Electronic Supplementary Information (ESI)

A water-soluble and fast-response mitochondria-targeted fluorescent probe for colorimetric and ratiometric sensing of endogenously generated SO_2 derivatives in living cells

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Contents

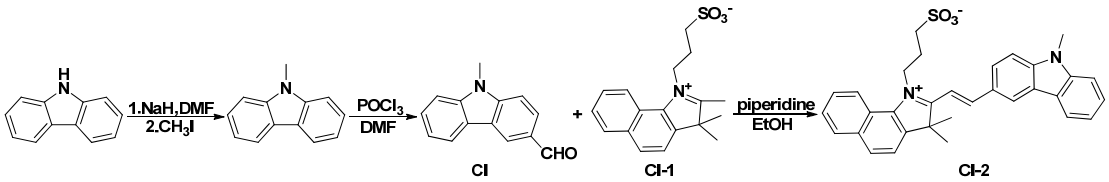
Experimental Section

General remarks for experimental

^{1}HNMR, ^{13}CNMR spectra were measured on an Agilent AM400 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a BrukerDaltonics Bio TOF mass spectrometer, respectively. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Fluorescence emission spectra were obtained using F7000 Spectrofluorophotometer (HITACHI) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

For ratio-imaging of SO_2 derivatives in living cells, we randomly selected several ROIs in the image (more than 15). The fluorescence intensity of both green channel and blue channel could be obtained via the confocal imaging instrument and the ratio of green channel and blue channel could be calculated. The average quantity of SO_2 in living cells could be calculated according to the calibration curve.

Synthesis of CI-2



Preparation and Characterization of CI

A solution of 9-methylcarbazole (3.00 g, 16.55 mmol) in DMF (12 mL) was treated with $POCl_3$ (3 mL) under N_2 and the reaction mixture was warmed at 125 °C for 1h. While still warm, the dark brown solution was poured into a 20% aqueous solution of NaOAc (60mL) and extracted with EtOAc. Flash chromatography (SiO_2, 50% EtOAc-hexanes) provided product as a colorless solid (2.86 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.70 (s, 1H), 8.28 (d, 1H,J = 8.0 Hz), 8.06 (dd, 1H, J = 8.7, 1.7 Hz), 7.52 (m, 3H), 7.32 (ddd, 1H, J = 7.5, 7.4, 1.0 Hz), 3.98 (s,1H).

Preparation and Characterization of CI-1

1,1,2-trimethyl-1H-benzo[e]indole (2.09 g, 10.0 mmol) was added a solution of 1,3-propane sultone (1.83 g, 30.0 mmol) in toluene (10 mL). The reaction mixture was stirred at 115°C for 18 h to give 3-(2,3,3-trimethyl-3H-benzo[g]indol-1-ium-1-yl),yield 2.48 g, 75%.

^{1}H NMR (400MHz ,CD_3OD) δ 8.34 (d, 1H, *J* = 8.4 Hz), 8.26 (dd, 1H, *J* = 8.8 Hz, *J* = 4.4 Hz), 8.18-8.14 (m, 2H), 7.82 (t, 1H, *J* = 8.0 Hz), 7.73 (d,1H, *J* = 8.0 Hz), 4.88 (t, 2H, *J* = 8.0 Hz), 3.37 (s, 3H), 3.07 (t, 2H, *J* = 6.4 Hz)2.49-2.42 (m, 2H), 1.86 (s, 6H); ^{13}C NMR (100MHz ,CD_3OD) δ 196.7, 138.5,137.3, 133.9, 196.7, 138.5, 137.3, 133.9, 131.1, 129.7, 128.2, 127.7, 127.3, 123.0, 112.5, 55.9, 47.2, 46.7, 23.4, 20.9. HRMS (ESI): m/z $[M + H]^+$calcd for $C_{18}H_{22}NO_3S$: 332.1315**;** found 332.1324.

Preparation and Characterization of CI-2

A mixture of **CI** (70 mg, 0.33 mmol), **CI-1** (122 mg, 0.37 mmol), piperidine (1 drop) in ethanol (10 mL) was heated to reflux and stirred overnight. The reaction mixture was cooled to rt and filtered. The solid was rinsed with 20 mL of cool methanol and dried over vacuum oven overnight to give 60 mg of desired product as a blue solid in 34% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.78 (d, *J* = 15.9 Hz, 1H), 8.43 (dd, *J* = 8.5, 3.0 Hz, 2H), 8.38 (d, *J* = 7.6 Hz, 1H), 8.28 (d, *J* = 8.9 Hz, 1H), 8.19 (t, *J* = 8.7 Hz, 2H), 8.03 (d, *J* = 16.1 Hz, 1H), 7.80 (dd, *J* = 7.9, 5.3 Hz, 2H), 7.73 – 7.67 (m, 2H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.36 (t, *J* = 7.2 Hz, 1H), 5.05 – 4.95 (t, *J* = 7.6 Hz, 1H), 3.98 (s, 3H), 2.82 – 2.74 (t, *J* = 6.0Hz, 1H), 2.29 (s, 2H), 2.09 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ182.15, 155.40, 144.39, 141.91, 139.00,138.03,134.20, 133.31, 133.89,131.34, 130.44, 128.70, 128.14, 127.32,127.18, 126.46 ,123.61, 123.36,122.80, 121.51, 120.93, 113.39, 110.50, 109.10, 53.68, 47.70, 45.63, 29.88, 26.36, 25.12. MS (ESI) m/z for $C_{32}H_{31}N_2O_3S$, 523.2055; found: 523.2053 $[M+H]^+$.

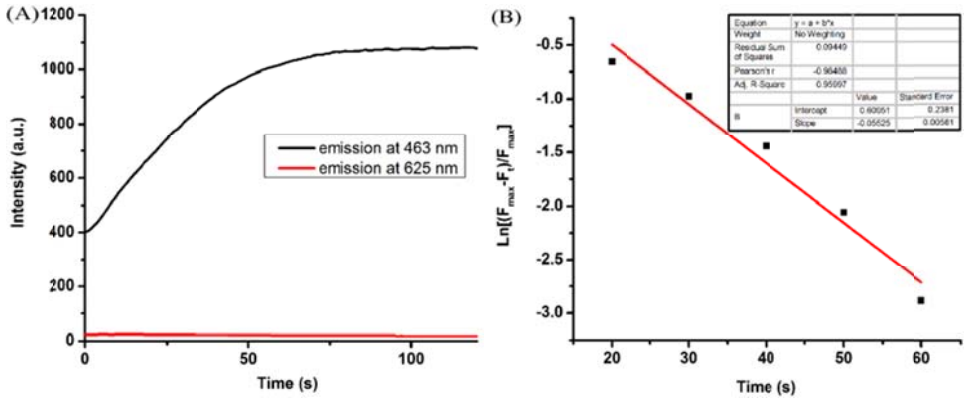


Figure S1. **(**A**)**The kinetic response of the bisulfite (15 equiv.) to **CI-2** in PBS buffer (pH 7.4, 10 mM); *(*B*)Pseudo* first-order kinetic plot of **CI-2** react with bisulfite (15 equiv.) in PBS buffer (pH 7.4, 10 mM). [**CI-2**] =10 μM. **CI-2**: Slope = -0.0552 s^{-1}.Slit: 5nm/5 nm.

