Supporting information for

A Fluorescent Probe for Ratiometic Imaging of SO_2 Derivatives in Mitochondria of Living Cells

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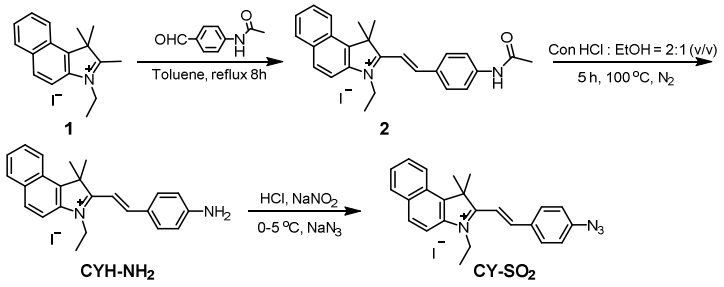
Materials and methods

All solvents and reagents used were reagent grade and were used without further purification unless otherwise stated. The solution of **CY-SO_2** was dissolved in DMSO at a concentration of 2 mM as the stock solution and stored in a refrigerator for use. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400(or a Bruker Avance II 400 MHz or 500 MHz) spectrometer. Chemical shifts (δ) were reported as ppm (in $CDCl_3$ or DMSO, with TMS as the internal standard). Fluorescence spectra were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). Mass spectrometric data were achieved with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. All pH measurements were performed using a Model PHS-3C meter. Slight pH variations in the solutions were achieved by adding the minimum volumes of HCl or NaOH (1 M). Flash column chromatography was performed using silica gel (100 - 200 mesh) and aluminum oxide (neutral. 100-200 mesh) obtained from Qingdao Ocean Chemicals. Mito Tracker Green FM, Lyso Tracker Green and Hoechst 33342 were purchased from Life Technologies Co. (USA). Doubly purified water used in all experiments was from Milli-Q systems. The response in a test solution containing CTAB is slight fast. The fluorescence quantum yields for compounds with Absolute PL Quantum Yield Spectrometer.

Synthesis of Compounds



Scheme S1 The synthesis of probe **CY-SO_2**.

The synthesis of 2

Ethyl-1,1,2-trimethyl-1H-benzo[e]indolium iodide **1** (365 mg, 1 mM) and 4-acetamidobenzaldehyde **2** (195.6 mg, 1.2 mM) were dissolved in 15 mL dry toluene. The mixture was stirred vigorously and heated at a reflux temperature for 6 h under the protection of N_2. After cooling to room temperature and then 30 mL of deionized water was poured into the reaction mixture, and the product was extracted with ethyl acetate, and the organic layer was washed by sodium chloride solution. The organic layer was dried with anhydrous $MgSO_4$ followed by evaporation of solvent, and the resulting was yellow crude product solid. The crude product was applied directly to the next step of experiment without purification. ESI-MS: 383.28 (cal. 383.21).

The synthesis of CY-NH_2

18 mL mixture (hydrochloric acid: ethanol = 1:1) was added to previous crude product, and the solution was cooled to room temperature after refluxing for 6 h. Then, the mixture was adjusted to the pH=7 with saturation NaOH, and red solid precipitation was out of solution. The solution was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts was washed with saturation NaCl, and the solution was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residual crude product was separated by column chromatography to get the desired black solid product. (149 mg, 32 %) ^{1}H NMR (400 MHz, MeOD) δ: 8.37 (dd, *J* = 16.4, 12.1 Hz, 2H), 8.15 (d, *J* = 8.9 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 2H), 7.85 (d, *J* = 8.9 Hz, 1H), 7.74 (t, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 15.6 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 2H), 4.64 (q, *J* = 7.2 Hz, 2H), 2.06 (s, 6H), 1.57 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, MeOD) δ: 12.28, 25.74, 40.81, 52.91, 102.86, 111.49, 114.29, 122.40, 122.80, 126.19, 127.55, 127.88, 129.85, 130.96, 133.22, 134.42, 136.79, 138.20, 154.67, 156.63, 180.61 ppm; HRMS-ESI: m/z calcd for $C_{24}H_{25}N_2^+$ $[M]^+$: 341.2012, found: 341.2024.

The synthesis of CY-SO_2

In a round-bottom flask equipped with a magnetic stirring bar, **CY-NH_2** (93.6 mg, 0.2 mM) was

dissolved with HCl (6 N, 0.5 mL) in an ice bath. $NaNO_2$ (3 mM) dissolved in 6 mL water was added dropwise. The reaction mixture was stirred for 30 min. Sodium azide (65 mg, 1 mM) was dissolved in 5 ml water was added dropwise. After that, the mixture was stirred for 4 hours at room temperature. The solution was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts was washed with saturation NaCl, and the solution was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was separated by column chromatography to get the desired crimson solid product. (79 mg, 80 %), ^{1}H NMR (400 MHz, DMSO) δ: 8.56 (d, *J* = 16.4 Hz, 1H), 8.45 (d, *J* = 8.4 Hz, 1H), 8.33 (t, *J* = 9.2 Hz, 3H), 8.24 (d, *J* = 8.1 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.77-7.70 (m, 2H), 7.37 (d, *J* = 8.45 Hz, 2H), 4.87 (q, *J* = 7.2 Hz,2H), 2.04 (s, 6H), 1.53 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO) δ:13.87, 25.30, 42.34, 53.74, 111.20, 113.07, 119.85, 123.05, 126.62, 127.18, 128.36, 129.92, 131.01, 131.24, 132.40, 133.11, 137.96, 138.59, 144.29, 151.55, 181.81 ppm; TOF HRMS: m/z calcd for $C_{24}H_{23}N_4^+$ $[M]^+$: 367.1917, found: 367.1934.

Determination of the detection limit

The detection limit was calculation based on the fluorescence titration (Figure 1b) of **CY-SO$_2$** in the presence of $NaHSO_3$ (1-5 μM). The fluorescence intensity of **CY-SO$_2$** was measured by three times and standard deviation of the blank measurement was achieved. The detection limit was calculated by using detection limit was calculated with the following equation[1]:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios ($F_{467\ nm}/F_{580\ nm}$) versus $NaHSO_3$ concentration.

Absolute PL Quantum Yield Spectrometer

Quantaurus-QY was developed as a compact, easy-to-system with a small footprint for measuring absolute photoluminescence quantum yields. Operating this system is simple. Load a sample and press the start button to measure the photoluminescence quantum yields, excitation wavelength dependence, PL excitation spectrum and other properties in a short time.

