Electronic Supplementary Information

Near-infrared Fluorescent Probe for Detecting Hydrogen Sulfide with High Selectivity in Cells and Ulcerative Colitis Mice

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Table of contents

1.The comparison of H_2S probes. S3

2. Experimental Section S5

3. Synthesis S9

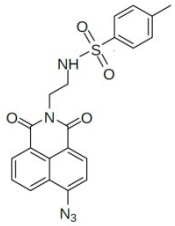
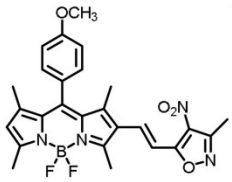
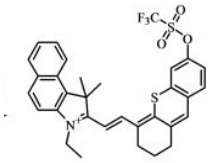
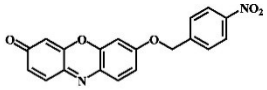
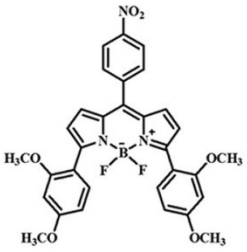
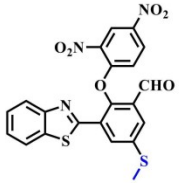
4. Spectral data. S12

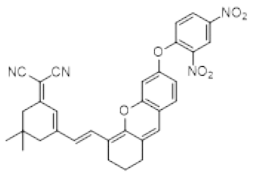
5. Response mechanism. S13

6. Biological assays. S14

1. The comparison of H_2S probes.

Table S1 Comparison of some reported probes for the detection of H_2S

Probe	λ_{ex} (nm)	λ_{em} (nm)	Stokes shift (nm)	Application	Response time (s)	Reference
	400	548	148	Cells imaging	1800	Ref. 20
	485	522	37	Cells imaging	55	Ref. 23
	380	455	75	Cells imaging	300	Ref. 24
	550	586	36	Paper chips and zebrafish imaging	/	Ref. 25
	575	625	50	cells imaging and tissues imaging	/	Ref. 26
	380	598	218	Cells imaging	720	Ref. 27

	584	770	186	Cells imaging and imaging of ulcerative colitis mice	200	This work

2. Experimental Section

Reagents and Instruments. Cyclohexanone, phosphorus tribromide (PBr_3), 2-hydroxy-4-methoxybenzaldehyde, cesium carbonate (Cs_2CO_3), boron tribromide (BBr_3), piperidine, isophorone, malononitrile were purchased from Aladdin (Shanghai, China). 2, 4-dinitrofluorobenzene, NaHS, 2-(aminooxy)acetic acid (AOAA) were bought from Macklin (Shanghai, China). Dextran sulfate sodium (DSS) were purchased from Aladdin (Shanghai, China). Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification.

Nuclear magnetic resonance (NMR) spectra were carried out on a Bruker Avance II NMR spectrometer (Germany). Mass spectra (MS) was acquired on a Bruker Autoflex MALDI-TOF mass spectrometer (Germany). Elemental analysis was obtained on PerkinElmer 2400 elemental analyzer (USA). The absorption spectra were collected on an Agilent CARY 60 UV-vis spectrophotometer (USA). The fluorescence spectra were determined by using a Hitachi F-4600 spectrophotometer (Japan). High-performance liquid chromatography (HPLC) experiments were conducted on LC-20A with a C18 column (Japan). The fluorescence images of cells were determined by using a Nikon confocal fluorescence microscope (Japan). The fluorescence images of mice were operated on an IVIS Lumina XR small animal optical in vivo imaging system (USA).

Fluorescence Detection of H_2S in Solution. First, IX-H_2S stock solution (100 μM) and H_2S stock solution (1 mM) were arranged in DMF and water, respectively. The

measured solutions were prepared by diluting IX-H_2S stock solution and H_2S stock solution with PBS buffer solution (pH 7.4) in volumetric flask to obtain the corresponding concentration. The final concentration of probe was 10 μM and the H_2S concentration was 5.0×10^{-5} to 1.0×10^{-6} M. The slit width was set to 10 nm/10 nm and fluorescence spectra in the range of 695.0-850.0 nm were recorded under the excitation at 584 nm at room temperature.