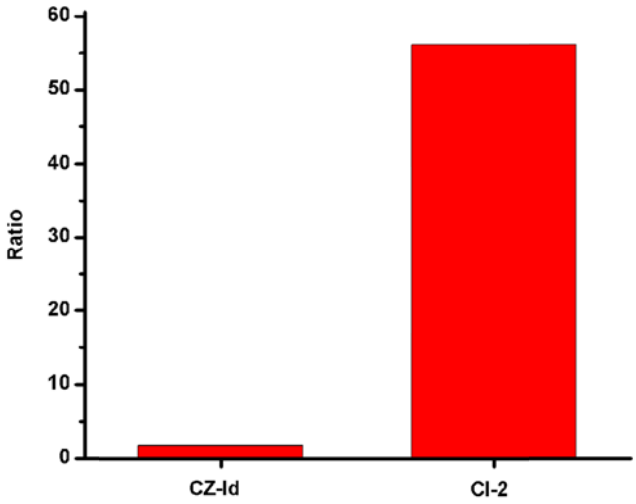


Figure S2. The fluorescence intensity ratio changes of these two probes to bisulfite in PBS buffer (pH 7.4, 10 mM). Slit: 5nm/5 nm. (Probe **CZ-Id** used from literature[1]).

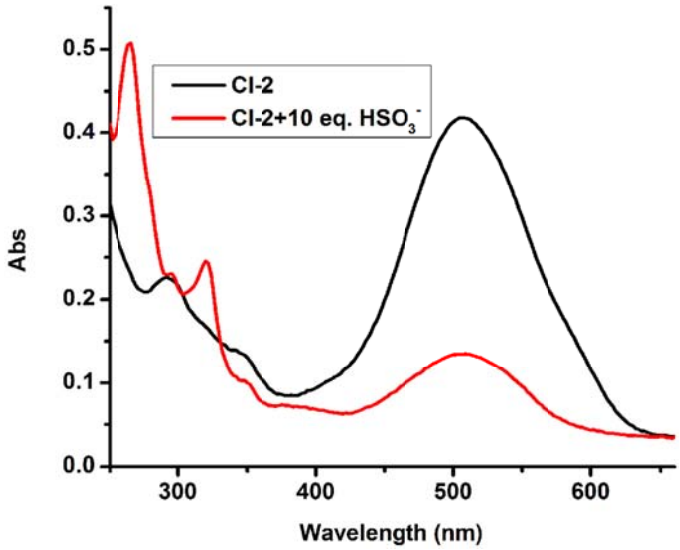


Figure S3. The absorption spectra of **CI-2** before and after reaction with bisulfite (10 equiv.) in PBS buffer (pH 7.4, 10 mM). [**CI-2**] =10 μM.

Figure S4. Image under visible light of **CI-2** in PBS (pH 7.4, 10 mM) to various anions（Left to Right：probe（10 μM），Na_2SO_3（10 equiv.），$NaHSO_3$（10 equiv.）, SO_2 releasing agent (15 equiv.N-benzyl-2,4-dinitrobenzenesulfonamide and 150 equiv. Cys), S^{2-}（20 equiv.），Cys（1mM），GSH（1mM）, Hcy（1mM），donor (15 equiv.), other anions（1mM）.

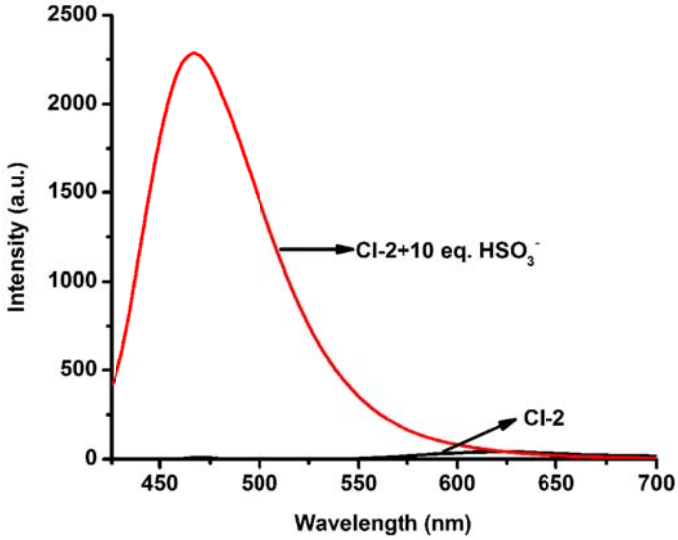


Figure S5. Fluorescence spectra of **CI-2** before and after reaction with bisulfite (10 equiv.) in PBS buffer (pH 7.4, 10 mM). [**CI-2**] =10 μM. λex = 405 nm. Slit: 5nm/5 nm.

