Supporting Information for

A novel fast-responsive fluorescent probe based on 1, 3, 5-triazine for endogenous H_2S detection with large Stokes shift and its application in cell imaging

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Materials and apparatus

Unless otherwise noted, all reagents and materials were purchased from commercial company and used without further purification. Twice-distilled water was applied to all experiments. High-resolution electronspray (ESI-HRMS) mass spectra were examined from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were obtained from AVANCE III 400 MHz Digital NMR Spectrometer with TMS as an internal standard; Electronic absorption spectra were recorded on a LabTech UV Power spectrometer; Photoluminescent spectra were obtained with a HITACHI F4700 fluorescence spectrophotometer; The fluorescence images were collected with Nikon A1MP confocal microscopy with a CCD camera; The pH measurements were implemented on a Mettler-Toledo Delta 320 pH meter; analysis was exhibited on silica gel plates and column chromatography was carried out over silica gel (mesh 200-300). Both TLC and silica gel were purchased from the Qingdao Ocean Chemicals.

The verification of the proposed sensing mechanism

To confirm the proposed mechanism is correct, the probe **TzAr-H_2S** (0.016 mmol, 9.7mg), excess NaHS (15.0mg) and 2.0 mL mixed solution of ethanol and PBS (1:1) were added into a 25mL reaction tube in sequence, and stirred at 37°C for 6 hours. After the reaction ended, the solution was extracted three times by dichloromethane. Then the organic phases were combined and dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The product of the H_2S nucleophilic reaction was obtained by Column chromatography.

Cell culture and cytotoxicity assays

Hela cells were provided by Jiangsu Kaiji Biotechnology Co., Ltd. The living HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% FBS) under the atmosphere containing 5%

CO_2 and 95% air at 37 °C. The cytotoxic effects of the probe **TzAr-H_2S** were tested by the MTT assay. The living cells line were treated in DMEM (Dulbecco's Modified Eagle Medium) supplied with fetal bovine serum (10%, FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) under the atmosphere of CO_2 (5%) and air (95%) at 37 °C. The HeLa cells were then seeded into 96-well plates, and 0, 1, 2, 3, 5, 10, 20μM (final concentration) of the probe **TzAr-H_2S** (99.9% DMEM and 0.1% DMSO) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Then the HeLa cells were washed with PBS buffer, and DMEM medium (500 μL) was added. Next, MTT (50 μL, 5 mg/mL) was injected to every well and incubated for 4 h. Violet formazan was treated with sodium dodecyl sulfate solution (500 μL) in the H_2O-DMF mixture. Absorbance of the solution was measured at 570 nm by the way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **TzAr-H_2S**.

Imaging of exogenous H_2S in HeLa cells

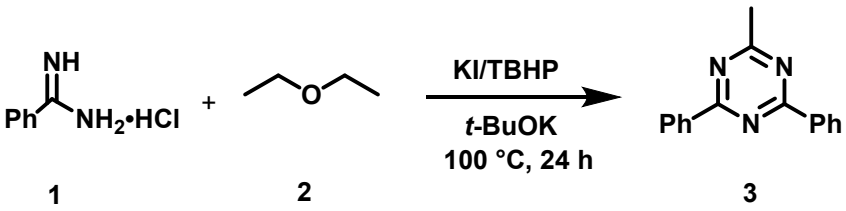
HeLa cells were grown in modified Eagle's medium (MEM) replenished with 10% FBS with the atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. For fluorescence imaging experiments, the cells were divided into two samples; one was incubated with the **TzAr-H_2S** probe (10 μM) only as a control, the other group was HeLa cells treated with **TzAr-H_2S** (10 μM) for 30 min, then with NaHS(100 μM) for 30 min at 37 °C. All HeLa cells were washed three times with PBS buffer after incubation. The experiments of cell imaging were acquired with a Nikon A1MP confocal microscopy

with the equipment of a CCD camera. Cell images were obtained using 350 nm excitation and signals were collected from 360 nm to 600 nm.

Imaging of endogenous H_2S in HeLa cells

Before the experiments, the well prepared cells were washed with PBS (pH = 7.4) buffer three times. HeLa cells were treated with 10 μM **TzAr-H_2S** for 20 min and then washed with PBS three times to remove the probe left in solution and optimize the back-ground signal, the cells incubated with 100 μM cysteine for 2 h in an atmosphere of 5% CO_2 and 95% air. For the control experiments, the cells were treated with 10 μM **TzAr-H_2S** for 20 min and then washed with PBS three times, and then cultivated for 2 h under the same conditions. For negative control group, the HeLa cells incubated with probe **TzAr-H_2S** (10 μM) for 20 min at 37◦C, washed by PBS buffer and subsequently incubated with 100 μM cysteine and DL-propargylglycine (PAG, 200 μM) for 2 h prior to imaging. Before imaging, cells were washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

