Supporting Information

Construction of a coumarin-based fluorescent probe for accurately visualizing hydrogen sulfide in live cells and zebrafish

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Contents:

1. General information

Unless otherwise noted, all reagents were purchased from commercial suppliers that were used without further purification. All the solutions were prepared with ultrapure water (18.2 M/cm). Fluorescence spectra were measured on a Horiba spectrofluorometer. All the spectra were performed in a quartz cuvette with 10.0 mm path length (volume: 2 ml). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker unit (400 MHz) using $CDCl_3$ as the solvent. Mass spectra were measured with Waters Xevo G2-XS Qtof mass spectrometer. Cell images were performed on an Olympus FV3000 confocal laser scanning microscopy with a 60 × oil objective lens. Zebrafish images were conducted on a stereomicroscope (Olympus SZX16, Japan).

2. Optical response of Cou-H_2S to H_2S

The stock solutions of Cou-H_2S (1 mM) were prepared in dimethyl sulfoxide (DMSO) and maintained at 4 °C. The fluorescence emission spectra of Cou-H_2S were measured in a PBS solution containing 100 μM CTAB at room temperature. Fluorescence emission spectra were obtained with Xenon lamp and 1.0 cm quartz cells. The fluorescence intensity was measured at $\lambda_{ex}/_{em}$ = 405/498 nm (slit width: 2.5 nm/2.5 nm).

3. Cytotoxicity of Cou-H_2S

The prepared DMEM medium was added to HeLa cells. The cell culture medium in the 96-well plate was aspirated, and medium containing different concentrations of Cou-H_2S (0 μM, 10 μM, 20 μM, 30 μM) was added, and incubated for 24 h in a cell culture incubator. CCK-8 solution (10 μL) was added to each well under light-avoidance conditions. Afterward, the 96-well plate was incubated in a cell culture incubator for 2 h. The endpoint kinetics at 450 nm was measured using a microplate reader.

4. Cell culture and fluorescence imaging

HeLa and RAW264.7 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). For the cell imaging experiment, configured Dulbecco's Modified Eagle's Medium (DMEM, low glucose) was added to HeLa cells and RAW264.7 cells, supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin, and 1 % streptomycin. HeLa and RAW264.7 cells were cultured in DMEM at 37 °C in a humidified incubator with 5% carbon dioxide. The cells were stained with 10 μM Cou-H_2S and CTAB (50 μM), then imaged. Cell images were obtained on an Olympus FV3000 laser confocal microscope with the excitation at 405 nm with an objective lens (× 60). λ_{ex} = 405 nm, λ_{em} = 450 - 550 nm.

5. Fluorescence imaging of H_2S in zebrafish

Zebrafish larvae were purchased from Eze-Rinka (Nanjing, China) and were maintained in embryonic medium containing 1-phenyl-2-thiourea at 30 ℃ after fertilization for 96 h. To study the effects of different treatments, zebrafish larvae were divided into two groups. The control group was 5-day-old zebrafish exposed to 10 μM Cou-H_2S for 30 min; 5-day-old zebrafish were stimulated with 2 μg/mL LPS for 12 h prior to the exposure to 10 μM Cou-H_2S for 30 min. After treatment, zebrafish were washed three times with PBS and imaged with a stereomicroscope (Olympus SZX16). λ_{ex} = 405 nm, λ_{em} = 450 – 550 nm.

6. Structure characterization of Cou-H_2S

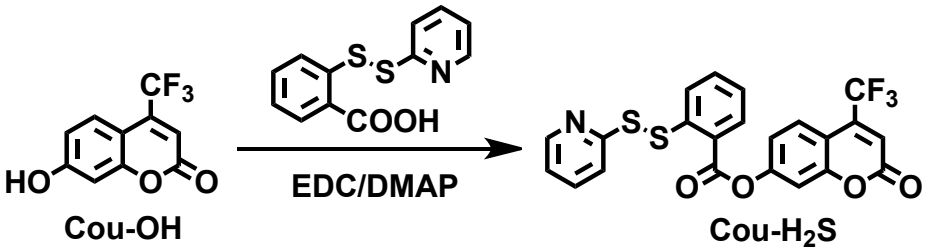

Figure S1. The general synthetic routes for Cou-H_2S.

