Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Mitochondria-targeted fluorescent probe for simultaneously imaging viscosity and sulfite in inflammation models

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1. General Information

General reagent information

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers and used as supplied without further purification.

General analytical information

Ultra-pure water was obtained from a water ultra-purification system. Silica gel (200-300 mesh) was used for flash column chromatography. Liquid nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV-400 NMR spectrometer. High-resolution mass spectra (HR-MS) were recorded on Thermo Scientific High-Resolution MS. UV-*vis* absorption spectra were obtained on a SHIMADZU UV-1800 spectrophotometer. Fluorescence measurements were performed with an Agilent Cary Eclipse Fluorescence spectrophotometer. The pH values were measured with a METTLER TOLEDO FiveEasy Plus pH meter. Laser confocal fluorescence imaging results were collected by Zeiss LSM 900. Fluorescence imaging experiments of mice were recorded by VISQUE In Vivo Smart-LF Imaging System.

General optical measurement

All spectra were measured in buffer solution (PBS/ glycerol = 2:8, v:v, 20 mM, pH = 7.4) at 37 °C. A stock solution of **ES** (5 mM) was prepared in DMSO and then diluted with PBS buffer solution to the desired concentration (10 μM). Na_2SO_3 was dissolved in ultra-pure water and other analytes were formulated with PBS buffer solution to the required concentration for fluorescence spectra. Optical tests were performed in quartz cuvettes (path length: 1.0 cm).

The Förster-Hoffmann equation

The relationship between the fluorescence emission intensity of the probe **ES** and the solvent viscosity could be formulated by the Förster-Hoffmann equation:

$$\log I = C + x \log \eta$$

Where η is the viscosity, I is the emission intensity, C is a constant, and x is the sensitivity of the probe to viscosity.

Cell imaging

Response of **ES** to viscosity: HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2.

For confocal imaging in Figure 4, (a) control cells; (b) cells were treated with **ES** (10 μM) for 1 h at 37 ℃; (c) cells were treated with **ES** (10 μM) for 1 h at 25 ℃; (d, e) cells were treated with Nys (20 μM)/LPS (1 μg/mL) for 20 min, sequentially incubated with **ES** (10 μM) for another 1 h at 37 ℃; (e-j) HepG2 cells were treated with LPS (1 μg/mL) for 20 min, and sequentially incubated **ES** (10 μM) for 1 h at 37 ℃, finally cultured with different concentrations of SO_3^{2-} (0-100 μM) for 10 min. Fluorescence collected in red channel: 627-680 nm. Data represent mean standard error (n = 3), λ_{ex} = 488 nm, scale bars: 50 μm.

Co-localization experiments in HeLa and HepG2 cells (Figures 3 and S9): cells were pretreated with Hoechst (1 μM) for 20 min, then incubated with MitoTracker green or LysoTracker green (1 μM) for another 20 min respectively, finally treated with **ES** (10 μM) for 1 h. Fluorescence collected in blue channel: 450-480 nm, in green channel: 500-550 nm, red channel: 627-680 nm, λ_{ex} = 488 nm.