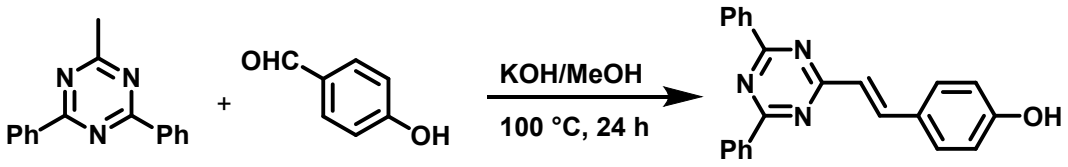
Synthesis of the compound 3



1 2 3

Compound **1** (2.0 mmol, 313.2mg), compound **2** (2.5 mL), potassium tert-butoxide (2.0 mmol, 224.4mg), potassium iodide (0.4mmol), and tert-butanol hydrogen peroxide (70%, 4.0 mmol, 515.0mg) were added into a 25mL reaction tube in sequence, and stirred at 100°C for 24 hours. Then the solution was cooled to room temperature and quenched with water, which were extracted three times by ethyl acetate. The organic phases were combined and dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Compound **3** was obtained by Column chromatography (petroleum ether: ethyl acetate = 100 :1～80:1) (23%, white solid, 115.3mg).

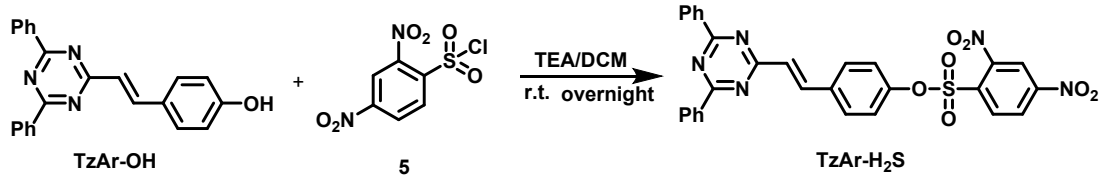
Synthesis of the compound TzAr-OH



Compound **3** (0.20 mmol, 49.5mg), compound **4** (0.20 mmol, 24.4mg) and potassium hydroxide (1.0 mmol) were dissolved in methanol (1.0 mL) into a 10mL reaction tube in turn and stirred at 100°C for 24 hours. Then reaction solution was cooled to room temperature and quenched with water, which were extracted three times with ethyl acetate. The organic phases were combined and dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure, and purified by column chromatography (dichloromethane: ethyl acetate = 80:1) to obtain **TzAr-OH** of the product (yellow- 26.21 % yield, green solid, 11.2 mg). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.08 (s, 1H), 8.67-8.65 (m, 4H), 8.37 (J = 15.8 Hz, 1H), 7.75-7.73 (m, 2H),

7.70-7.67 (m, 2H), 7.65-7.61 (m, 4H), 7.14 (J = 15.8 Hz, 1H), 6.89-6.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.1, 170.5, 159.9, 142.6, 135.6, 132.9, 130.6, 129.0, 128.6, 126.2, 122.4, 116.0; HRMS (ESI): calcd for $C_{23}H_{18}N_3O$ $[M+H]^+$ 352.1444, found 352.1451.

Synthesis of the probe TzAr-H_2S



The compound **TzAr-OH** (0.20 mmol, 70.3mg) and 2,4-dinitrobenzenesulfonyl chloride (0.20 mmol, 53.3mg) were dissolved in dichloromethane (5.0 mL). After that triethylamine (0.20 mmol, 20.238mg) was added slowly at 0 °C. The reaction was moved to room temperature overnight. The crude product was purified by silica gel column chromatography to obtain the probe **TzAr-H_2S**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, 5H), 8.52 (d, 1H), 8.35 (d, 1H), 8.24 (d, 1H), 7.73 (d, 2H), 7.60 (td, 6H), 7.30 (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 171.2, 151.0, 149.4, 149.1, 139.6, 135.8, 135.7, 133.9, 133.6, 132.8, 129.9, 129.0, 128.7, 128.2, 126.4, 122.6, 120.4; HRMS (ESI): calcd for $C_{29}H_{20}N_5O_7S$ $[M+H]^+$ 582.1078, found 582.1072.

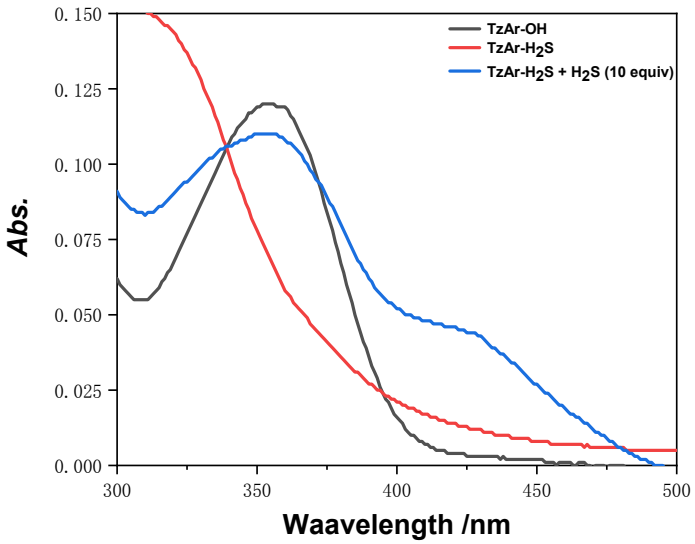


Fig. S1. The absorption spectra of **TzAr-OH** (10 μM) and **TzAr-H_2S** (10 μM) in pH 7.4 PBS/EtOH (v/v = 1/1) in the absence or presence of NaHS (10.0 equiv).

