Supporting Information

Mitochondria-targeted long-wavelength fluorescent probe for imaging sulfur dioxide in ferroptosis

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Contents

1. Reagents and apparatus

All other chemicals were obtained from commercial suppliers and used without further purification. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance III at 400MHz, δ values are in parts per million relatives to TMS in DMSO-*d6*. High Resolution Mass spectra (HRMS) were measured with Bruker Apex IV FTMS using electrospray ionization (ESI). pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30). Absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10mm quartz cuvette. Fluorescence imaging was observed under an Olympus IX81 confocal fluorescence microscope. Images of mouse liver tissue sections were observed under a ZEISS Axio Imager D2 fluorescence microscope.

2. General procedure for analysis

Stock solutions (10 mM) of **CMP** were prepared in DMSO. The test solution was prepared by placing 100 μL of parent stock solution of **CMP** into the PBS solution (containing 10% EtOH, pH 7.4, 20 mM, 200 mL). All spectra were obtained in a quartz cuvette (path length=1 cm). The fluorescence spectra excitation wavelength is 550 nm.

A series of standard pH buffer solutions were prepared by mixing 20 mM Na_2HPO_4 and NaH_2PO_4 at varied volume ratios, and the accurate pH values were measured by a Mettler Toledo FE-30 pH acidometer. Other analytes were prepared by dissolving salts in the ultrapure water.

3. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of probe **CMP** were measured by ten times and the standard deviation of blank measurement was obtained. To gain the slope, the fluorescence intensity ratios (at 622 nm) were plotted as the increasing concentrations of HSO_3^-. The detection limit was calculated with the following equation:

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

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity ratios versus the concentrations of HSO_3^-.

4. Cell culture and imaging

HeLa cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin with 5% CO_2 at 37°C.

The cells were seeded into 96-well plates at a density of 3×10^3 cells per well in culture media, then 0, 1, 2, 5 and 10 μM (final concentration) probe **CMP** were added, respectively. Next, the cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. Finally, 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added and cultured for another 4 h, respectively. Finally, the medium was replaced by DMSO and spectrophotometrically quantified at 490 nm.

In the colocalization experiment, the mitochondrial tracer **Mito Traker Green** (0.25 μM) and probe **CMP** (5 μM) were added to the cells and incubated for 30 min. After washing the cells with PBS buffer three times, fluorescence imaging was performed.

In the exogenous cell experiment, HeLa cells were incubated **CMP** (5 μM) at 37°C

for 30 min. After washing three times with PBS buffer, 10 μM, and 25 μM HSO_3^- was respectively incubated for 30 min at 37 °C. After washing the cells with PBS buffer three times, fluorescence imaging was performed.

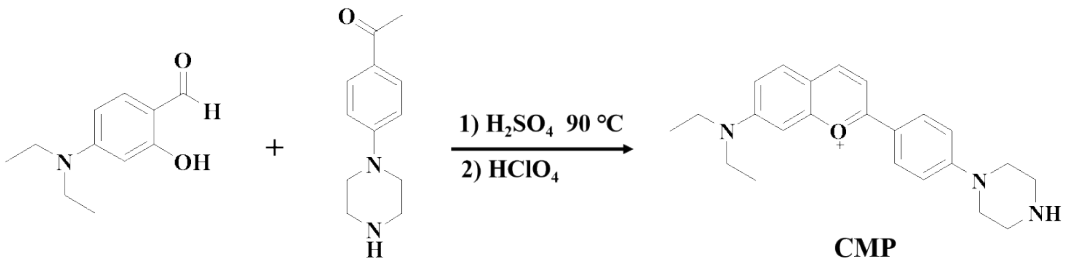
In the CCCP cell experiment, HeLa cells were incubated 10 μM, and 30 μM CCCP was respectively incubated at 37°C for 60 min. After washing three times with PBS buffer, **CMP** (5 μM) was incubated for 30 min at 37 °C. After washing the cells with PBS buffer three times, fluorescence imaging was performed.

In the ferroptosis cell experiment, HepG2 cells were respectively preincubated with Erastin (10 μM) for 2 h, Erastin (10 μM) and Fer-1 (20 μM) for 2 h. Then L-02 cells were incubated with 5 μM **CMP** for 30 min at 37 °C and washed with PBS thrice.

5. Zebrafish imaging

Wild type zebrafishes were purchased from the Jinan Pengyue Experimental Animal Breeding Co., Ltd. (Shandong, China). This study strictly adhered to international ethical standards for laboratory animal use, following the European Community guidelines (Directive 2010/63/EU). All experimental were approved by the Animal Care and Use Committee of Institute of Biomedicine of Shandong University of Technology. In the zebrafish experiment, zebrafish were incubated **CMP** (10 μM) for 30 min. After washing three times with PBS buffer, 20 μM HSO_3^- was incubated, fluorescence imaging was performed at 10 and 30 min, respectively.