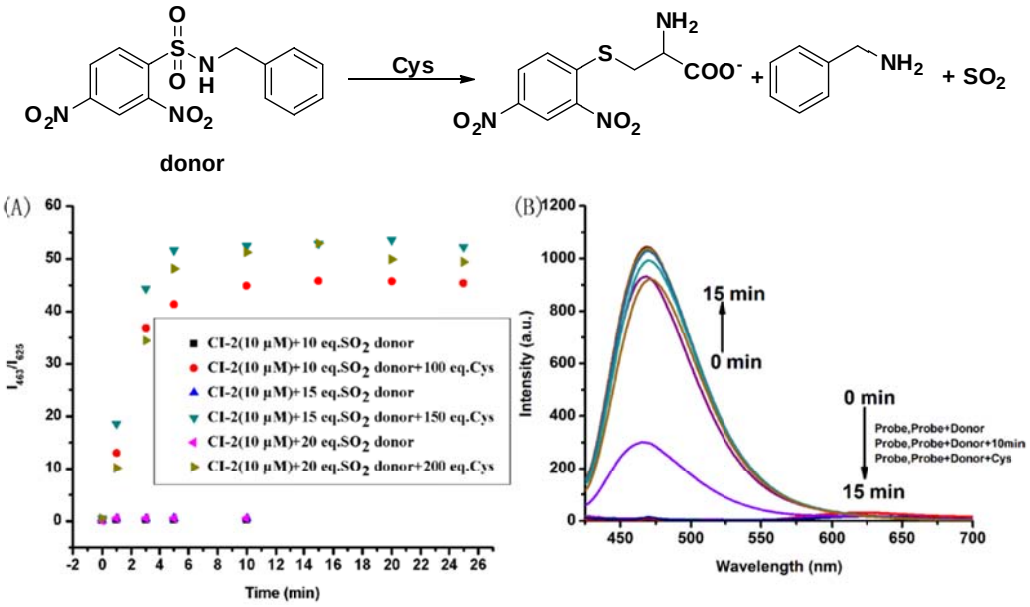


Figure S6. Time-dependent (0-25 min) emission spectra of **CI-2** (10 μM) + SO_2 donor (150 μM) upon addition of Cys (1.5 mM) in pH 7.4 PBS buffer (pH 7.4, 10 mM). λex = 405 nm.

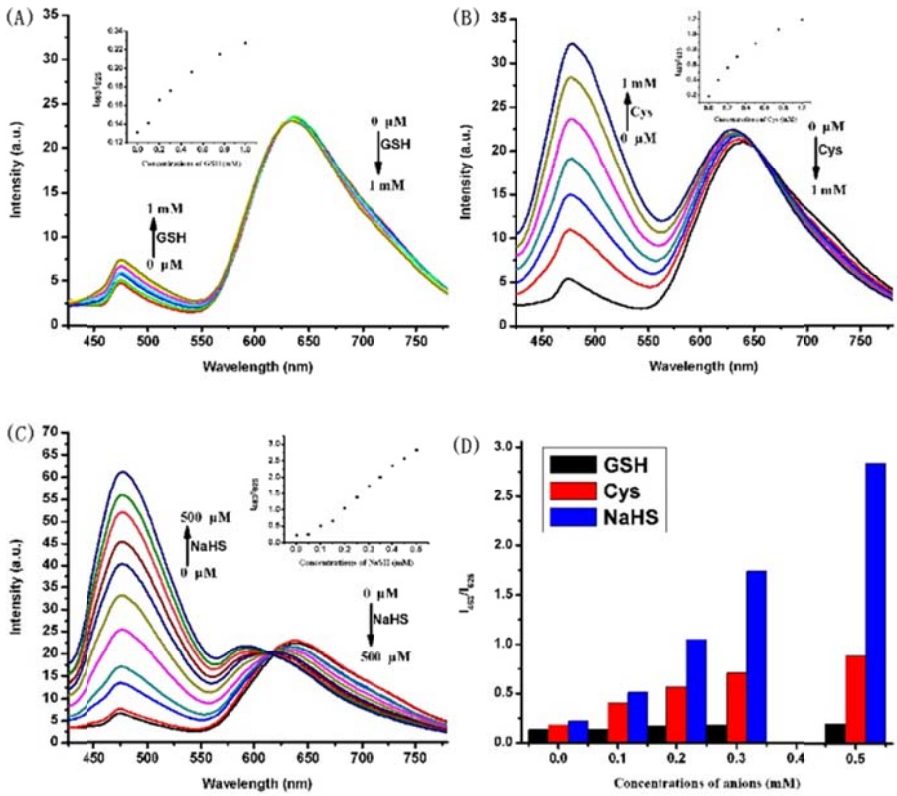


Figure S7. (a-c) Fluorescence titration spectra of **CI-2** upon addition of anions in PBS buffer (pH 7.4, 10 mM). [**CI-2**] =10 μM. Inset: the titration curve plotted with the fluorescence intensity ratio of CI-2 as a function of anions concentration, respectively. (d) Fluorescence intensity ratio changes of **CI-2** to GSH, Cys and NaHS. Data were acquired in PBS buffer (p**H** 7.4,10 mM). λex = 405 nm.

The detection limit of probe to sulfite

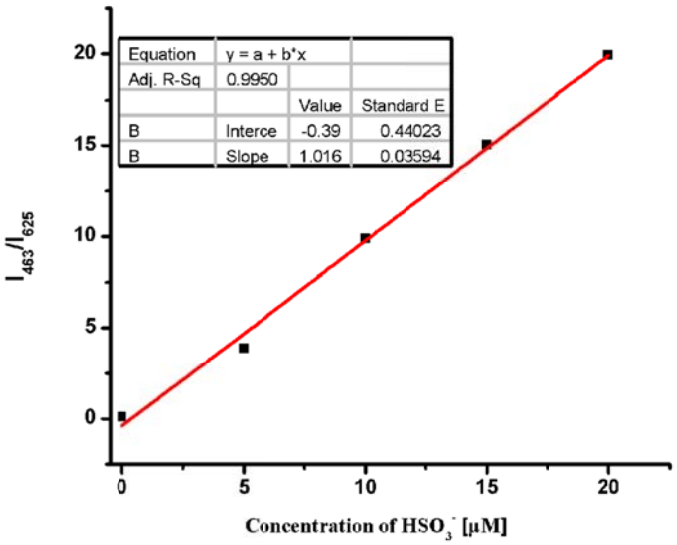


Figure S8. The line relationship between the fluorescent intensity ratios of **CI-2** and the concentration of bisulfite in PBS buffer (p**H** 7.4, 10 mM). [**CI-2**] =10 μM. λex = 405 nm.

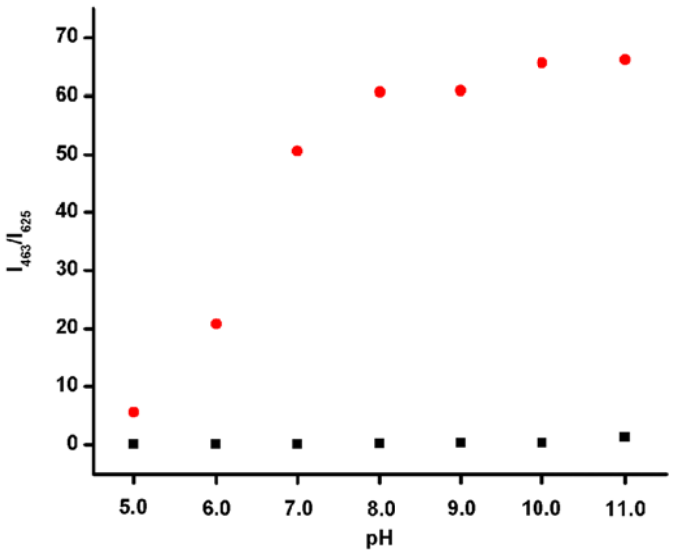


Figure S9. The influence of pH value to the reaction of **CI-2** (10 μM) with bisulfite (100 μM), λex = 405 nm. Slit: 5nm/5 nm.