Cell Culture and Cytotoxicity Assay. The cells utilized were obtained from the State Key Laboratory of Chemistry/Biosensing and Chemometrics, Hunan University, and were grown in DMEM (Dulbecco's modified Eagle's medium) medium supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 at 37 °C. To evaluate cytotoxicity, MTT assay were performed after incubation with different concentrations of probe (0, 5, 10, 15, 20, 25, 30 μM).

Fluorescence Imaging of H_2S in Cells. 293T cells (human embryonic kidney cell line) and HCT116 cells (human colorectal carcinoma cells) were exploited to image H_2S in cells.

For imaging exogenous H_2S, 293T cells were first directly subjected to confocal imaging as a cell blank to assess background fluorescence. Then, one group of cells were staining with IX-H_2S for 15 min to obtain fluorescence images. Meantime, another two groups of cells were pretreated with NaHS (20 μM and 50 μM, individually) for 30 min and then treated with IX-H_2S for 15 min.

For endogenous H_2S imaging with IX-H_2S, HCT116 cells were treated differently as follows. One group of cells were stained with IX-H_2S for 15 min. Another two group of cells was pretreated with different concentrations of Cys (100 and 200 μM, individually) for 60 min, and then treated with IX-H_2S. In addition, the last group of cells were sequentially treated with AOAA (200 μM) and Cys (200 μM) for 60 min, and then incubated with IX-H_2S.

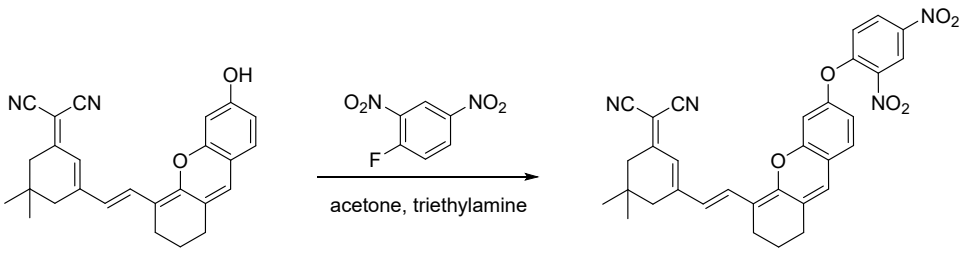
Fluorescence Imaging of of H_2S in Mice. All animal manipulations were performed in accordance with the regulations issued by the Ethics Committee of Hunan Slack Jingda Experimental Animal Co., Ltd. (Changsha, China). BALB/c nude mice and C57BL/6 mice were used and maintained in good condition for all experiments.

To perform imaging of exogenous H_2S, the BALB/c nude mice was subcutaneously injected with IX-H_2S (200 μM) at the dark blue dotted circle in the left hind leg, followed by PBS buffer (pH 7.4, 100 μL) injection in the same area for imaging. Similarly, IX-H_2S (200 μM) was injected subcutaneously in the right hind leg with the red dotted circle, and fluorescence images were obtained at distinct time points (0, 5, 10, 15 and 20 min) after injection of NaSH (1 mM, 100 μL) at the same location.

To image endogenous H_2S in vivo, C57BL/6 mice were divided into two groups. One group of mice were drank deionized water as a control group. Another group of mice were drank 5% dextran sodium sulfate (DSS) solution for one week to establish the mice model of ulcerative colitis (UC). Two groups of mice were subjected to intraperitoneal injection

of the IX-H_2S, and the images were acquired at 0, 5, 10, 15, 20, 25 and 30 min, respectively.

3. Synthesis



IX-OH **IX-H_2S**

Scheme S1. Synthesis of probe IX-H2S.

