Supporting Information

Synthesis of a fluorescent probe based on Rhodol's highly selective recognition of H_2S and its application in cells

Contents

Materials and Instruments

Phthalic anhydride (99.7%), m-hydroxy-N,N-diethylbenzeneamine (95%), catechol (99%), and 2-chloro-1,4-naphthoquinone (98%) along with other analytical reagents were commercially obtained and used as received. NMR data were collected using $CDCl_3$ as solvents on a 600 MHz NMR spectrometer, with high-resolution mass spectra (HR-MS) measured at 298K on a WNMR-I 500MHz spectrometer. UV-visible absorption spectra were recorded using a UV-2550 spectrophotometer (Shimadzu, Japan), and fluorescence spectra at room temperature were recorded using a Cary Eclipse fluorescence spectrometer (Agilent Technologies, USA), with an excitation wavelength set at 490 nm. The cytotoxic effects of compound **TF-1** on cells were determined using the CCK-8 assay. the excitation and emission wavelength band passes were set at 1 nm and 2.5 nm, excitation voltage was 700 V.

Methods for synthesis of compounds TF-1

Weigh m-hydroxy-N,N-diethylbenzeneamine (1.65 g, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol), first dissolve the phthalic anhydride in toluene, and react at 80°C for 30 minutes. After 30 minutes, add m-hydroxy-N,N-dimethylbenzeneamine and heat to 110°C for a further 8 hours. Monitor the reaction by TLC. Upon completion, remove the solvent by rotary evaporation and recrystallize from n-butanol. Purify the intermediate 2-(4-Diethylamino-2-hydroxybenzoyl)benzoic acid (86% yield) via column chromatography. Weigh intermediate 2-(4-Diethylamino-2-hydroxybenzoyl)benzoic acid (1.56 g, 5 mmol) and catec

hol (0.55 g, 5 mmol), dissolve in 5 mL of trifluoroacetic acid, and reflux at 80°C for 12 hours. Monitor the reaction by TLC. After completion, remove the solvent by rotary evaporation and recrystallize using ethyl acetate. Purify the compound to obtain intermediate ROA-H (80% yield). Weigh the obtained intermediate ROA-H (193.7 mg, 0.5 mmol) and 2-chloro-1,4-naphthoquinone (96.3 mg, 0.5 mmol), dissolve in 8 mL of DMSO under nitrogen protection, and react at room temperature for 12 hours. Monitor the progress of the reaction by TLC. After completion, extract with ethyl acetate and saturated salt water, collect the organic phase, dry over anhydrous sodium sulfate, and reduce the pressure to dry the solvent, obtaining a crude product. Purify the crude product via column chromatography, using a mobile phase system of dichloromethane to methanol 100:1. The product is a brown solid, namely the fluorescent probe **TF-1**, with a yield of 74.3%. ESI-MS m/z calcd for $C_{34}H_{25}NO_6$ $[M+H]^+$ 544.1755 , found 544.1768.

