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A Dual-Emission Fluorescence-Enhanced Probe for Hydrogen Sulfide and Its Application in Biological Imaging

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Contents

1. Synthesis and characterization of compounds

*Synthesis of Compound **1***[1]

A mixture of 7-Hydroxycoumarin-3-carboxylic acid (1.03 g, 5.0 mmol), *N*-Boc-piperazine (0.931 g, 5.0 mmol), EDC·HCl (1.44 g, 7.5 mmol), HOBT (1.01 g, 7.5 mmol) in THF (20 mL) was stirred for 12 h at room temperature. The solvent was removed by evaporation, and the residue was washed with water. The obtained solid was redissolved in CH_2Cl_2 (2 mL) and TFA (2 mL), and the mixture was stirred for 30 min at room temperature. The solvent was evaporated and dried in vacuo to give compound **1** as a colorless powder (0.623g, 45.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 9.07 (s, 1H), 8.14 (s, 1H), 7.62 (d, J = 8.5 Hz, 1H), 6.86 (d, J = 8.6 Hz, 1H), 6.78 (s, 1H), 3.86 – 2.98 (m, 8H).

*Synthesis of Compound **2***[2]

To a solution of 4-Chloro-7-chlorosulfonyl-2,1,3-benzoxadiazole (0.506 g, 2.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise a solution of dimethylamine hydrochloride (0.204 g, 2.5 mmol) dissolved in CH_2Cl_2 (20 mL) with triethylamine (700 μL, 5.0 mmol). The mixture was stirred at room temperature for 30 min before the solvent was removed under reduced pressure. The residue was purified through column chromatography (PE: EA = 5: 1) to give compound **2** as a colorless powder (0.302 g, 57.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 7.3 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 2.96 (s, 6H).