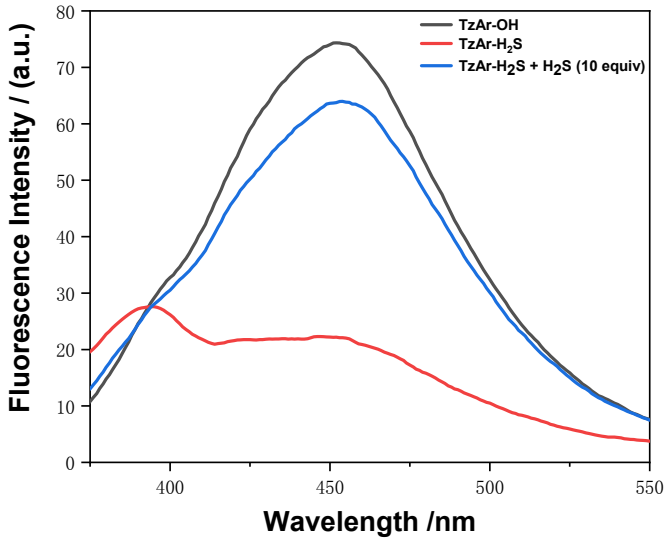


Fig. S2. The fluorescence spectra of **TzAr-OH** (10 μM) and **TzAr-H_2S** (10 μM) in pH 7.4 PBS/EtOH (v/v = 1/1) in the absence or presence of NaHS (10.0 equiv).

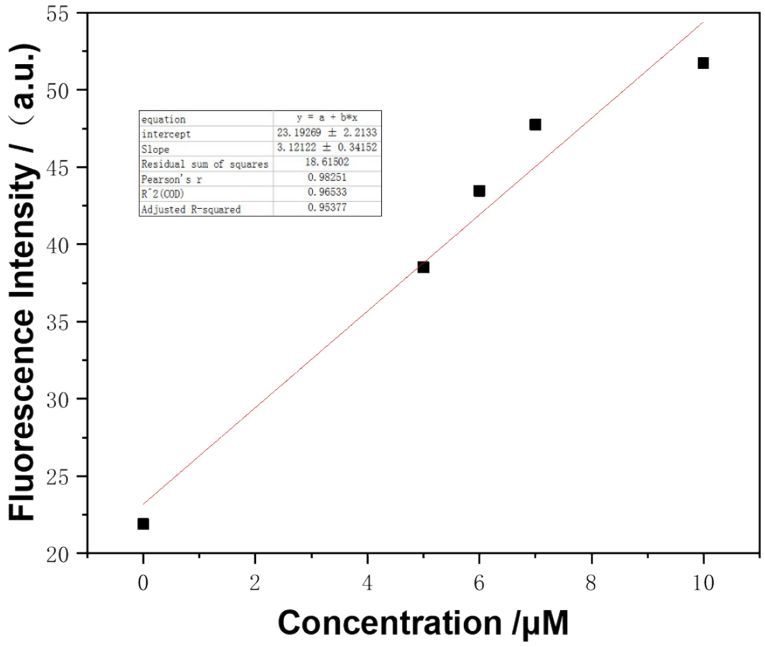


Fig. S3. The linear fit of **TzAr-H_2S** (10 μM) in pH 7.4 PBS/EtOH (v/v = 1/1) in the absence or presence of NaHS (0-1 equiv).

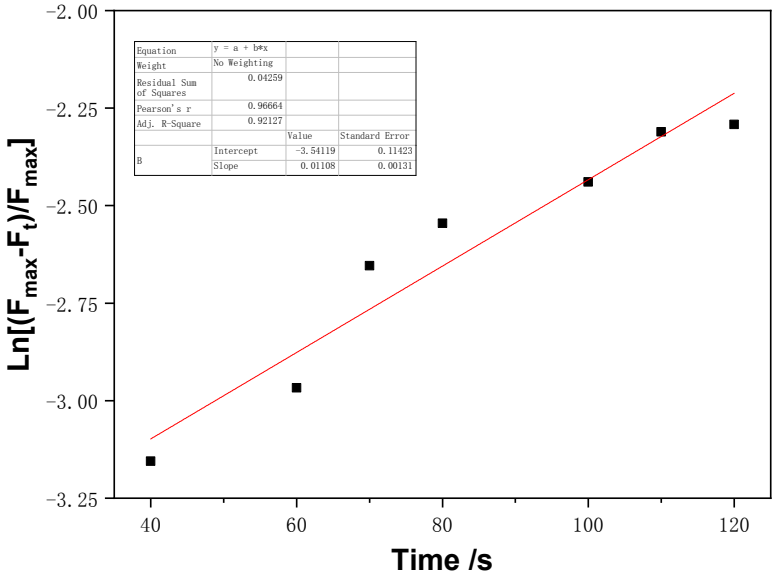


Fig. S4. *Pseudo*-first-order kinetic plot of the reaction of **TzAr-H_2S** in the presence of NaHS (10.0 equiv) at room temperature.

