Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

A turn-on fluorescent probe for selective detection of H_2S in environmental samples and bio-imaging in human breast cancer cells

Dipanjan Banik[a], Anirban Karak[a], Satyajit Halder[b], Shilpita Banerjee[a], Moumi Mandal[a] Anwesha Maiti[a], Kuladip Jana[b], Ajit Kumar Mahapatra[a]*

[a]Molecular Sensor and Supramolecular Chemistry Laboratory, Department of Chemistry, Indian Institute of Engineering Science and Technology, Shibpur, Howrah-711103, West Bengal, India.

[b]Division of Molecular Medicine, Bose Institute, P 1/12, CIT Scheme VIIM, Kolkata 700054, India.

*Author to whom correspondence should be addressed; electronic mail:

akmahapatra@chem.iiests.ac.in; Tel.: +91 – 9434508013

Contents

1. Experimental section

Materials and Methods

The chemicals of Sigma-Aldrich company were used directly for synthesis purposes. Commercially supplied analytical grade solvents were used for all experiments. 60-120 mesh Silica gel (Spectrochem Pvt. Ltd. Mumbai, India) was used in column chromatography for the purification of products and Merck silica gel 60 F_{254} plates were used for Thin layer chromatography (TLC). UV-vis spectra were done by using Shimadzu UV-1800 UV-vis spectrophotometer. A fluorescence spectrophotometer (Model 1057, Fluorolog, Horiba Scientific, USA) was used for recording Fluorescence spectra. ^{1}H and ^{13}C NMR spectra were performed by using Bruker Avance 400 MHz instrument where DMSO-d_6 and $CDCl_3$ were used as solvent and TMS as an internal standard. Mass spectra were carried out by using Waters Q-TOF YA 263 mass spectrometer.

The general method of UV-vis and fluorescence titration

Both spectral measurements were done by preparing a stock solution of the probe **TPB-NO_2** (4×10^{-5}M) in a DMSO/H_2O solution (1:1, v/v; 10 mM HEPES buffer, pH 7.4) by filling a quartz optical cell having a 1cm optical path length was filled with 2 ml probe solution (4×10^{-5}M) and both the titration experiments were done by continuous addition of a 10 μM analyte stock solution (4×10^{-4}M) to this probe solution (4×10^{-5}M) by using a micropipette.

Kinetic Study

To investigate the reaction kinetics of the probe **TPB-NO_2** with H_2S, first fluorescence intensity was plotted against time(seconds) to find out the response time and then the apparent rate constant (k_{obs}) was calculated by putting the fluorescence intensity values in the pseudo-first-order equation:

$$\ln [(F_{max} - Ft)/F_{max}] = - k_{obs}t$$

where F_t and F_{max} denote the fluorescence intensities at 483 nm at time t and the maximum value acquired after the completion of the reaction respectively.

Detection limit calculation

The fluorescence spectra of the blank probe **TPB-NO_2**(1×10^{-5} M) were recorded ten times to obtain the standard deviation. Then the fluorescence intensity was measured upon gradual addition of Na_2S and a linear fit curve was found when intensity was plotted against the concentration of Na_2S. Then by putting the value of standard deviation and slope in the equation 3σ/K (where σ is the standard deviation of the blank solution and K is the slope of the calibration curve), the detection limit was calculated.

Synthesis of the probe

Synthesis of compounds 2 and 3

Compounds 2 and 3 were synthesized according to the reported method.[1]

Synthesis and characterization of 2-(benzo[d]thiazol-2-yl)-5-(diphenylamino)phenol (TPB-OH)

Compound 3 (210 mg, 0.726 mmol) was dissolved in anhydrous EtOH (20 ml). Then 2-amino thiophenol (0.117 ml,1.08 mmol) and a catalytic amount of $KHSO_4$ were added to the mixture successively and refluxed for 5h. A yellow precipitate appeared. The precipitate was filtered and washed with cold EtOH to afford the yellowish-white powder of compound TPB-OH (197 mg, yield~ 69%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.98 (dd, J = 8.2, 5.7 Hz, 2H), 7.50 (t, J = 7.3 Hz, 1H), 7.43 – 7.36 (m, 5H), 7.19 (t, J = 7.8 Hz, 6H), 6.53 – 6.45 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d_6): δ 165.69 (s), 157.84 (s), 152.06 (s), 151.63 (s), 146.54 (s), 134.16 (s), 130.31 (s), 130.00 (s), 126.78 (s), 126.47 (s), 125.23 (s), 124.96 (s), 122.29 (s), 121.98 (s), 112.40 (s), 111.99 (s), 107.06 (s).