Zebrafish imaging

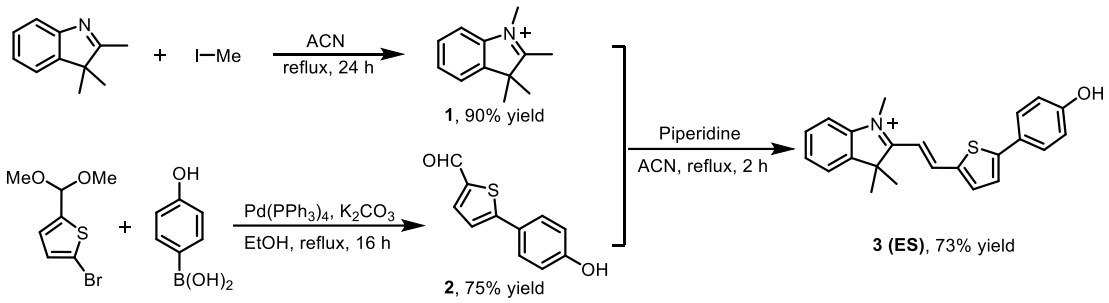
These zebrafish pretreated with 1-phenyl-2-thiourea (PTU) were cultured in the embryonic medium for 3 to 7 days at 25 °C. For confocal imaging in Figure 5, (a) control; (b) zebrafish larvae were incubated with **ES** (10 μM) for 1 h at 37 ℃; (c) zebrafish larvae were pretreated at 10 ℃ for 20 min, and sequentially incubated with **ES** (10 μM) for another 1 h at 10 ℃; (d, e) zebrafish larvae were treated with Nys (20 μM) /LPS (1 μg/mL) for 20 min, and sequentially incubated with **ES** (10 μM) for another 1 h at 37 ℃; (e-j) zebrafish larvae were treated with LPS (1 μg/mL) for 20 min, sequentially incubated **ES** (10 μM) for 1 h at 37 ℃, finally cultured with different concentrations of SO_3^{2-} (0-100 μM) for 10 min. Fluorescence collected in red channel: 627-680 nm. Data represent mean standard error (n = 3), λ_{ex} = 488 nm.

Mice imaging

5-week-old nude mice average body weight of 30 ± 2 g were purchased from Hengyisai Biotech Co. Inc. (Wuhan, China). A group of five-week-old KM mice were treated with subcutaneous (s.c.) injection of HepG2 cells. After 14-day incubation, mice imaging was conducted. For mice imaging in Figure 6, tumor mice were

peritumorally injected with **ES** (10 μM), and then treated with SO_3^{2-} (100 uM) at the 10th minute. Fluorescence images were collected at different time (0-15 min) in red channel: 620-650 nm. Data represent mean standard error (n = 3), λ_{ex} = 510 nm.

2. Synthesis of probe ES



Synthesis of compounds 1 and 2: Compounds **1** and **2** were synthesized following the previous method (*Chem Commun* **2017,** *53*, 10406-10409; *J Mater Chem B* **2017,** *5*, 3940-3944).

Synthesis of probe ES: To a 100 mL round-bottomed flask equipped with a magnetic stirring bar were added 1,2,3,3-tetramethyl-3H-indole iodide (200.0 mg, 1.14 mmol), 5-(4-hydroxyphenyl)-thiophene-2-carbaldehyde (116.3 mg, 0.57 mmol), and piperidine (300 μL) in 10 mL of acetonitrile. The reaction was refluxed for 2 h. Then, the mixture was cooled to room temperature, and treated with vacuum distillation. The obtained residual was purified by flash column chromatography over silica gel (100% CH_2Cl_2:MeOH = 50:1) to afford a red solid (150 mg, 73% yield). ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 8.59 (d, *J* = 15.6 Hz, 1H), 7.95 (d, *J* = 4.2 Hz, 1H), 7.73 (dd, *J* = 6.2, 4.2, 1.4 Hz, 4H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.63 – 7.56 (m, 2H), 7.54 (d, *J* = 4.2 Hz, 1H), 7.15 (d, *J* = 15.6 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.07 (s, 3H), 1.83. (s, 6H). ^{13}C NMR (100 MHz, Methanol-*d*$_4$) δ 181.03, 159.82, 156.80, 146.36, 143.07, 141.93, 140.30, 138.18, 129.01, 128.81, 127.86, 124.39, 124.14, 122.42, 115.90, 113.89, 108.28, 51.89, 32.70, 25.17. HRMS (ESI): m/z calcd for $C_{24}H_{14}N_2O_2S^-$ [M-H]$^-$: 360.14166, Found: 360.14166.

3. Supporting results

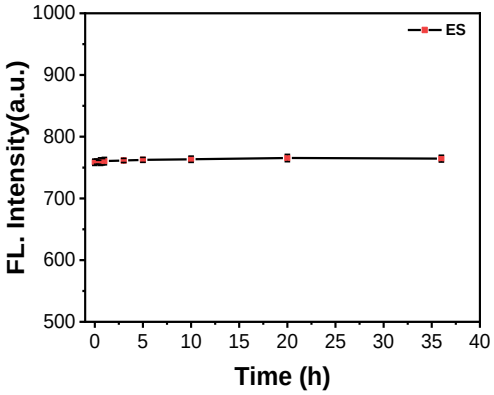


Figure S1. The fluorescence stability of probe **ES**. Data represent mean standard error (n = 3).