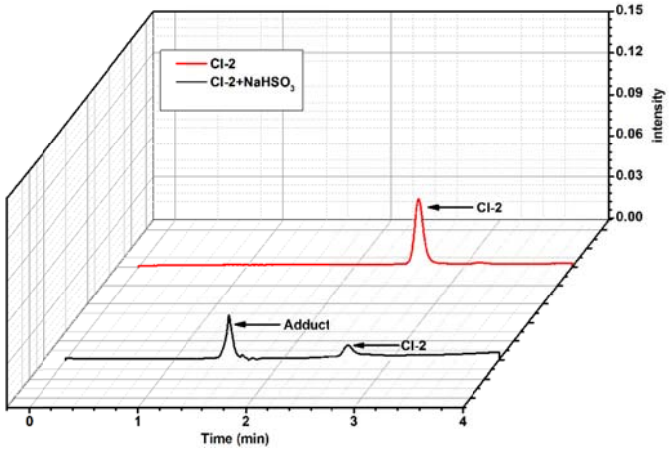


Figure S10. Mechanistic studies of probes reacting with SO_2 and identification of adduct using HPLC method.**CI-2**: Water 20% and methanol 80% were used as eluents with a flow rate of 1 ml/min (254 nm).

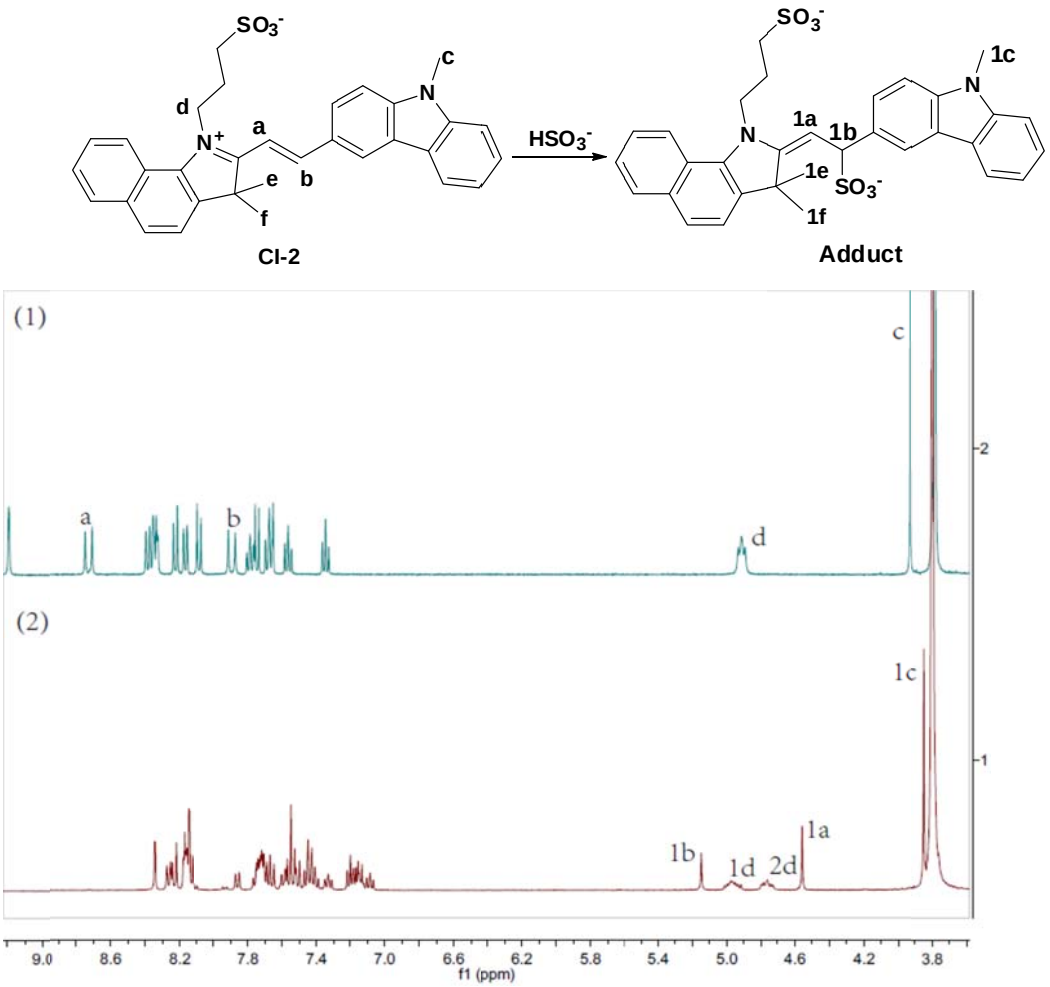
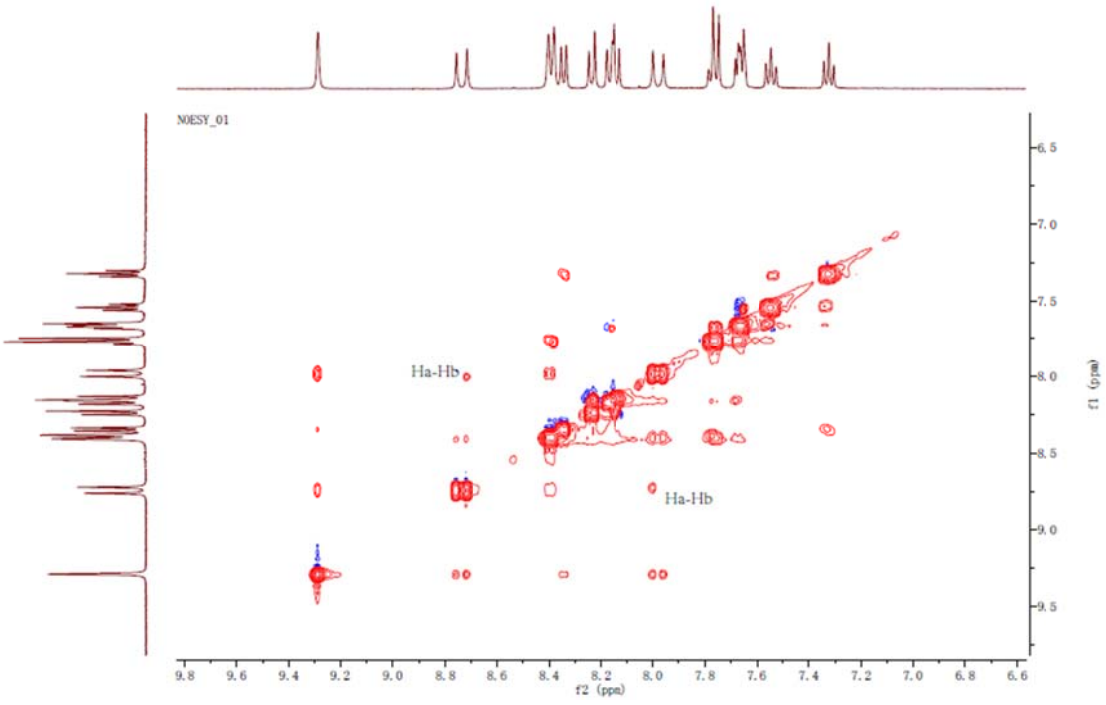