HRMS (ESI): *m/z* (%): 395.1210 [**TPB-OH** + H]$^+$ (Calc. for $C_{25}H_{19}N_2OS^+$: 395.1213)

Synthesis and characterization of 4-(benzo[d]thiazol-2-yl)-3-(2,4-dinitrophenoxy)-N, N-diphenylaniline (TPB-NO_2)

First, compound TPB-OH (150 mg, 0.38 mmol) was dissolved in dry acetonitrile (18 ml). Then, K_2CO_3 (63 mg, 0.456 mmol) was added to it, and stirred the mixture for 1h at room temperature. After that 2,4-dinitrofluorobenzene (85 mg, 0.456 mmol) was added to the reaction mixture and was stirred at room temperature for 6 h. After completion of the reaction (TLC monitored), the solvent was removed under vacuum. The residue was then washed with water and the organic part of the mixture was separated by using dichloromethane (30 ml×2). The collected organic layer was dried over anhydrous Na_2SO_4 and then purified by column chromatography using DCM/hexane (1:1, V/V). The obtained product was then recrystallized from diethyl ether to give yellow crystals of TPB-NO_2 as pure product (108 mg, yield~ 50.67%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88 (d, J = 2.7 Hz, 1H), 8.24 (d, J = 9.0 Hz, 2H), 7.94 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.44 (t, J = 7.1 Hz, 1H), 7.39 – 7.29 (m, 6H), 7.18

(dd, *J* = 14.2, 7.4 Hz, 7H), 7.07 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.00 (d, *J* = 9.3 Hz, 1H), 6.75 (d, *J* = 2.3 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 161.20 (s), 155.81 (s), 152.71 (s), 151.77 (s), 151.39 (s), 145.85 (s), 141.56 (s), 139.25 (s), 134.95 (s), 131.82 (s), 129.89 (s), 128.93 (s), 126.38 (s), 126.03 (s), 125.32 (s), 125.15 (s), 122.90 (s), 122.29 (s), 121.36 (s), 118.59 (s), 117.98 (s), 117.34 (s), 112.38 (s) ppm.

HRMS (ESI): *m/z* (%): 561.1226 [**TPB-NO_2** + H]$^+$. (Calc. for $C_{31}H_{21}N_4O_5S^+$: 561.1227

2. Emission spectra of TPB-OH

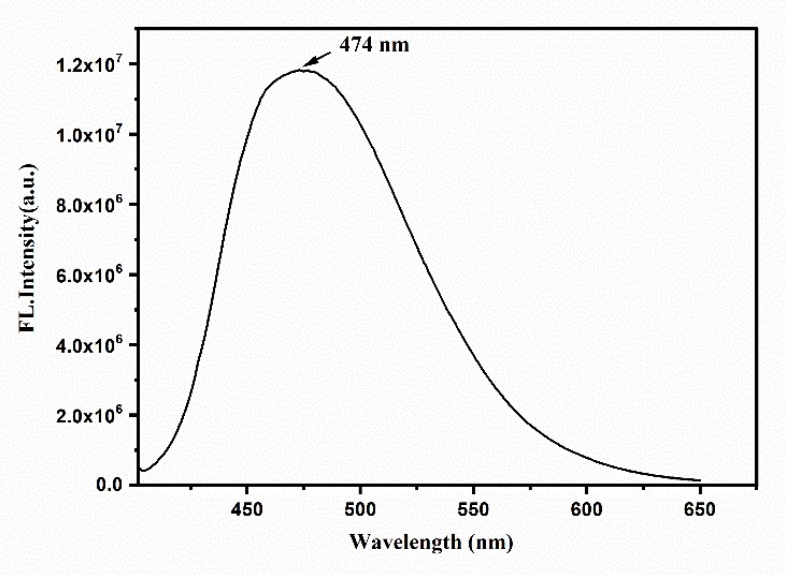


Figure S1. Emission spectra of **TPB-OH** (1×10^{-5} M) in DMSO/H_2O solution (1:1, v/v; 10 mM HEPES buffer, pH 7.4) (λ_{ex}= 390 nm, λ_{em}= 474 nm).