The PL Quantum Yield (Φ) is expressed as the ratio of the number of photons emitted from molecules (PN_{em}) to that absorbed by molecules (PN_{abs}).

$$\Phi_F = PN_{em}/\ PN_{abs}$$

Live cell imaging experiments

Human breast cancer cells (MCF-7) were purchased from Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen). The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 ºC under 5% CO_2. Before imaging, the live cells were incubated with **CY-SO_2** (5 μM) for another 30 min and then washed with phosphate-buffered saline (PBS) three times. Fluorescence imaging was performed using an OLYMPUSFV-1000 inverted fluorescence microscope with a 60 × objective lens. Under the confocal fluorescence microscope, **CY-SO_2** was excited at 405 nm and emission was collected at 450-510 nm (green channel) and 540-600 nm (red channel). For the detection of exogenous $NaHSO_3$, MCF-7 cells were incubated with **CY-SO_2** (5 μM) at 37 ºC for 30 min and then $NaHSO_3$ (20 equiv) was added at 37 ºC for 15 min.

MTT assay

Measurement of cell viability was evaluated by reducing of MTT (3-(4, 5)-dimethylthiahiazo (-2-yl)-3, 5-diphenytetrazoliumromide) to formazan crystals using mitochondrial dehydrogenases (Mosmann, 1983). MCF-7 cells were seeded in 96-well microplates (Nunc, Denmark) at a density of 1×10^5 cells/mL in 100 μL medium containing 10 % FBS. After 24 h of cell attachment, the plates were then washed with 100 μL / well PBS. The cells were then cultured in medium with 1, 5 and 10 μM of **CY-SO_2** for 12 h. Cells in culture medium without **CY-SO_2** were used as the control. Six replicate wells were used for each control and test concentration. 10 μL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37ºC for another 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple crystals were lysed in 200 μL DMSO. Optical density was determined on a microplate reader (Thermo Fisher Scientific) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm. Cell viability was expressed as a percent of the control culture value, and it was calculated using the following equation:

Cells viability (%) = $(OD_{dye} - OD_{K\ dye}) / (OD_{control} \cdot OD_{K\ control}) \times 100$

Samples preparation

Soft sugar and granulated sugar purchased from a supermarket in the samples analysis. The solution of samples were prepared by dissolving 5.0 g of sugars with ultrapure water in 10 mL volumetric flask. 50 μL of the sugar solution was added directly to the PBS buffer (10 mM, pH 7.4) containing the probe **CY-SO_2** (10 μM), and the emission intensities at 467 and 580 nm were recorded.

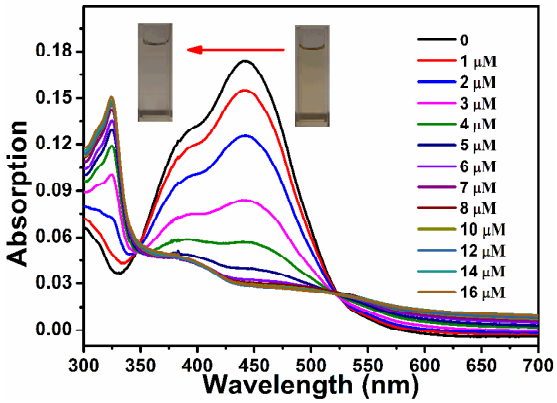


Figure S1 Absorption spectra of **CY-SO$_2$** (10 μM) upon the titration of $NaHSO_3$ (0-16 μM) in PBS buffer (0.01 M, pH 7.4, containing 25 μM CTAB). Inset: the color of **CY-SO$_2$** (10 μM) in absence and presence of $NaHSO_3$.

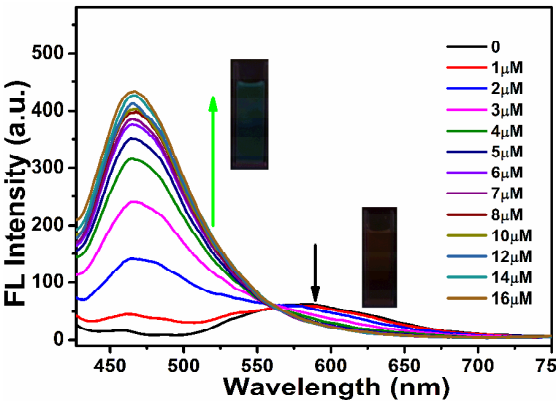


Figure S2 Fluorescence spectra of **CY-SO$_2$** (10 μM) upon the titration of $NaHSO_3$ (0-16 μM) in PBS buffer (0.01 M, pH 7.4, containing 25 μM CTAB). Inset: the fluorescent color of **CY-SO$_2$** (10 μM) in absence and presence of $NaHSO_3$.

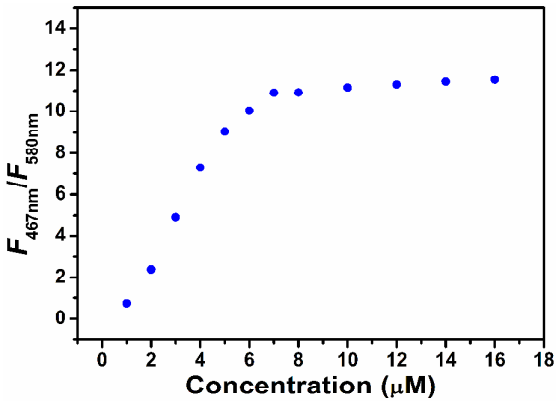


Figure S3 Fluorescence ratios ($F_{467\ nm}/F_{580\ nm}$) of **CY-SO$_2$** (10 μM) were displayed upon the titration of $NaHSO_3$ (0-16 μM) in PBS buffer (0.01 M, pH 7.4, containing 25 μM CTAB).

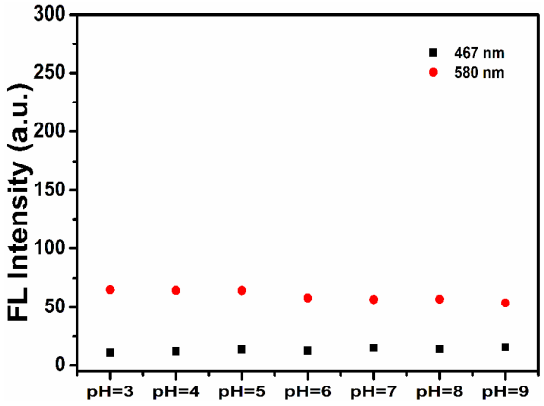


Figure S4 Fluorescence ($F_{467\ nm}$ and $F_{580\ nm}$) of **CY-SO$_2$** (10 μM) were displayed in different pH PBS buffer.