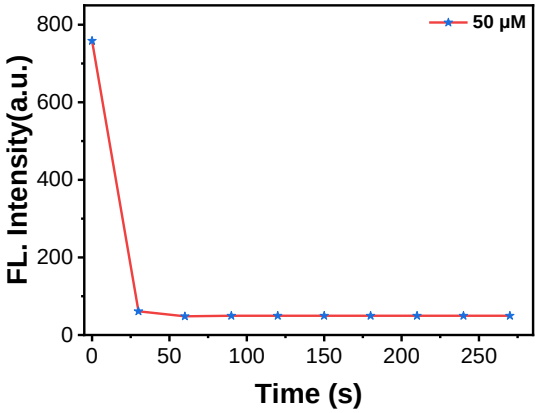


Figure S2. Fluorescence intensity changes at 628 nm of probe **ES** (10 μM) with time in the presence of SO_3^{2-} (50 μM) in glycerol-PBS solution (glycerol:PBS = 8:2, v/v, 20 mM, pH = 7.4) at 37 °C. λ_{ex} = 510 nm.

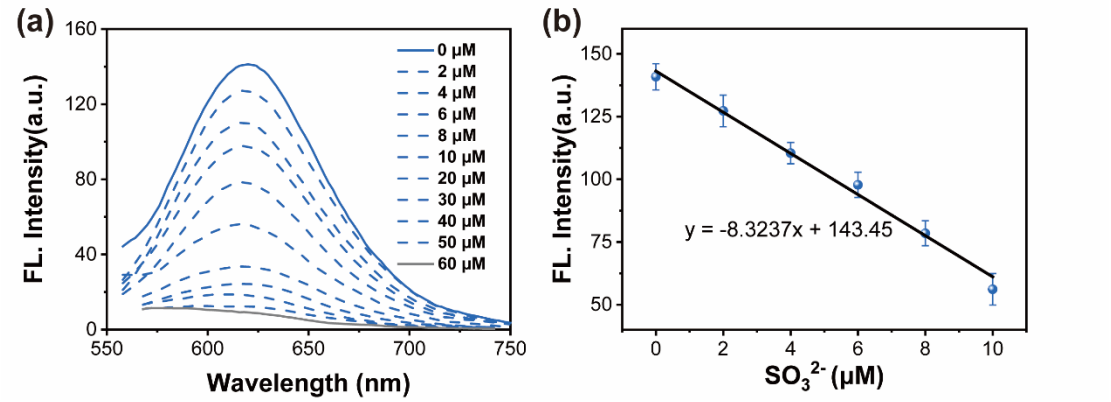


Figure S3. (a) Fluorescence spectra of probe **ES** (10 μM) to different concentrations of SO_3^{2-} (0-60 μM) in PBS solution (DMSO:PBS = 2:98, v/v, pH = 7.4). (b) The linear relationship between the fluorescence intensity at 628 nm and the concentration of SO_3^{2-} (0-10 μM). Data represent mean standard error (n = 3). λ_{ex} = 510 nm.

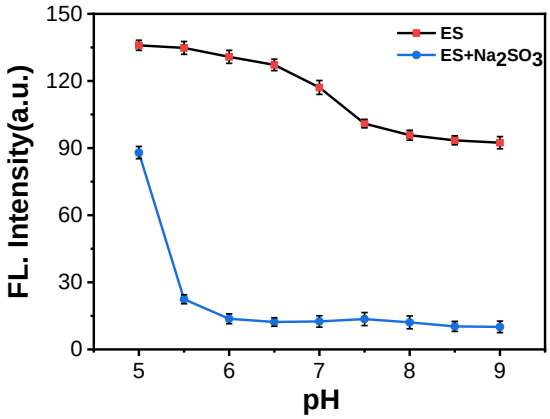


Figure S4. The pH effects of probe **ES** (10 μM) in the presence of SO_3^{2-} (50 μM) in PBS solution (DMSO:PBS = 2:98, v/v, pH = 7.4). Data represent mean standard error (n = 3).