3. Plot of fluorescence change of TPB-NO_2 against different concentrations of H_2S

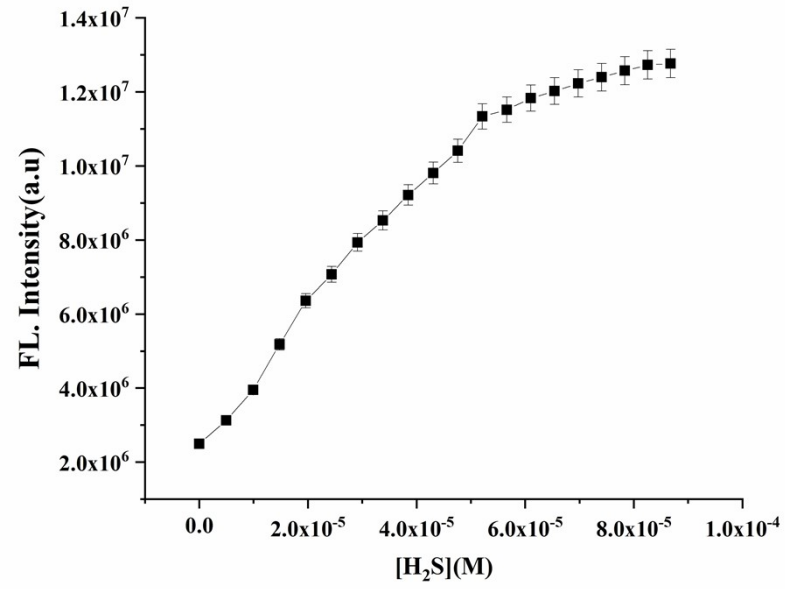


Figure S2. The plot of fluorescence intensity vs concentration of H_2S

4. Calculation of fluorescence quantum yield of TPB-NO_2 in the presence and absence of H_2S

Here, the fluorescence quantum yield Φ was calculated by using the following equation:

$\Phi_x = \Phi_s (F_x / F_s) (A_s / A_x) (\eta_X{}^2 / \eta_S{}^2)$

Where,

X and S indicate the unknown and standard solution respectively, Φ = quantum yield

F = Area under the emission curve, A= Absorbance at the excitation wavelength,

η = Refractive index of solvent. Here Φ measurements were performed using fluorescein in ethanol as standard [Φ = 0.79]

η_S = 1.361 (for ethanol); η_x = 1.479 (for DMSO)

In the absence of H_2S, the calculated quantum yield (Φ_x) for probe **TPB-NO_2** = 0.0189. where, Φ_s = 0.79, F_x =2.64×10^8, F_s =4.34×10^8, A_s = 0.008, A_x = 0.239.

In the presence of H_2S the calculated quantum yield (Φ_x) = 0.0931. Where, Φ_s = 0.79, F_x =1.38×10^9, F_s =4.34×10^8, A_s = 0.008, A_x = 0.254.