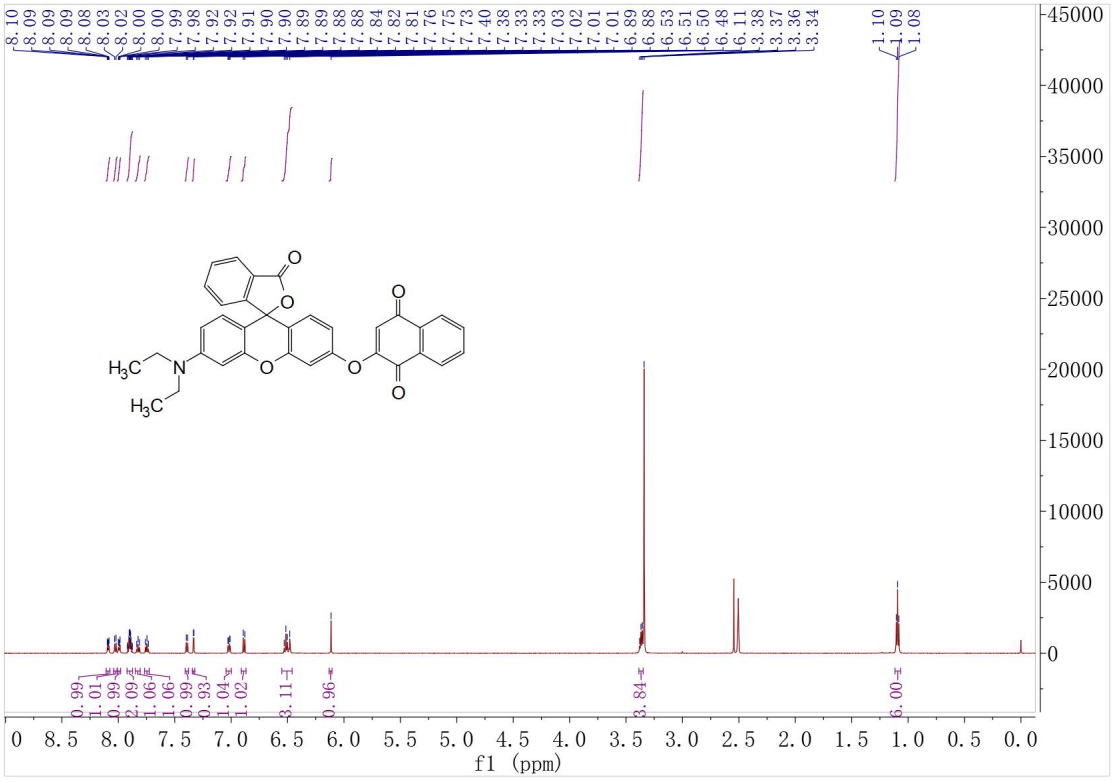


Fig. S1. ^{1}H NMR of compound **TF-1** in DMSO-d_6

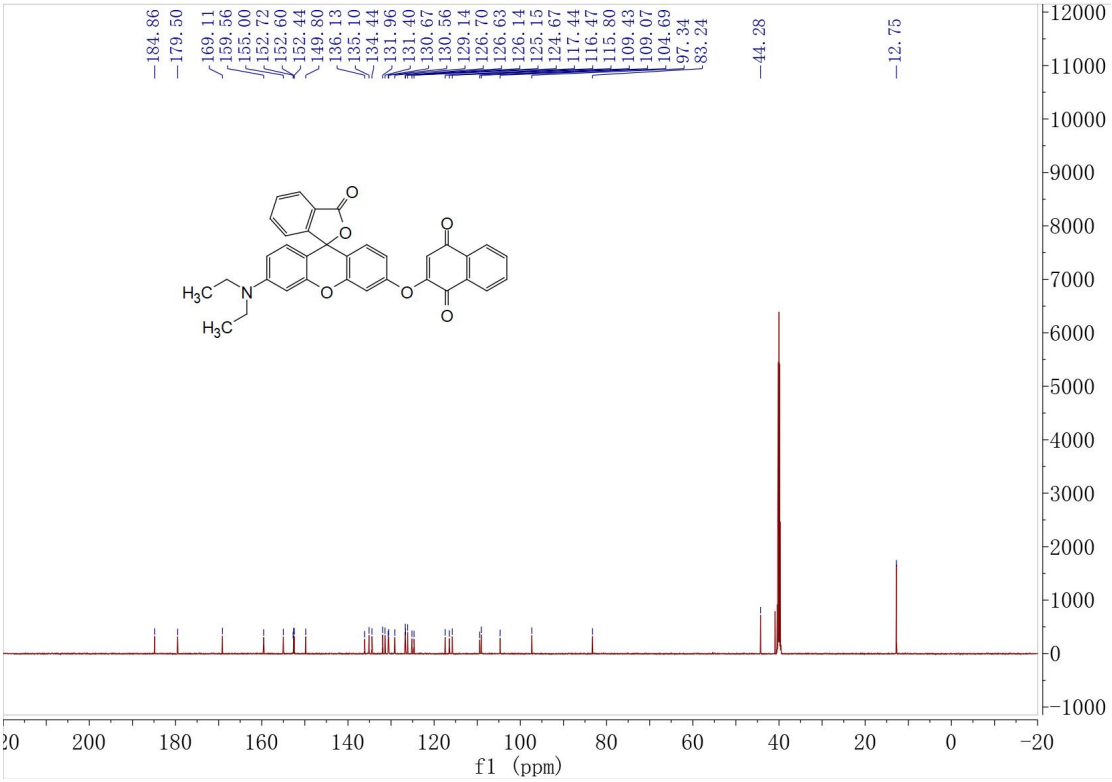


Fig. S2. ^{13}C NMR of compound **TF-1** in DMSO-d_6

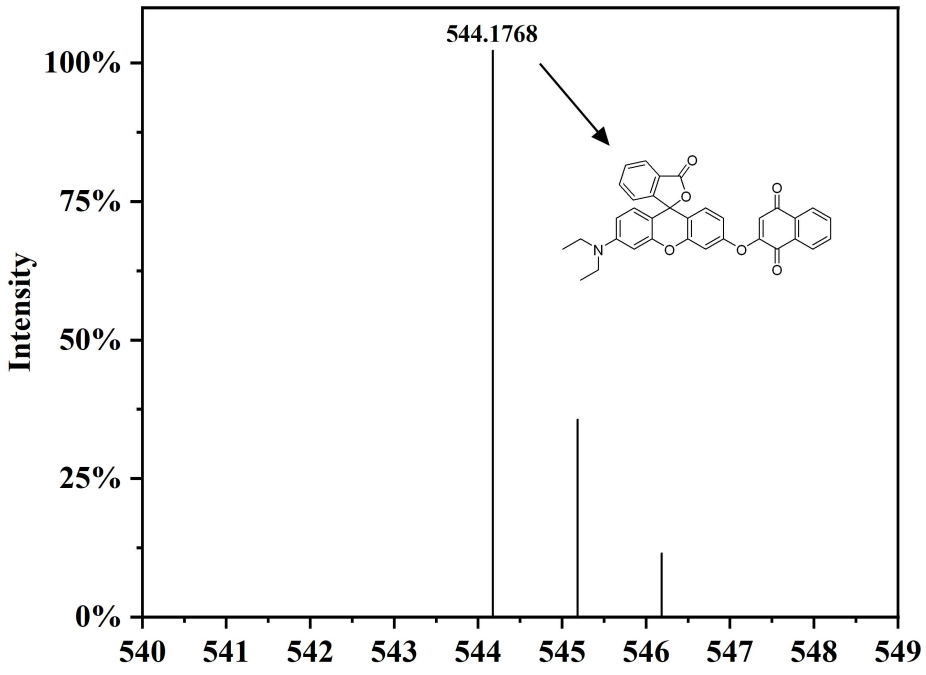


Fig. S3. High-resolution mass spectrometry of probe **TF-1**

Methods for synthesis of compounds TF-2

Weigh 1.65 g (10 mmol) of meta-hydroxy-N,N-diethyl-aniline and 1.48 g (10 mmol) of phthalic anhydride. Dissolve the phthalic anhydride in toluene and react at 80°C for 30 minutes. After 30 minutes, add meta-hydroxy-N,N-dimethyl-aniline and heat to 110°C for an additional 8 hours, monitoring the reaction by thin-layer chromatography (TLC). After c ompletion, remove the solvent by rotary evaporation and recrystallize from n-butanol. Purif y the intermediate, 2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid, by column chromatogr aphy (yield 86%).Weigh 1.56 g (5 mmol) of the intermediate, 2-(4-diethylamino-2-hydroxy benzoyl)benzoic acid, and 0.62 g (5 mmol) of 4-methylresorcinol. Dissolve them in 5 mL of trifluoroacetic acid and reflux at 80°C for 12 hours. Monitor the reaction by TLC. Afte r completion, remove the solvent by rotary evaporation and purify the compound by colu mn chromatography to obtain the intermediate ROA-CH_3 (yield 88%). Weigh 200.7 mg (0. 