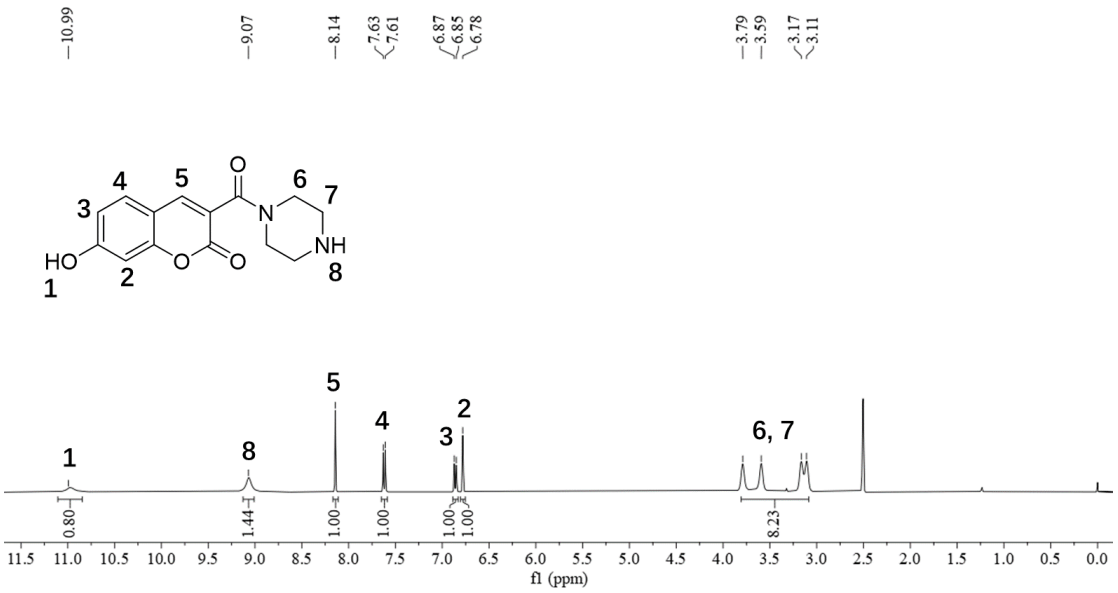


Fig. S1. ^{1}H NMR spectrum of compound **1** in DMSO-d_6

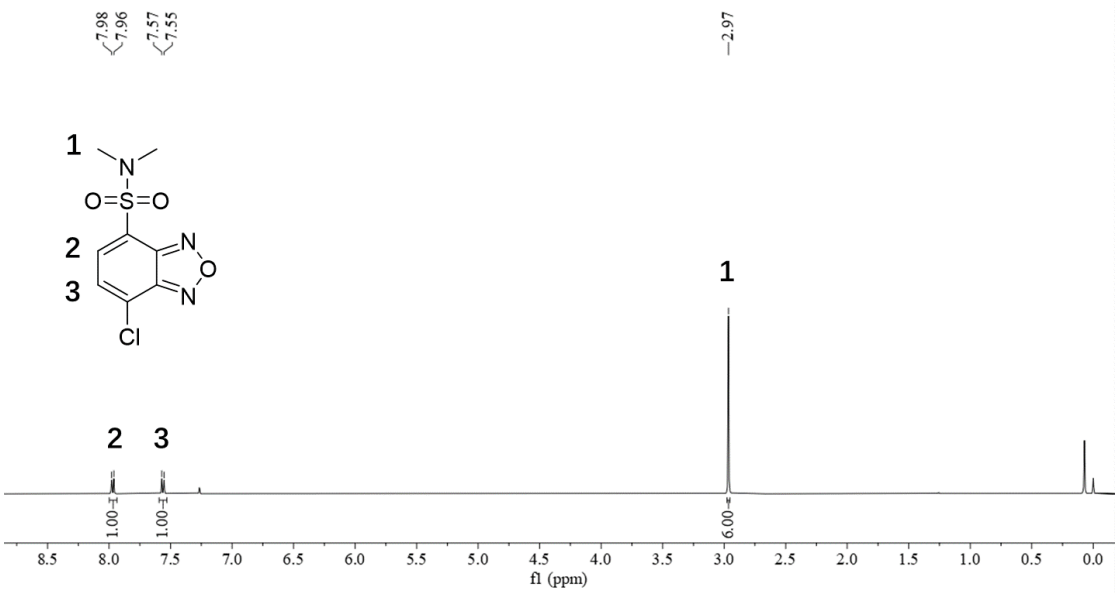


Fig. S2. ^{1}H NMR spectrum of compound **2** in $CDCl_3$

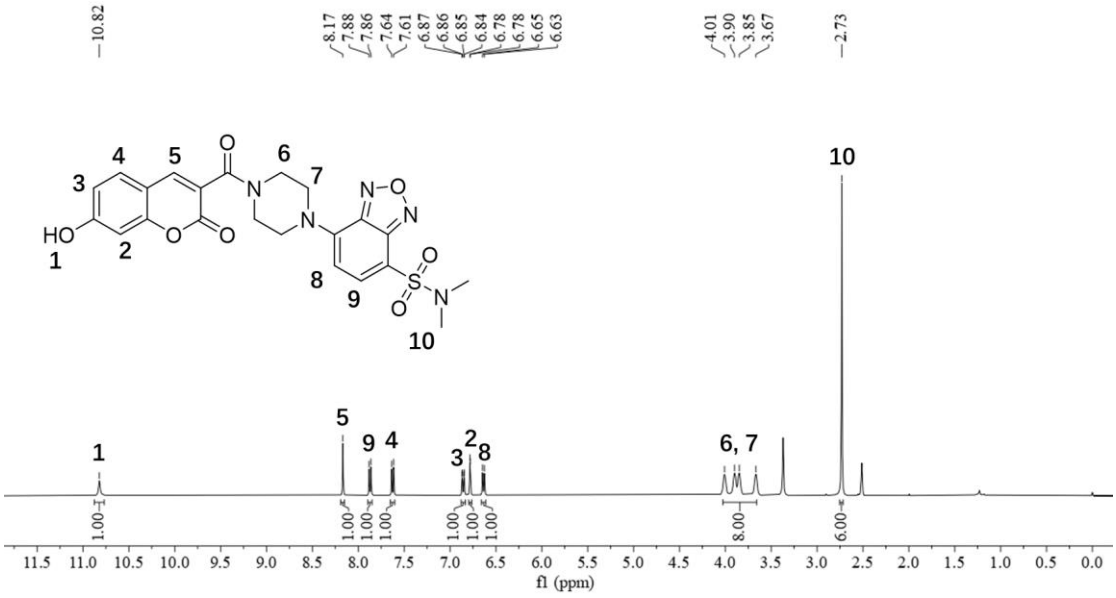


Fig. S3. ^{1}H NMR spectrum of compound **DCH** in DMSO-d_6

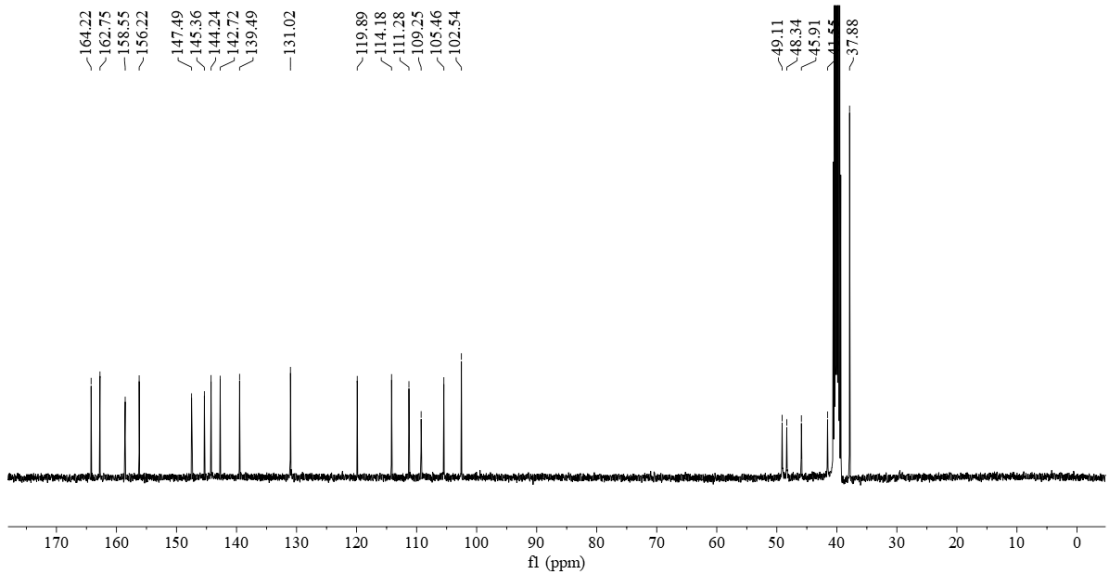


Fig. S4. ^{13}C NMR spectrum of compound **DCH** in DMSO-d_6

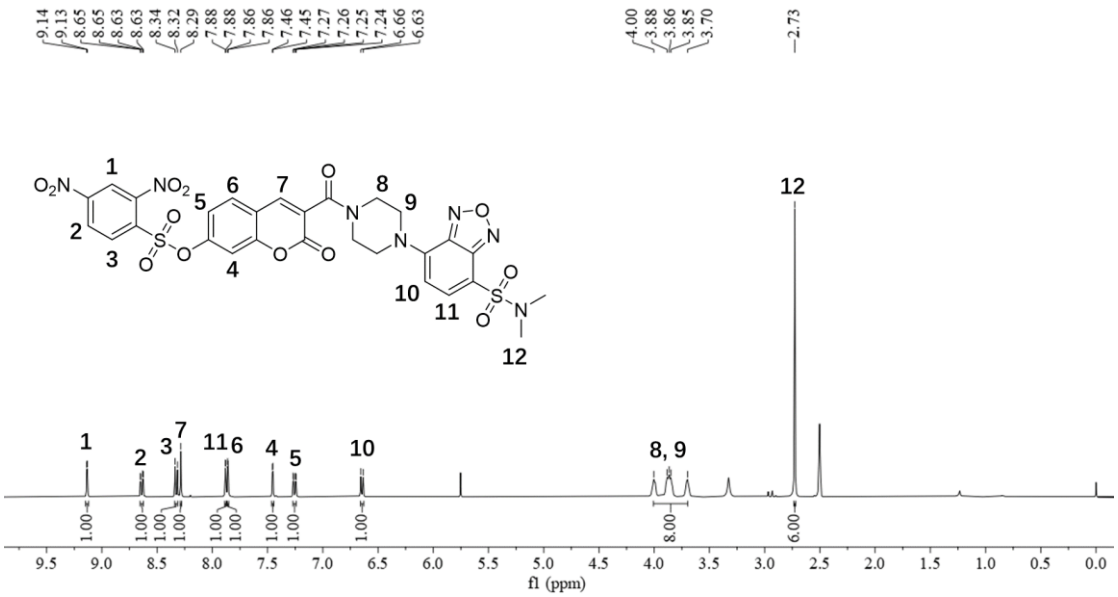