5. Determination of the response time of probe TPB-NO_2

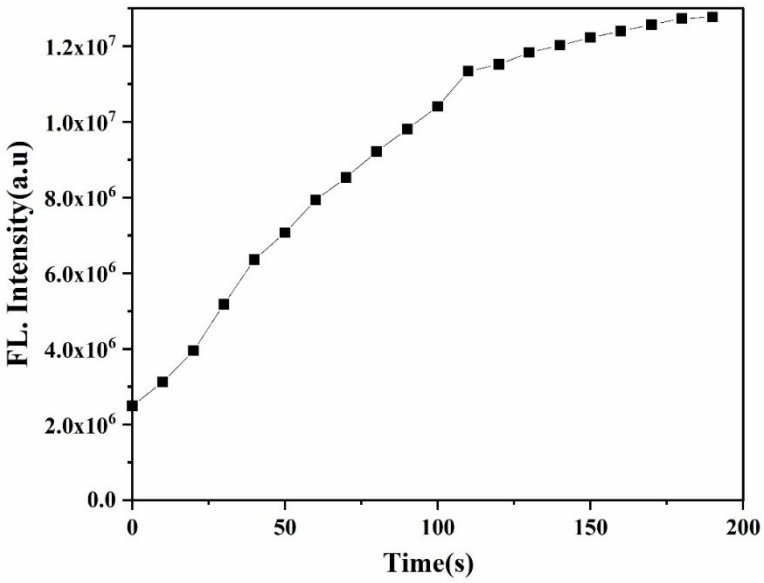


Figure S3. Time course (0–180 s) of fluorescence enhancement of **TPB-NO_2** (10.0 μM) in DMSO/H_2O solution (1:1, v/v) solution upon addition of 80 μM of Na_2S (λ_{ex} = 390 nm; λ_{em} = 483 nm).

6. Investigation of the reaction mechanism by using HRMS spectra

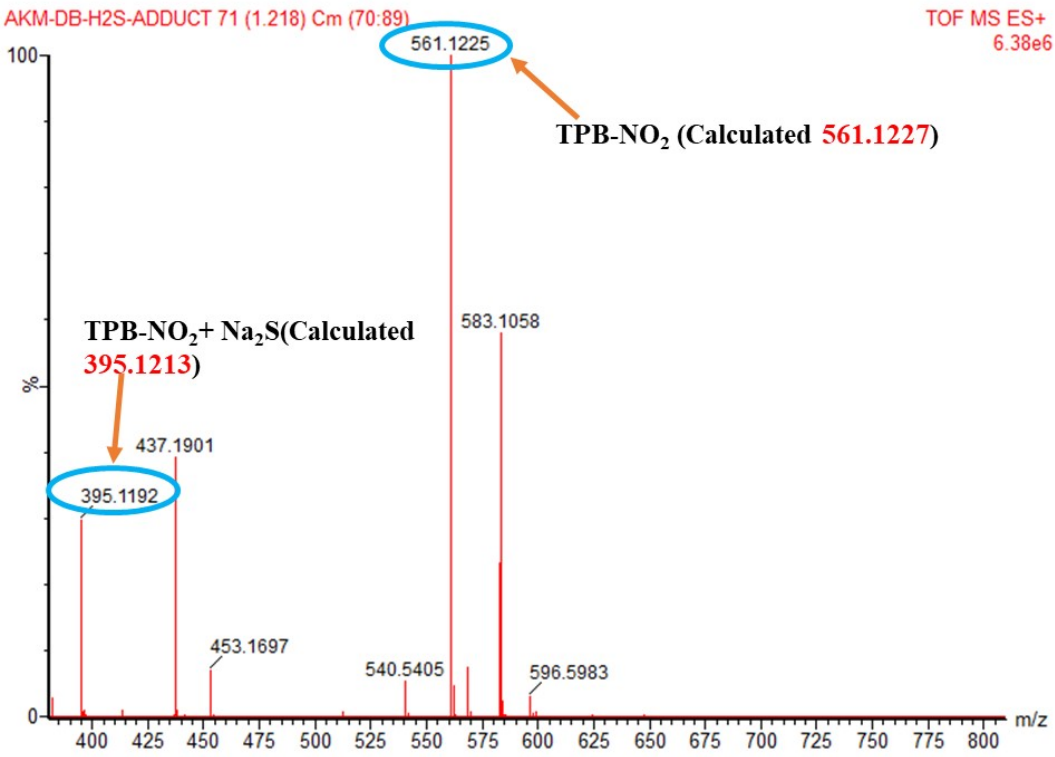


Figure S4. HRMS spectra were taken after a few minutes of reaction between probe **TPB-NO_2** and Na_2S.