Cou-OH (115 mg, 0.5 mmol) and 2-(pyridin-2-yldisulfanyl) benzoic acid (158 mg, 1.2 mmol) were dissolved in dry dichloromethane (20 ml). Subsequently, EDC (383 mg, 2.0 mmol) and DMAP (25 mg) were added. The reaction mixture was

stirred at room temperature for 10 h. After completion of the reaction, the solvent was evaporated in vacuo. The resulting product was purified by silica gel column chromatography The reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, the solvent was evaporated in vacuo. The resulting product was purified by silica gel column chromatography (eluent: 20 % ethyl acetate/80 % petroleum ether) to afford the desired compound (76 mg, 32 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49-8.47 (m, 1H), 8.30 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.01 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.82(dd, *J* = 8.8, 1.8 Hz, 1H), 7.61-7.53 (m, 3H), 7.41-7.32 (m, 3H), 7.13-7.10 (m, 1H), 6.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.83, 158.57, 158.44, 155.09, 153.87, 149.72, 142.14, 137.35, 134.34, 132.14, 126.40, 126.35, 125.97, 125.32, 121.14, 119.97, 119.78, 119.23, 115.55, 115.49, 111.59, 111.32; HRMS m/z: $C_{22}H_{12}F_3NO_4S_2$ $[M+H]^+$ calcd for 476.0238 found 476.0282.

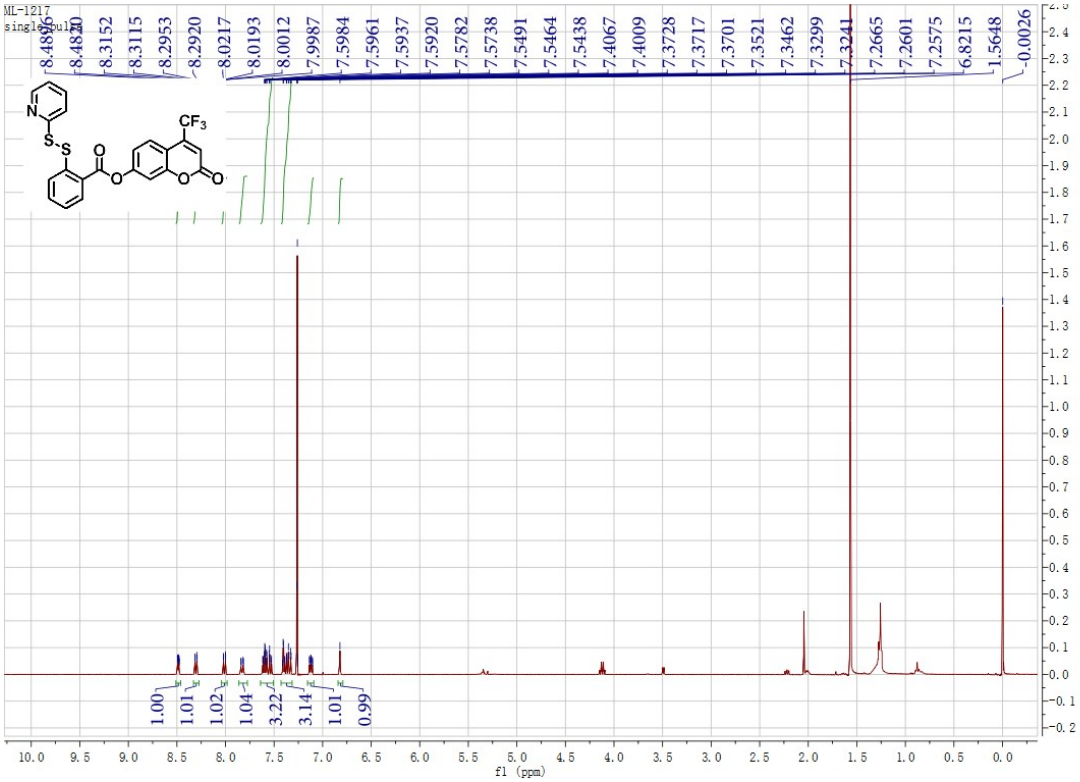

Figure S2. ^{1}H NMR (400 MHz) spectra of Cou-H_2S in $CDCl_3$.