5 mmol) of the intermediate ROA-CH_3 and 96.3 mg (0.5 mmol) of 2-chloro-1,4-naphthoqu inone. Dissolve them in 8 mL of DMSO under nitrogen atmosphere and react at room te mperature for 12 hours. Monitor the reaction by TLC. After completion, extract with ethyl acetate and saturated brine. Collect the organic phase, dry over anhydrous sodium sulfate, and remove the solvent under reduced pressure to obtain the crude product. Purify the cr ude product by column chromatography using a dichloromethane and methanol mobile pha se system. The product, a brown solid, is the fluorescent probe **TF-2** with a yield of 81. 6%.^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.23 – 8.19 (m, 1H), 8.08 – 8.05 (m, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.79 – 7.74 (m, 2H), 7.70 (td, *J* = 7.6, 1.0 Hz, 1H), 7.65 – 7.60 (m, 1H), 7.26 (s, 1H), 6.73 (d, *J* = 8.7 Hz, 1H), 6.71 – 6.68 (m, 1H), 6.59 (d, *J* = 9.0 Hz, 1H), 6.49 (d, *J* = 2.6 Hz, 1H), 6.39 (dd, *J* = 9.0, 2.6 Hz, 1H), 5.86 (s, 1H), 3.38 (q, *J* = 7.0 Hz, 4H), 2.35 (s, 3H), 1.18 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, Chlorofo rm-*d*) δ 184.81 , 179.62 , 169.45 , 159.29 , 152.90 , 152.70 , 151.53 , 151.30 , 149.74 , 134.97 , 134.52 , 133.61 , 131.96 , 131.11 , 129.68 , 128.80 , 127.10 , 126.81 , 126.73 , 126.30 , 125.00 , 124.16 , 118.86 , 117.83 , 115.80 , 113.24 , 108.84 , 104.65 , 97.64 , 83.90 , 44.48 , 12.54 , 9.37 . ESI-MS m/z calcd for $C_{35}H_{27}NO_6$ $[M+H]^+$ 558.1911 , fo und 558.1909.