6. Figures



Scheme S1 Synthesis of fluorescent probe **CMP**

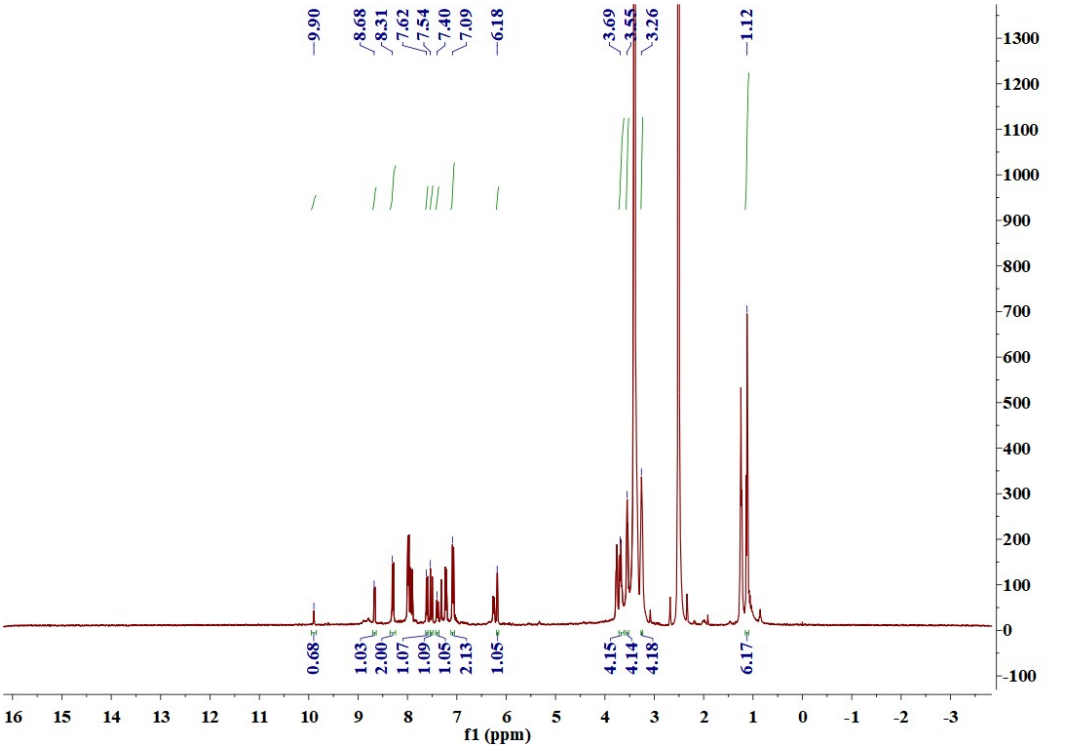


Figure S1 ^{1}H NMR of **CMP**

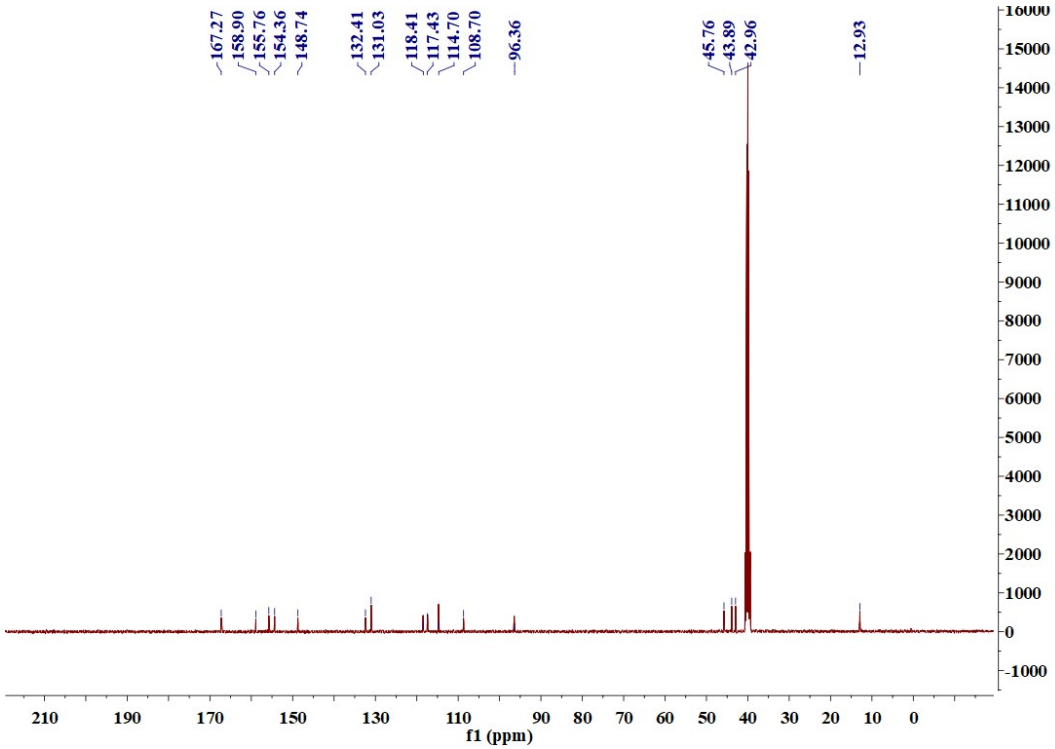


Figure S2 ^{13}C NMR of **CMP**

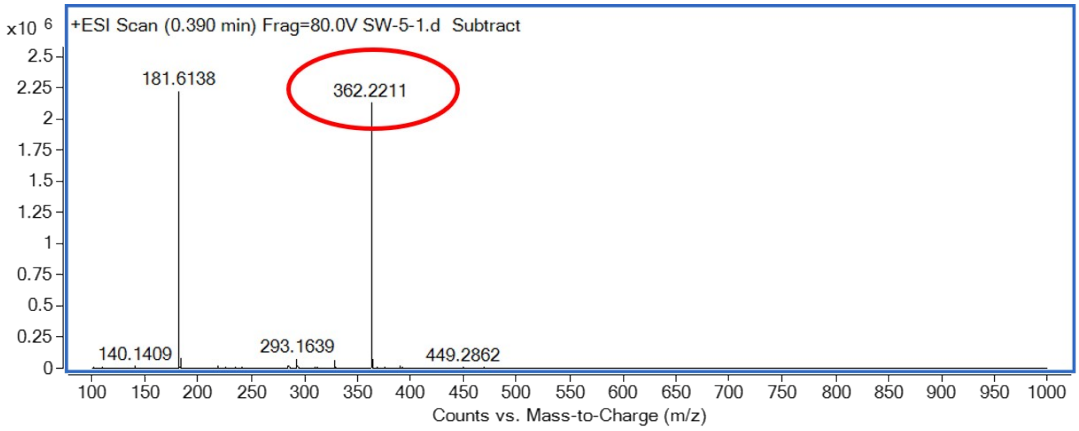