IX-OH was synthesized according to previous work (Analyst. 2021, 146, 118-123). The fluorescence quantum yield of IX-OH is 0.27. IX-H_2S was synthesized through a route described in Scheme S1.Compound IX-OH (0.10 g, 0.25 mmol) and 2,4-dinitrofluorobenzene (0.05 g, 0.25 mmol) was dissolved in acetone (5 mL), and triethylamine (0.5 mL) was pipetted into the reaction flask subsequently. Then the mixed solution was refluxed at 65°C for 0.5 h. Acetone was removed by evaporation, followed by the addition of 10 mL of 5% HCl solution. The precipitate was filtered and washed several times with water. The crude product was purified by recrystallization in acetone to yield a dark green solid. Yield: 0.11 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.56-7.45 (m, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.86 (s, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.40 (t, *J* = 8.0 Hz, 2H), 2.55 (d, *J* = 4.0 Hz, 4H), 2.47 (s, 2H), 2.42 (s, 2H), 1.88-1.80 (m, 2H), 1.04 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 155.5, 154.9, 154.2, 153.8, 150.0, 141.9, 139.7, 131.3, 130.2, 128.8, 127.5, 127.0, 122.5, 121.6, 119.8, 118.8, 114.9, 113.2, 107.7, 100.0, 76.2, 43.0, 39.2, 32.0, 29.9, 28.0, 24.7, 20.7. MS (TOF):

562.1. Elem. anal. (%) calcd. for $C_{32}H_{26}N_4O_6$: C, 68.32, H, 4.66, N, 9.96. Found: C, 68.28, H, 4.52, N, 10.02.

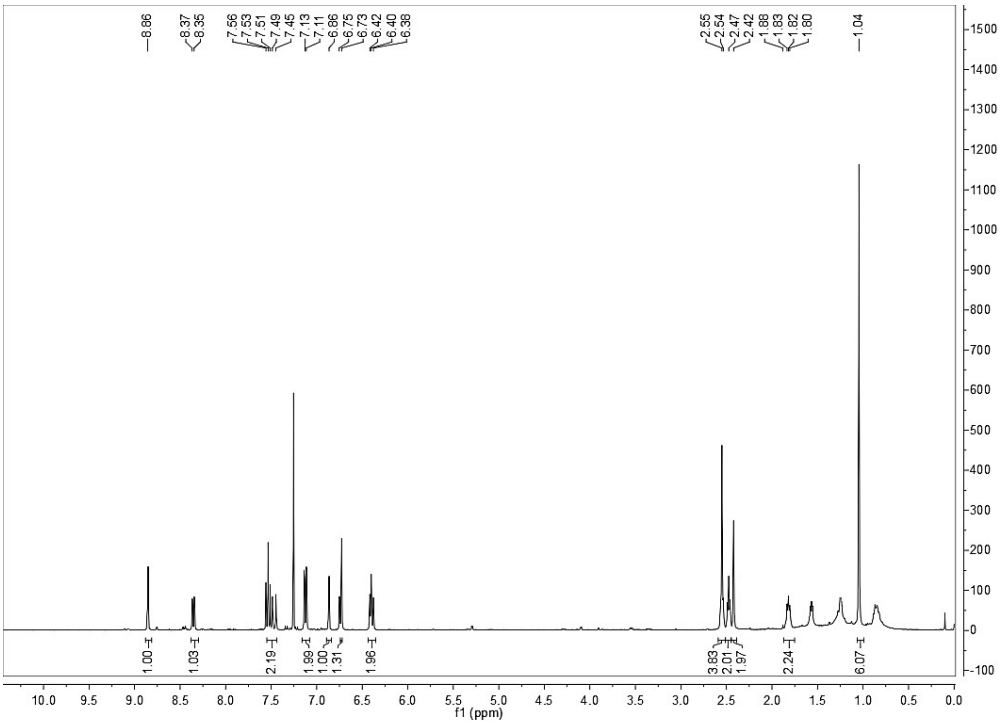


Fig. S1. ^{1}H NMR spectra of IX-H_2S in $CDCl_3$.

