Supporting Information for

A PET-based lysosome-targeted turn-on fluorescent probe for the detection of H_2S and its bioimaging application in living cells and zebrafish

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Instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Uv/Vis absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Fluorescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the detection limit

The detection limit was determined from the fluorescence titration data based on a reported method. **CMDN** (10.0 μM) was titrated with different concentrations of H_2S, the linear relationship between the values of emission intensity at 460 nm and the concentration of H_2S was fitted based on the fluorescence titration.

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

Where σ is the standard deviation of the blank sample and ‘k’ is the slope of the linear regression equation.

Cells culture

HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sijiqing) at 37 ℃ and 5% CO_2. Before the imaging experiments, 1 mL of HeLa cells were subcultured and seeded in the glass bottom culture dishes at a density of 1×10^5. About 36 h later, the cells reached about 75 % confluence for the further experiments.

Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 ℃ and 5% CO_2 atmosphere for overnight and then incubated for 24 hours in the presence of **CMDN** at different concentrations (0, 1, 2, 5, 10 and 20 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/ mL) was added to each well and incubated for 4 hours. Violet formazan was dissolved in 500 μL sodium dodecyl sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **CMDN**.

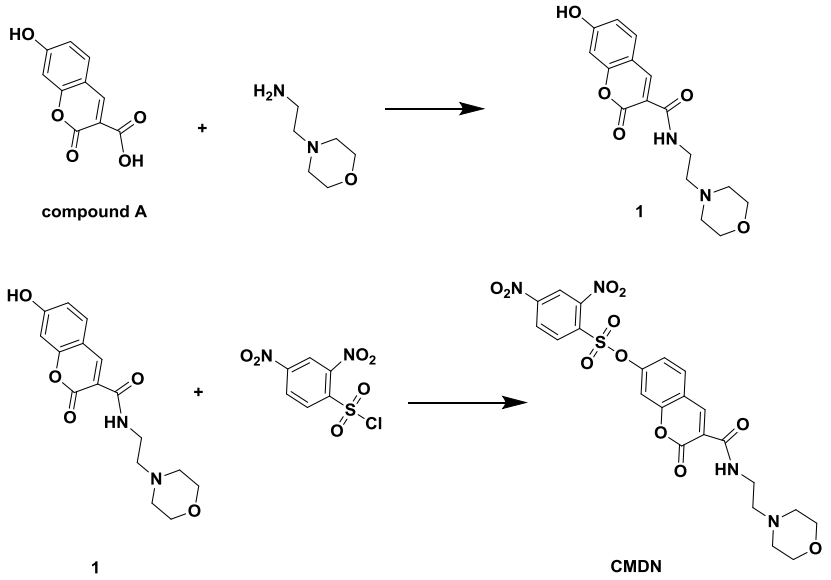
Fluorescence imaging of H_2S in living cells

About the cell imaging experiments, as the untreated group, HeLa cells were incubated with **CMDN** (10 μM) for 30 min, then washed by PBS buffer before imaging. For control group, HeLa cells were preincubated with 500 μM N-Ethylmaleimide (NEM) for 30 min, then incubated with **CMDN** (10 μM) for 30 min and washed by PBS

buffer before imaging. As the experimental groups, HeLa cells were pretreated with 500 μM N-Ethylmaleimide (NEM) for 30 min and 200 μM H_2S for 30 min successively, then incubated with **CMDN** (10 μM) for 30 min and washed by PBS buffer before imaging. The confocal microscopic imaging uses Nikon A1MP confocal microscope with an excitation filter of 405 nm and the collection wavelength range is from 425-475 nm (blue channel).

Fluorescence imaging of H_2S in zebrafish

About the zebrafish imaging experiments, as the control group, zebrafish was incubated with **CMDN** (10 μM) for 30 min, then washed by PBS buffer before imaging. As the experimental groups, zebrafish was preincubated with 200 μM H_2S for 30 min, incubated with **CMDN** (10 μM) for 30 min, and then washed by PBS buffer before imaging. The confocal microscopic imaging uses Nikon A1MP confocal microscope with an excitation filter of 405 nm and the collection wavelength range is from 425-475nm (blue channel).



Scheme S1. Synthesis of the chemosensor **CMDN** and the structure of compound **A** and **1**.