Fig. S5. ^{1}H NMR spectrum of compound **DCH-S** in DMSO-d_6

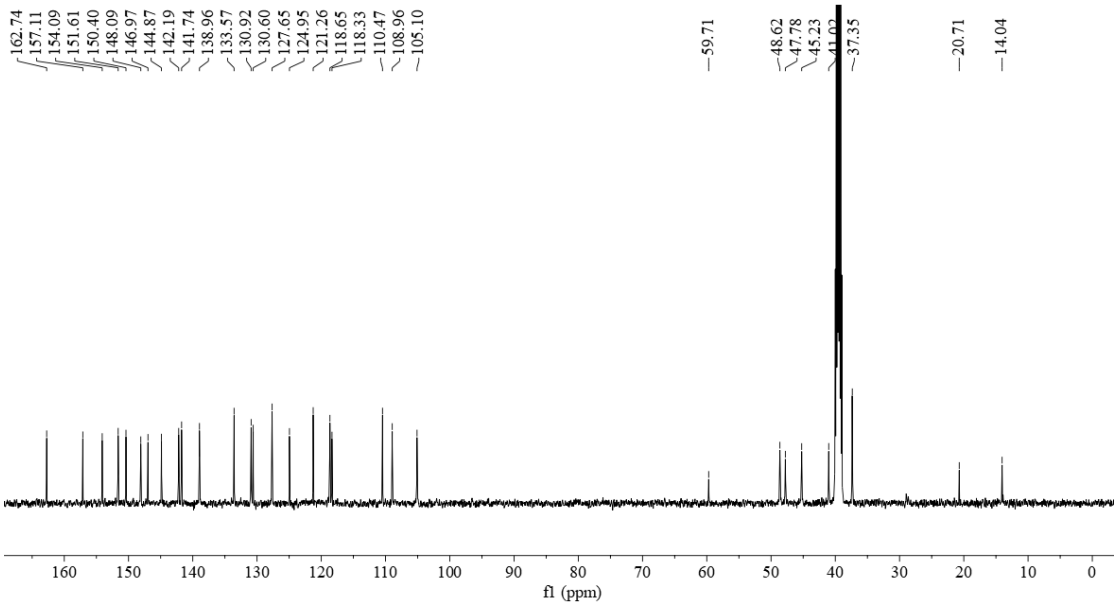


Fig. S6. ^{13}C NMR spectrum of compound **DCH-S** in DMSO-d_6

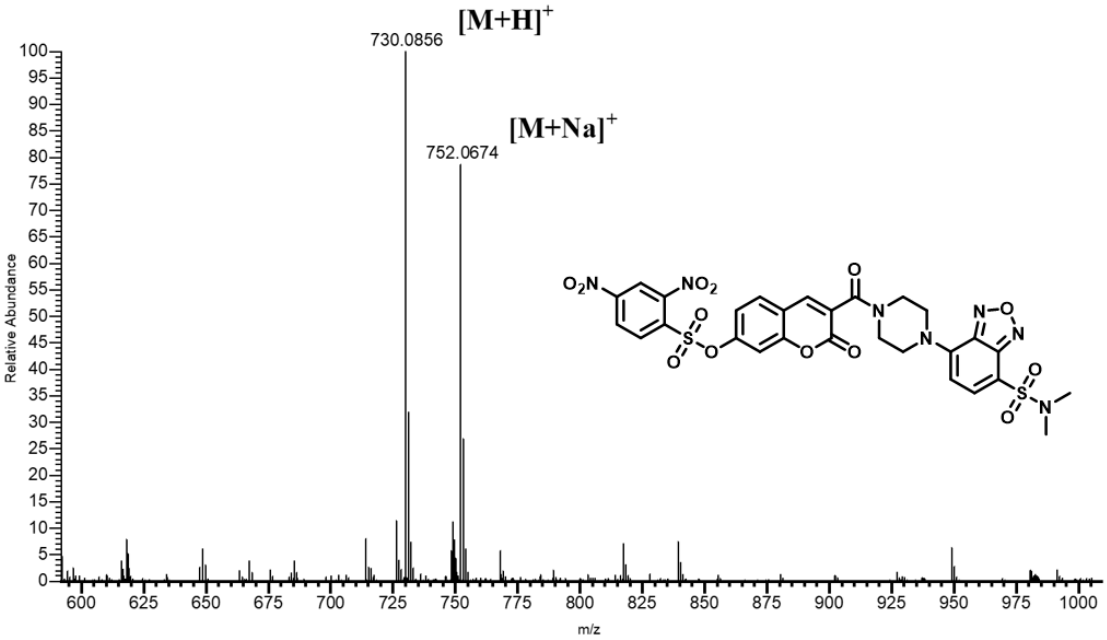


Fig. S7. ESI-MS spectrum of compound **DCH-S**

2. Selectivity and anti-interference ability of **DCH-S** for NaHS

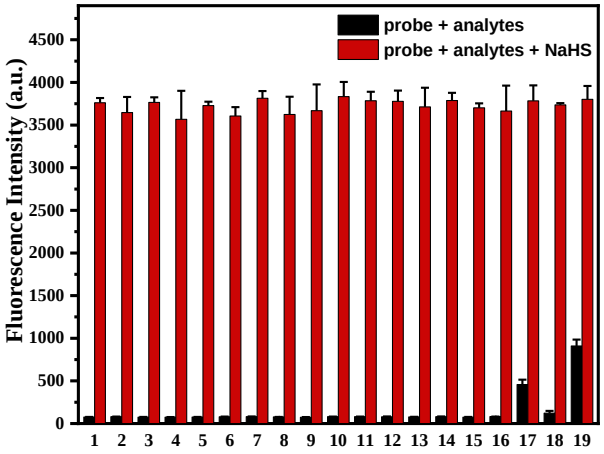