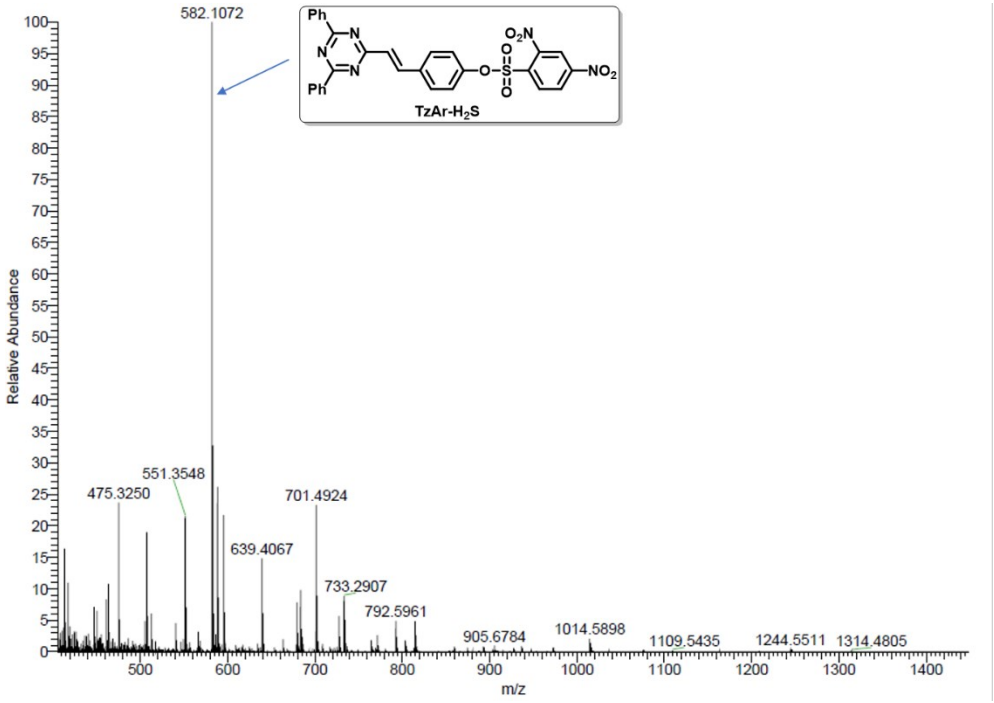


Fig. S5. HRMS (positive ion mode) spectrum of **TzAr-H_2S**.