Figure S3 HRMS of **CMP**

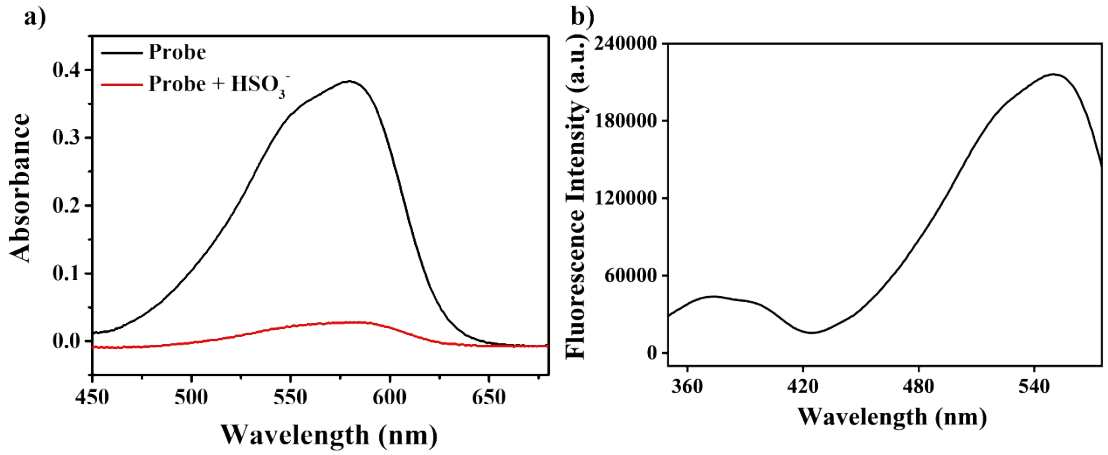


Figure S4 (a) The absorption spectra of **CMP** (10 μM) in the presence and absence of 15 μM HSO_3^-. (b) Excitation spectra of the **CMP** solutions.