Fig. S8. Fluorescence responses of **DCH-S** (5 μM) at 600 nm towards NaHS (50 μM) and various analytes. Black bars represent the solution of **DCH-S** in the presence of various analytes. Red bars represent the addition of NaHS to the above solution, respectively. Analytes 1-19: None, $NaNO_3$, $NaNO_2$, Na_2SO_4, Na_2SO_3, $Na_2S_2O_3$, NaCl, KCl, $CaCl_2$, H_2O_2, L-ascorbic acid, Gluocose, Phe, Pro, Ala, His, Cys, Hcy, GSH.

3. The capability of **DCH-S** for detecting NaHS at different pH

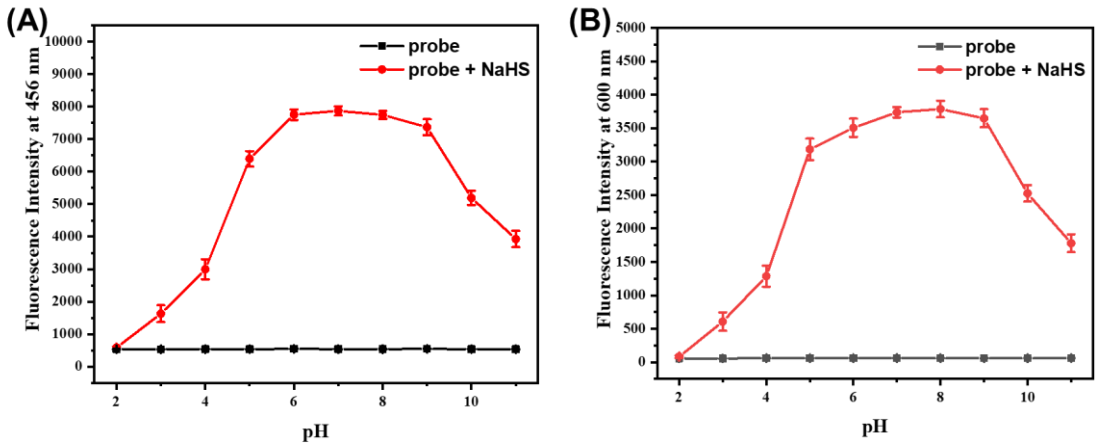


Fig. S9. Fluorescence responses of **DCH-S** (5 μM) at (A) 456 nm and (B) 600 nm in the presence and absence of NaHS (50 μM) under different pH values.

4. Kinetic studies

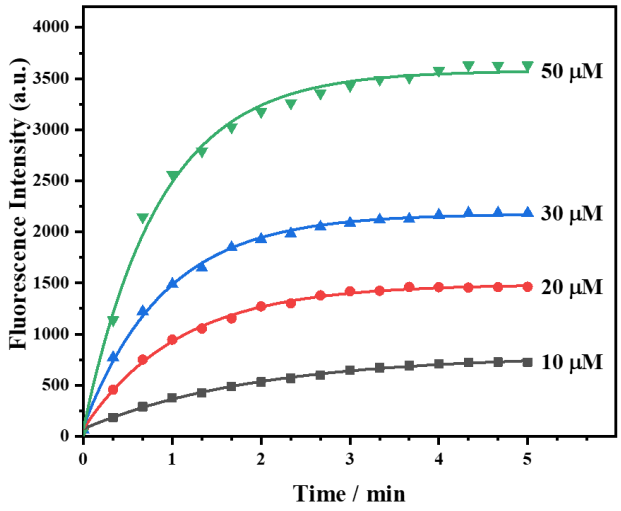


Fig. S10 Time-dependent fluorescence intensities of **DCH-S** (5 μM) at 600 nm in the presence of different concentration of NaHS (10, 20, 30, 50 μM).