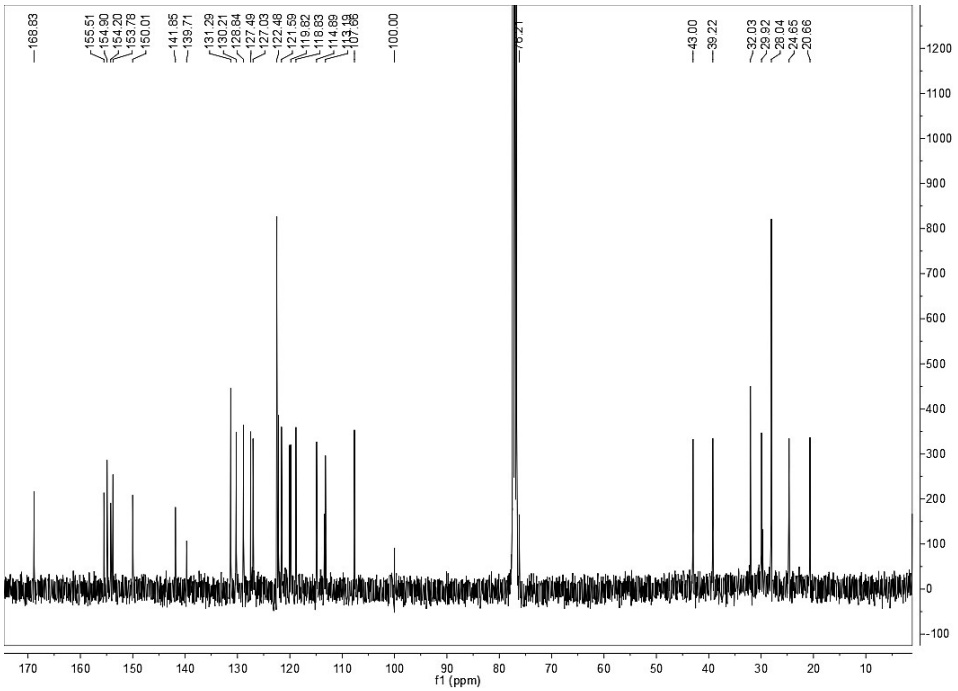


Fig. S2. ^{13}C NMR spectra of IX-H_2S in $CDCl_3$.

