Supporting Information for

Development of a New Hydrogen Sulfide Fluorescent Probe Based on Coumarin–Chalcone Fluorescence Platform and Its Imaging Application

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Experimental Section

1. Experimental instruments and reagents

Common reagents or materials were obtained from commercial suppliers without further purification except as otherwise noted. ^{1}H NMR and ^{13}C NMR data were obtained on Bruker Avance III HD 500 MHz NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal reference. Agilent 6520a Q-TOF LC/ MS was used to obtain high-resolution mass spectrometry data (HRMS). The UV absorption spectra were measured by a Shimadzu UV-2700 UV–visible spectrophotometer, and the fluorescence emission spectra were recorded by a Hitachi F-4700 fluorescence spectrophotometer. Fluorescence imaging experiments were performed with a TCS-SP8 DIVE confocal fluorescence microscope in Leica, Germany. PHS-3e in China was used to measure pH values.

2. Synthesis and characterization of C-HS.

Synthesis of Compound 1. 4-(diethylamino)salicylaldehyde (6.3 mmol, 1.2 g) and ethyl acetate (9 mmol, 1.2 g) were dissolved in 15 mL ethanol, 0.5 mL piperidine was added, and then the mixture was refluxed for 2 h. Cool to room temperature and extract under reduced pressure to obtain yellow solid compound **1** (1.42 g, 97%). The crude product is directly used in the next reaction without

further purification.

Synthesis of Compound 2. Compound **1** (6 mmol, 1.55 g) and *p*-hydroxybenzaldehyde (6 mmol, 0.732 g) were dissolved in 30 mL acetonitrile, 200 μL piperidine was added, and the mixture was reflux for 11 h. After cooling to room temperature, the mixture was filtered, washed with 10 mL acetonitrile, and dried under pressure to obtain yellow powder compound **2** (1.23 g, 56%, m. p. 264℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.57 (s, 1H), 7.79 (d, *J* = 15.7 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.81 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.60 (d, *J* = 2.1 Hz, 1H), 3.50 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). HRMS (ESI): m/z calculated for $[M+H]^+$ $C_{22}H_{22}NO_4^+$, 364.1543; found, 364.1544.

Synthesis of Probe C-HS. Compound **2** (0.363 g, 1 mmol) and triethylamine were dissolved in 20 mL trichloromethane. After refluxing for 5 min, 2,4-dinitrobenzenesulfonyl chloride (0.535 g, 2 mmol) was added and refluxed for 5 h. After cooling to room temperature, filtration, ethanol washing, and drying were performed to obtain crude products. The crude product was purified on a silica gel column ($V_{\text{ethyl acetate}}$: $V_{\text{petroleum ether}}$ = 1:1) to obtain a red solid probe **C-HS** with a yield of 40% (0.300 g, m. p. 225℃). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.12 (d, J = 2.3 Hz, 1H), 8.61 (m, 1H), 8.60 (s, 1H), 8.27 (d, *J* = 8.7 Hz, 1H), 7.94 (d, *J* = 15.8 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 9.1 Hz, 1H), 7.66 (d, *J* = 15.8 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 2H), 6.82 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.61 (d, *J* = 2.3 Hz, 1H), 3.51 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.33, 159.92, 158.26, 153.10, 151.54, 149.33, 148.54, 148.09, 139.68, 135.05, 133.63, 132.43, 130.62, 130.32, 127.50, 126.66, 122.60, 121.09, 115.19, 110.28, 107.91, 95.90, 44.47, 12.34. HRMS (ESI): m/z calculated for $[M+H]^+$ $C_{28}H_{24}N_3O_{10}S$, 594.1177; found, 594.1177.

3. General procedure for spectral measurement of C-HS in response to H_2S.

Dissolve the PBS (phosphate buffered saline) powder in ultrapure water and transfer it to a volumetric flask with a constant volume of 2 L. After standing, the pH was measured as 7.42 with a pH meter and sealed for use.

A 1.2 mg compound of **C-HS** was accurately weighed, dissolved in 2 mL DMSO, and evenly shaken to prepare 1 mM probe mother liquor, which was sealed for later use.

4. The detection limit

According to the absorption titration curve of **C-HS** in the presence of different concentrations of H_2S, the detection limit can be calculated by the following equation:

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Here, σ represents the standard deviation of the blank measurements, and k represents the slope of the curve of absorption intensity as a function of H_2S concentrations. The absorption spectrum of the blank sample was measured eleven times to calculate the standard deviation.

5. Calculation of fluorescence quantum yield

For fluorescence quantum yield experiments in DMSO solution, the fluorescence quantum yields of the probe and the product were evaluated by using fluorescein (Φf =79% in 0.1M NaOH at 25°C) as a reference standard (J. Am. Chem. Soc., 1945, 1099). The quantum yield calculation formula is as follows:

$$\Phi_x=\Phi_s(n_x/n_s)^2(A_s/A_x)(F_x/F_s)$$

where Φ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and the unknown, respectively.