Synthesis of compound 1. Compound A (323.4 mg) with 3 mL DMF, puts it in flask, adds 1-hydroxybenzotriazole (0.2 g), adds 1-(3-dimethylaminopropyl)

-3-ethylcarbodimethylamine hydrochloride (0.3 g), stirs at room temperature for 15 minutes, then mixes evenly, adds 215 uL N-(2-aminoethyl) morpholine, stirs overnight at 50 ℃. After the reaction, absolute ethanol was added to the flask, yellow solid precipitated, vacuum drainage, and the solid was dried to obtain the crude product, which was separated and purified by silica gel column. (153.9 mg, yield: 65.8%).^{1}H NMR (400 MHz, DMSO) δ 11.07 (s, 1H), 8.86 (t, J = 5.3 Hz, 1H), 8.81 (s, 1H), 7.83 (d, J = 8.6 Hz, 1H), 6.88 (dd, J = 8.6, 2.2 Hz, 1H), 6.80 (d, J = 2.1 Hz, 1H), 3.64 - 3.54 (m, 4H), 3.44 (dd, J = 11.9, 6.1 Hz, 2H), 3.35 (s, 4H), 2.48 (d, J = 6.3 Hz, 2H), 2.43 (s, 2H). HRMS m/z calculated for $C_{16}H_{18}N_2O_5$ $[M + H]^+$: 319.1288. Found 319.1291.

Synthesis of CMDN. compound **1** (1 mmol), 2,4-dinitrobenzenesulfonyl chloride (1 mmol) and sodium carbonate (1 mmol) are placed in a flask, stirred for 5 h under the condition of tetrahydrofuran (5 mL), solid is precipitated, filtered and dried. The obtained solid is purified by column chromatography and the white product is **CMDN**. (94.7 mg, yield: 43.7%) ^{1}H NMR (400 MHz, DMSO) δ 9.15 (d, J = 2.3 Hz, 1H), 8.95 (s, 1H), 8.63 (s, 1H), 8.57 (s, 1H), 8.41 (dd, J = 8.6, 2.3 Hz, 1H), 8.35 (d, J = 8.7 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 7.53 (s, 1H), 7.31 (d, J = 8.6 Hz, 1H), 4.00 (d, J = 11.7 Hz, 2H), 3.71 (d, J = 6.0 Hz, 2H), 3.63 (d, J = 12.2 Hz, 2H), 3.55 (d, J = 12.1 Hz, 2H), 3.42 (s, 2H), 3.34 (d, J = 10.9 Hz, 2H).^{13}C NMR (101 MHz, DMSO) δ 162.14, 159.84, 154.88, 152.15, 151.92, 148.58, 147.88, 147.42, 145.27, 134.05, 132.88, 131.25, 131.14, 128.20, 126.09, 121.77, 119.67, 119.60, 118.78, 118.74, 110.87, 63.75, 55.78, 55.16, 52.03, 34.42, 30.96, 26.57, 22.10, 14.31. HRMS m/z calculated for $C_{22}H_{20}N_4O_{11}S$ $[M+H]^+$: 549.0922. Found: 549.0926.

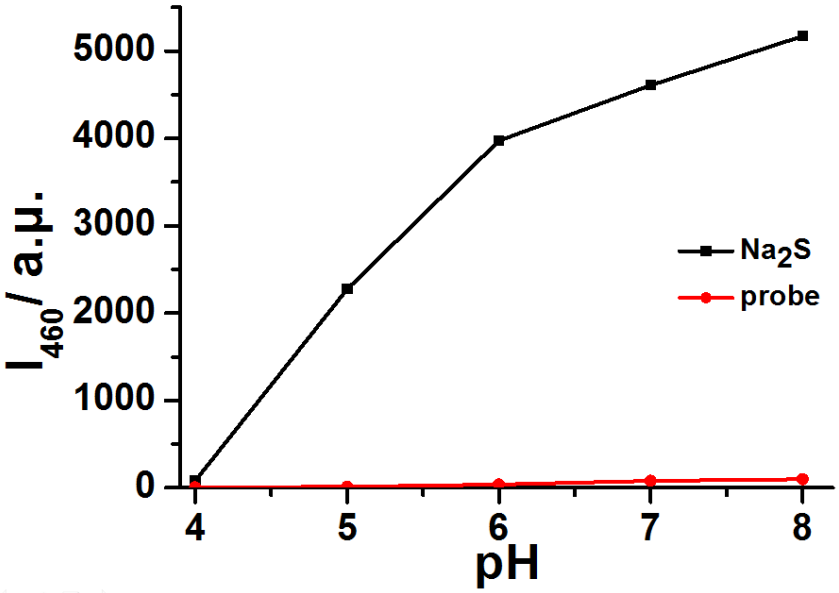


Fig. S1 The pH influence on the fluorescence intensity (I_{460}) of **CMDN** (10.0 μM) in the absence or presence of Na_2S (100 μM).