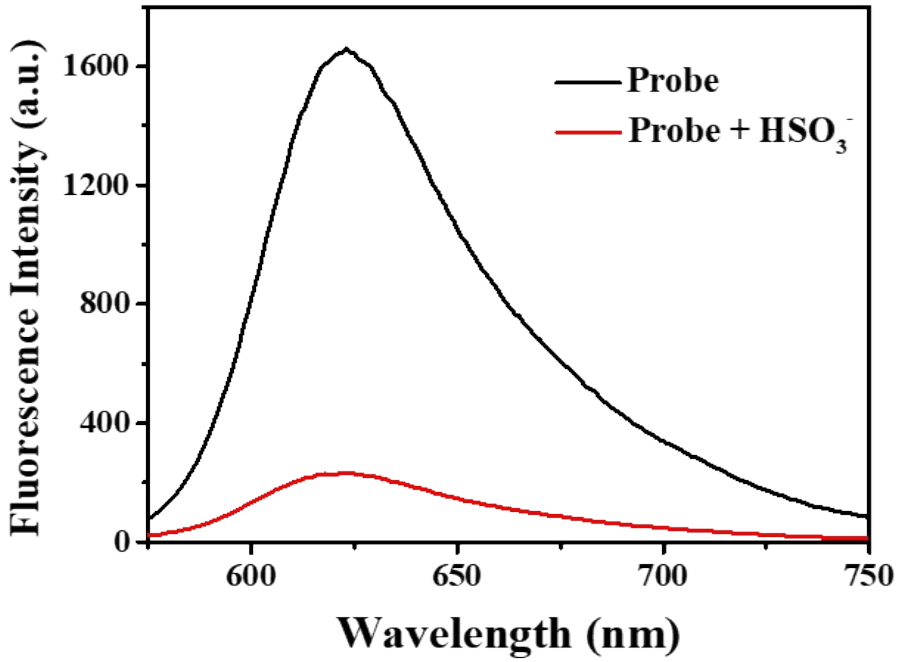


Figure S5 The fluorescence spectra of **CMP** (5 μM) in the presence and absence of 5 μM HSO_3^-.

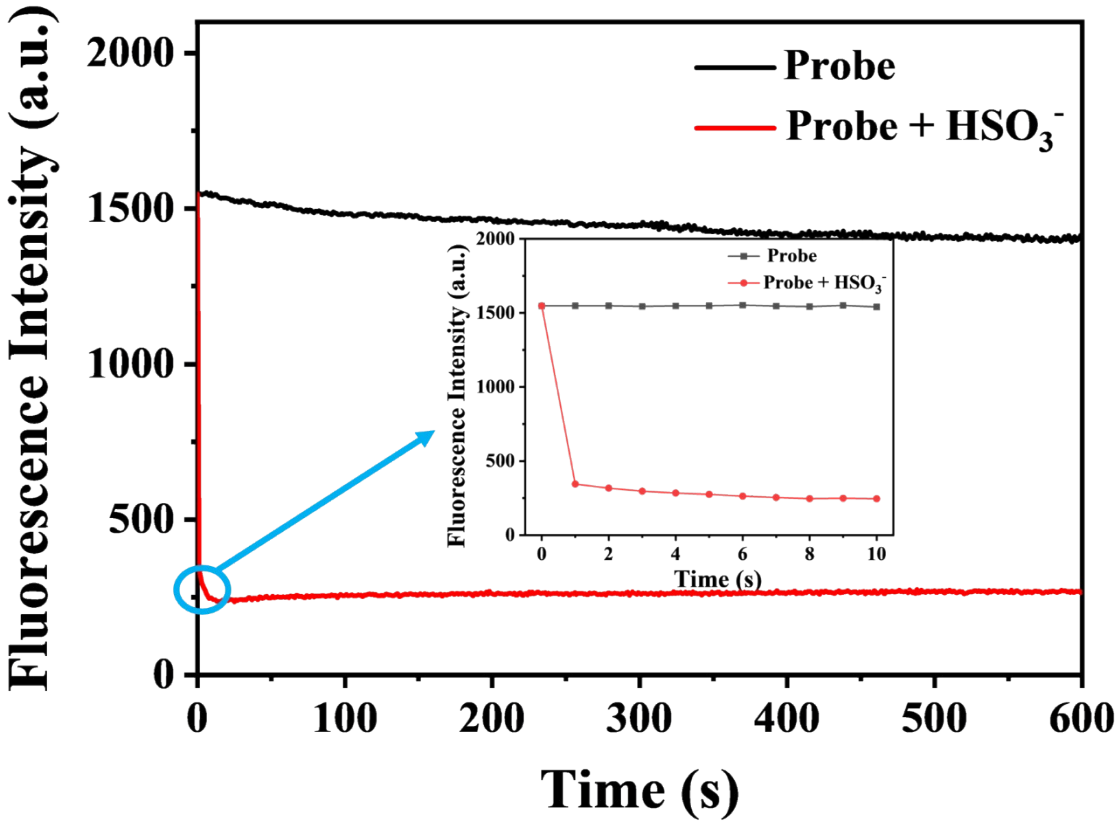


Figure S6 The fluorescent intensity of **CMP** (5 μM) in the presence and absence of 15 μM HSO_3^- at different time. Inset: The fluorescent intensity of **CMP** (5 μM) in the presence and absence of 15 μM HSO_3^- over a period of 0-10 s. The excitation wavelength was 550 nm. Excitation and emission slit widths were 5 nm.

