig. S7: (a)Absorption spectra of **In-1** (10 μM) in phosphate buffer at different pH range from 2 to 8. (b)The changes of the fluorescence intensity of probe at 430nm within 1 hour in PBS and PBS/glycerol =1:1 (pH= 5.0).

4. Bioimaging of In-1

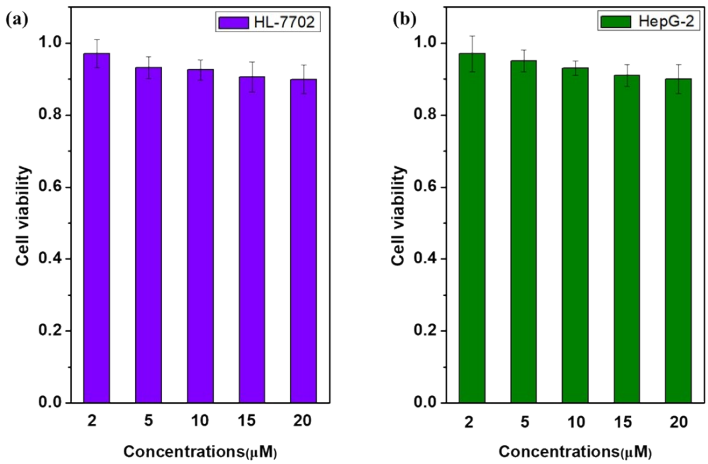


Fig. S8: Cytotoxicity data of probe **In-1** (2 μM, 5 μM, 10 μM, 15 μM, 20 μM) in HL-7702 cells. (b) Cytotoxicity data of probe **In-1** (2 μM, 5 μM, 10 μM, 15 μM, 20 μM) in HepG-2 cells.

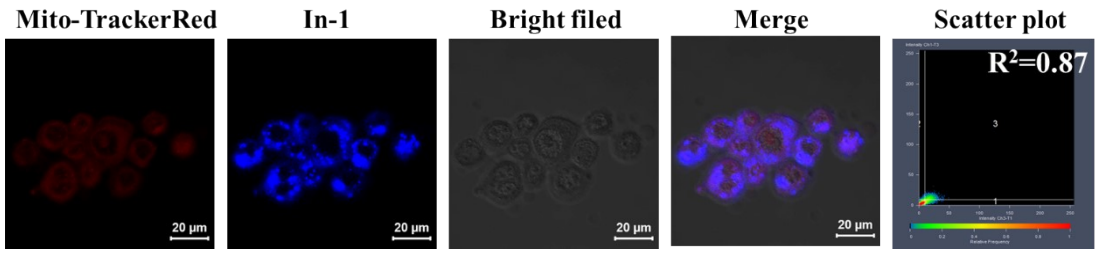


Fig. S9: The colocalization fluorescence imaging of HL-7702 cells co-treated with Mito-Tracker Red (500 μM) and CCCP. Scale bar: 20 μm.

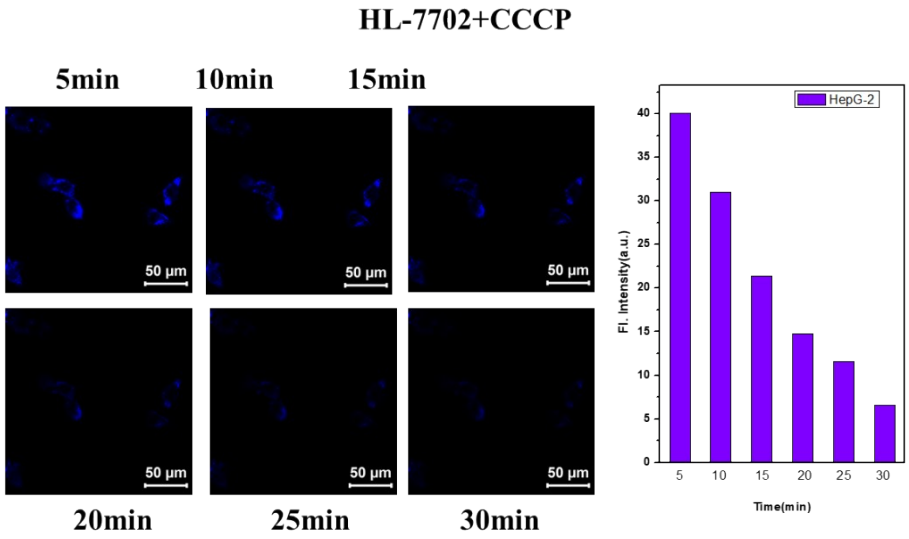


Fig. S10: (a) Confocal microscopy images of HL-7702 cells induced by CCCP for 0-30 min. Scale bar: 50 μm. (b) Quantification of fluorescent intensity from blue channel.

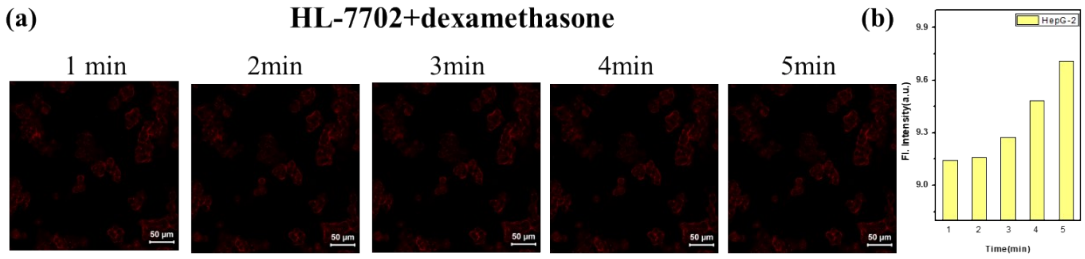


Fig. S11: (a) Confocal microscopy images of HL-7702 cells induced by nystatin for 0-5min. Scale bar: 50 μm. (b) Quantification of fluorescent intensity from red channel.

5. References

1 Kamoun P, Amino Acids, 2004, **26**, 243.