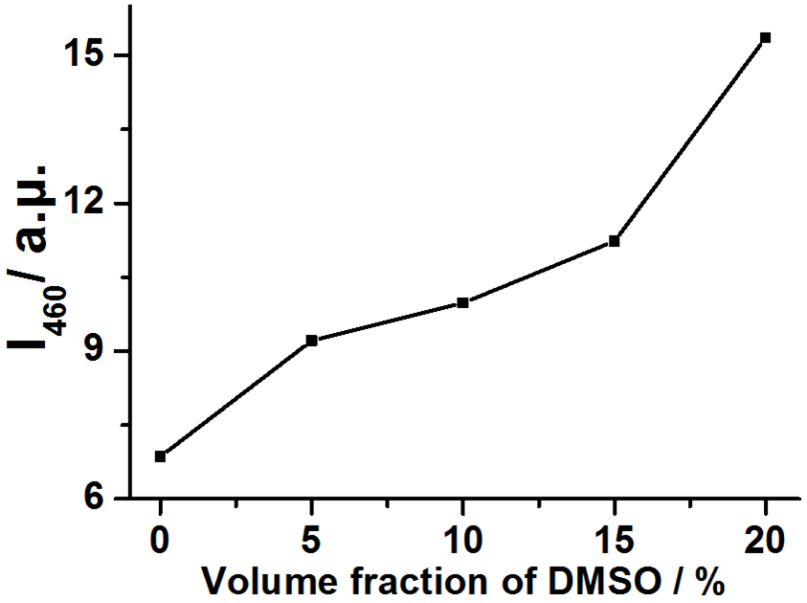


Fig. S2 The fluorescence intensity of probe varies with the volume ratio of DMSO and PBS buffer solution. λ_{ex} = 405 nm and λ_{em} = 460 nm.

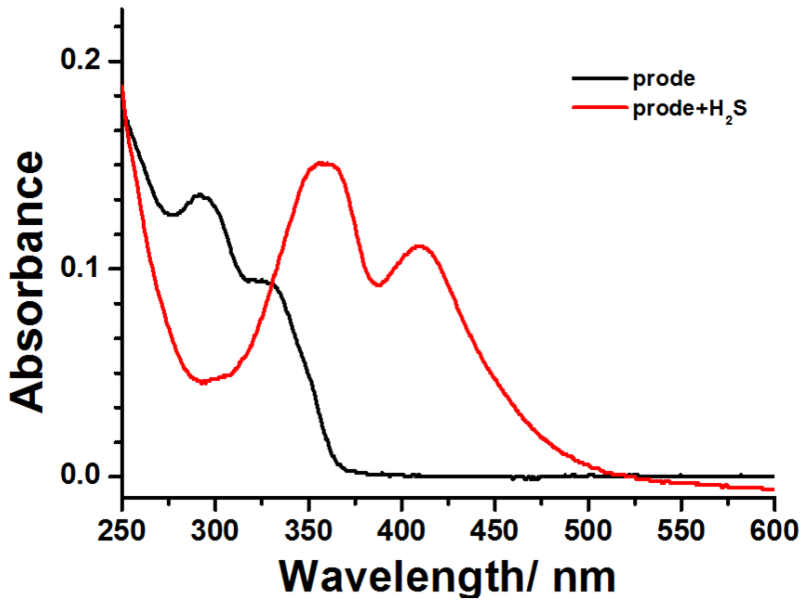


Fig. S3 UV/Vis spectra of **CMDN** and in the prescence of 10 μM Na_2S in PBS buffer (pH 5, 20% DMSO).