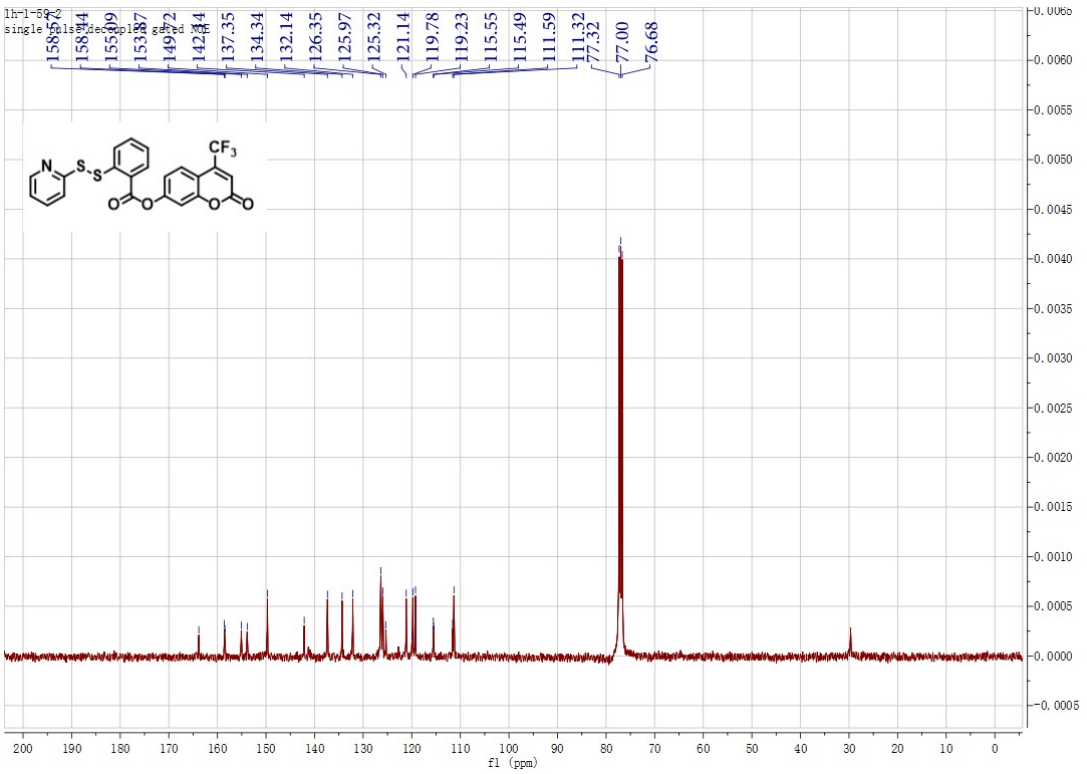

Figure S3. ^{13}C NMR (400 MHz) spectra of Cou-H_2S in $CDCl_3$.