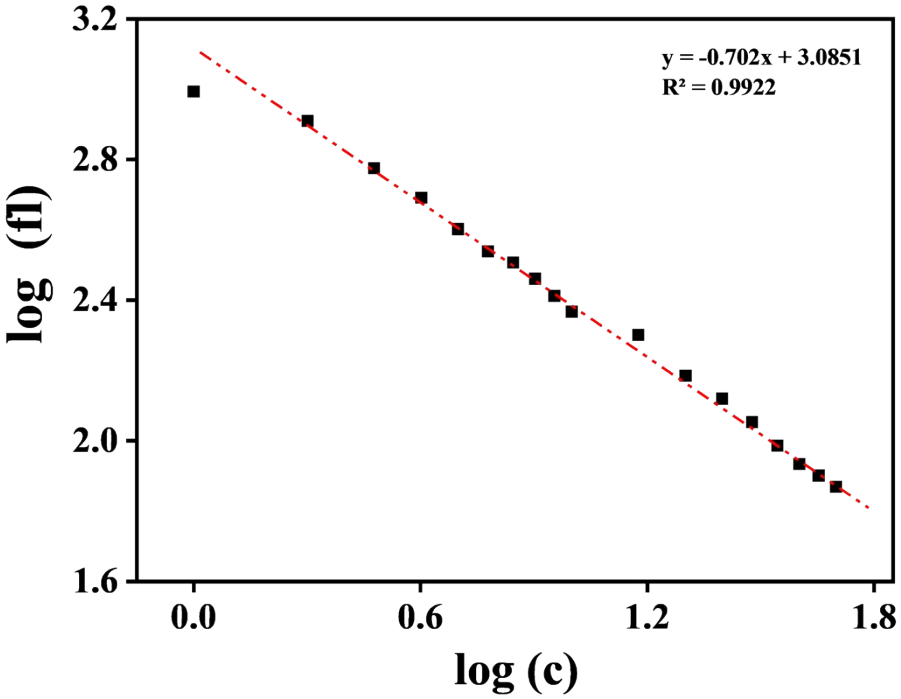


Figure S7 The log-linear relationship between log(fl) and log(c).

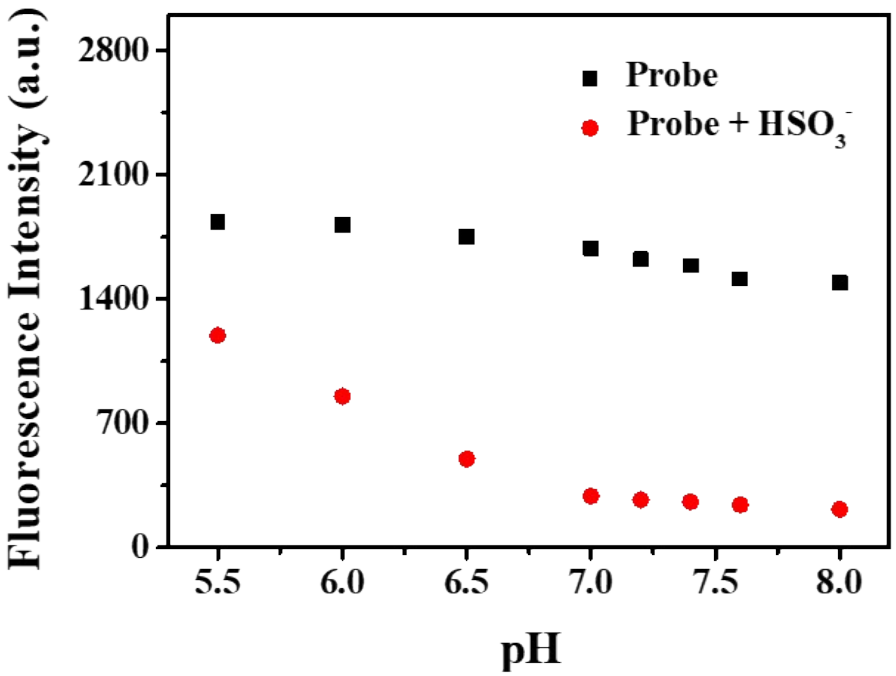


Figure S8 The fluorescence intensity of **CMP** (5 μM) in the presence and absence of 5 μM HSO_3^- at different values of pH.