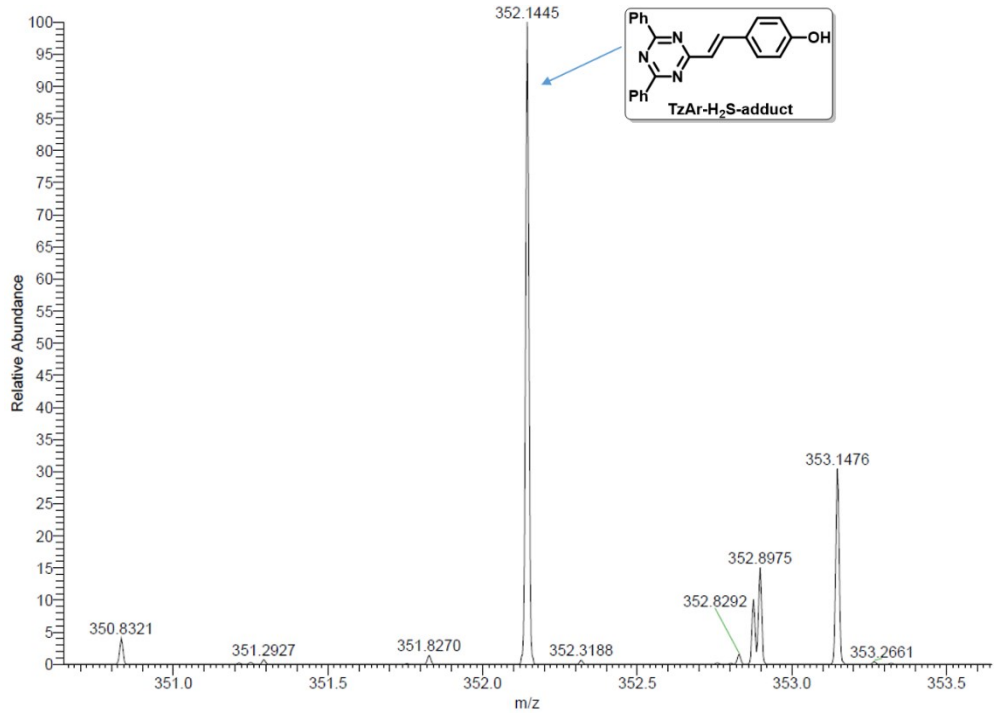


Fig. S6. HRMS (positive ion mode) spectrum of **TzAr-H_2S** (10 μM) after treatment with NaHS (100 μM) in pH 7.4 PBS/EtOH (v/v = 1/1) for 20 min. The peak at m/z 352.1445 corresponds to **TzAr-OH**.

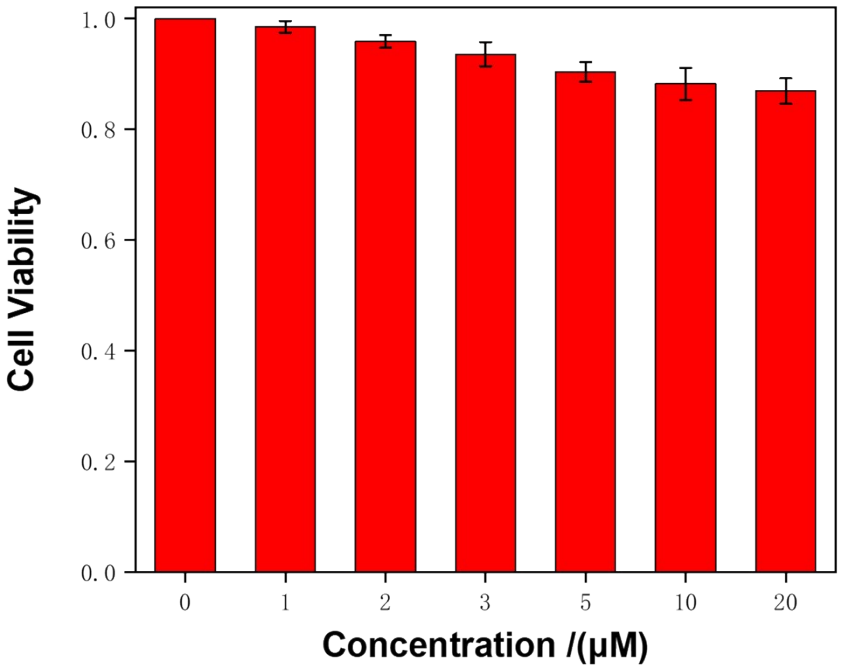


Fig. S7. Cytotoxicity assays of **TzAr-H_2S** at different concentrations (0 μM; 1μM; 2μM; 3μM; 5 μM; 10 μM; 20 μM) for HeLa cells