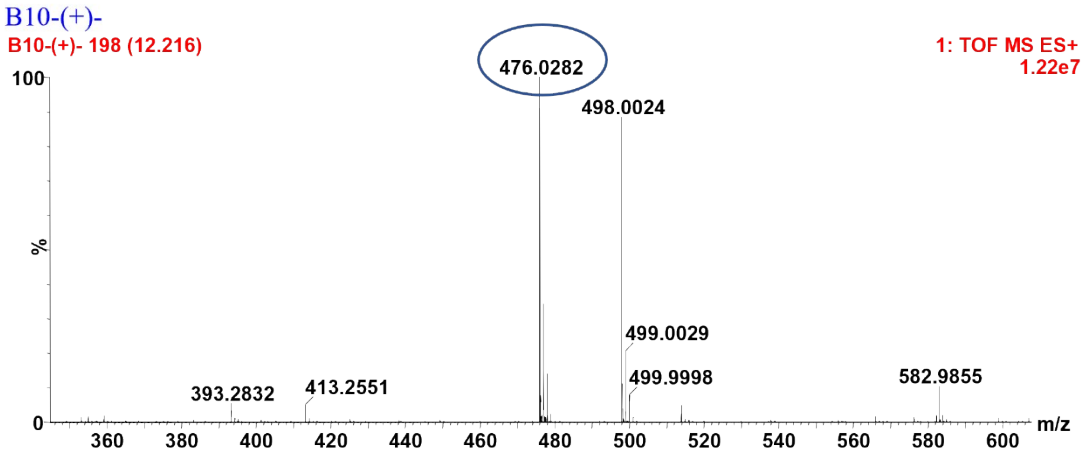

Figure S4. HRMS of Cou-H_2S.

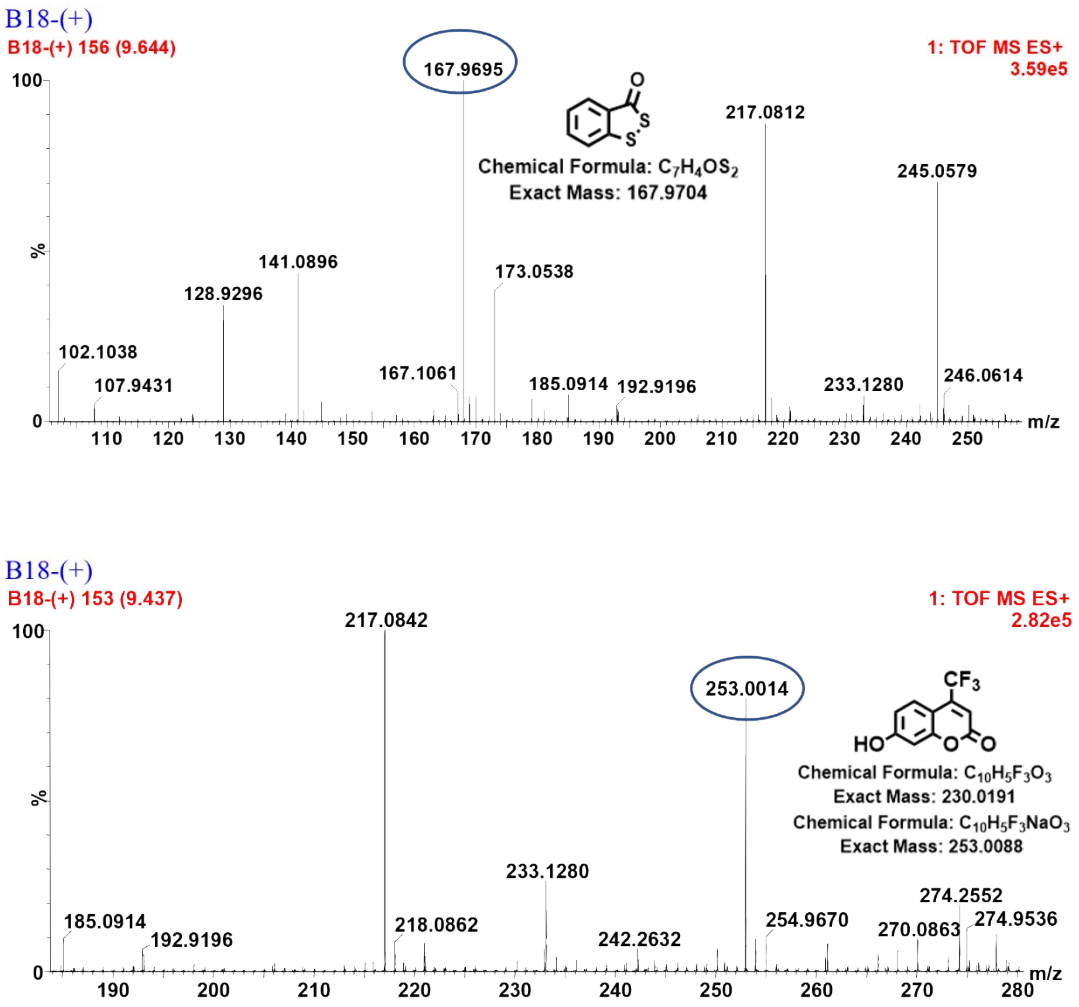

Figure S5. HRMS analysis of the reaction product of Cou-H_2S and Na_2S.