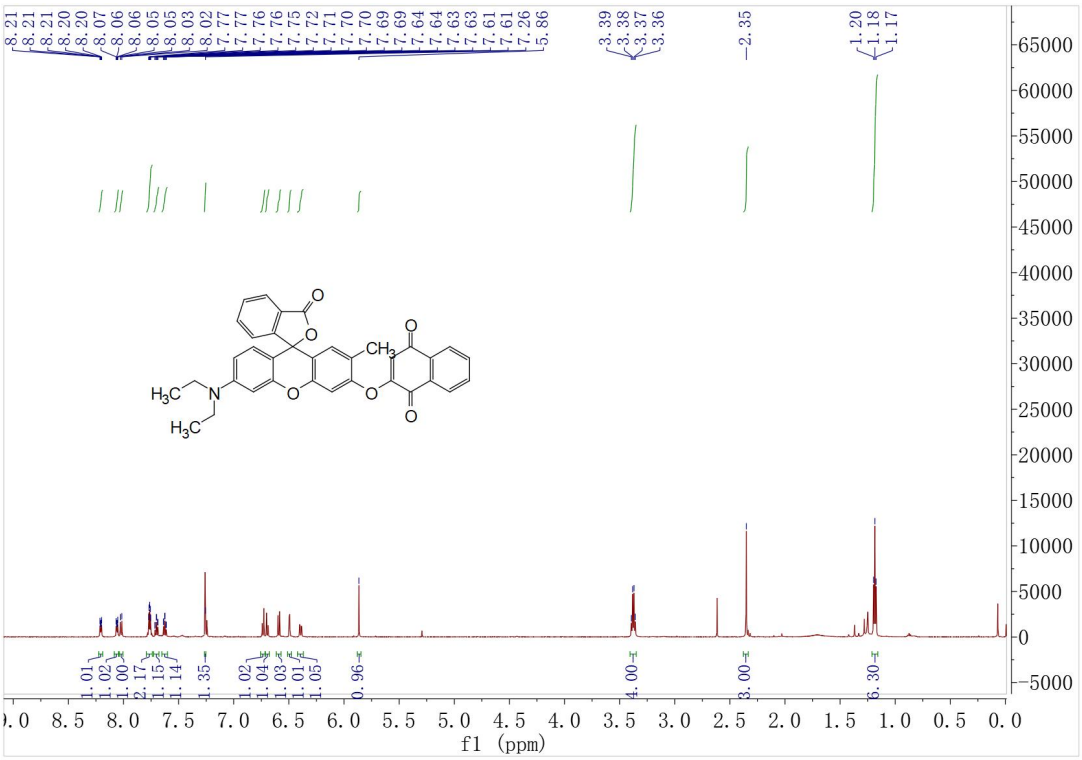


Fig. S4. ^{1}H NMR of compound **TF-2** in $CDCl_3$

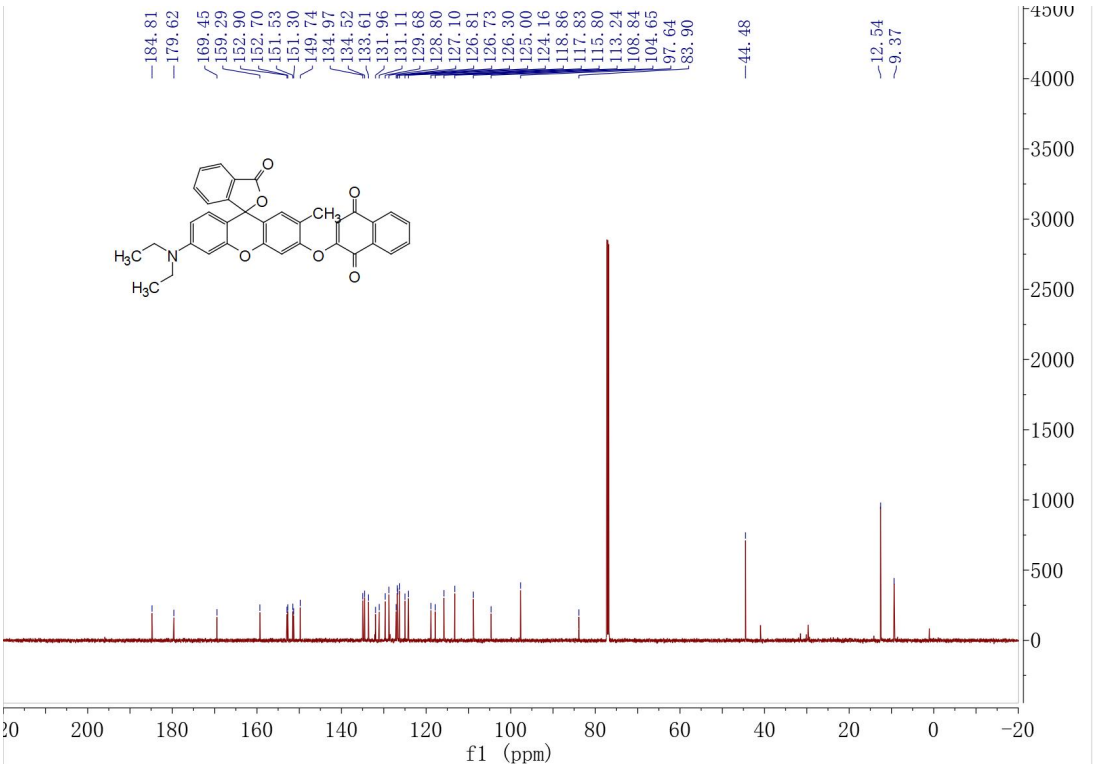


Fig. S5. ^{13}C NMR of compound **TF-2** in $CDCl_3$

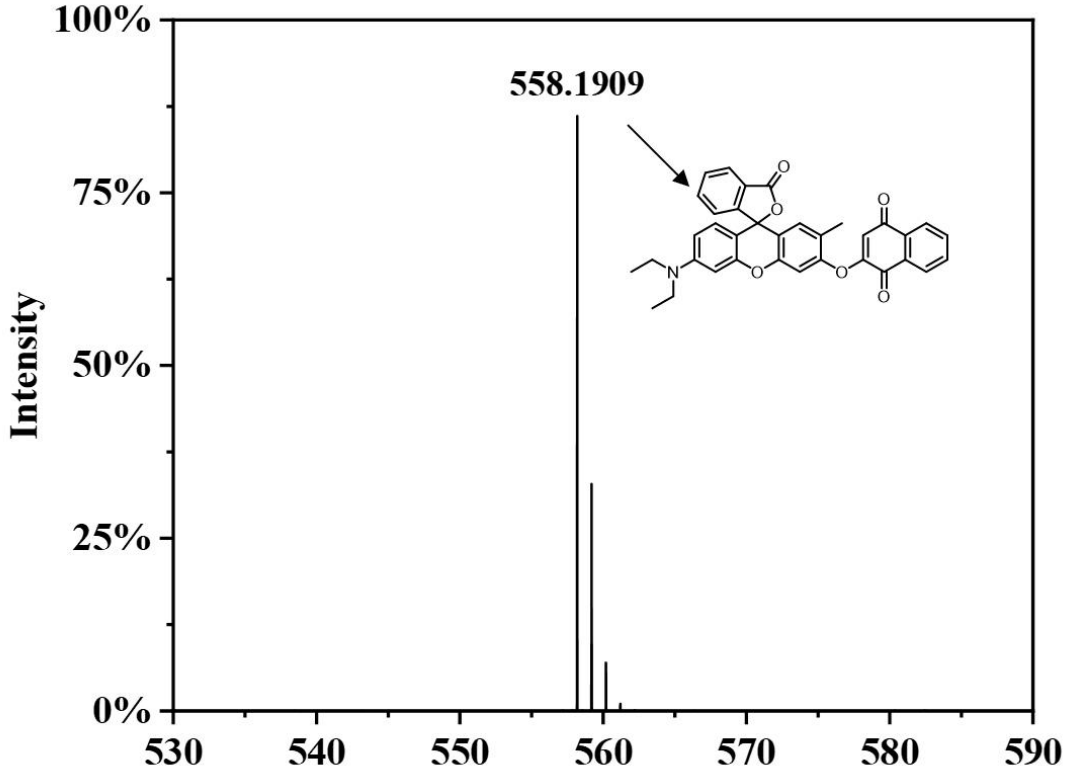


Fig. S6. High-resolution mass spectrometry of probe **TF-2**

Methods for synthesis of compounds TF-3

Weigh 1.65 g (10 mmol) of meta-hydroxy-N,N-diethyl-aniline and 1.48 g (10 mmol) of phthalic anhydride. Dissolve the phthalic anhydride in toluene and react at 80°C for 30 minutes. After 30 minutes, add meta-hydroxy-N,N-dimethyl-aniline and heat to 110°C for an additional 8 hours, monitoring the reaction by thin-layer chromatography (TLC). After completion, remove the solvent by rotary evaporation and recrystallize from n-butanol. Purify the intermediate, 2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid, by column chromatography (yield 86%).Weigh 1.56 g (5 mmol) of the intermediate, 2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid, and 0.72 g (5 mmol) of 4-chlororesorcinol. Dissolve them in 5 mL of trifluoroacetic acid and reflux at 80°C for 12 hours. Monitor the reaction by TLC. After completion, remove the solvent by rotary evaporation and purify the compound by column chromatography to obtain the intermediate ROA-Cl (yield 70.3%). Weigh 210.9 mg (0.5 mmol) of the intermediate ROA-Cl and 96.3 mg (0.5 mmol) of 2-chloro-1,4-naphthoquinone. Dissolve them i