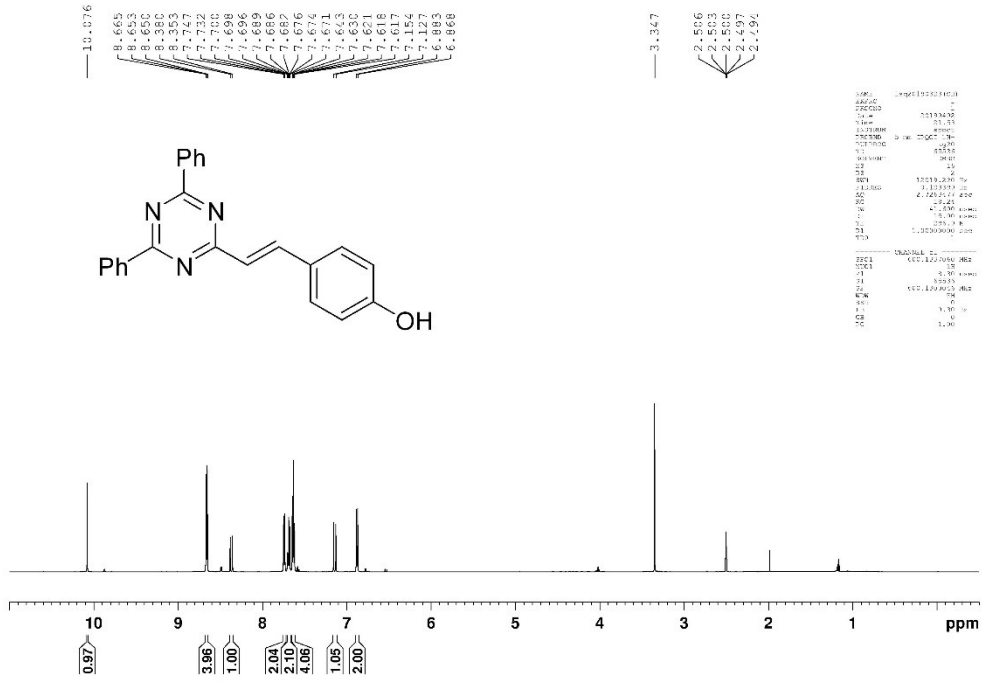


Fig. S8. ^{1}H NMR (DMSO-d_6) spectrum of **TzAr-OH.**

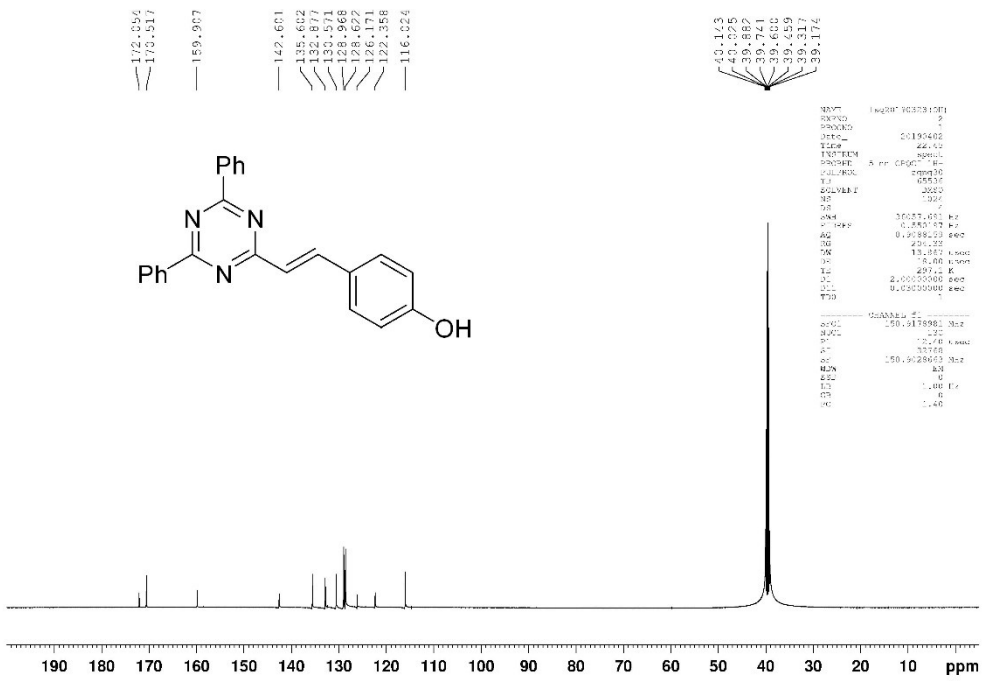


Fig. S9. ^{13}C-NMR (DMSO-d_6) spectrum of **TzAr-OH**.