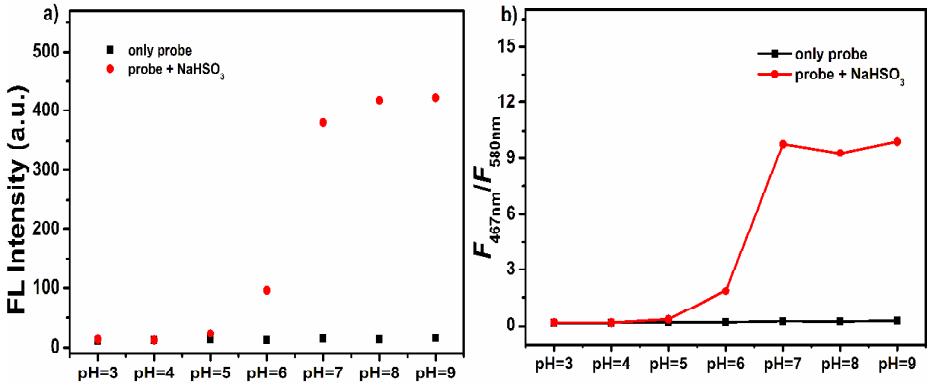


Figure S5 The influence of pH on fluorescence intensity at 467 nm and 580 nm of **CY-SO$_2$** in PBS buffer (0.01 M, pH 7.4, containing 25 μM CTAB).

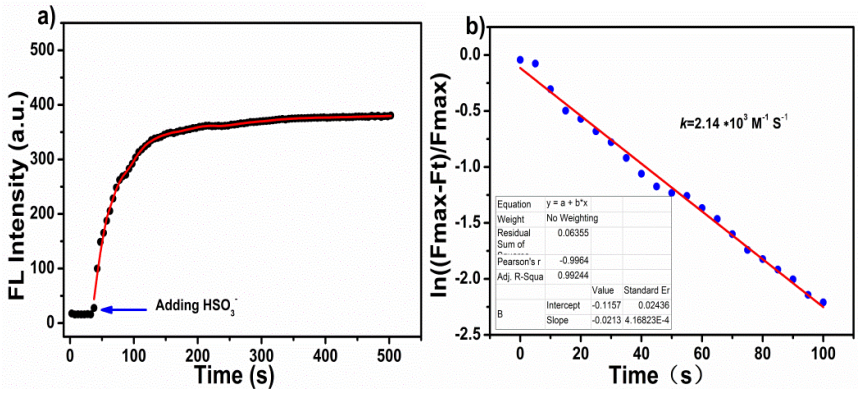


Figure S6 (a)Time-dependent fluorescence changes of **CY-SO$_2$** (10 μM) upon addition of HSO_3^- (10 μM) in PBS buffer (10 mM, pH 7.4, containing 25 μM CTAB). (b)Pseudo-first-order kinetic plot of reaction of sensor **CY-SO$_2$** (10 μM) with HSO_3^-, for $k = 2.14 \times 10^3$ M^{-1} S^{-1}.

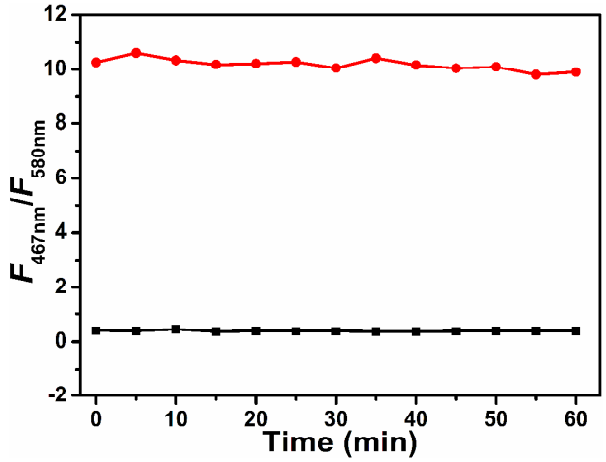


Figure S7 The stability of time dependence of **CY-SO$_2$** (10 μM) with 1 equiv (red line) or not (black line) in PBS buffer (10 mM, pH 7.4, containing 25 μM CTAB) were measured with a spectrophotometer every 6 min from 0 to 60 min. λ_{ex} = 400, slit: 10/10 nm.