6. Culture and preparation of the HeLa cells

The HeLa cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37°C. Before the experiments, seed the HeLa cells in 35 mm glass-bottomed dishes at a density of 2×10^5 cells per dish in 2 mL of culture medium and incubate them inside an incubator containing 5% CO_2 and 95% air at 37°C. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

7. Cytotoxicity assay.

Hela cells were inoculated into 96-well plates, 100 μL PBS was added to the periphery of 96-well plates, and 0–50 μM **C-HS** (99.9% DMEM and 0.1% DMSO) was added to the rest of the plates, respectively. Subsequently, the cells were cultured at 37°C in 5% CO_2 and 95% air for 24 h. Then, the liquid was sucked up, and MTT and fresh culture medium were added into the well for further incubation for 4 h. Finally, the culture medium was removed. Add 100 μL DMSO to each well and shake well to dissolve the crystal completely. The absorbance of the solution was measured at 492 nm with a microplate reader. The toxicity of **C-HS** is calculated as follows.

$$\text{Cell survival rate (\%)} = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\ \%$$

OD_{sample} refers to cells incubated with different concentrations of probes, $OD_{control}$ refers to cells without probes, and OD_{blank} refers to pores containing only media.

8. Imaging of exogenous H_2S.

The first group: cells were cultured with 10 μM **C-HS** for 30 min. The second group: cells were cultured with 10 μM **C-HS** for 30 min and then cultured with 20 μM NaHS for 30 min. The third group: cells were cultured with 10 μM **C-HS** for 30 min and then cultured with 50 μM NaHS for 30 min. The fourth group: cells were cultured with 10 μM **C-HS** for 30 min, then 100 μM NaHS for 30 min.

9. Imaging of endogenous H_2S.

The first group: cells were cultured with 10 μM **C-HS** for 30 min. The second group: cells were cultured with 100 μM $ZnCl_2$ for 30 min. The third group: cells were cultured with 100 μM $ZnCl_2$ for 30 min, then 10 μM **C-HS** for another 30 min. Group 4: Cells were co-cultured with 100 μM $ZnCl_2$, 10 μM **C-HS,** and 100 μM NaHS for 30 min.

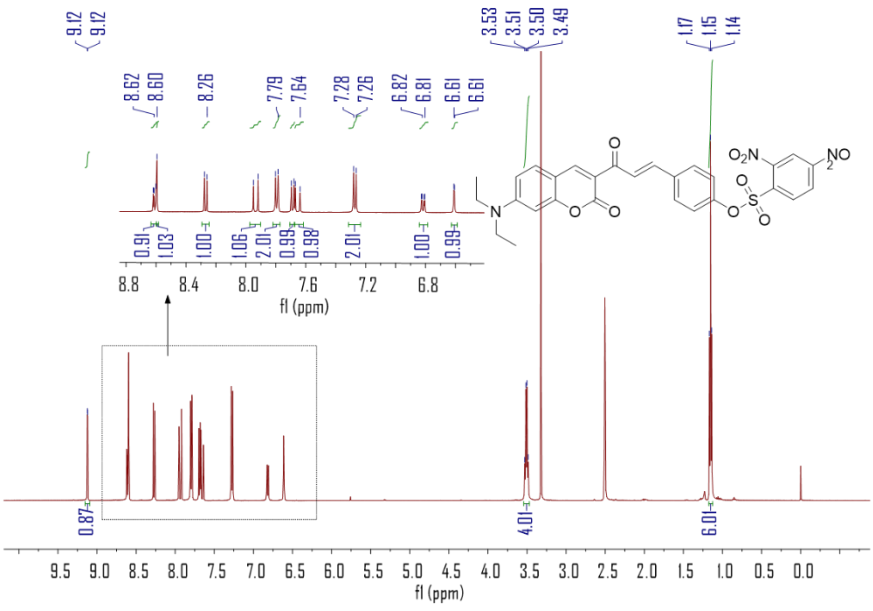

Figure S1. The ^1H NMR of **C-HS**.