n 8 mL of DMSO under nitrogen atmosphere and react at room temperature f or 12 hours. Monitor the reaction by TLC. After completion, extract with ethyl acetate and saturated brine. Collect the organic phase, dry over anhydrous sod ium sulfate, and remove the solvent under reduced pressure to obtain the crude product. Purify the crude product by column chromatography using a dichloro methane and methanol mobile phase system. The product, a brown solid, is the fluorescent probe **TF-3** with a yield of 61.6%.1H NMR (600 MHz, Chlorofor m-d) δ 8.23 – 8.20 (m, 1H), 8.10 – 8.04 (m, 2H), 7.80 – 7.76 (m, 2H), 7.76 – 7.72 (m, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.27 (s, 1H), 7.14 (s, 1H), 6.89 (s, 1H), 6.57 (d, J = 9.0 Hz, 1H), 6.44 (d, J = 2.5 Hz, 1H), 6.39 (dd, J = 9.0, 2.6 Hz, 1H), 5.94 (s, 1H), 3.36 (q, J = 7.1 Hz, 4H), 1.17 (t, J = 7.1 Hz, 6H). 13C NMR (151 MHz, CDCl3) δ 184.58, 179.02, 169.13, 158.32, 152.37, 152.3 0, 151.36, 149.84, 149.47, 135.30, 134.57, 133.76, 131.89, 131.03, 130.22, 130. 07, 128.84, 126.90, 126.82, 126.38, 125.26, 124.10, 120.63, 119.74, 114.10, 111. 56, 109.02, 104.11, 97.50, 82.71, 44.54, 12.47. ESI-MS m/z calcd for $C_{34}H_{24}ClNO_6$ $[M+H]^+$ 578.1365 , found 578.1351.