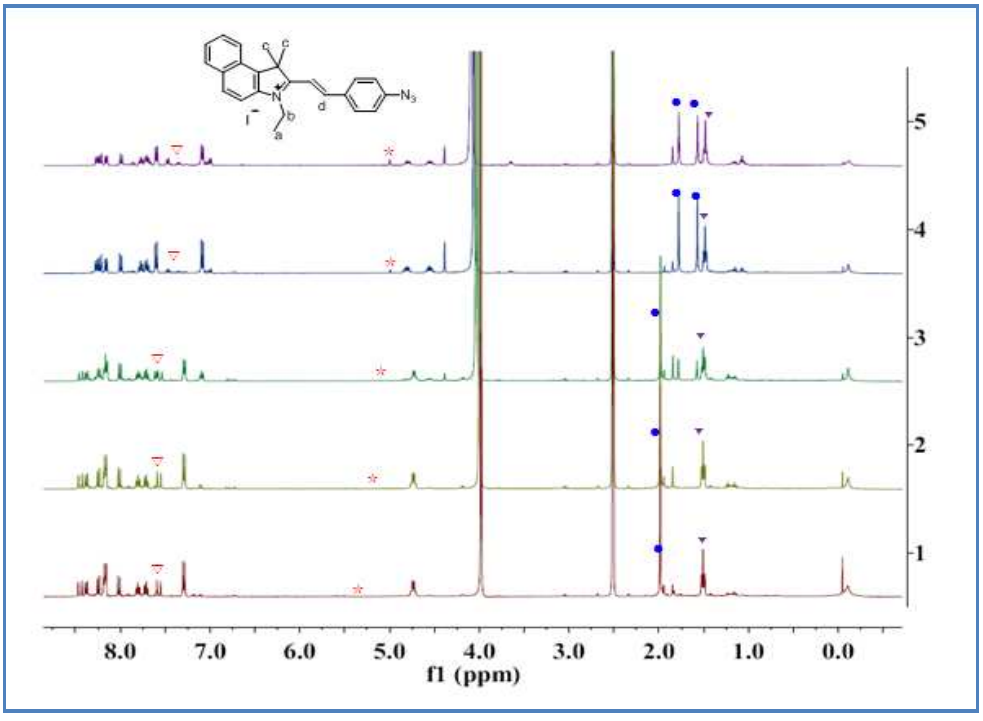


Figure S8 ^{1}H NMR spectra of **CY-SO$_2$** (3.5 mM) in presence of different concentrations of $NaHSO_3$ in DMSO-d_6 solution were shown in upside. Peaks marked with (▽) (☆) (●) (▼) represent different chemical environment of hydrogen, H_d, H_d', H_b, H_a, respectively. 1-only probe, 2- probe + 4 μL $NaHSO_3$ solution, 3- probe + 8 μL $NaHSO_3$ solution, 4- probe + 18 μL $NaHSO_3$ solution,5- probe + 28 μL $NaHSO_3$ solution. The $NaHSO_3$ dissolved in D_2O made into 300 mM solution.

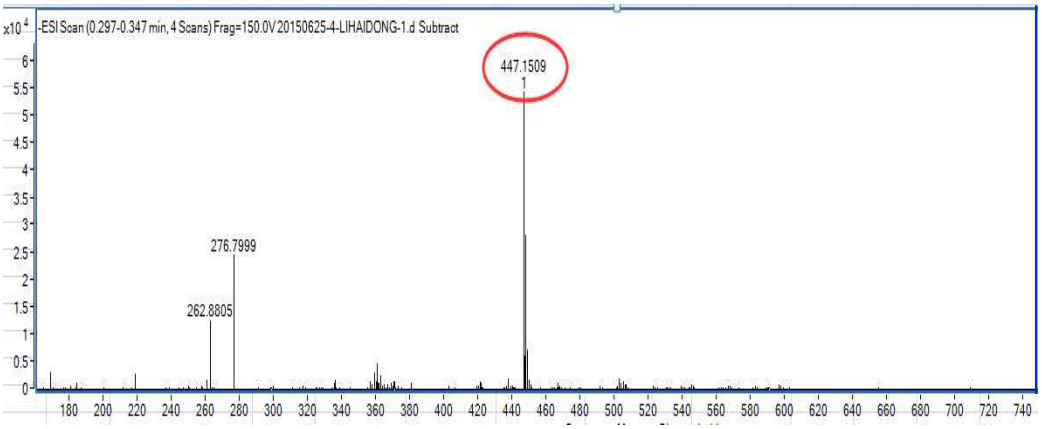


Figure S9 TOF-ESI-MS of the **CY-HSO$_3$** adduct.

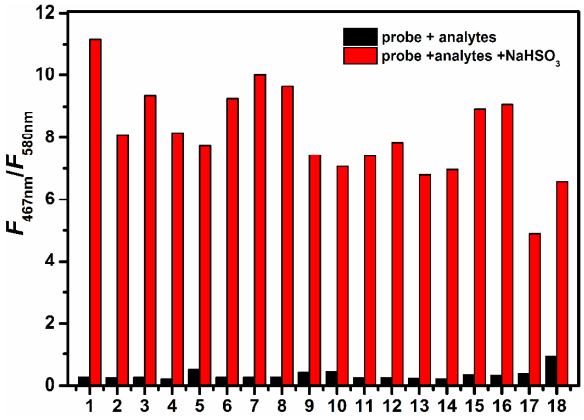


Figure S10 The Fluorescence responses (F_{467}/F_{580}) of **CY-SO$_2$** (10 μM) toward various anlytes (1 mM except for notation).Black bar: 1, blank; 2, F^-; 3, Cl^-; 4, Br^-; 5, I^-; 6, NO_2^-; 7, NO_3^-; 8, SCN^-; 9, HPO_4^{2-}; 10, CH_3COO^-; 11, CO_3^{2-}; 12, HCO_3^{2-}; 13, ClO^-; 14, H_2O_2; 15, Glutathione (GSH); 16, Cysteine (Cys); 17, Ascorbic Acid (AA); 18, S^{2-} (60 μM); Red bar: 1, blank+ HSO_3^- (10 μM); 2, F^- + HSO_3^- (10 μM); 3, Cl^- + HSO_3^- (10 μM); 4, Br^- + HSO_3^- (10 μM); 5, I^- + HSO_3^- (10 μM); 6, NO_2^-

+ HSO_3^- (10 μM); 7, NO_3^- + HSO_3^- (10 μM); 8, SCN^- + HSO_3^- (10 μM); 9, HPO_4^{2-} + HSO_3^- (10 μM); 10, CH_3COO^- + HSO_3^- (10 μM); 11, CO_3^{2-} + HSO_3^- (10 μM); 12, HCO_3^{2-} + HSO_3^- (10 μM); 13, ClO^- + HSO_3^- (10 μM); 14, H_2O_2 + HSO_3^- (10 μM); 15, Glutathione (GSH) + HSO_3^- (10 μM); 16, Cysteine (Cys) + HSO_3^- (10 μM); 17, Ascorbic Acid (AA) + HSO_3^- (10 μM); 18, S^{2-} (60 μM) + HSO_3^- (10 μM). Each measurement was performed after 3 min of mixing. λ_{ex} = 400 nm, slit: 10/10 nm.

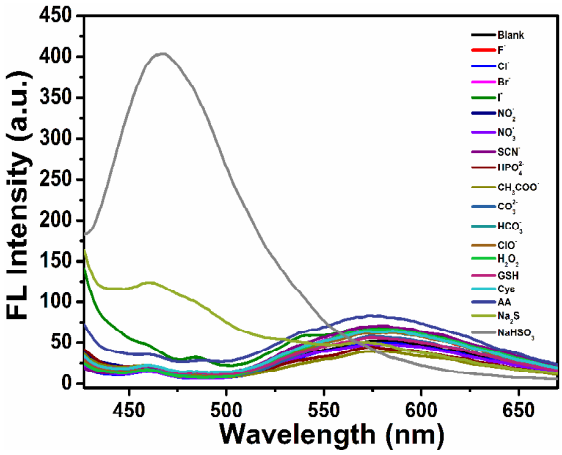


Figure S11 Fluorescence responses of **CY-SO_2** (10 μM) toward various anlytes (1 mM except for notation).1, blank; 2, F^-; 3, Cl^-; 4, Br^-; 5, I^-; 6, NO_2^-; 7, NO_3^-; 8, SCN^-; 9, HPO_4^{2-}; 10, CH_3COO^-; 11, CO_3^{2-}; 12, HCO_3^-; 13, ClO^-; 14, H_2O_2; 15, Glutathione (GSH); 16, Cysteine (Cys); 17, Ascorbic Acid (AA); 18, S^{2-} (60 μM); 19, HSO_3^-. Each measurement was performed after 3 min of mixing. λ_{ex} = 400 nm, slit: 10/10 nm.