Figure S11. [1]HNMR spectral change of **CI-2** (5 μM) in the absence (1) and presence of 15 equiv of $NaHSO_3$ (2) in DMSO /D_2O = 1:1.



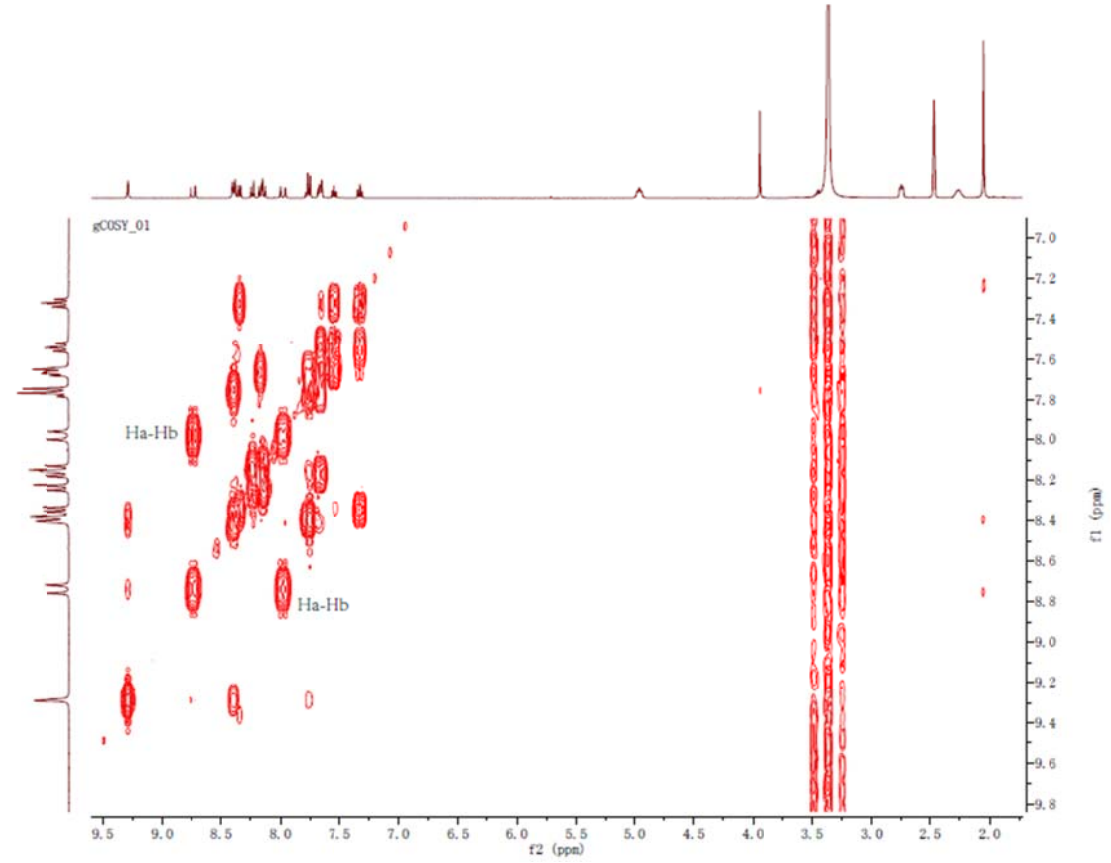
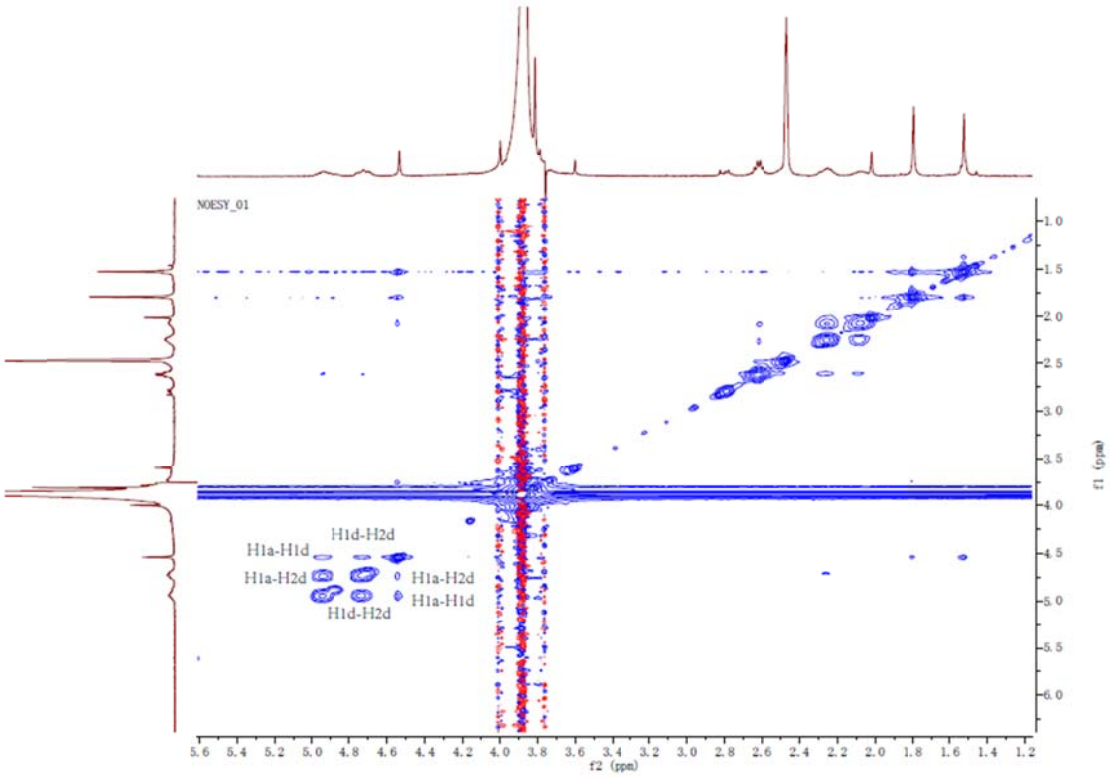