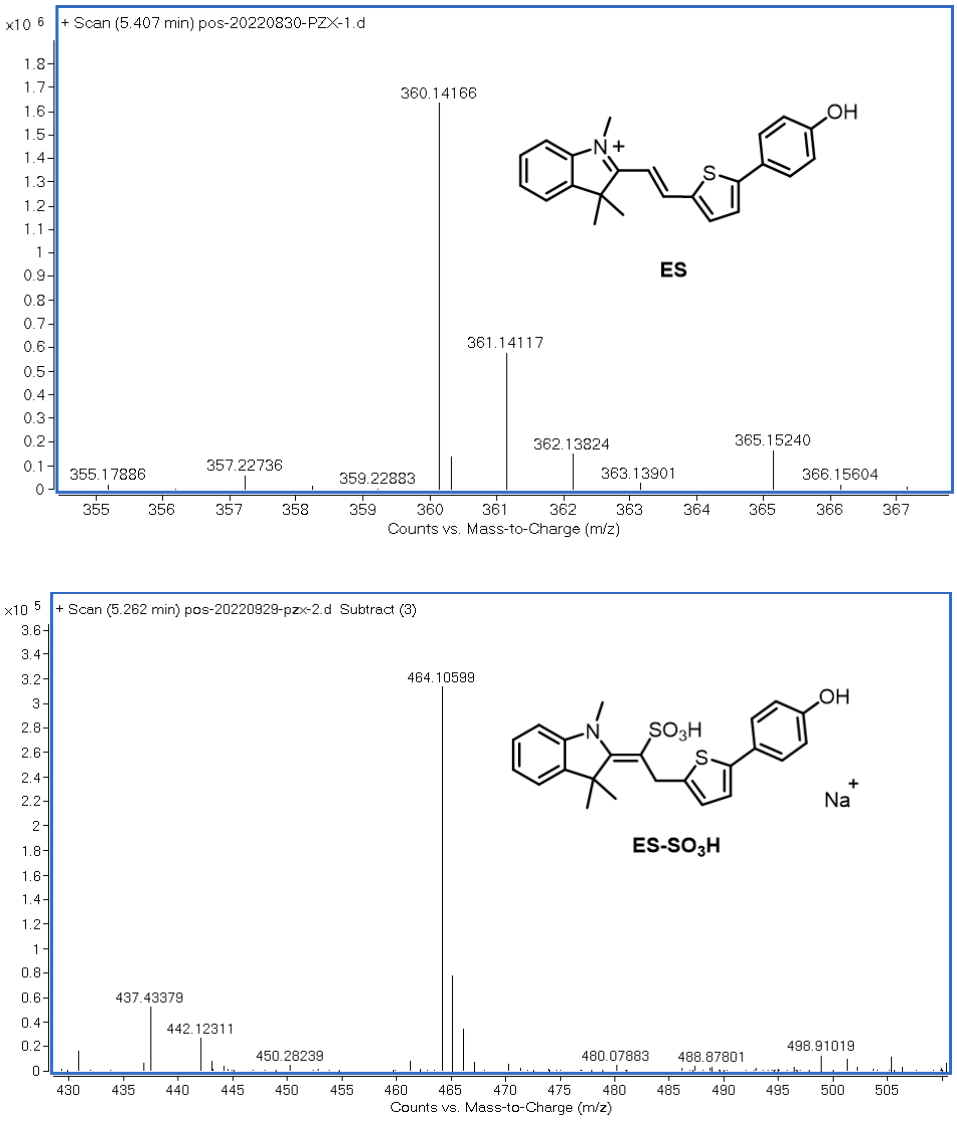


Figure S5. HRMS spectra of probe **ES** in the absence (a) and presence (b) of SO_3^{2-}.