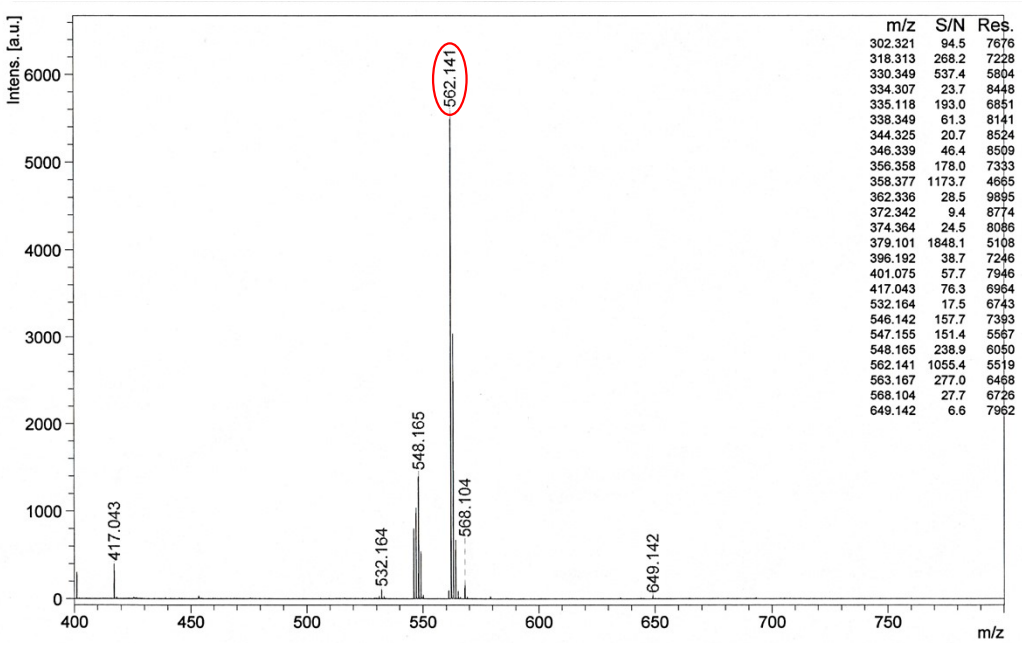


Fig. S3. Mass spectra of IX-H_2S. MS (TOF) for $C_{32}H_{26}N_4O_6$: m/z Found, 562.1 (Calcd, 562.1).

4. Spectral data.

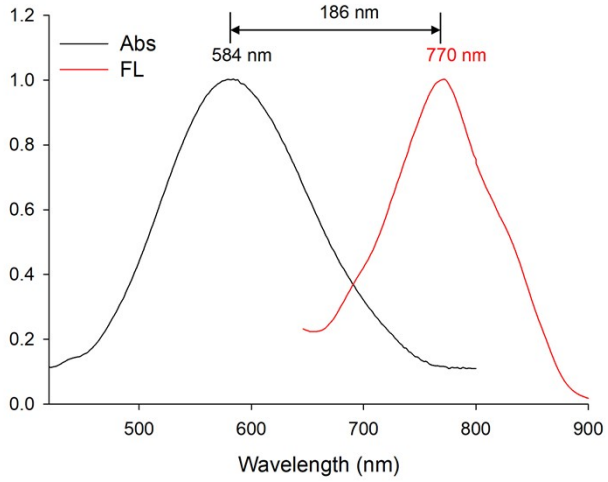


Fig. S4. The normalized absorption (black line) and fluorescence (red line) spectra of IX-OH at pH 7.4.

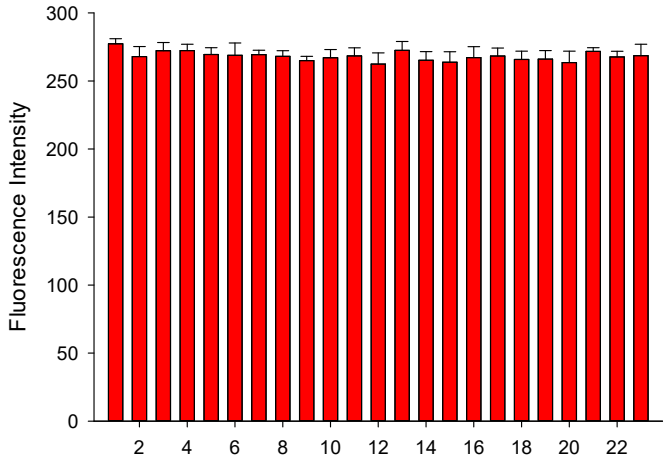


Fig. S5. Fluorescence intensity of IX-H_2S (10 μM) with various analytes in the presence of H_2S (50 μM): 1.Blank; 2.Cys; 3.Hcy; 4.GSH; 5.$S_2O_3^{2-}$; 6.SO_4^{2-}; 7.CO_3^{2-}; 8. HCO_3^-; 9.ClO^-; 10.NO_3^-; 11.NO_2^-; 12.$ONOO^-$; 13.Cl^-; 14.Br^-; 15.I^-; 16.NH_4^+; 17.K^+; 18.Na^+; 19.Mg^{2+}; 20.Ca^{2+}; 21.Zn^{2+}; 22.Fe^{2+}; 23.Cu^{2+}. Cys, Hcy and GSH are 5 mM and other analytes are 200 μM.