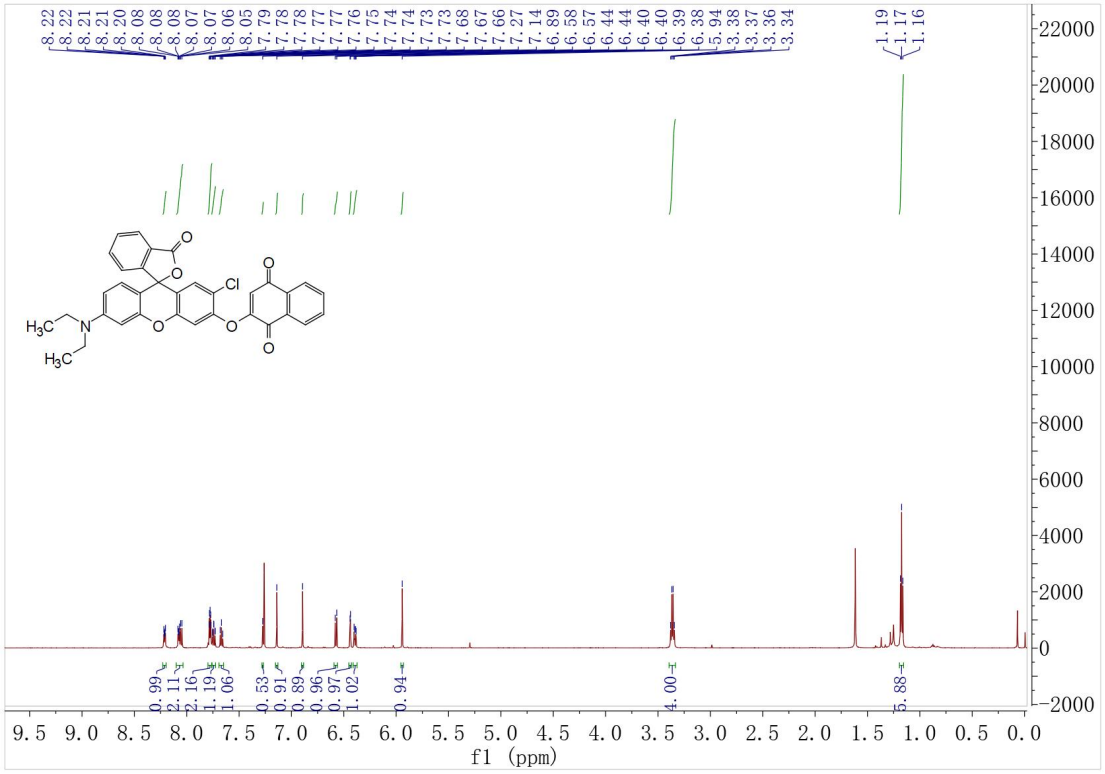


Fig. S7. ^{1}H NMR of compound **TF-3** in $CDCl_3$

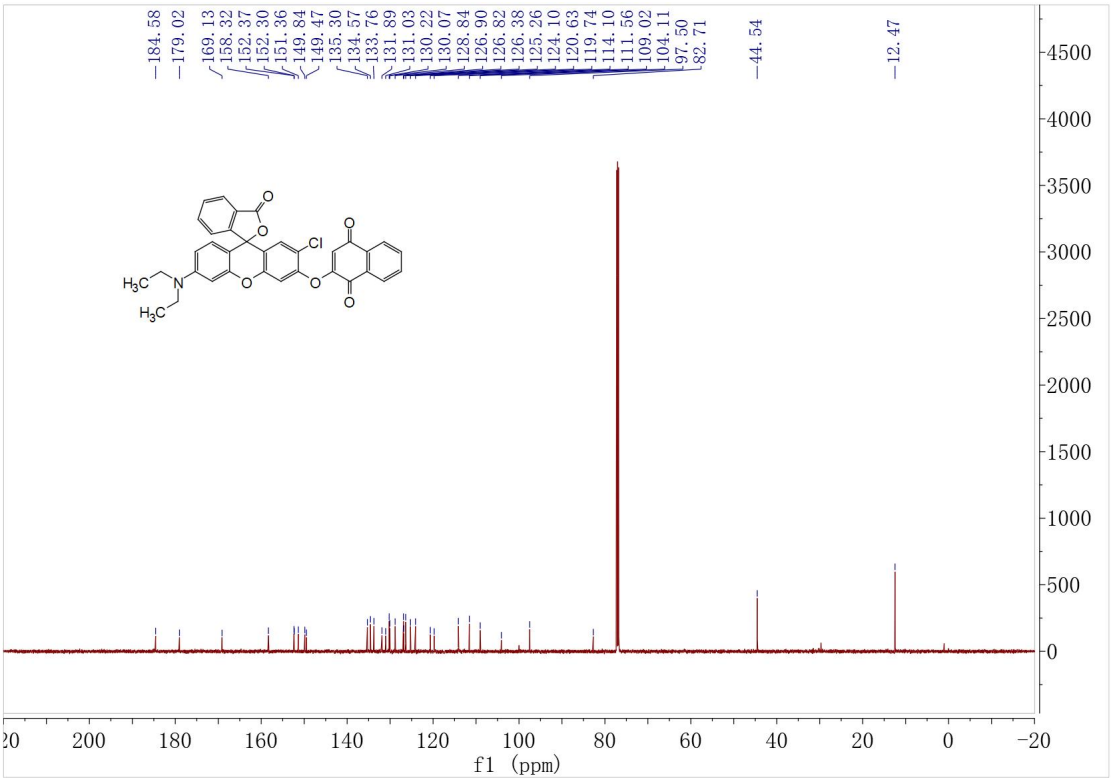
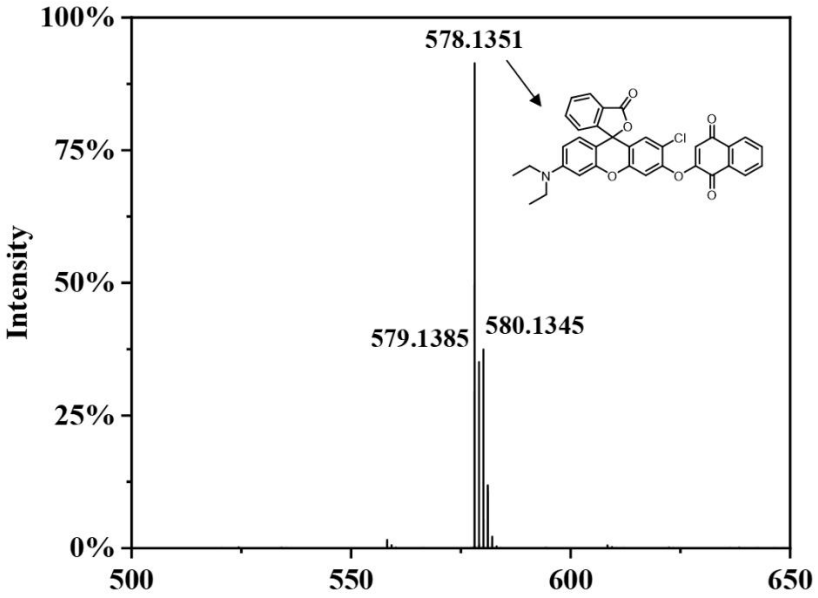