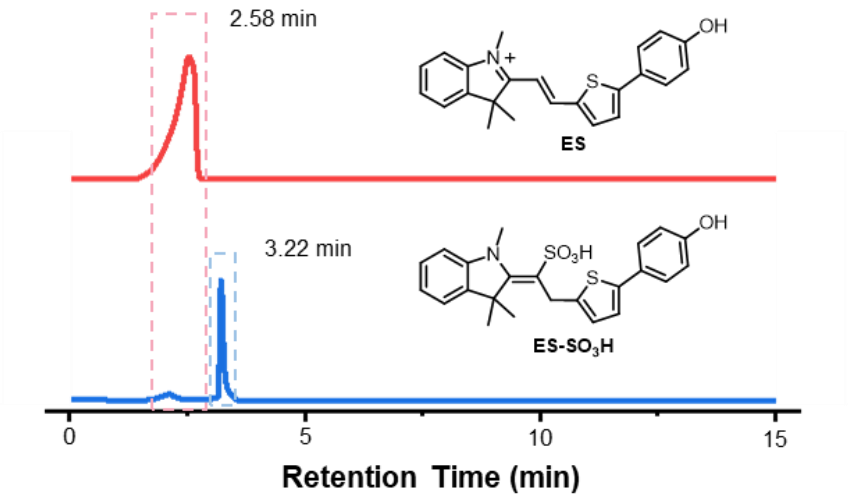


Figure S6. HPLC spectra of probe **ES** in the absence (a) and presence (b) of SO_3^{2-}.

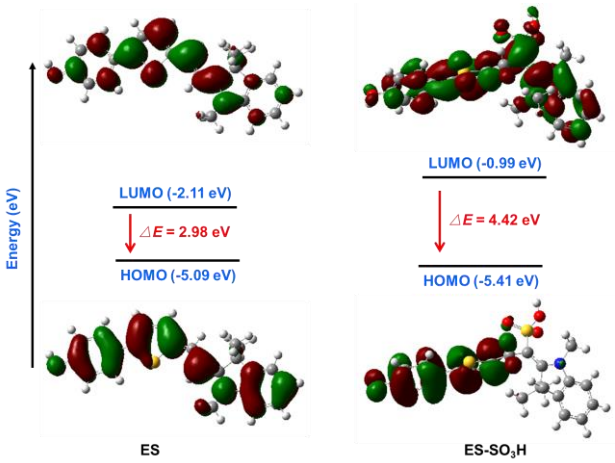


Figure S7. DFT calculation results for ES and ES-SO_3H at the B3LYP/6-31G(d) level.

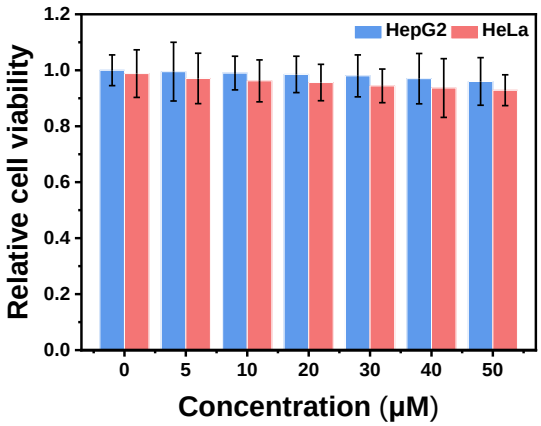


Figure S8. Cytotoxicity assays of probe **ES** at different concentrations for HepG2 and HeLa cells. Data represent mean standard error (n = 3).