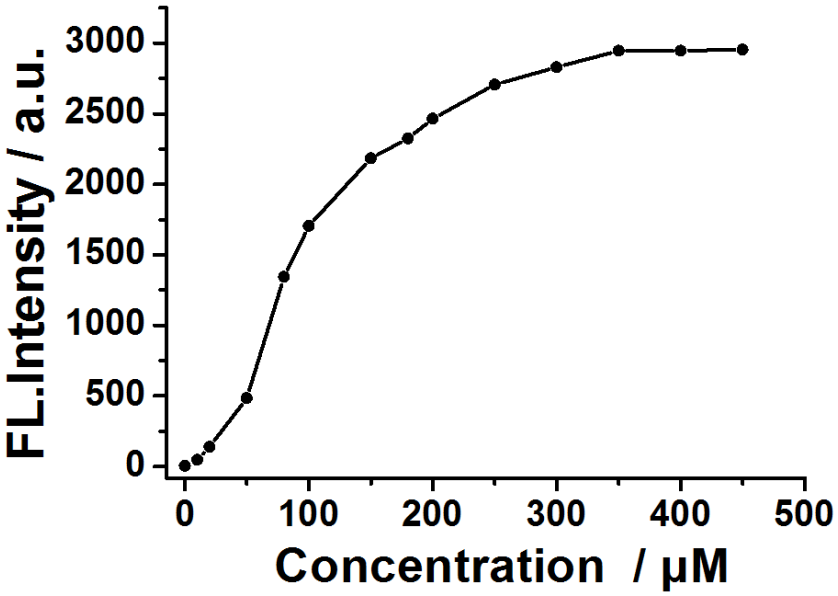


Fig. S4 The fluorescent intensity changing with Na_2S to **CMDN** ratio. 10 μM of **CMDN** titrated with increasing concentrations of Na_2S. λ_{ex} = 405 nm and λ_{em} = 460 nm.

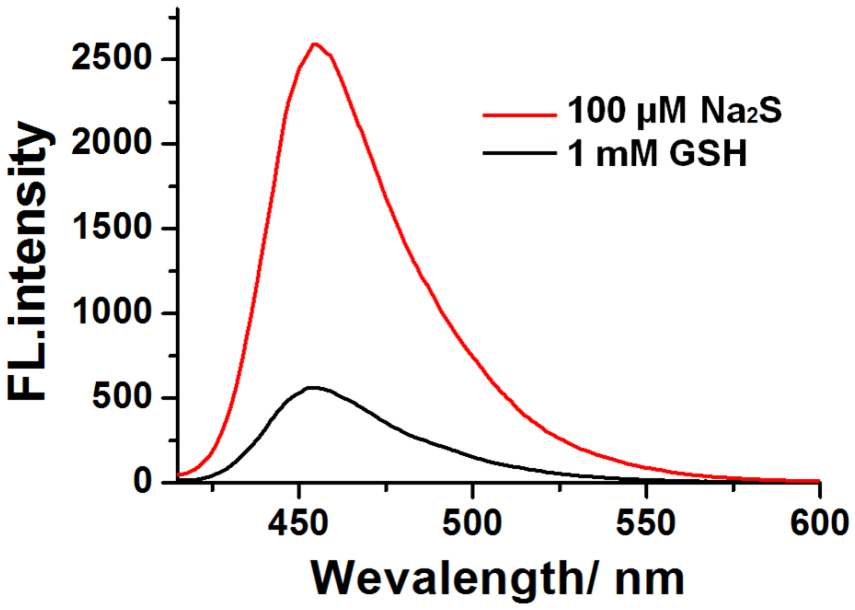


Fig. S5 Fluorescent spectra of the probe **CMDN** (10.0 μM) in the presence of 100 μM Na_2S and 1 mM GSH in PBS buffer (pH 5, 20% DMSO).