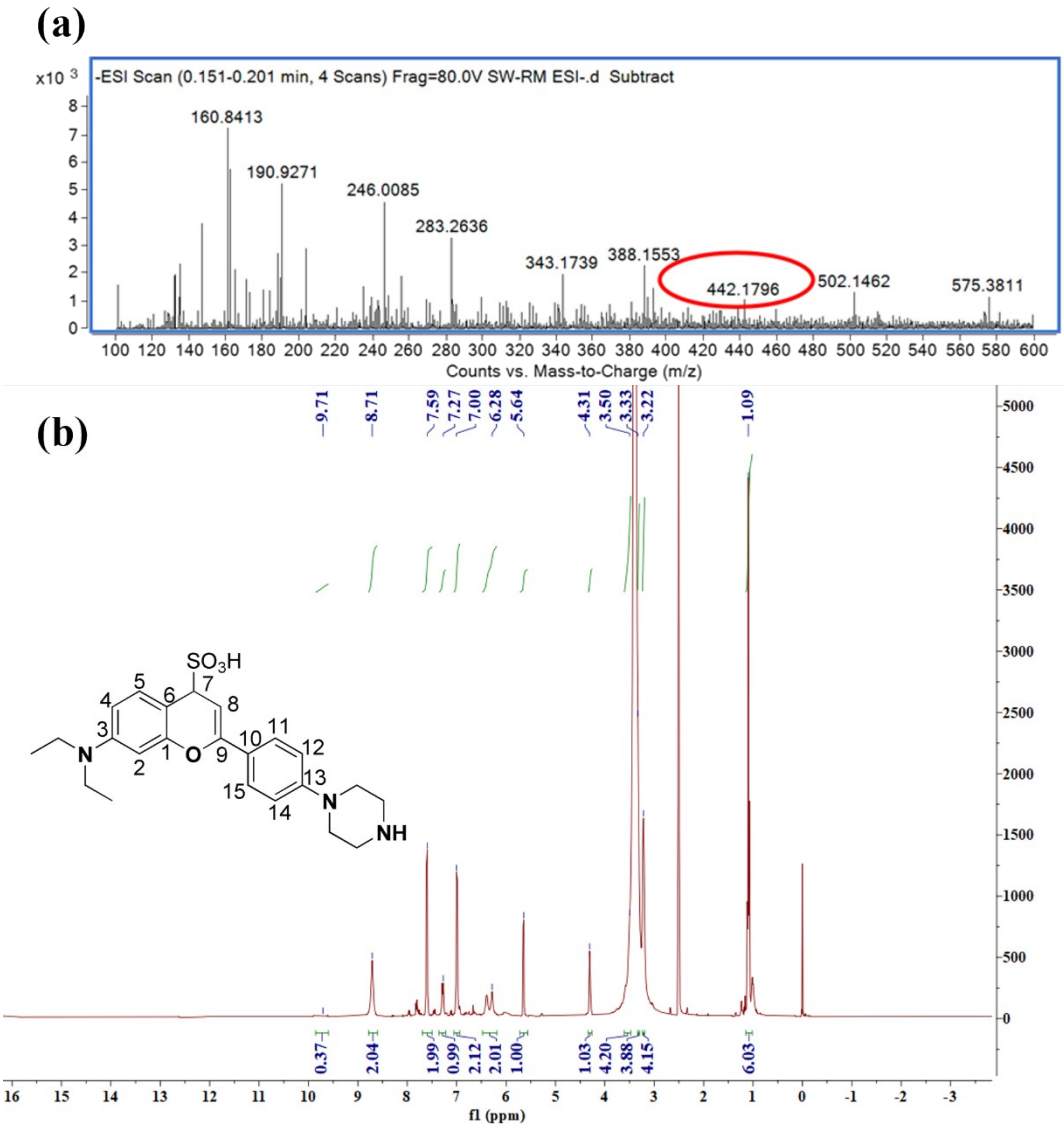


Figure S9 (a) The HRMS of the product after reaction between **CMP** and HSO_3^-. (b) The ^1H NMR of the product after reaction between **CMP** and HSO_3^-.