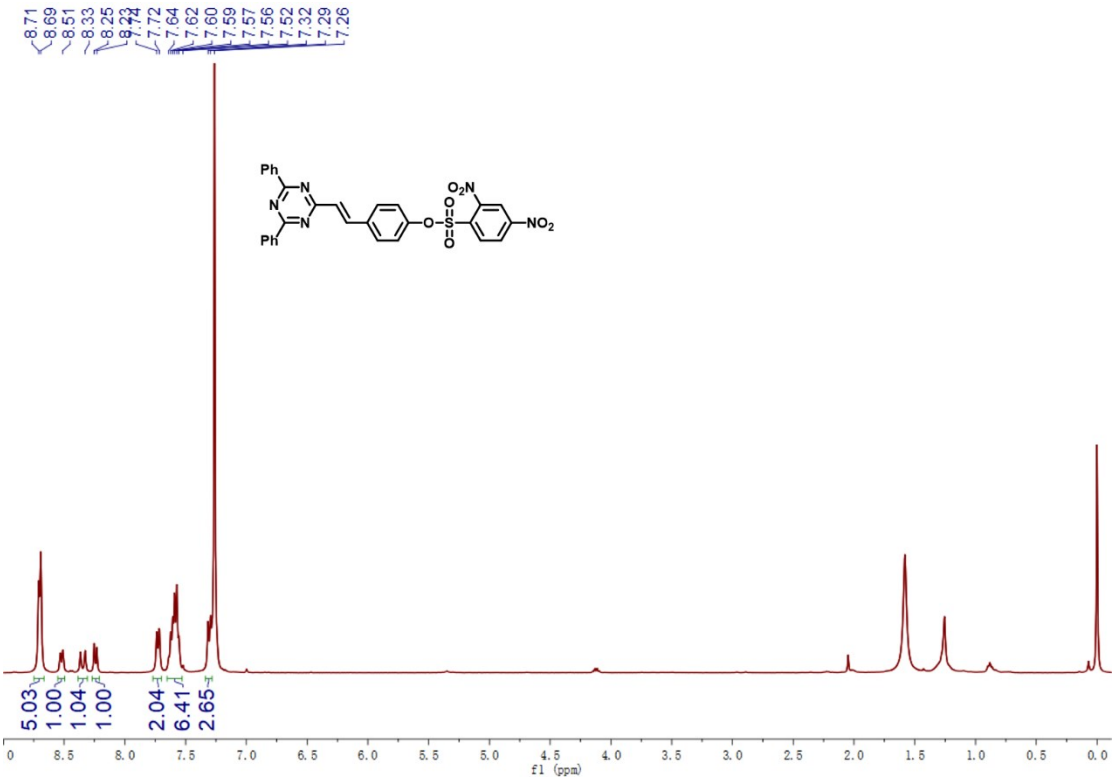


Fig. S10. ^{1}H NMR ($CDCl_3$) spectrum of **TzAr-H_2S.**

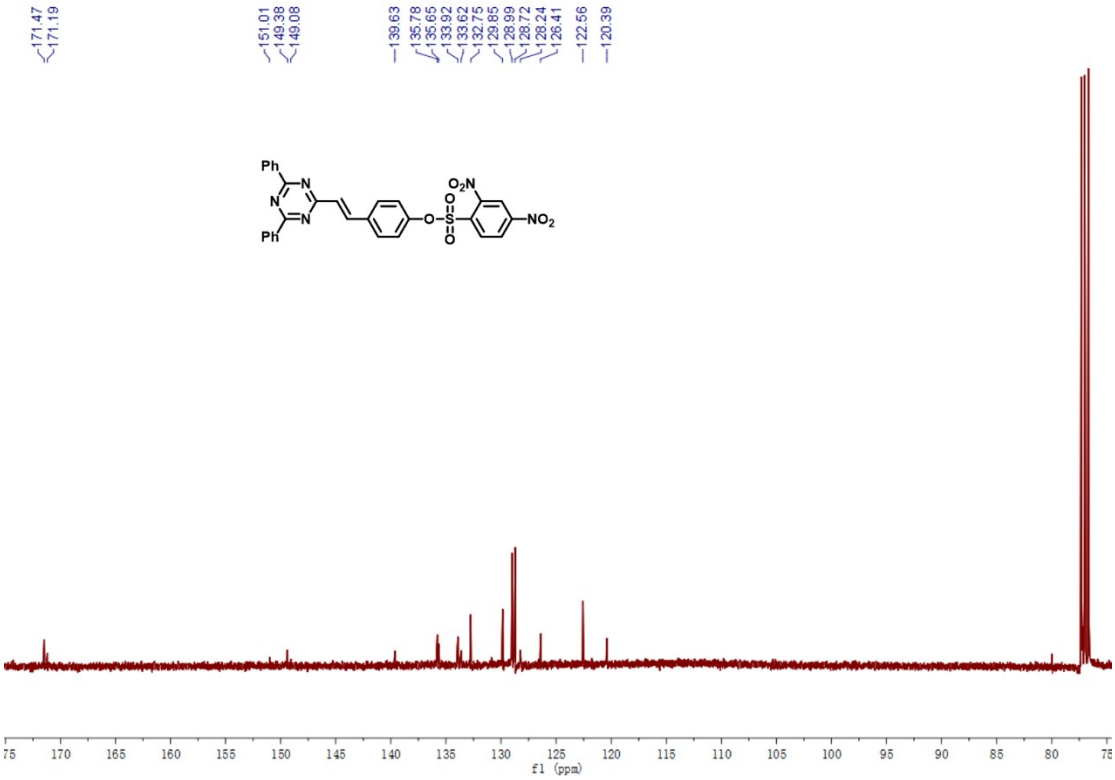


Fig. S11. ^{13}C-NMR ($CDCl_3$) spectrum of **TzAr-H_2S**.