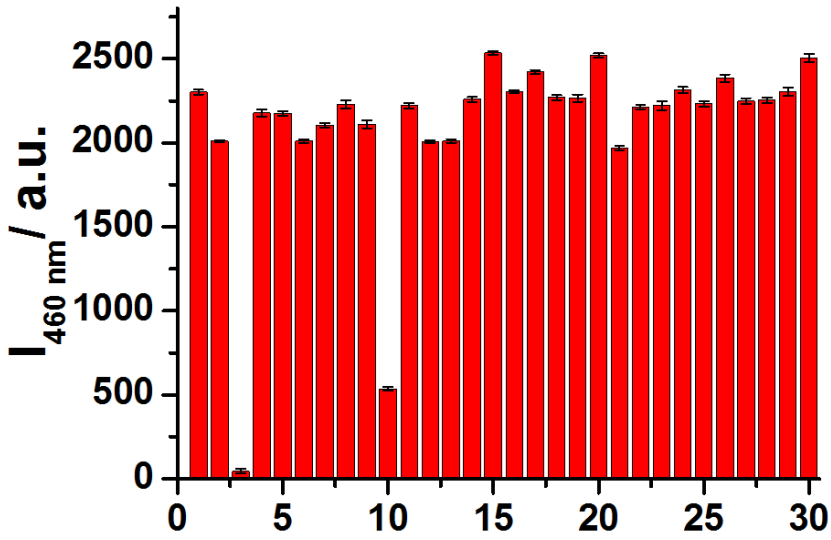


Fig. S6 Fluorescence intensity ratios of CMDN treated with various species in the presence of interferers (1, PBS; 2, $CaCl_2$; 3, $AlCl_3$; 4, KCl; 5, KI; 6, KNO_3; 7, NaF; 8, NaCl; 9, NaBr; 10, $BaCl_2$; 11, NaI; 12, $MgCl_2$; 13, Na_2SO_3; 14, Ser; 15, Ala; 16, Thr; 17, Glu; 18, Gln; 19, Arg; 20, Val 21, Asp; 22, Leu; 23, LLe; 24, His; 25, Gly; 26, HClO; 27, Cys; 28, GSH; 29, Hcy; 30, Na_2S) in PBS buffer (pH 5, 20% DMSO) under λ_{ex} at 405 nm.

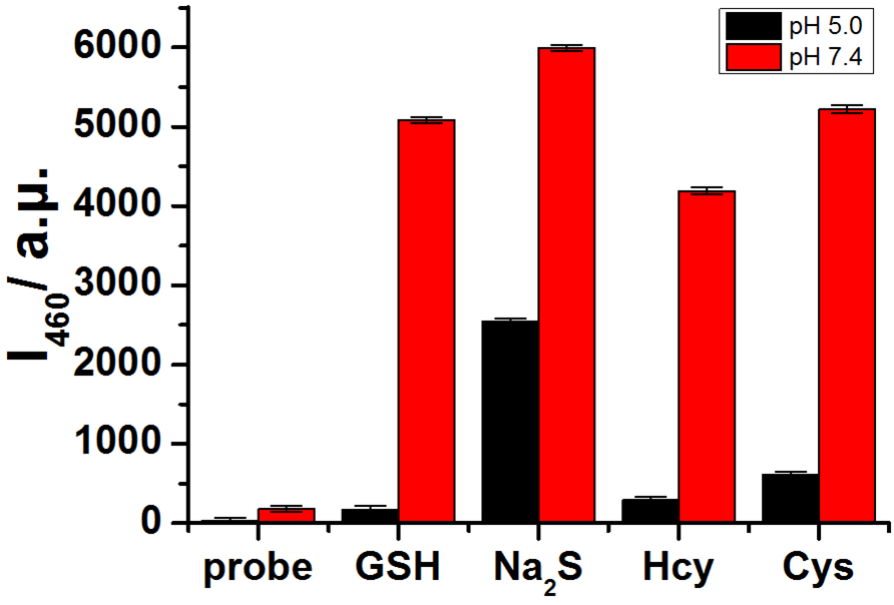


Fig. S7 Fluorescence intensity of **CMDN** detected in PBS buffer solutions at pH 5.0 and 7.4 in the presence of Na_2S, Cys, Hcy and GSH. λ_{ex} = 405 nm and λ_{em} = 460 nm.

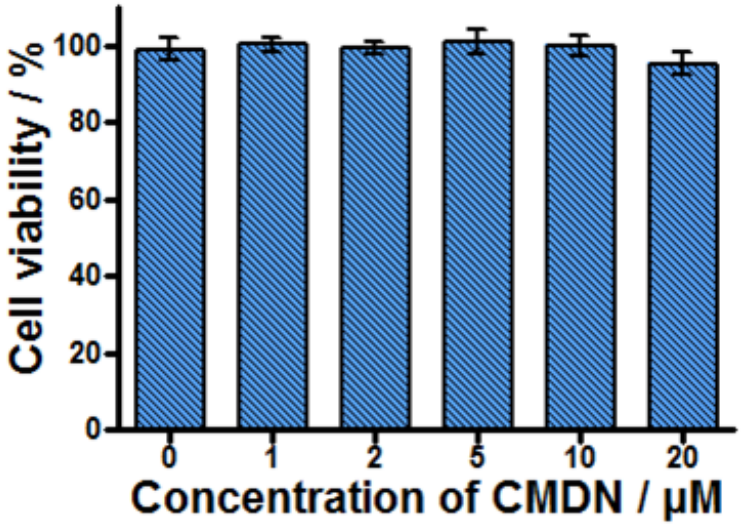


Fig. S8 Cell viability of HeLa cells incubated with probe **CMDN** of different concentration (0, 1, 2, 5, 10 or 20 μM) for 24 h.