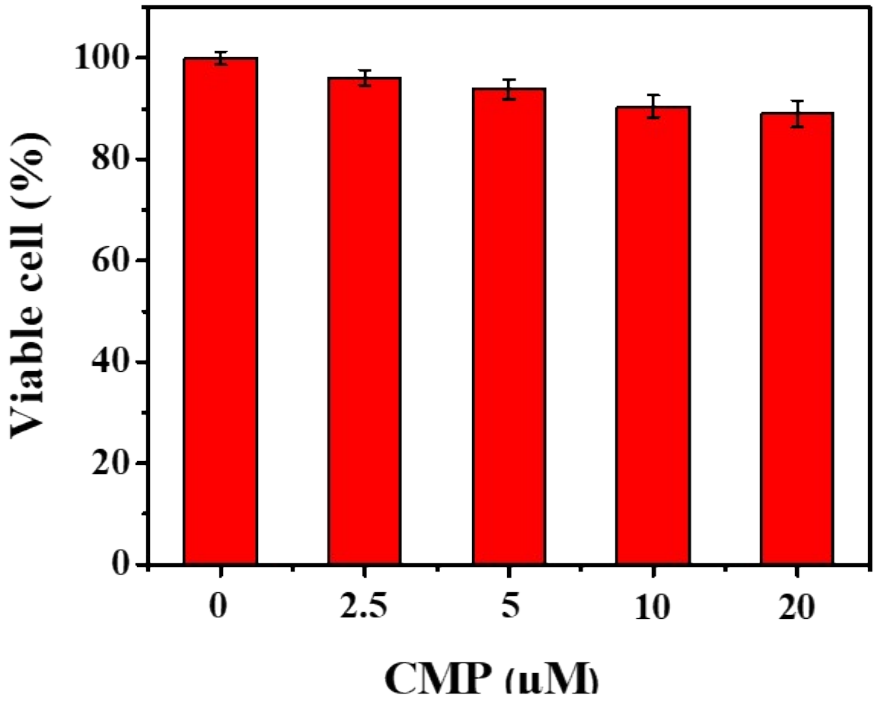


Figure S10 Cytotoxicity assays of **CMP** at different concentrations.

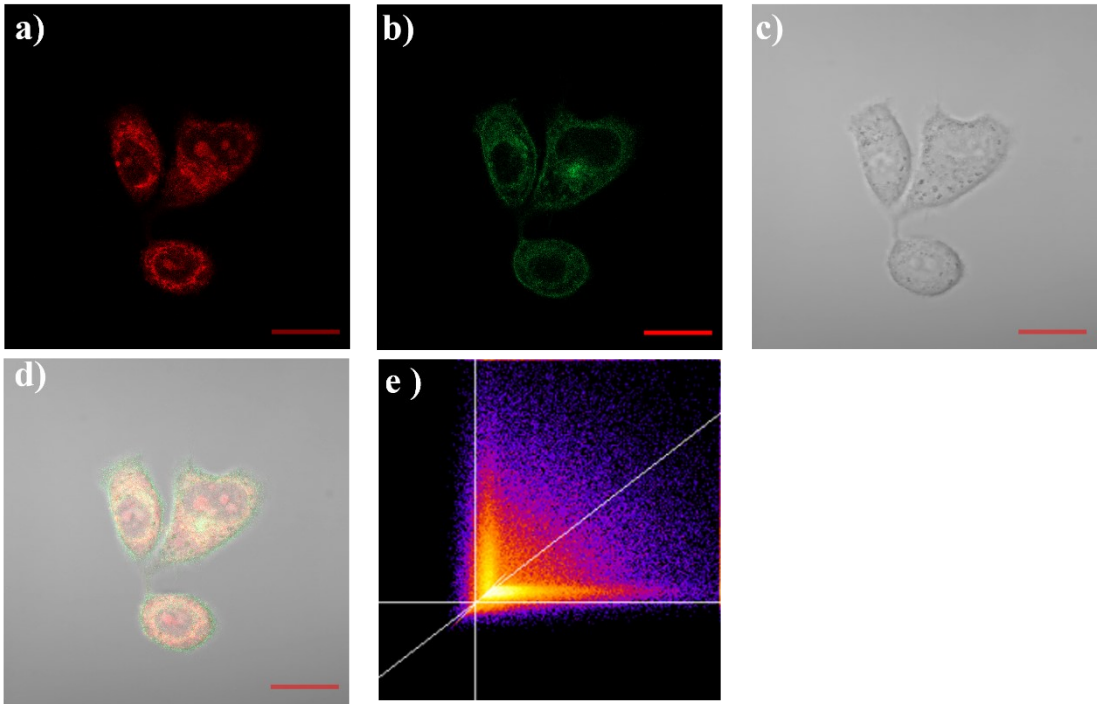


Figure S11 Co-localization of **CMP** in HeLa cells. (a) **CMP** channel (10 μM, λ_{ex}= 561 nm, λ_{em}= 600-700 nm). (b) Lyso-tracker Green channel (1 μM, λ_{ex}= 405 nm, λ_{em}= 450-550 nm). (c) Bright field. (d) Merged. (e) Intensity correlation plot. Scale bar: 20 μm.