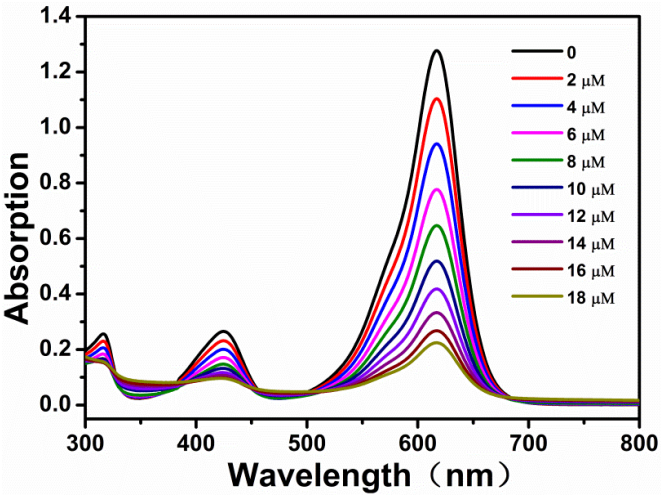


Figure S12 The Absorption responses of Malachite green (40 μM) toward various the concentration of HSO_3^-. Insert: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 μM HSO_3^-.

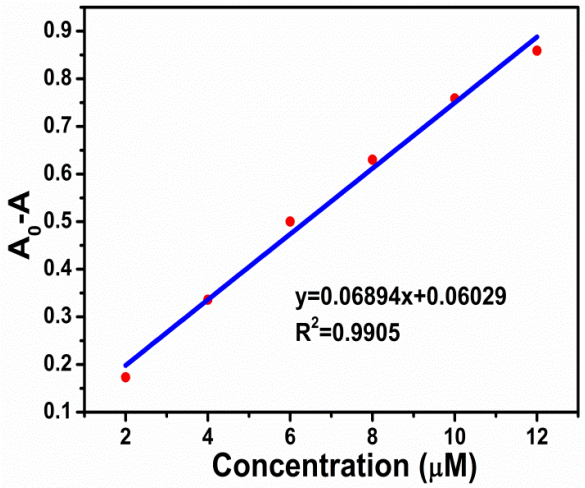


Figure S13 The Absorption (A_0-A) responses of Malachite green (40 μM) toward various the concentration of HSO_3^-.

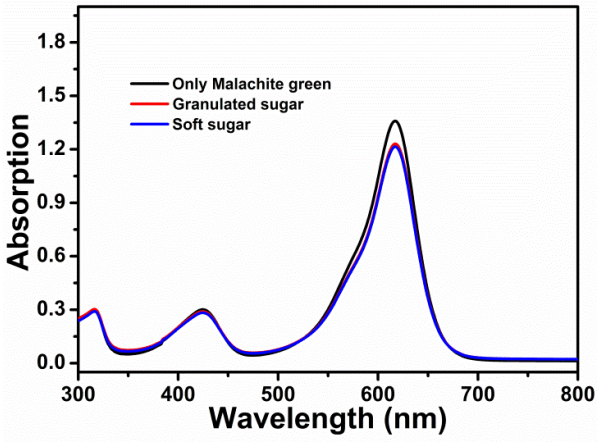


Figure S14 The absorption of Malachite green in PBS buffer. Black line: only Malachite green (40 μM); Red line: Malachite green (40 μM) + Granulated sugar (50 μL); Blue line: Malachite green (40 μM) + Soft sugar (50 μL).

Table S1 Results for the detection of HSO_3^- in various samples via different methods

Method	Malachite green method[2]		Fluorescence detection method		Standard deviation
Soft sugar	1.0837 μM[a]	10.53mg/kg	0.9665 μM	9.40mg/kg[b]	0.025
granulated sugar	0.9851 μM	9.58mg/kg	0.9610 μM	9.34mg/kg	0.026

[a] the concentration of HSO_3^- in various. [b] the HSO_3^- levels was shown by per kilogram of different samples. Each experiment was carried out in three replicates (n = 3).

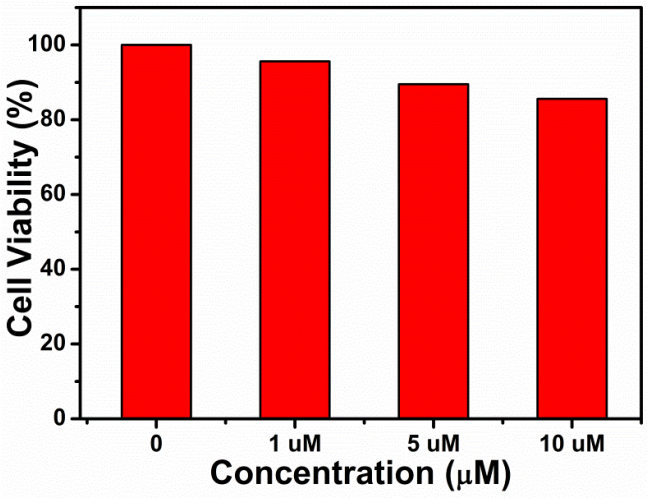


Figure S15 Biological toxicity of **CY-SO$_2$** in living MCF-7 cells for 12 h.