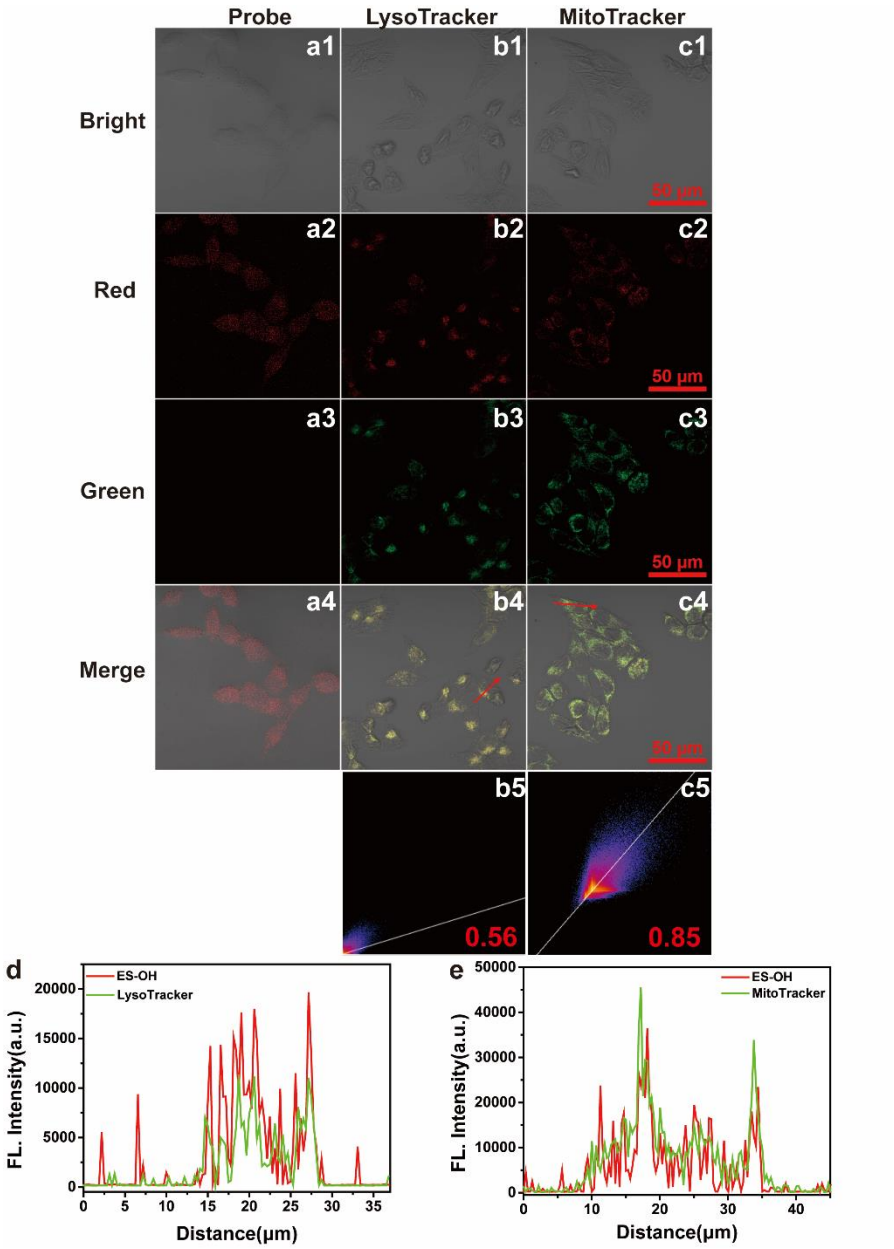


Figure S9. Confocal imaging of probe **ES** in HeLa cells. (a) cells were treated with **ES** (10 μM) for 1 h; (b, c) cells were first treated with MitoTracker (1 μM) /LysoTracker (1 μM) for 20 min, after that treated with **ES** (10 μM) for another 1 h; (d, e) Fluorescence intensity/distance plots, data were collected from b4 and c4. Fluorescence collected in green channel: 500-550 nm, red channel: 627-680 nm. Data represent mean standard error (n = 3), λ_{ex} = 488 nm, scale bars: 50 μm.

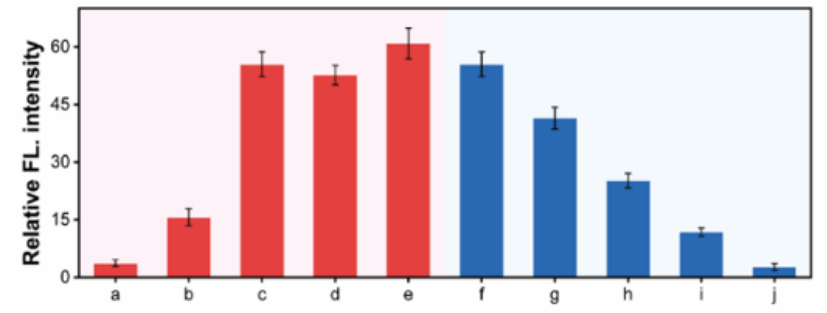


Figure S10. Relative fluorescence intensity of Figures 4a-j. Fluorescence collected in red channel: 627-680 nm. Data represent mean standard error (n = 3), λ_{ex} = 488 nm, scale bars: 50 μm.