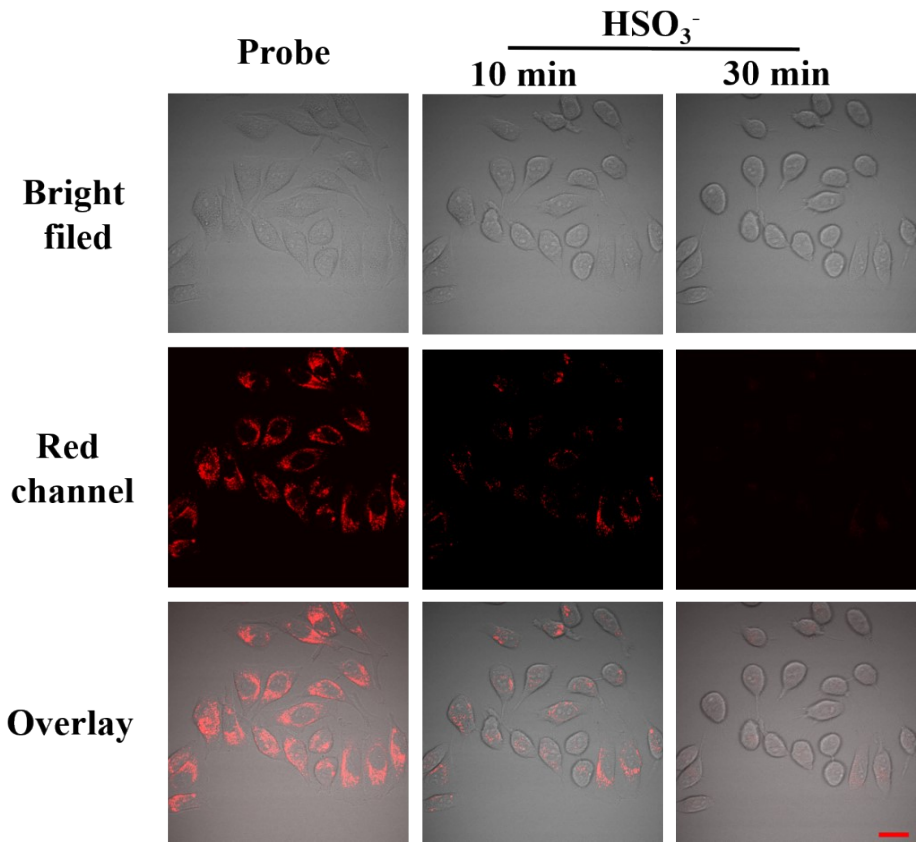
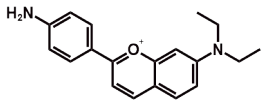
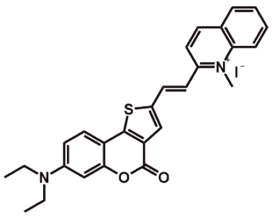
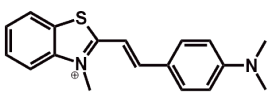
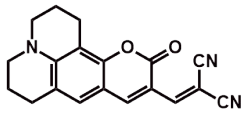
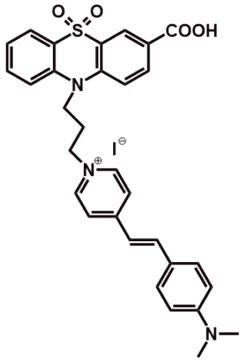
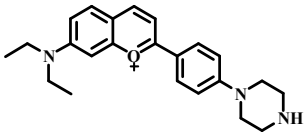


Figure S12 Confocal imaging of **CMP** (5 μM) towards 25 μM HSO_3^- at different time. (λ_{ex}=543 nm, λ_{em}=590-660 nm). Scale bar 20 μm.

Table S1 Key Performance Comparison of HSO_3^- Probes

Probe	LOD (μM)	Response time	Mito-targeting	Application environment	References
	0.14	120 s	No	Brain of living animals	*Chem Sci,* 2024, 15 (13), 4824-4832.
	0.038	40 s	Yes	Waste-water, Food samples, Living cells, Zebrafish, Mouse models,	*J Hazard Mater,* 2024, 465, 133165.
	2.01	10 min	No	Living cells	*J Photochem Photobiol, A,* 2020, 395, 112498.

Probe	LOD (μM)	Response time	Mito-targeting	Application environment	References
	0.87	<5 min	No	Living cells	*Dyes Pigm,* 2017, 136, 830-835.
	11.3	80 min	Yes	Living cells	*Dyes Pigm,* 2020, 181, 108639.
	0.034	5 s	Yes	Living cells, CCCP-induced apoptosis, Erastin-induced ferroptosis	This work