Figure S12. The NOESY and COSY of **Cl-2.**



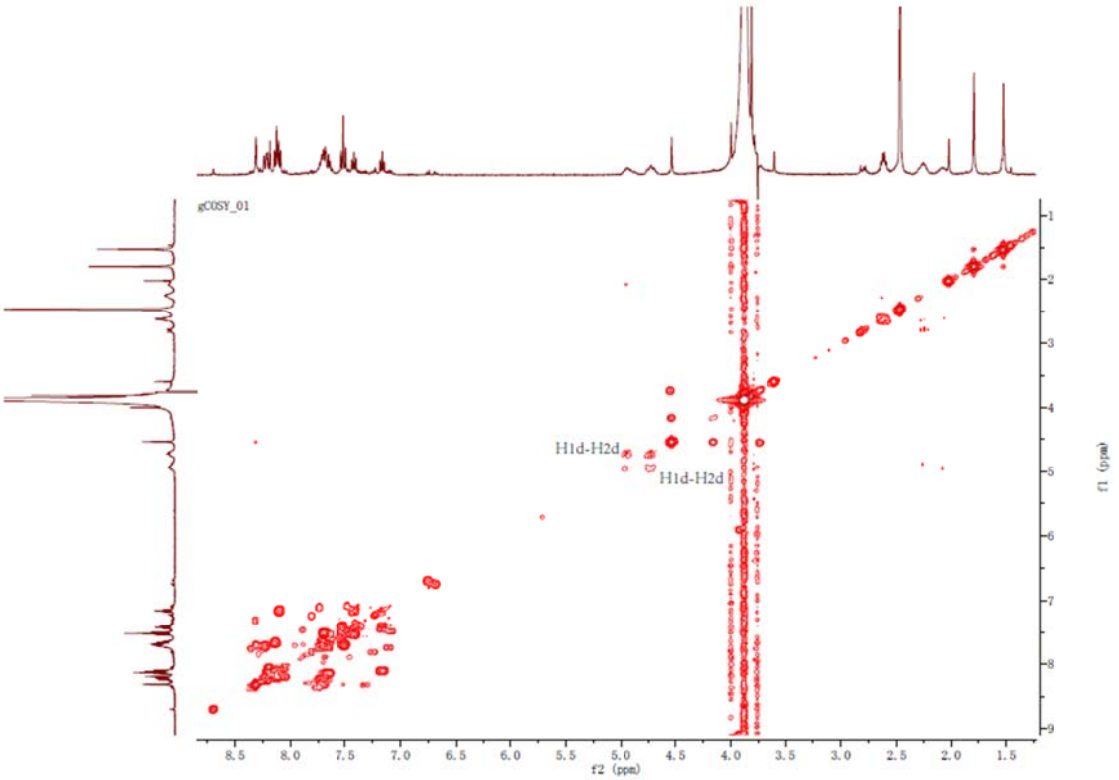


Figure S13 The NOESY and COSY of **Cl-2-SO_2.**

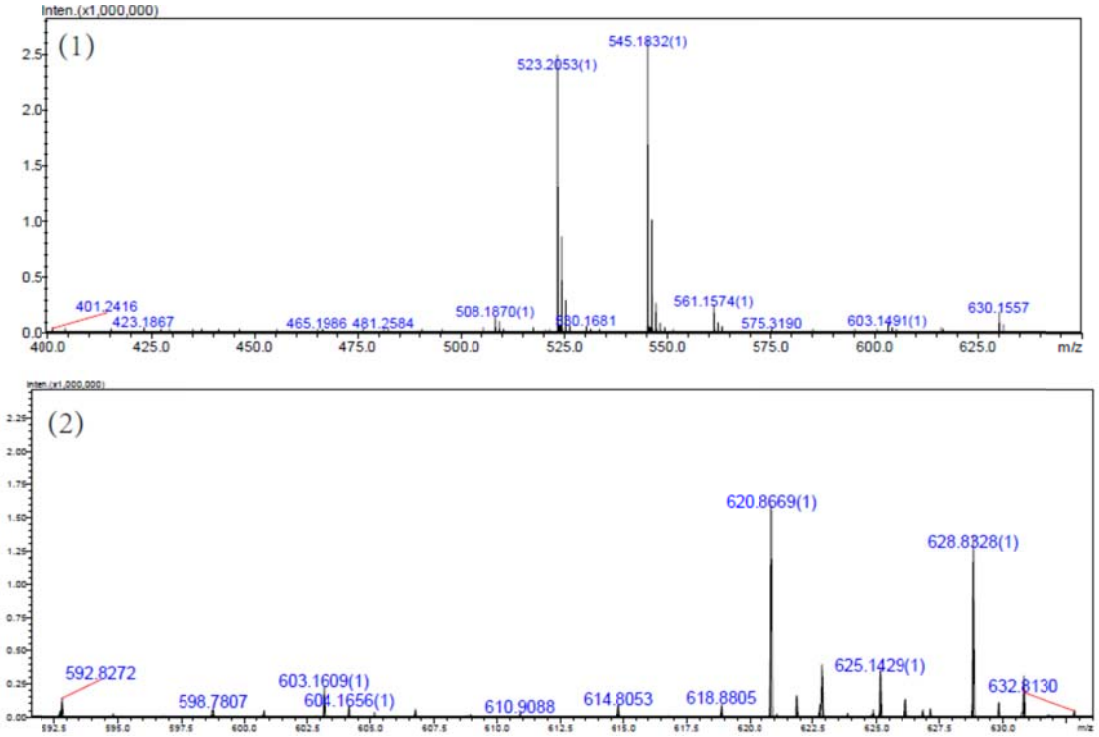


Figure S14. HRMS spectral change of **CI-2** in the absence (1) and presence of 15 equiv of $NaHSO_3$ (2) in DMSO /D_2O = 1:1.