5. LC-MS analysis of the reaction system

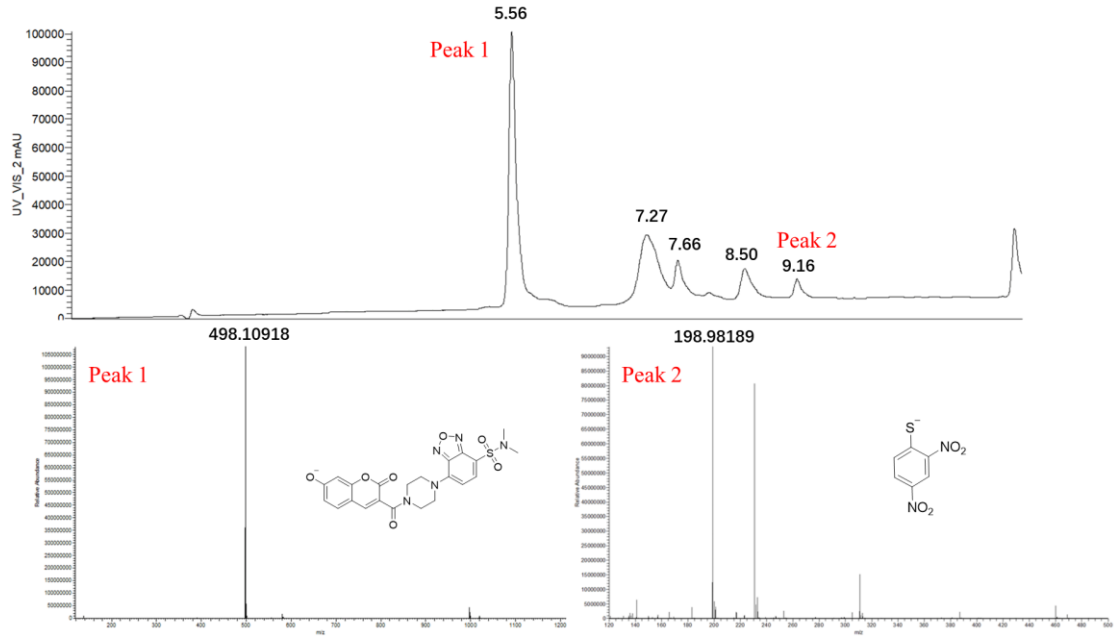


Fig. S11. LC-MS analysis of the mixture of **DCH-S** and NaHS (10 equiv.). The peak at m/z = 498.10918 can be assigned to the produced **DCH** (calculated for $[M–H]^-$: 498.10889). The peak at m/z = 198.98189 can be assigned to the produced 2,4-dinitrothiophenol (calculated for $[M–H]^-$: 198.98190).

6. Cytotoxicity assay

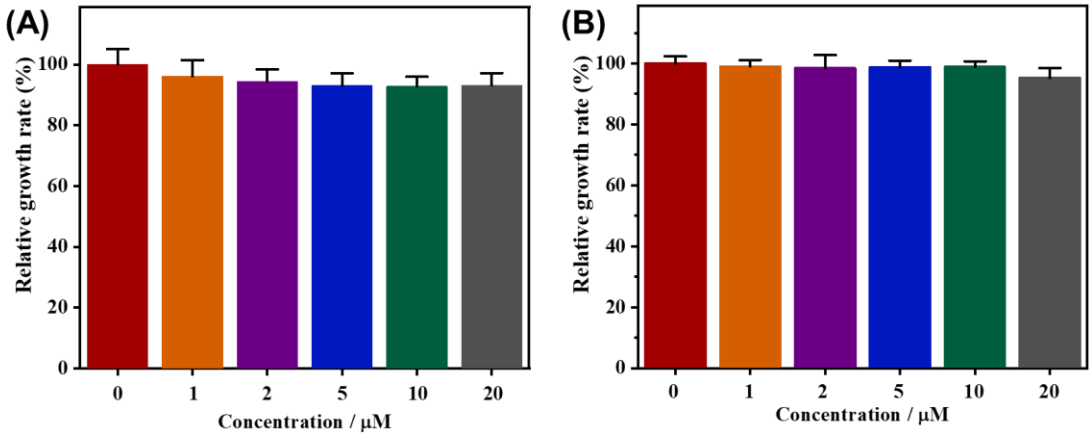


Fig. S12. Relative growth rate (%) estimated by the MTT assay. The MCF-7 cells were cultured in the presence of 0–20 μM of (A) **DCH-S** and (B) **DCH** for 24 h, respectively.