Fig. S8. ^{13}C NMR of compound **TF-3** in



$CDCl_3$

Fig. S9. High-resolution mass spectrometry of probe **TF-3**

Mechanistic studies

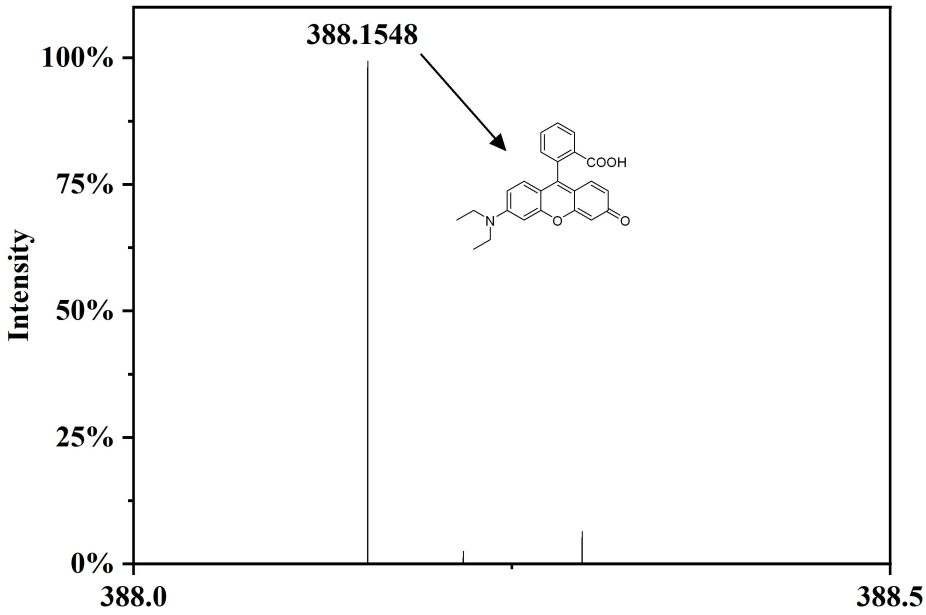


Fig. S10. High-resolution mass spectrometry of probe **TF-1-HS**

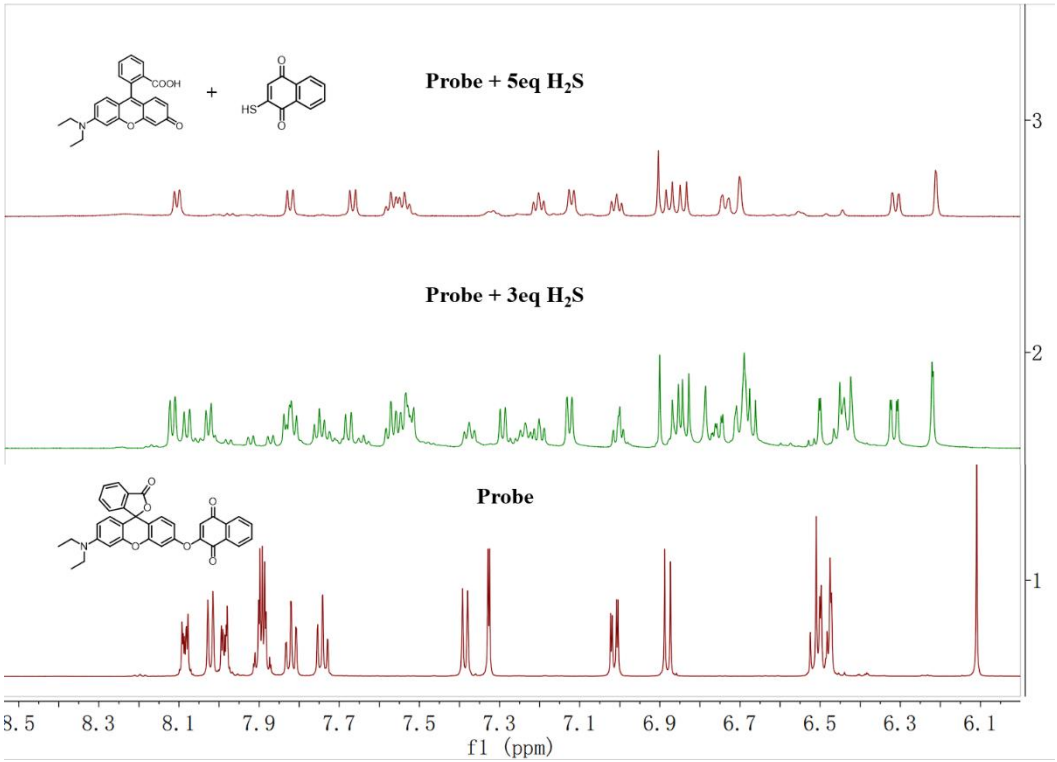


Fig. S11. ^{1}H-NMR of probe **TF-1** at 0/3/5eq H_2S

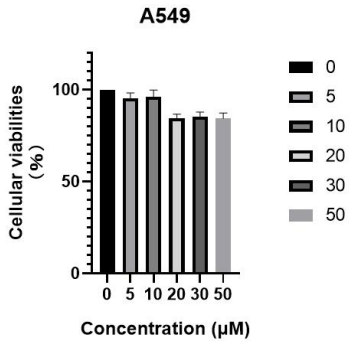


Fig.S12. Probe **TF-1** for A549 cytotoxicity test

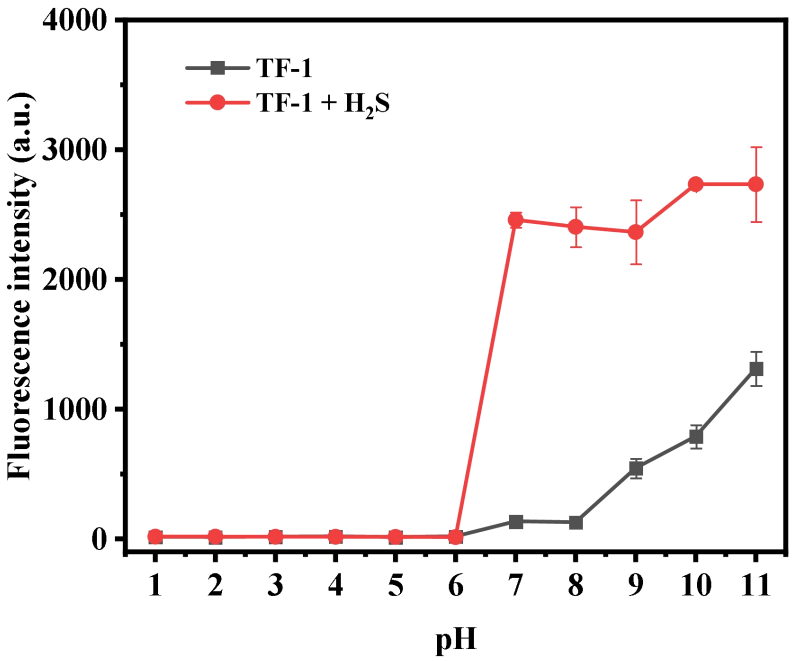


Fig.S13. Changes in fluorescence intensity of probe **TF-1** (10μM) before and after addition of H2S (100μM) at different pH