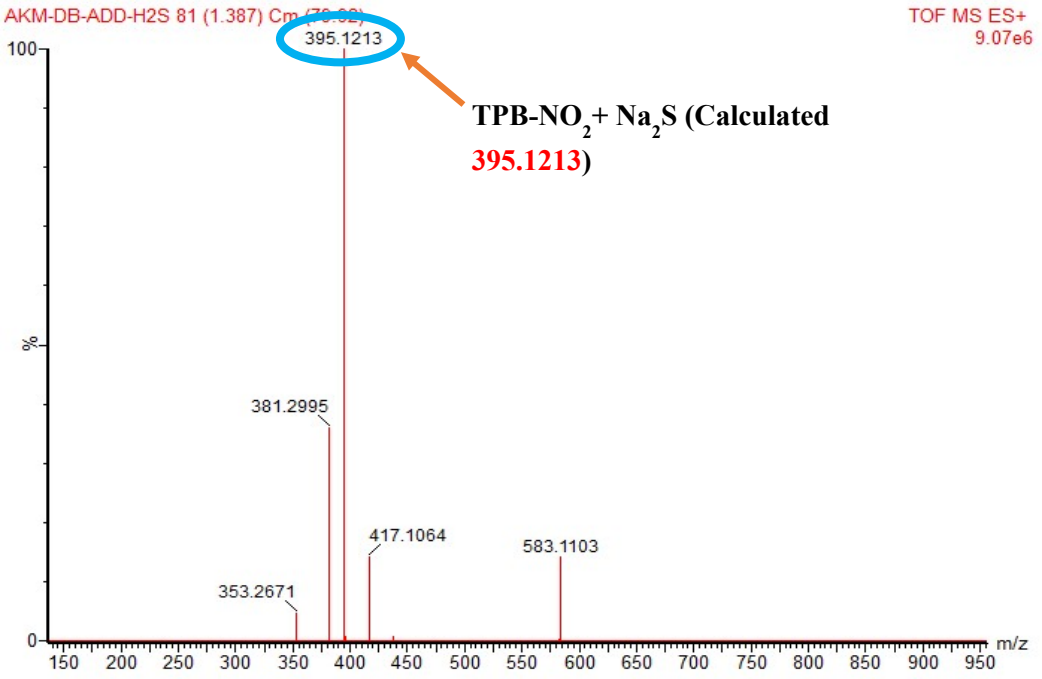


Figure S5. HRMS spectra were taken after 1 hour of the reaction between probe **TPB-NO_2** and Na_2S.

7. Table S1 Crystal Data and Structure Refinement Parameters of the probe TPB-NO_2

Empirical formula	$C_{31}H_{20}N_4O_5S$
Formula Weight	560.57
Temperature (K)	188
Wavelength (Å)	0.71073
Crystal system	Triclinic
space group	$P\bar{1}$
a, b, c (Å)	8.090(4), 11.340(6), 15.677(8)
α, β, γ (°)	73.356(17), 87.625(16), 79.487(17)
Volume ($Å^3$)	1354.7(12)
Z / Density (calc.) (Mg/m^3)	2/ 1.374
Absorption coefficient (mm^{-1})	0.169
F(000)	580.0
Crystal size (mm^3)	0.18 x 0.13 x 0.08
θ range for data collection	2.634 to 27.263
Completeness to θ (%)	100%
Absorption correction	Multi-scan
Max. and min. transmission	0.987 and 0.974
Refinement method	Full-matrix least-squares

	on F^2
Data/parameters	6095/ 370
Goodness-of-fit on F^2	1.008
Final R indices [I > 2σ(I)]	$R_1 = 0.0711$, $wR_2 = 0.2105$
R indices (all data)	$R_1 = 0.0929$, $wR_2 = 0.2420$
Largest diff. peak and hole ($e.Å^{-3}$)	0.375 and -0.508

$R_1=\sum||F_o|-|F_c||/\sum|F_o|$, $wR_2 = [\sum\{(F_o^2-F_c^2)^2\}/\sum\{w(F_o^2)^2\}]^{1/2} w = 1/\{\sigma^2(F_o^2) + (aP)^2 + bP\}$, $P = (F_o^2 + 2F_c^2)/3$, where, a = 0.1739 and b =0.0766.