5. Response mechanism.

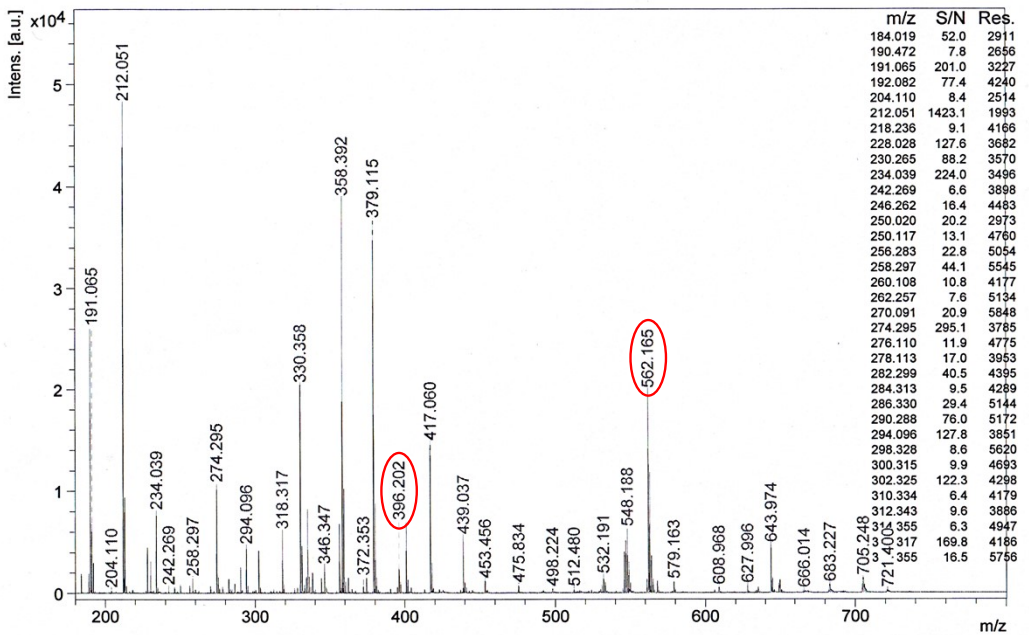


Fig. S6. Mass spectra of IX-H_2S with H_2S. MS (TOF) for $C_{26}H_{24}N_2O_2$: m/z Found, 396.2 (Calcd, 396.2).

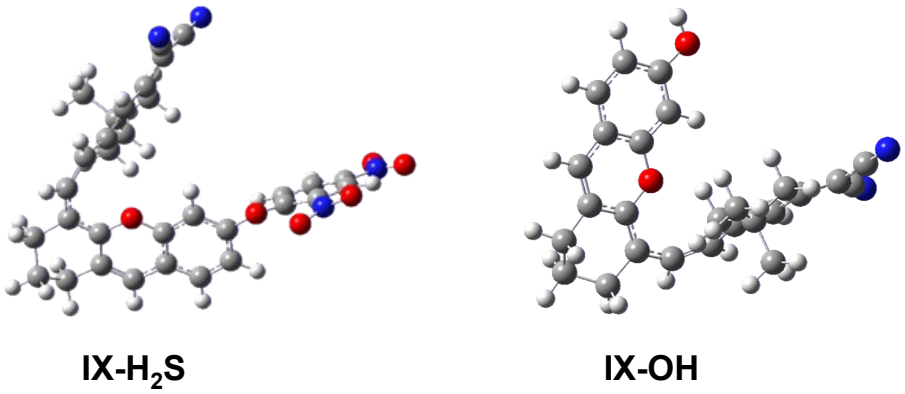


Fig. S7. The optimized structures of IX-H_2S and IX-OH. In the ball-and-stick model, carbon, oxygen and nitrogen atoms are colored in gray, red and blue, respectively.