7. Determination of the detection limit

The detection limit was calculated from the titration experiments according to the following equation[3]:

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

Where σ is the standard deviation of blank measurements, k is the slope of the linear regression equation (Fig. S13). The detection limit was calculated to be 47 nM.

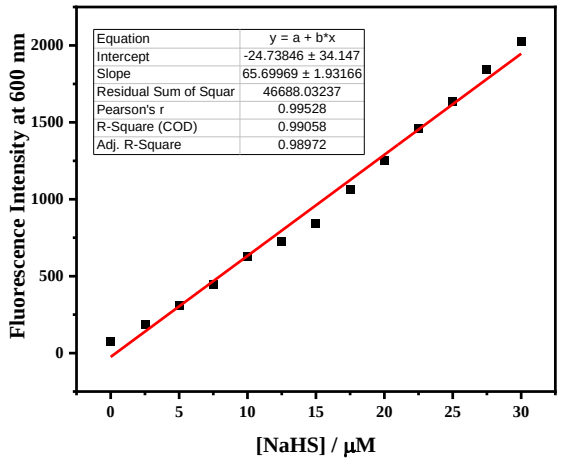


Fig. S13 Determination of detection limits from the fluorescence intensity data.

8. Theoretical calculations

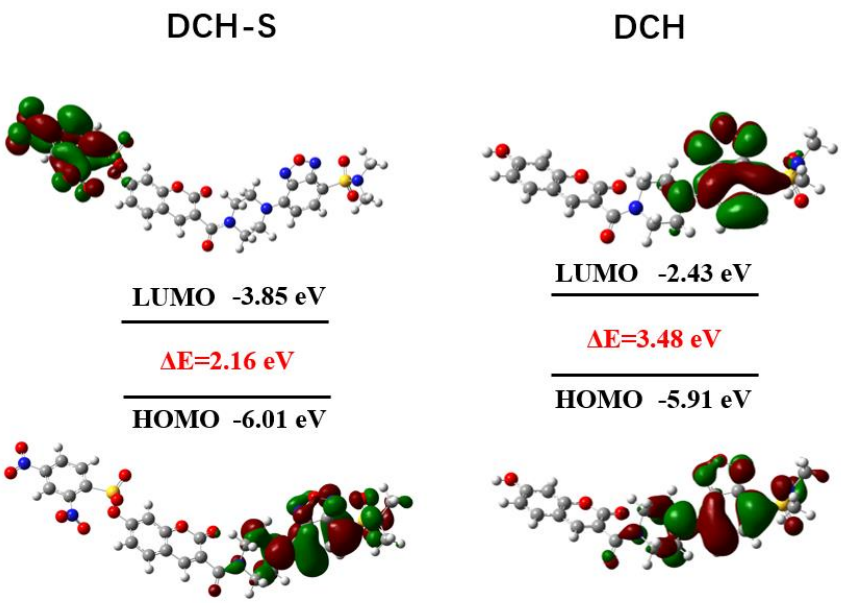


Fig. S14 The HOMO and LUMO of **DCH-S** and **DCH**. The geometries of the molecules were optimized and their frontier molecular orbital energies were calculated using Gaussian 16 (density functional theory/time-dependent density functional theory (DFT/TDDFT) at B3LYP/6-31G(d) level).