8. Computational method

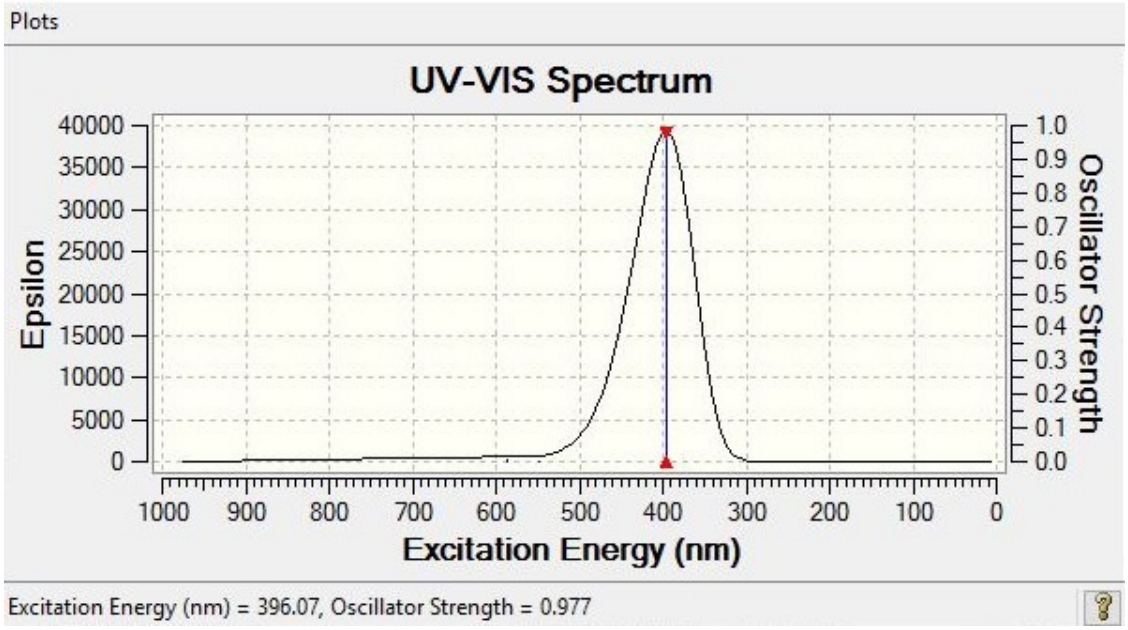


Figure S6. Absorption spectra of the Probe **TPB-NO_2**

Table S2: The main vertical orbital transition of the Probe **TPB-NO_2** calculated by TD-DFT method:

Energy (eV)	Wavelength (nm)	Osc. Strength (f)	Transition
3.1303	396.07	0.9770	**HOMO → LUMO+2**
2.2630	547.87	0.0003	**HOMO → LUMO+1**
2.1123	586.97	0.0069	**HOMO → LUMO**

9. Live cell imaging study

Cytotoxicity assay

The cytotoxic impact of the ligand TPB-NO_2 was assessed by means of the MTT cell proliferation assay[2,3] conducted on both the MDA-MB-231 cancer cell line and the NKE normal cell line. To elucidate, cells were initially seeded in 96-well plates with a density of 1 × 10^4 cells per well, allowing 24 hours for adherence before exposure to varying concentrations of the TPB-NO_2 ligand (0 µM, 10 µM, 20 µM, 40 µM, 80 µM, 100 µM) for the duration of 24 hrs. Post-treatment media was discarded from each well and washed with 1x PBS, following each well underwent treatment with 0.5 mg/ml of MTT solution and incubated for 4 hours, followed by a rinse with 1x PBS. The ensuing formazan crystals were dissolved in DMSO, and the microplate reader recorded absorbance at 570 nm. The measurement of cell viability was expressed as a percentage of the control experimental setup.