7. Absorption spectra of Cou-H_2S in the presence of Na_2S

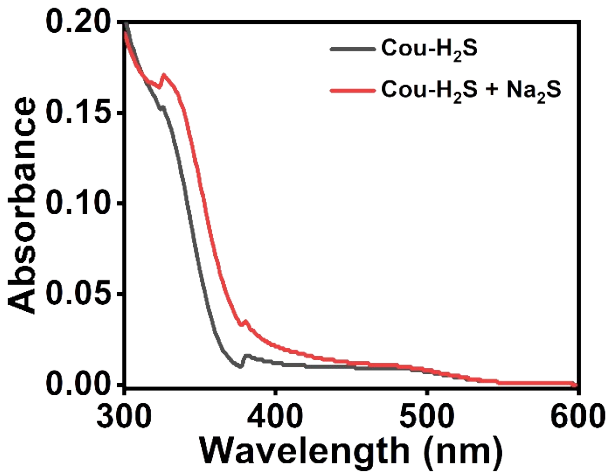

Figure S6. Absorption spectra of Cou-H_2S (10 μM) in the presence of Na_2S (20 μM).

7. Comparison of fluorescent probe for H_2S

Table S1. Comparison of fluorescent probe for H_2S

Probes	Detection system	Linear response range	Detection limit	Reference
Mito-HS-1	DMSO/PBS buffer = 3:10 (pH=7.4, v/v)	3 - 25 μM	170 nM	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, **2023,** 298, 122767
Mito-HS-2	DMSO/PBS buffer = 3:10 (pH=7.4, v/v)	3 – 150 μM	160 nM	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, **2023,** 298, 122767
SS-N_3	DMSO/PBS buffer = 3:2 (pH=7.4, v/v)	0 - 80 μM	10 nM	Talanta, 2022, 250, 123741
NT-SH	PBS buffer (pH 7.4)	0 - 50 μM	80.01 nM	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2021, 254, 119620
Gol-H_2S	DMSO/PBS buffer = 1:4 (pH=7.4, v/v)	0 - 30 μM	110 nM	Analytical Chemistry, 2020, 92, 1883−1889
SNARF-SeSPy	PBS buffer (pH 7.4, containing 100 μM CTAB)	0 - 20 μM	34 nM	Analytica Chimica Acta, 2020, 1109, 37-43
Probe	DMF/PBS buffer = 1:9 (pH=7.4, v/v)	1 - 6 μM	36 nM	Sensors Actuators: B. Chemical, 2019, 297, 126773
NDCM-1	CH_3CN/PBS buffer = 1:1 (pH=7.4, v/v)	0 - 7 μM	58.797 nM	Journal of Materials Chemistry B, 2018, 6, 7916-7925
Cou-H_2S	PBS buffer (pH 7.2, containing 100 μM CTAB)	4 – 10 μM	25 nM	This work

9. The cytotoxicity of Cou-H_2S in live cells

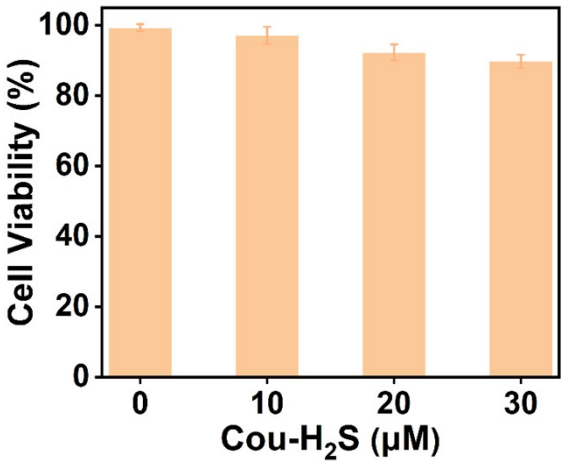

Figure S7. Cell viability of HeLa cells in different concentrations of Cou-H_2S for 24 h was determined by CCK-8 assay. The experiments were repeated three times and the data were shown as mean (± S.D.).