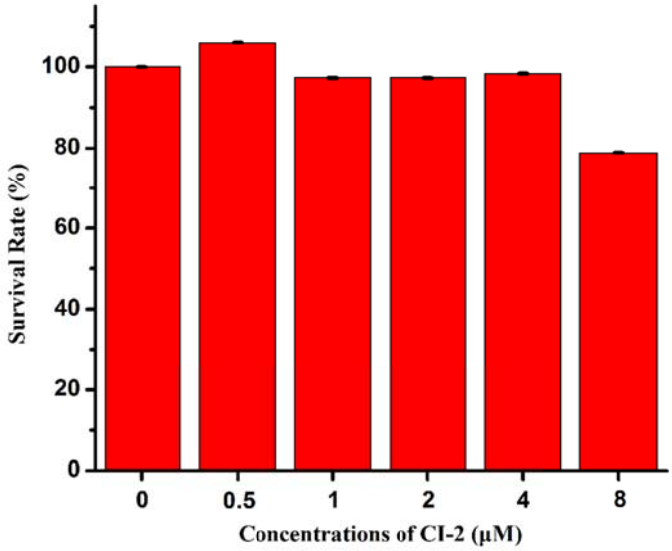


Figure S15. Effects of **CI-2** at varied concentrations on the viability of Hela cells. The results are the mean standard deviation of three separate measurements.

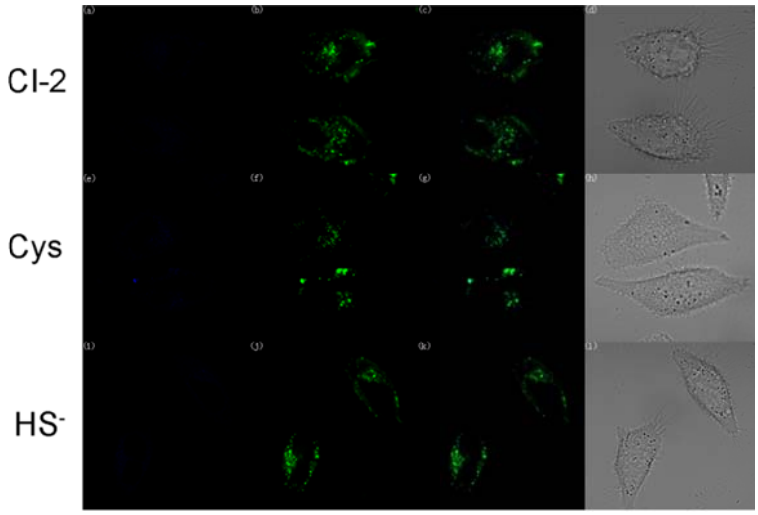


Figure S16 (a) Fluorescence imaging of HeLa cells incubated with **CI-2** (4 μM) from the blue channel; (b) Fluorescence imaging of (a) from the green channel; (c) overlay of (a) and (b); (e),(i) Fluorescence imaging of HeLa cells incubated with **CI-2** (4 μM) for 30 min and further incubated with Cys (1mM) and HS^- (60 μM), for 15 min from the blue channel; (f),(j) Fluorescence imaging of (e),(i) from the green channel; (g),(k) overlap of(e) and (f), (i) and (j); (d),(h),(l) bright field of (a),(e),(i). Ex@405 nm for the blue channel (425–520 nm), Ex@488 nm for the green channel (540–680 nm).

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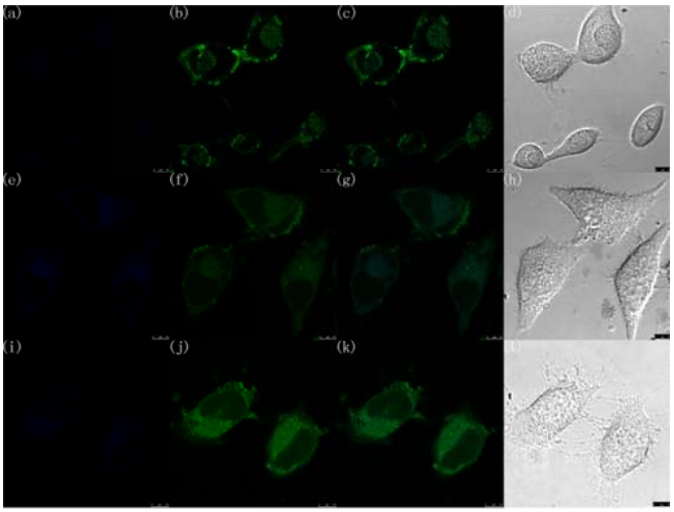