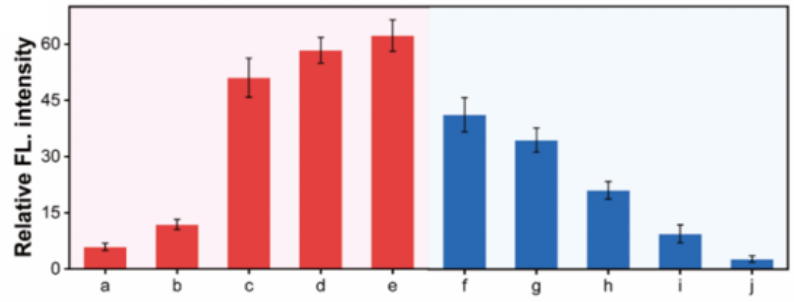


Figure S11. Relative fluorescence intensity of Figures 5a-j. Fluorescence collected in red channel: 627-680 nm. Data represent mean standard error (n = 3), λ_{ex} = 488 nm, scale bars: 50 μm.

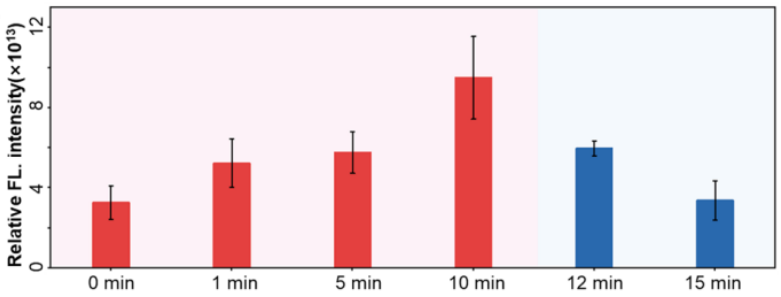


Figure S12. Fluorescence intensity inside mouse tumors with time (0-15 min) . Data represent mean standard error (n = 3), Fluorescence images were collected at different time (0-15 min) in red channel: 620-650 nm. λ_{ex} = 510 nm.