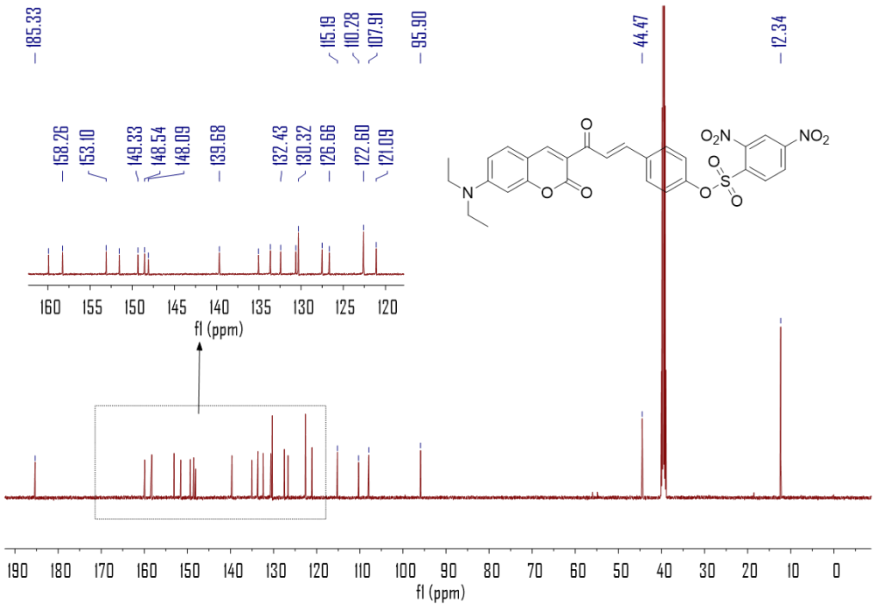

Figure S2. The ^{13}C NMR of **C-HS**.

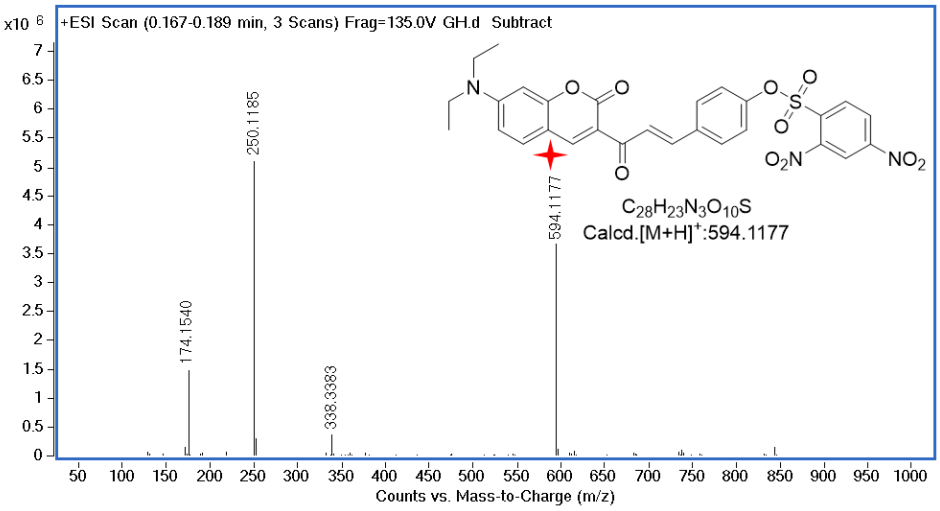

Figure S3. ESI-MS spectrum of **C-HS**.

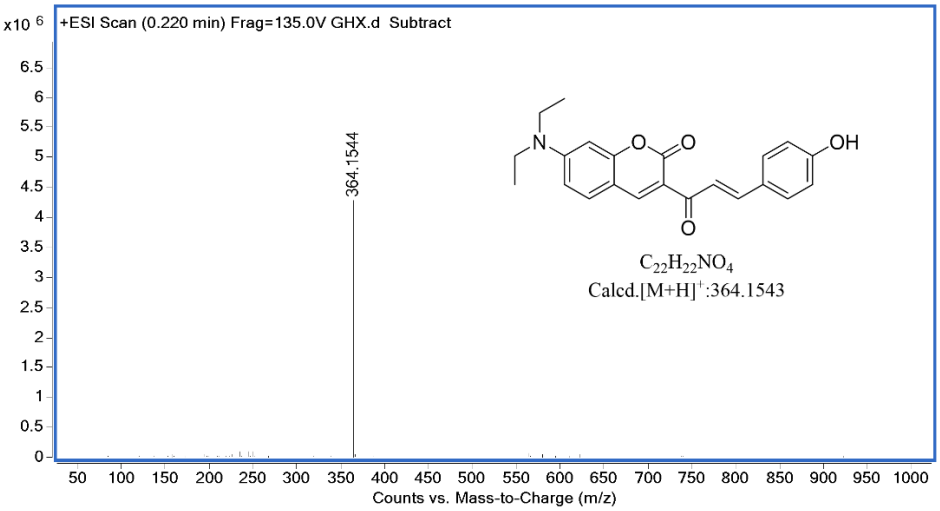

Figure S4. HRMS (ESI) spectra of **C-HS** and H_2S after reaction.