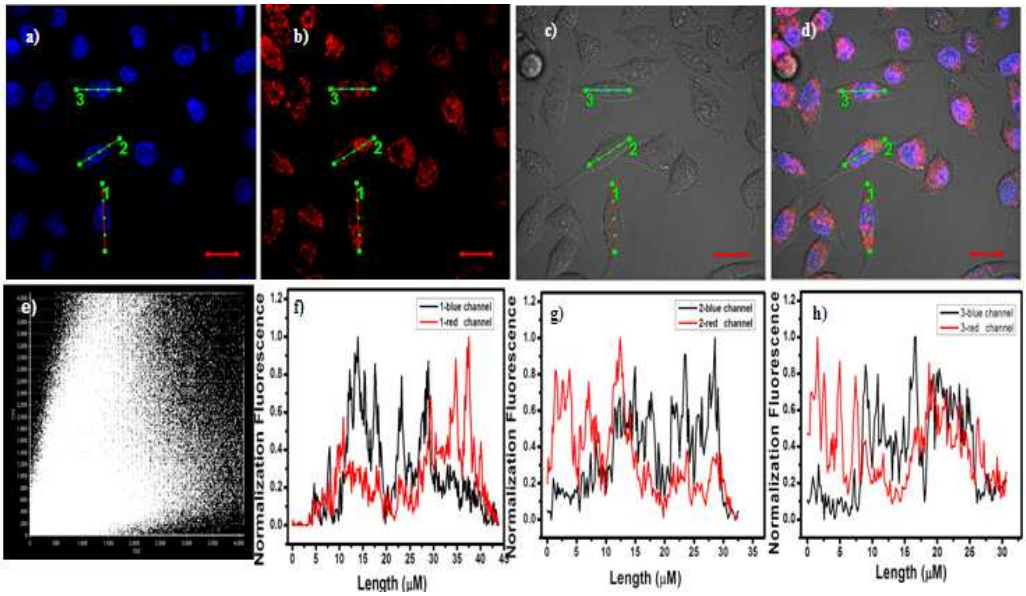


Figure S16 Colocalization fluorescence imaging of MCF-7 cells incubated with **CY-SO$_2$** (5 μM) for 30 min and Hoechst 33342 (1.0 μM) for 10 min at 37 ^{o}C. (a) Confocal image from Hoechst 33342 (1.0 μM) on green channel (λ_{ex} = 405 nm). (b) Confocal image from **CY-SO$_2$** on red channel (λ_{ex} = 405 nm). (c) Bright image. (d) Merged image of (a), (b) and (c). (e) Correlation plot

of the intensities of **CY-SO$_2$** and Hoechst 33342 (R_r = 0.71). (f), (g) and (h) Normalized intensity profile of regions of interest (ROIs) across MCF-7 cells. Scale bar = 20 μm.

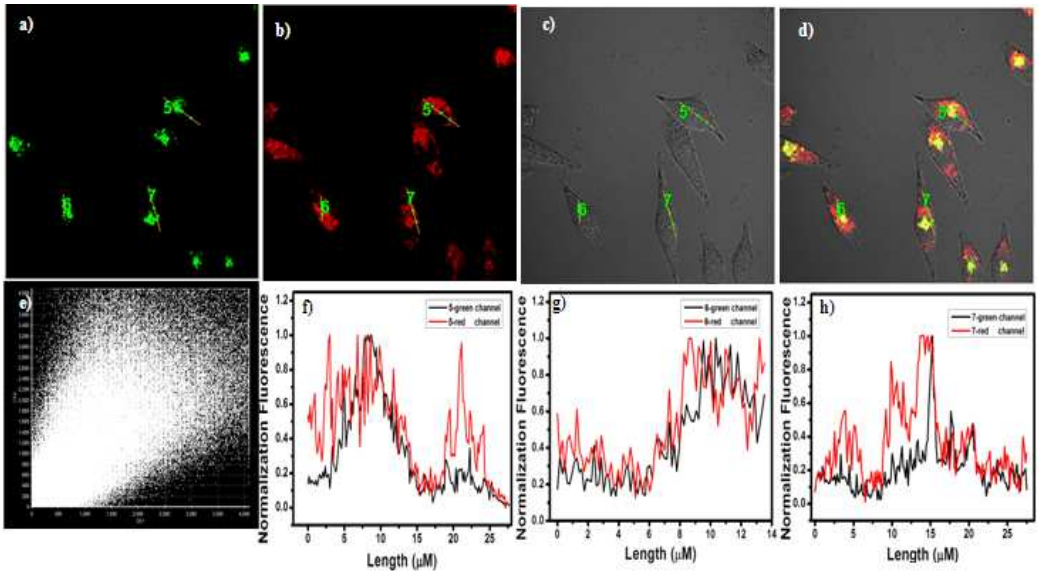


Figure S17 Colocalization fluorescence imaging of MCF-7 cells incubated with **CY-SO$_2$** (5 μM) for 30 min and Lyso Tracker Green DND-26 (1.0 μM) for 10 min at 37 ^{o}C. (a) Confocal image from Lyso Tracker Green DND-26 (1.0 μM) on green channel (λ_{ex} = 488 nm). (b) Confocal image from **CY-SO$_2$** on red channel (λ_{ex} = 405 nm). (c) Bright image. (d) Merged image of (a), (b) and (c). (e) Correlation plot of the intensities of **CY-SO$_2$** and Mito Tracker Green FM (R_r = 0.81). (f), (g) and (h) Normalized intensity profile of regions of interest (ROIs) across MCF-7 cells. Scale bar = 20 μm.