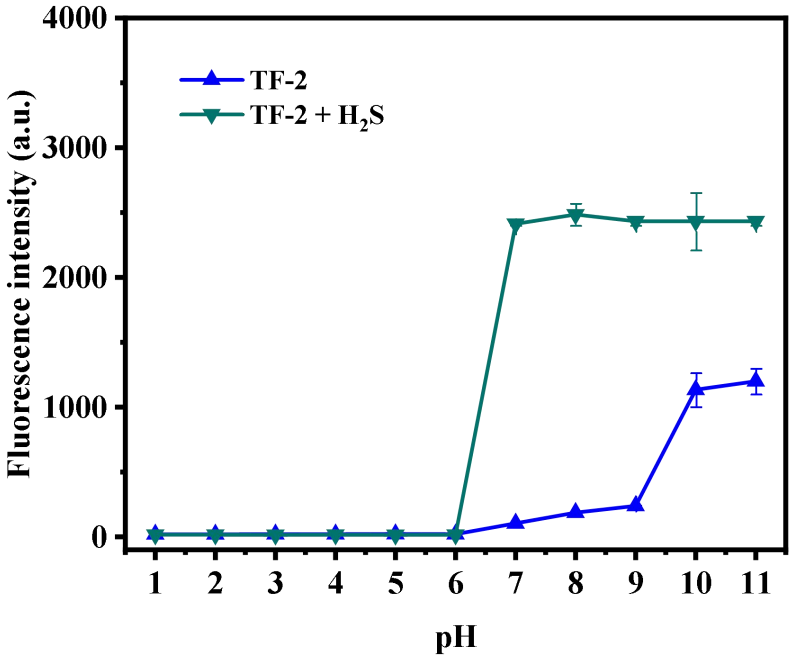


Fig.S14. Changes in probe **TF-2** (10μM) before and after addition of H_2S (100μM) at different pH

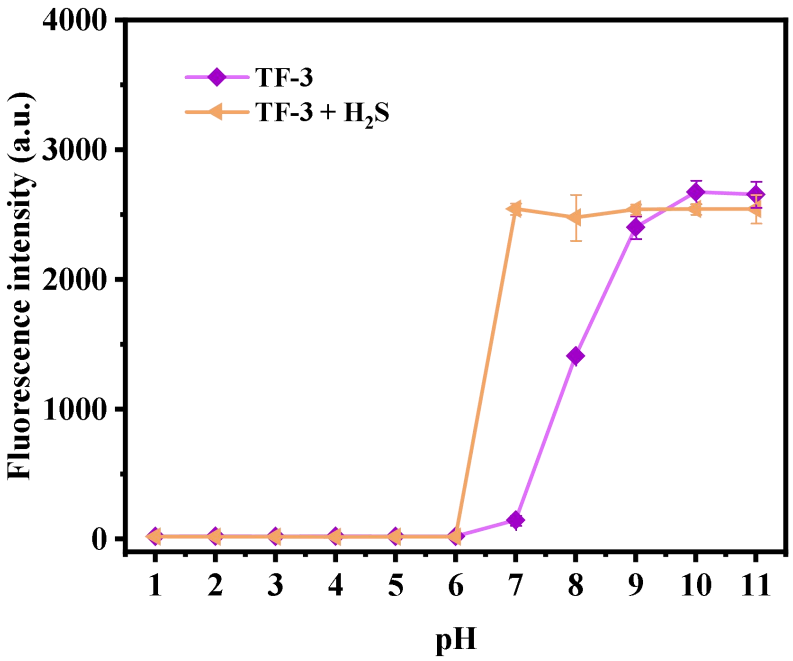
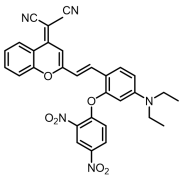
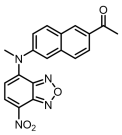
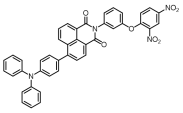
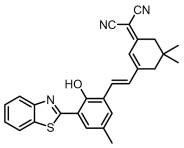
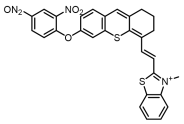
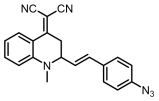
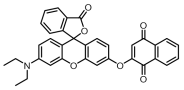


Fig.S15. The fluorescence intensity changes of probe **TF-3** (10μM) before and after adding H_2S (100μM) at different pH

Comparison with other reported probes for determination of H_2S

Table S1.

Probe	Limit of detection (nM)	Response time (min)	Reaction media	Practical application	Reference
	25.3	3	PBS/ DMSO (9/1, v/v)	Cell	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 257 (2021) 119764
	6.8	300	PBS/ DMSO (7/3, v/v)	Water sample and human blood	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 273 (2022) 121043
	80	10	HEPES/DMSO (9/1, v/v)	Cell	Journal of Molecular Structure 1308

					(2024): 138125.
	28.3μM	0.2	CH_3CN/PBS (1/1, v/v)	Cell	Microchemical Journal,(2024) 111143.
	90	120	PBS/ DMSO (95/5, v/v)	Cell	Sensors and Actuators: B. Chemical 369 (2022) 132297
	10	60	PBS/ DMSO (2/3, v/v)	Water sample	Microchemical Journal 191 (2023) 108856
	31.8	<1	CH_3CN/ PBS (4/1, v/v)	Cell	This work