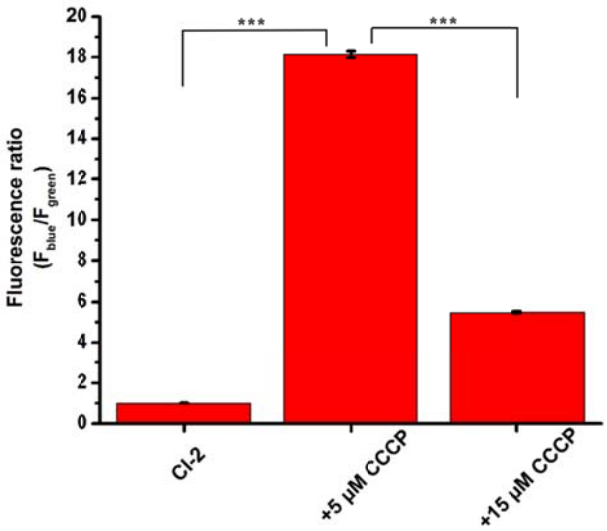


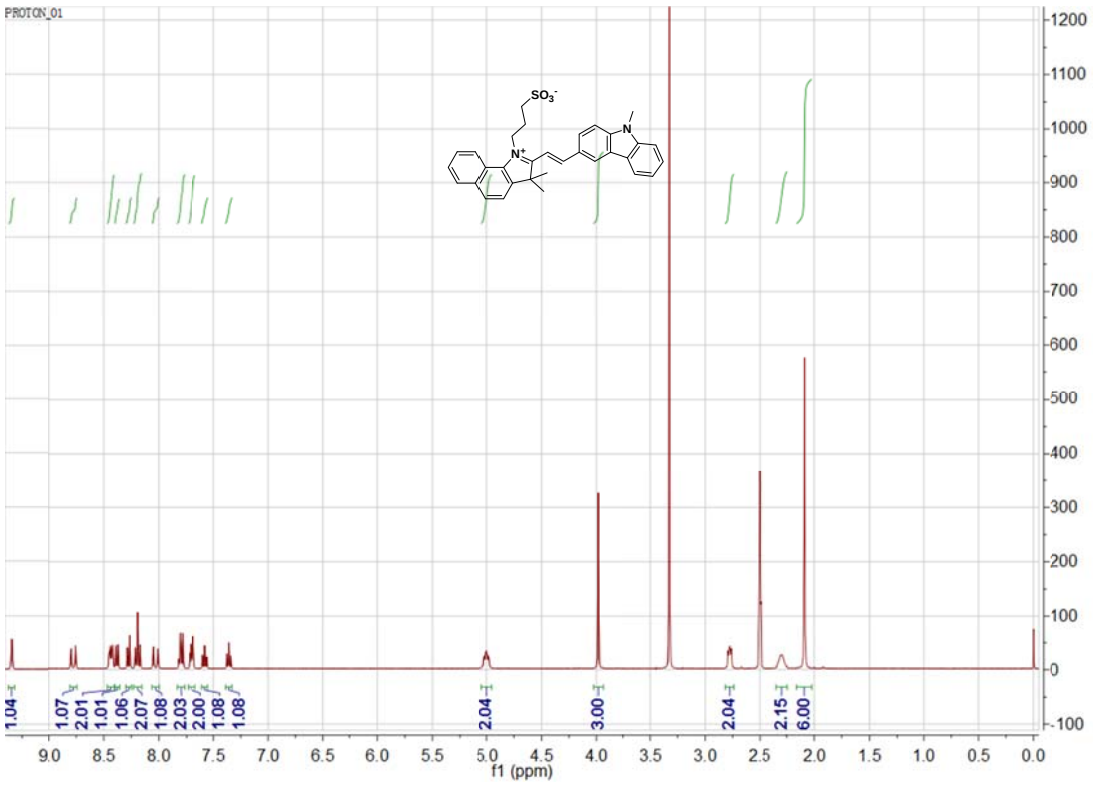
Figure S17. (a) Fluorescence imaging of HeLa cells incubated with **CI-2** (4 μM) from the blue channel; (b) fluorescence imaging of (a) from the green channel; (c) overlay of (a) and (b); (d) bright field of (a). (e) Confocal image of **CI-2** (4 μM) incubated with 5 μM CCCP and SO_2 donor (60 μM) in Hela cells from blue channel; (f) fluorescence imaging of (e) from the green channel; (g) overlay of (e) and (f); (h) bright field of (e). (i) Confocal image of **CI-2** (4 μM) incubated with 15 μM CCCP (Carbonyl cyanide m-chlorophenylhydrazone) and SO_2 donor (60 μM) in Hela cells from blue channel; (j) fluorescence imaging of (i) from the green channel; (k) overlay of (i) and (j); (l) bright field of (i). Ex@405 nm for the blue channel (425–520 nm), Ex@488 nm for the green channel (540–680 nm). (m) statistical analysis were performed with Student's t-test (m = 10 fields of cells), $***P < 0.001$. Bars: 10 μm.

References:

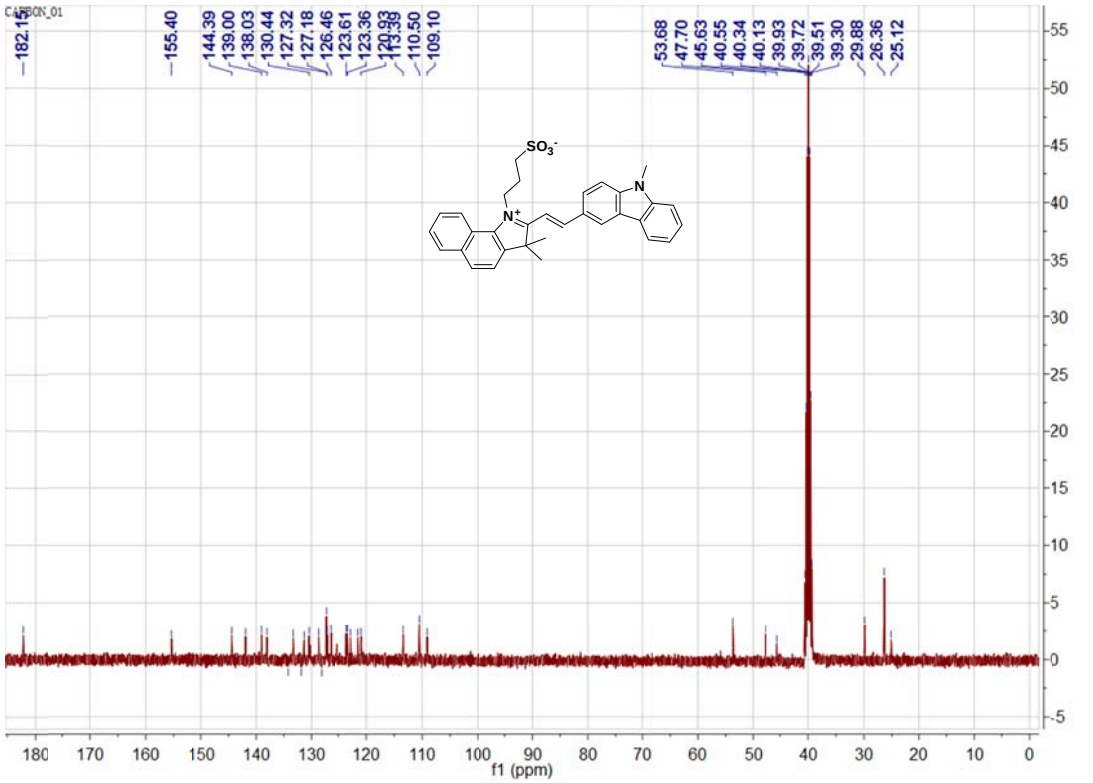
1. Y. Liu, K. Li, M.-Y. Wu, Y.-H. Liu, Y.-M. Xie, X.-Q. Yu, *Chem. Commun.*, 2015, **51**, 10236-10239.

Spectra

^{1}H-NMR Spectrum of **CI-2** in DMSO-d_6 (400 MHz)



^{13}C-NMR Spectrum of **CI-2** in DMSO-d_6 (100 MHz)



HRMS Spectrum of **CI-2**