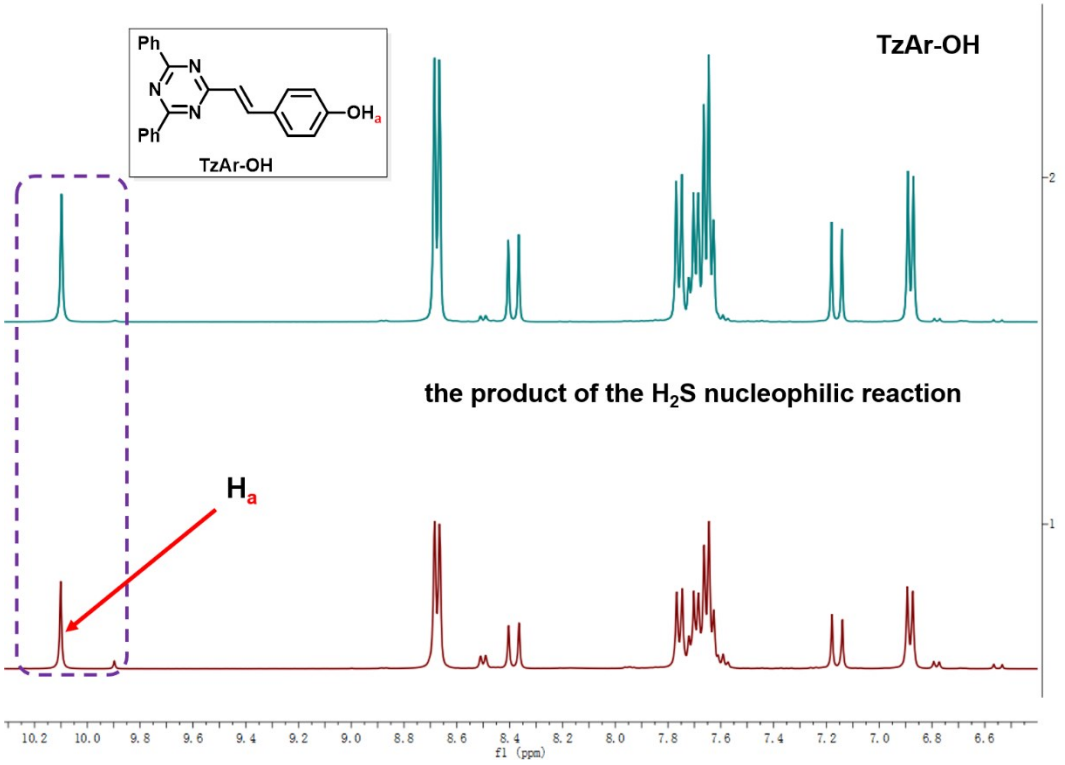


Fig. S12. ^{1}H NMR (DMSO-d_6) spectrum of the products of the H_2S nucleophilic reaction and **TzAr-OH.**

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

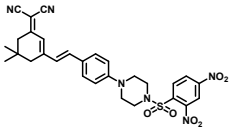
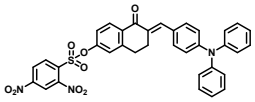
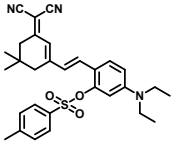
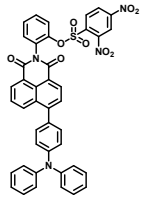
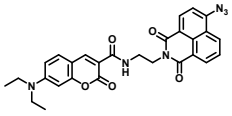
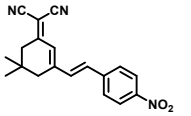
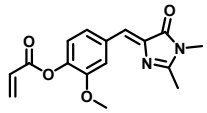
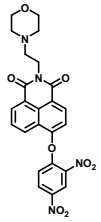
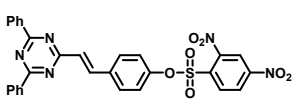
Probes	Response time	Detection limition	Stokes shift	Imaging	References
	60 s	6 nM	221 nm	Living cells mice	*Sensor. Actuat. B. -Chem.*, 2018, **262**, 837-844.
	30 s	27.3 nM	130 nm	Living cells	*Spectrochim. Acta. A.*, 2020, **243**, 118775.
	0.73 min	0.17 μM	160 nm	Living cells	*Dyes Pigments.*, 2019, **165**, 31-37.
	3 min	0.206 μM	135 nm	-	*Tetrahedron., 2021,**81**,131923.*
	150 s	9.1×10^{-8} M	109 nm	Living cells	*Chem.Commun.*, 2015, **51**, 1510-1513.
	120 s	1.08 nM	70 nm	Living cells mice	*Sens. Actuat. B-Chem.*, 2019, **288**, 507-511.
	3500 s	0.48 μM	68 nm	Living cells	*Sens. Actuat. B-Chem.*, 2019, **298**, 126875.
	20 min	0.48 μM	105 nm	Living cells	*Org. Lett.*, 2013, **15**, 2310-2313.
	20 s	2.13 μM	100 nm	Living cells	This work

Table S2 Optical properties and quantum yields of the **TzAr** dyes in different solvents

dyes	solvents	$\lambda_{Abs, max}$	$\lambda_{Em, max}$	Stokes shift	φ
TzAr-H_2S	DMSO	317/0.228	451/6.520	134	0.017
TzAr-OH		361/0.156	463/14.150	102	0.053
TzAr-H_2S	THF	317/0.213	451/7.988	134	0.010
TzAr-OH		355/0.148	432/28.840	77	0.050
TzAr-H_2S	CH_3CN	318/0.199	449/6.590	131	0.012
TzAr-OH		346/0.156	453/19.230	107	0.044
TzAr-H_2S	EtOH	319/0.218	438/5.495	119	0.013
TzAr-OH		354/0.166	389/3.079	35	0.010
TzAr-H_2S	MeOH	327/0.175	457/4.117	130	0.016
TzAr-OH		353/0.150	458/11.720	105	0.054
TzAr-H_2S	DMF	318/0.184	448/6.773	130	0.017
TzAr-OH		359/0.147	457/15.350	98	0.049
TzAr-H_2S	EtOH:PBS=1:1	317/0.238	447/2.120	130	0.011
TzAr-OH		355/0.163	447/5.200	92	0.040