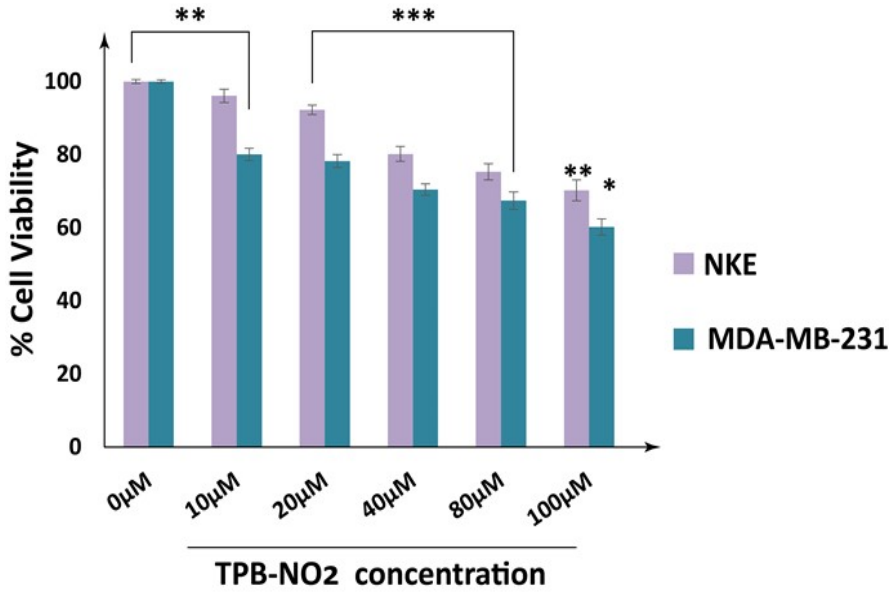


Figure S7. Cell survivability of MDA-MB 231 and NKE cells exposed to different ligand TPB-NO_2 concentrations. Data are representative of at least three independent experiments and the bar graph shows mean ± SEM, ***$p < 0.0001$, **$p < 0.001$, *$p < 0.01$ were interpreted as statistically significant, as compared with the control.

Fluorescence imaging:

Fluorescence imaging was conducted on the MDA-MB 231 cell line to visualize the fluorescence capability of the TPB-NO_2 in the presence of H_2S (10 µM). In brief, cells were cultured on coverslips for 24 hours at 37°C in a humidified environment with 5% CO2. Subsequently, pretreatment with N-Ethylmaleimide (NEM) was carried out to eliminate thiol-containing molecules and endogenous Cys. Then cells were washed with PBS and mock-treated with 10 µM of the TPB-NO_2 ligand and on another set-up NEM pre-treated cells were then incubated with 10 µM of the TPB-NO_2 ligand for the time period of 1 hour, following this, the cells underwent treatment with H_2S (10 µM) and incubated for 10 minutes and 20 minutes. Later cells were washed with PBS to remove any unbound TPB-NO_2 and transferred onto a glass slide for examination using an Olympus fluorescence microscope.[4,5]