6. Biological assays.

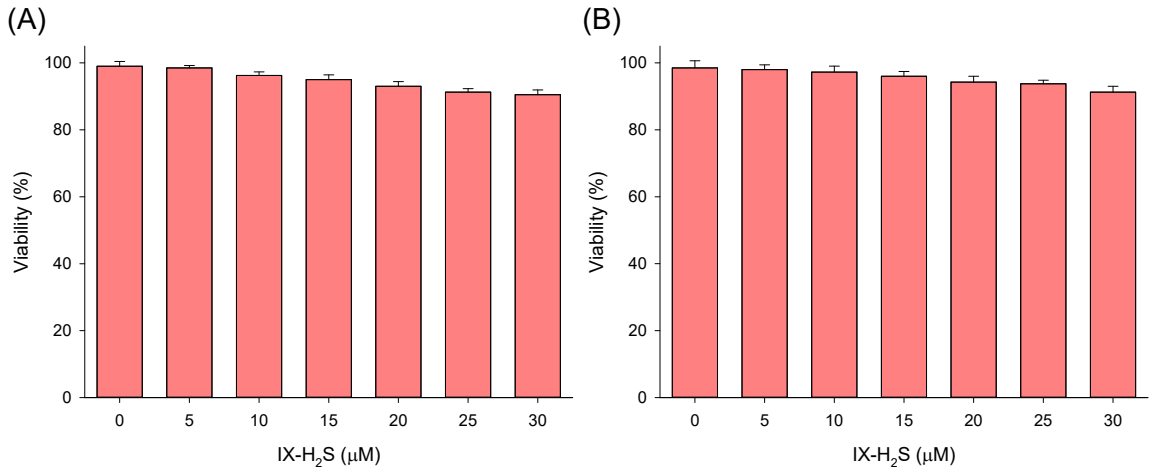


Fig. S8. MTT assay for estimating cell viability (%) of (A) 293T cells and (B) HCT116 cells treated with various concentrations of IX-H_2S (0-30 μM) after 24 h incubation.

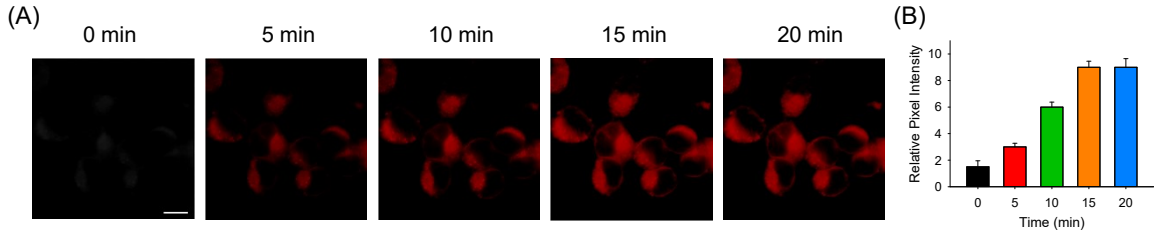


Fig. S9. (A) Fluorescence imaging of exogenous H_2S in 293T cells. The cells were pretreated with NaHS (50 μM) for 30 min, and then incubated with IX-H_2S (10 μM) at different time points: 0, 5, 10, 15, 20 min. (B) Relative pixel intensity in (A). λ_{ex} = 568 nm, λ_{em} = 750-850 nm; Scale bar: 10 μm.

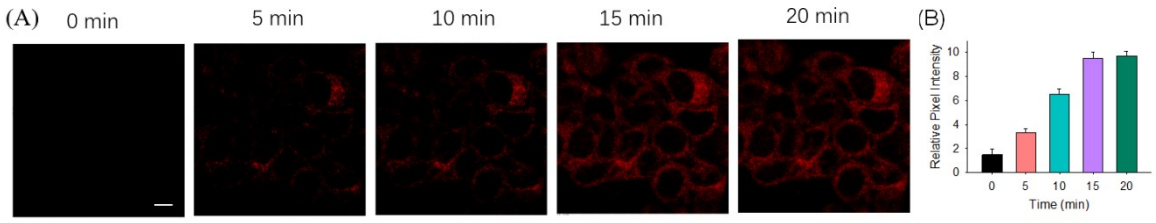


Fig. S10. (A) Fluorescence imaging of endogenous H_2S in HCT116 cells. The cells were stimulated with Cys (200 μM) for 60 min and then incubated with IX-H_2S (10 μM) at different time points: 0, 5, 10, 15, 20 min. (B) Relative pixel intensity in (A). λ_{ex} = 568 nm, λ_{em} = 750-850 nm; Scale bar: 10 μm.