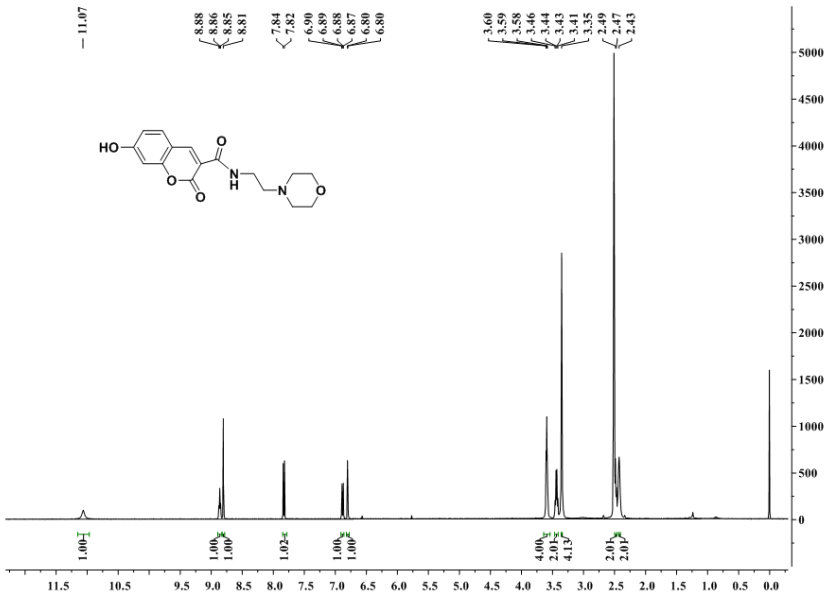


Fig. S9 ^{1}H NMR spectrum of compound **1** in DMSO-*d6*.

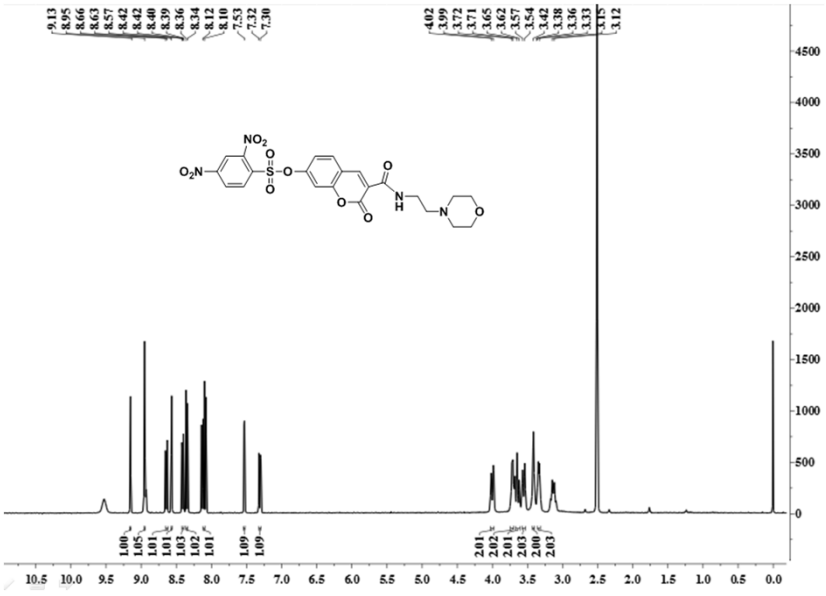


Fig. S10 ^{1}H NMR spectrum of compound **CMDN** in DMSO-*d6*.