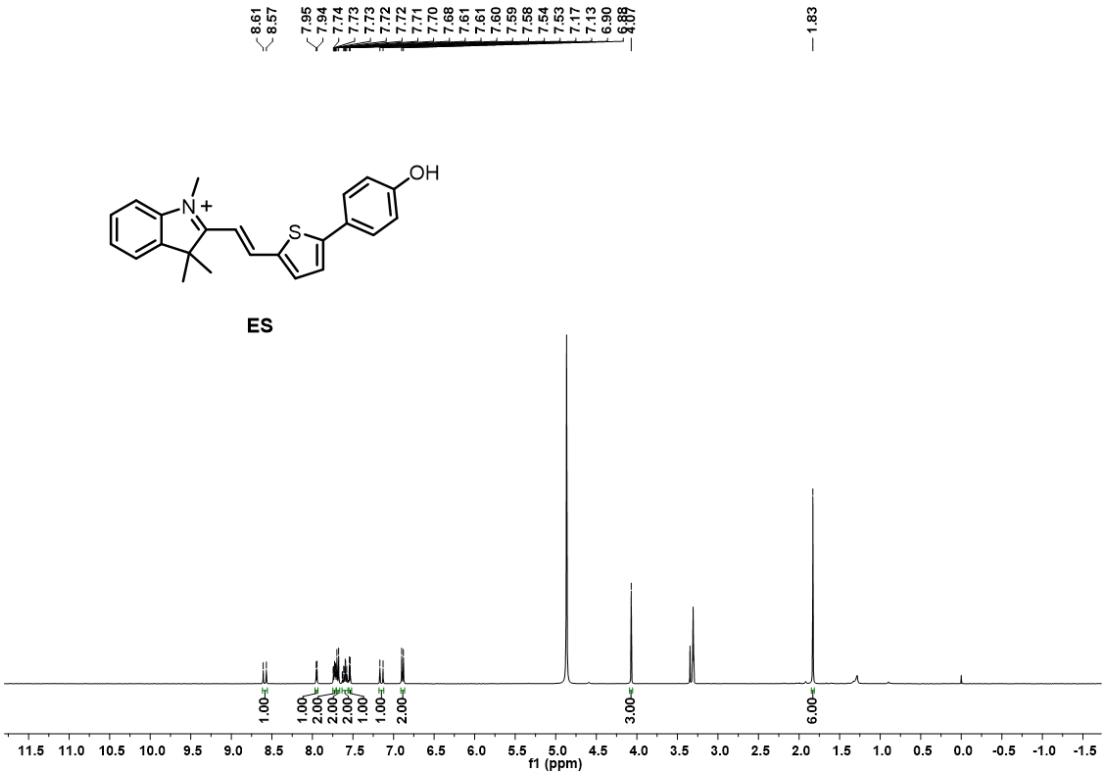


Figure S13. ^{13}H NMR spectrum of probe **ES** in Methanol-*d*$_4$.

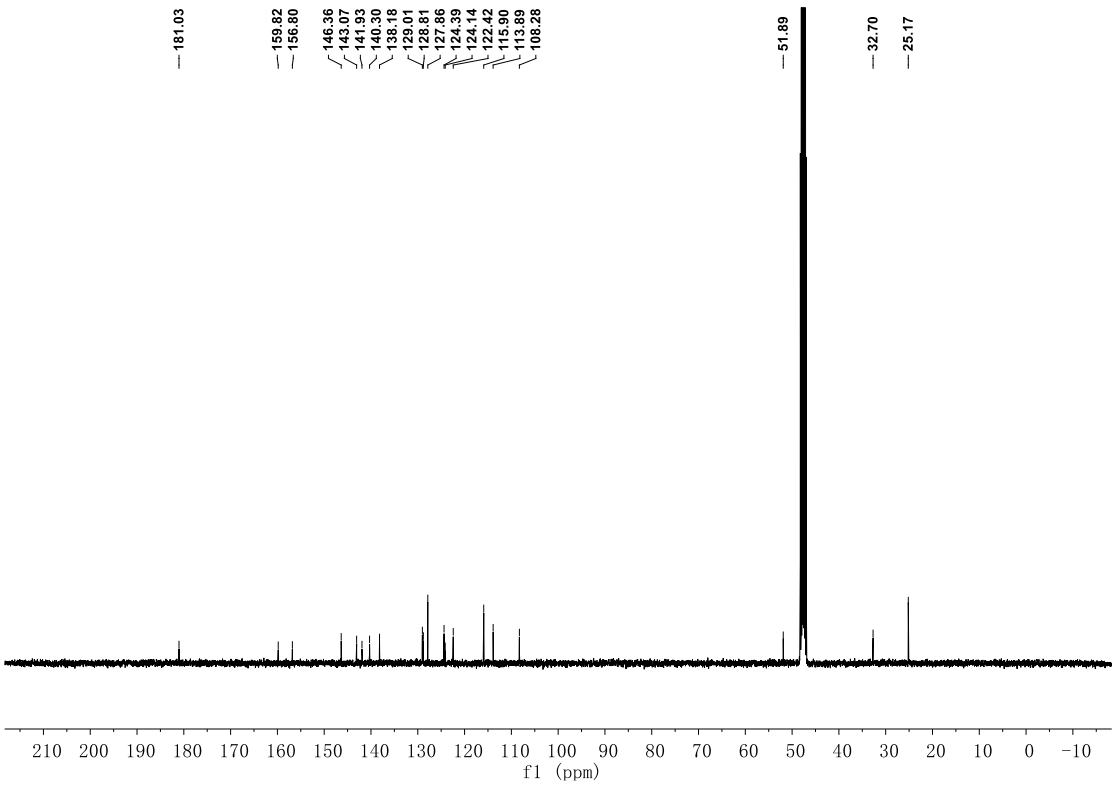


Figure S14. ^{13}C NMR spectrum of probe **ES** in Methanol-*d*$_4$.