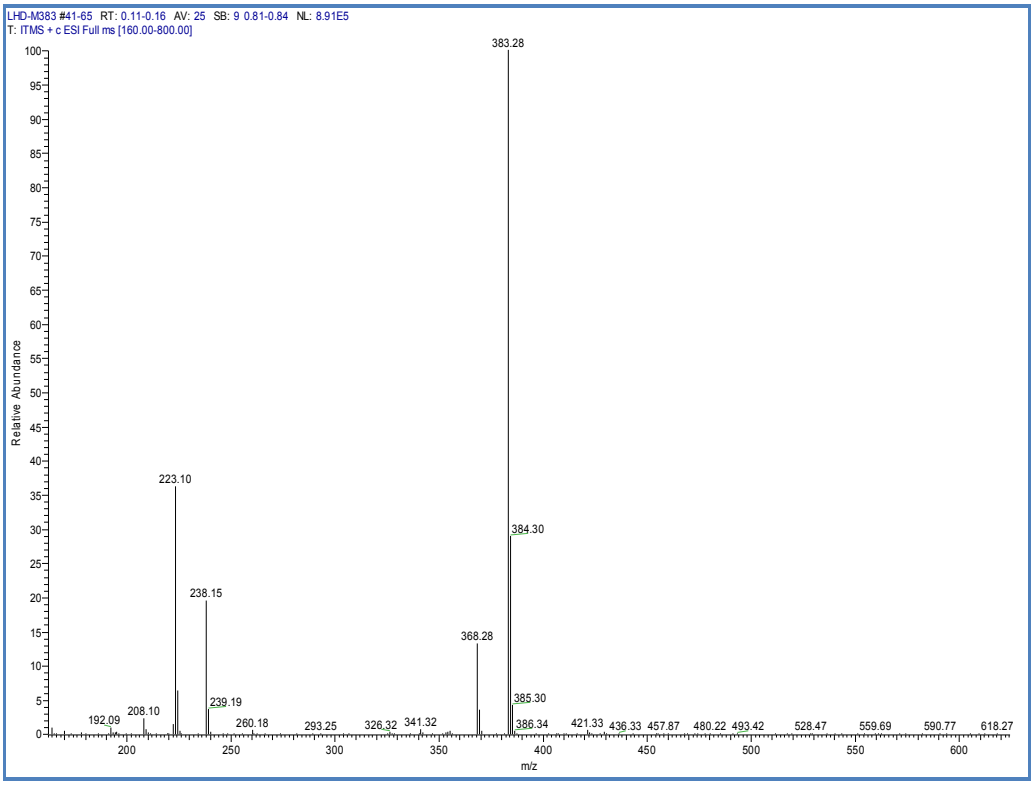


Figure S18 HRMS spectra of **2**.

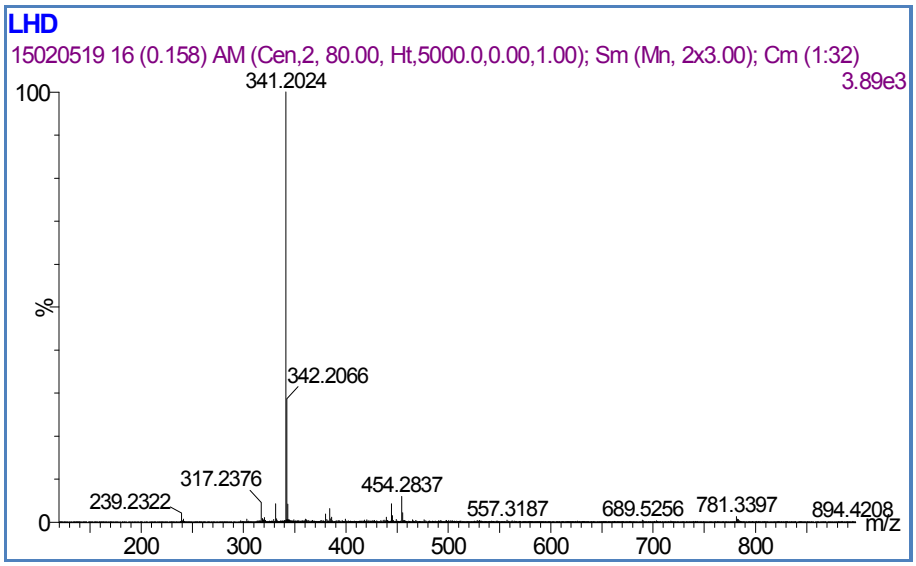
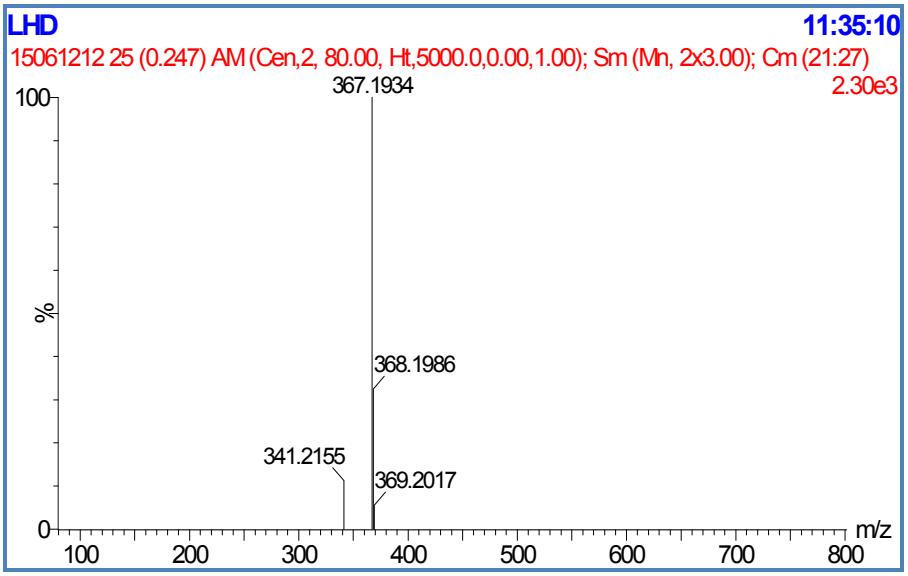


Figure S19 HRMS spectra of **CY-NH$_2$**.



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-80 H: 0-100 N: 4-4

Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
367.1934	367.1923	1.1	3.0	15.5	8.4	C24 H23 N4

Figure S20 HRMS spectra of **CY-SO$_2$**.

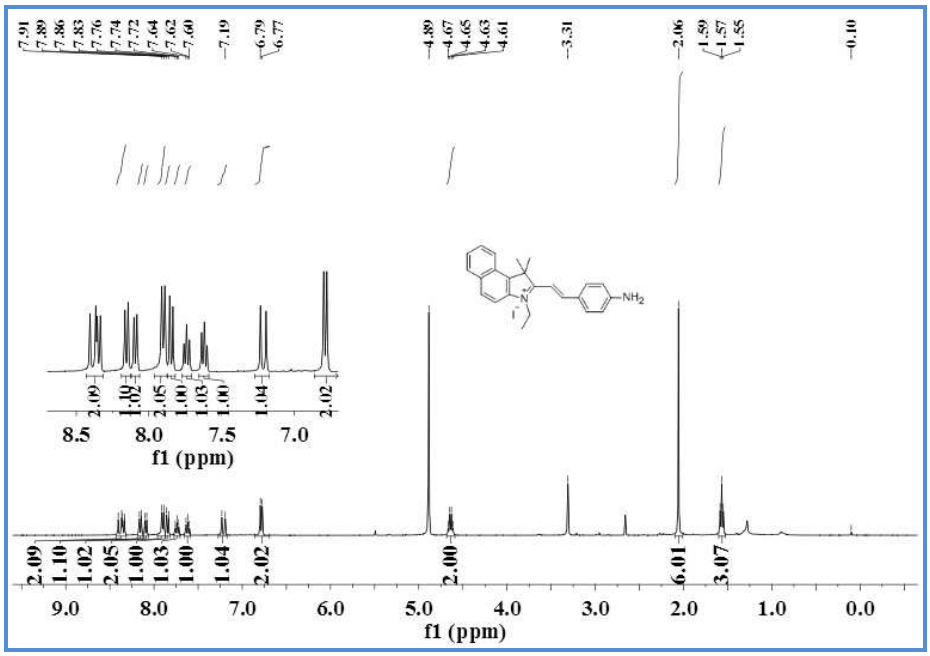


Figure S21 ^{1}H NMR spectra of probe **CY-NH$_2$**.