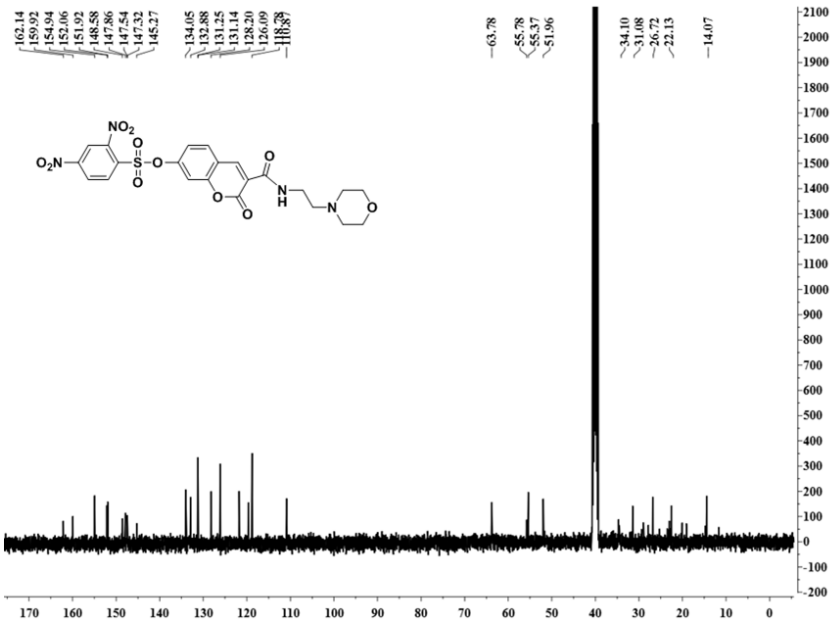


Fig. S11 ^{13}C NMR spectrum of compound **CMDN** in DMSO-*d6*.

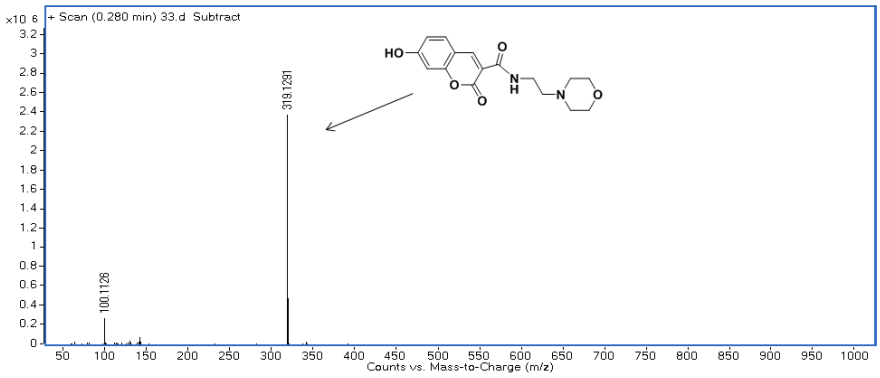


Fig. S12 HRMS spectrum of compound **1**. Compound **1**: $C_{16}H_{18}N_2O_5$; HRMS m/z calculated for $C_{16}H_{18}N_2O_5$, $[M + H]^+$: 319.1288. Found 319.1291.

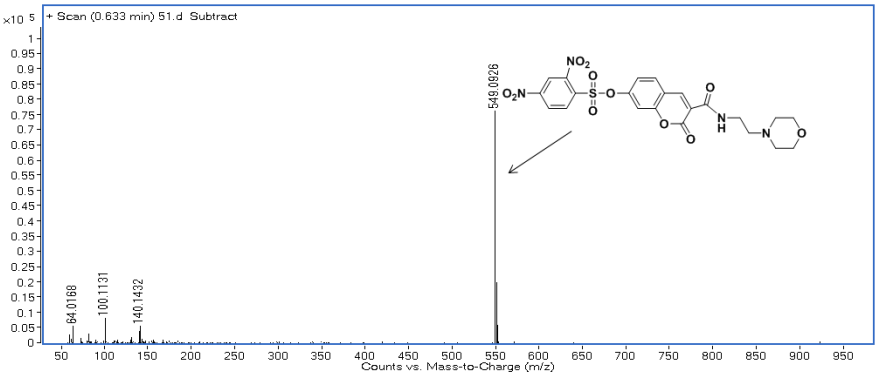


Fig. S13 HRMS spectrum of compound **CMDN**. Compound **CMDN**: $C_{22}H_{20}N_4O_{11}S$;

HRMS m/z calculated for $C_{23}H_{22}N_4O_{10}S$, $[M + H]^+$: 549.0922. Found 549.0926.