9. Mean fluorescence intensities of confocal imaging in MCF-7 cells

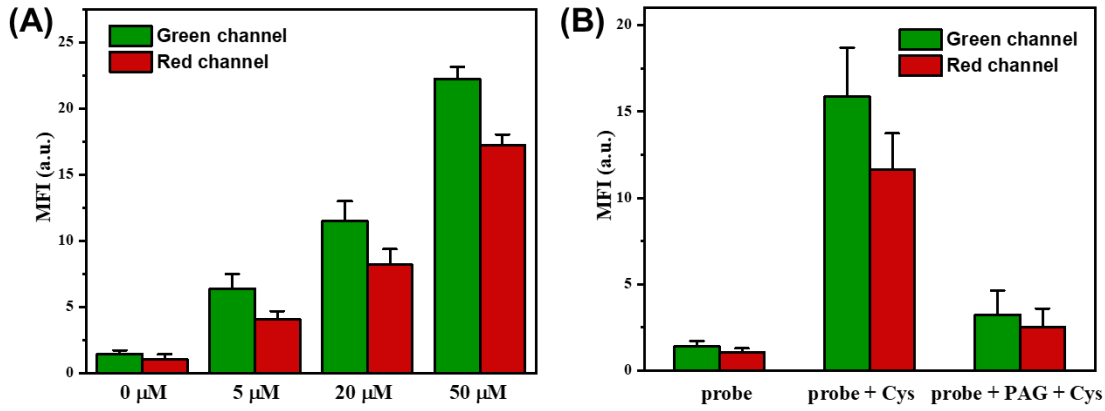
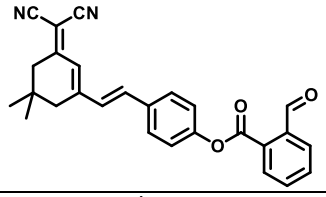
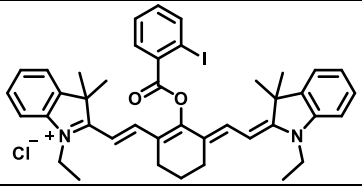
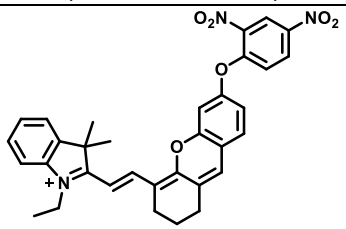
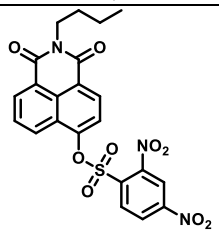
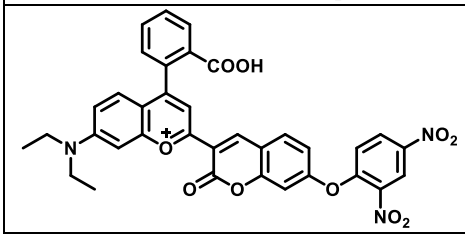
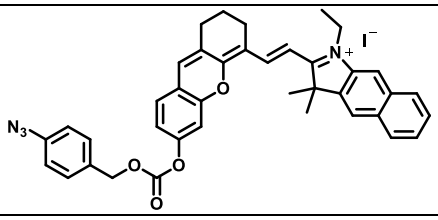
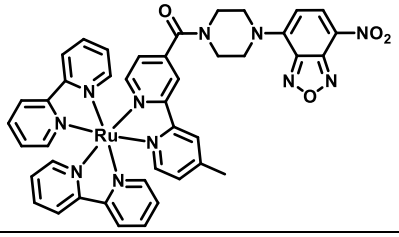
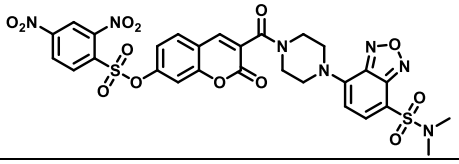


Fig. S15 Mean fluorescence intensities (MFI) of confocal imaging of (A) exogenous and (B) endogenous H_2S in MCF-7 cells. The error bars represent the standard deviation (± SD).

Table S1 Properties of the reported fluorescent probes for H_2S in recent years

Probe structure	Solution	λ_{em}/nm	Response time	Detection limit	Ref.
	DMSO/PBS buffer (10 mM, pH 7.4, 1:9, v/v)	560/650	12 min	39.1 nM	4
	HEPES buffer (20 mM, pH 7.4, containing 20% DMSO)	630/805	10 min	0.5 μM	5
	DMF/PBS buffer (10 mM, pH 7.4, 1:1, v/v)	741	5 min	96 nM	6
	PBS buffer (10 mM, pH 7.4)	560	30 min	80 nM	7
	PBS buffer (10 mM, pH 7.4, containing 1% DMSO)	668	10 min	14.8 nM	8
	EtOH/HEPES buffer (10 mM, pH 7.4, 3:7, v/v)	736	20 min	20 nM	9
	DMSO/PBS buffer (pH 7.4, 2:8, v/v)	644	3 min	88 nM	10
	PBS buffer (10 mM, pH 7.4)	456/600	5 min	47 nM	This work

References

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