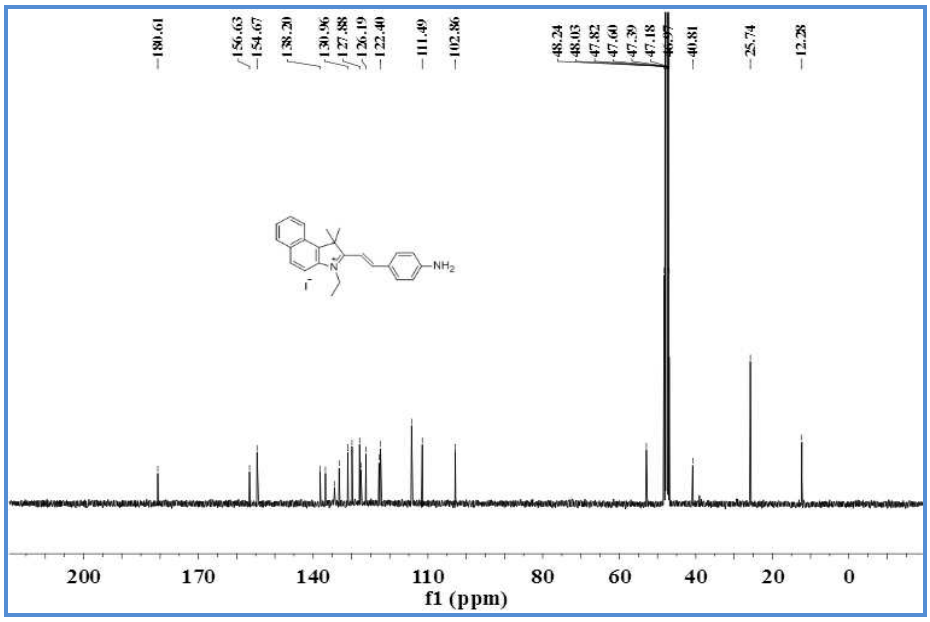


Figure S22 ^{13}C NMR spectra of probe **CY-NH$_2$**.

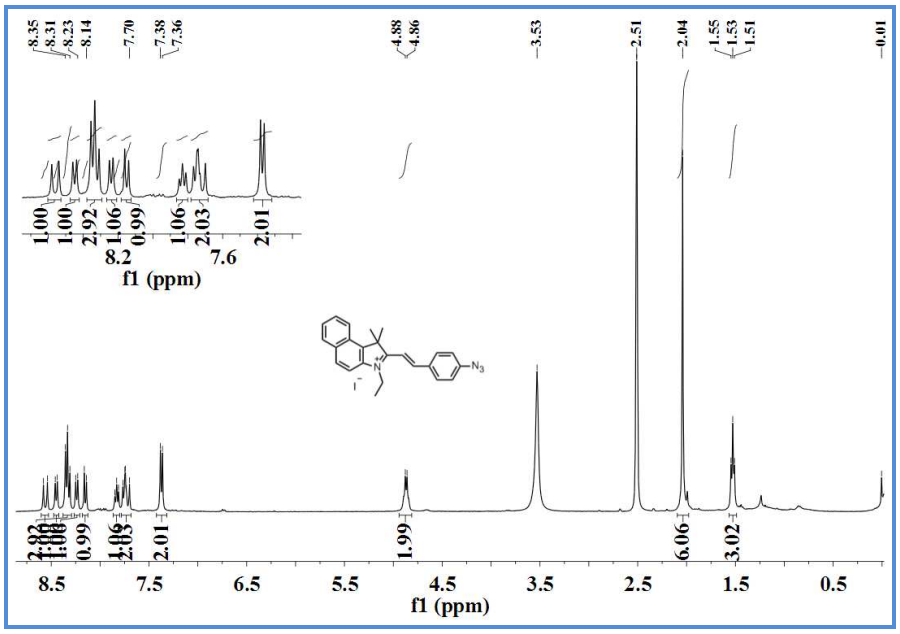


Figure S23 ^{1}H NMR spectra of probe **CY-SO$_2$**.

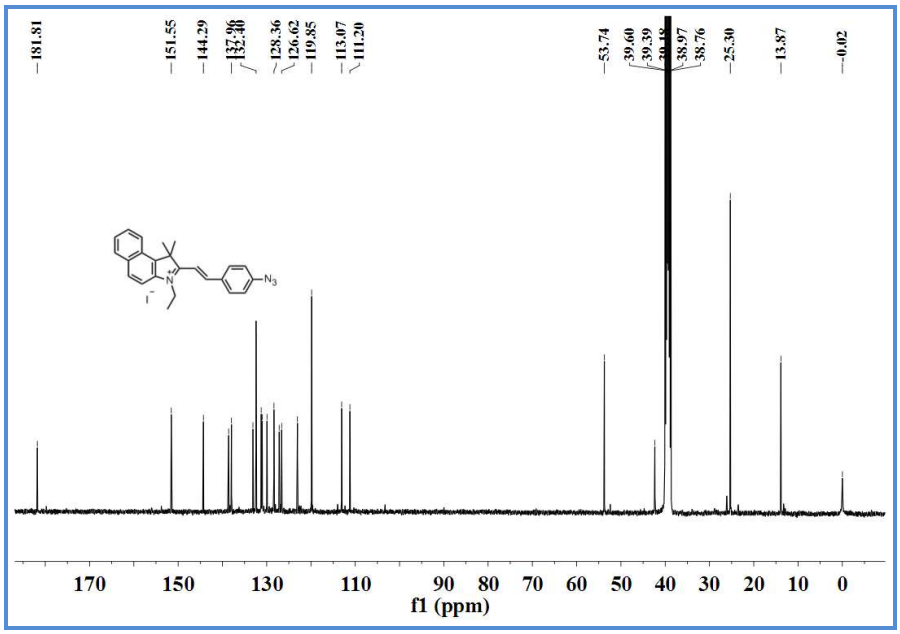


Figure S24 ^{13}C NMR spectra of probe **CY-SO$_2$**.

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