10.NMR Spectra: ^{1}H-NMR, ^{13}C-NMR

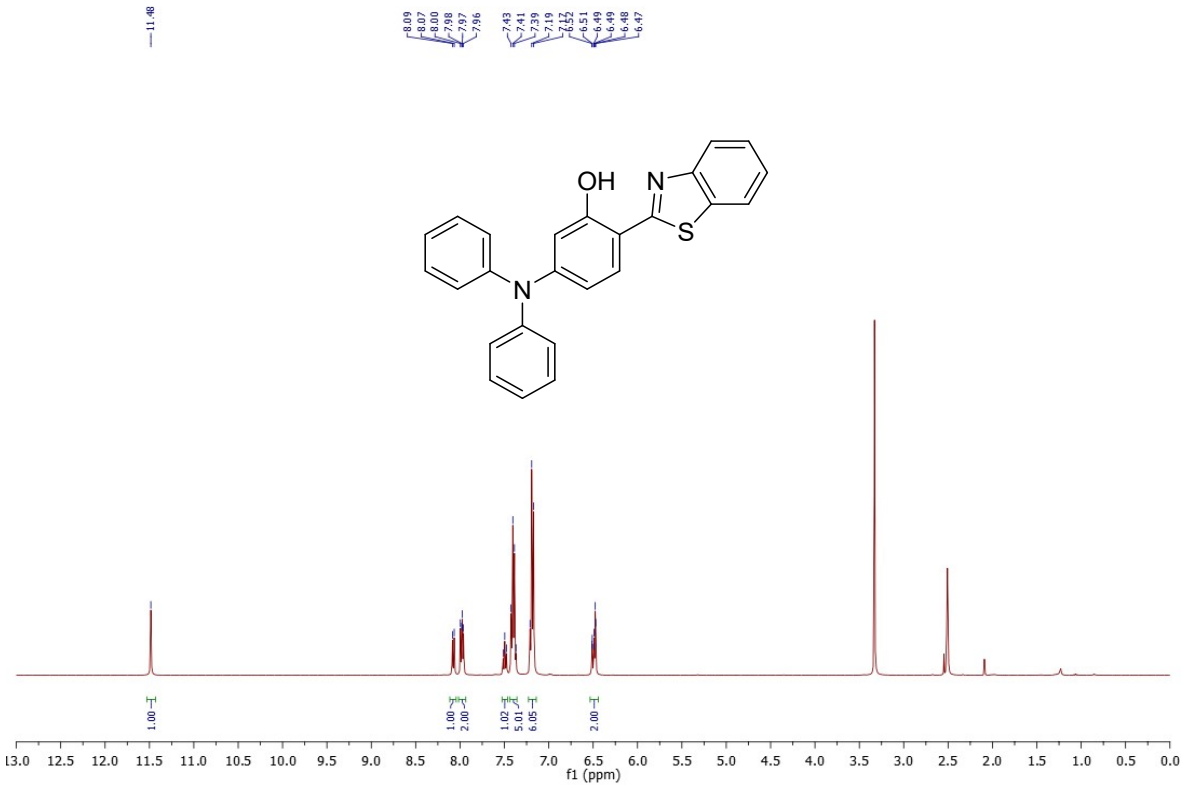


Figure S8. ^{1}H NMR spectrum of the compound **TPB-OH** in DMSO-d_6

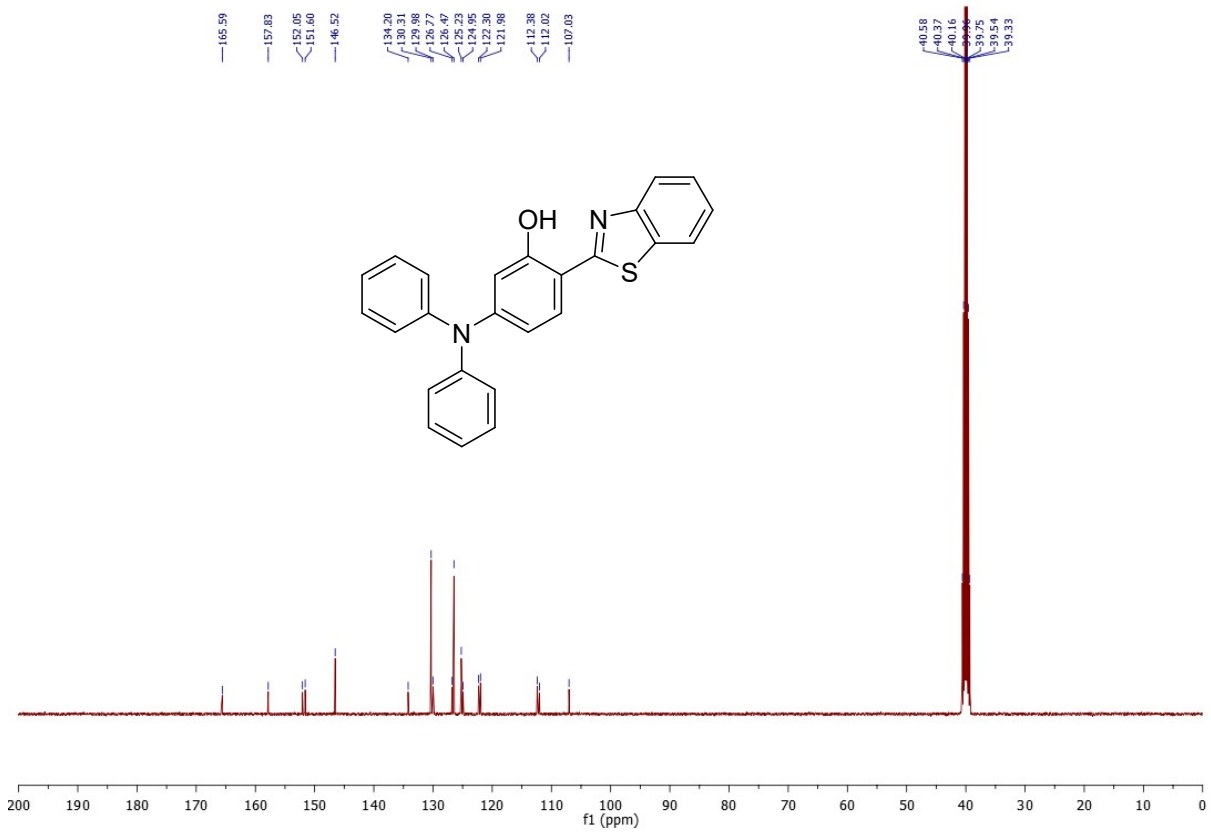


Figure S9. ^{13}C NMR spectrum of the compound **TPB-OH** in DMSO-d_6

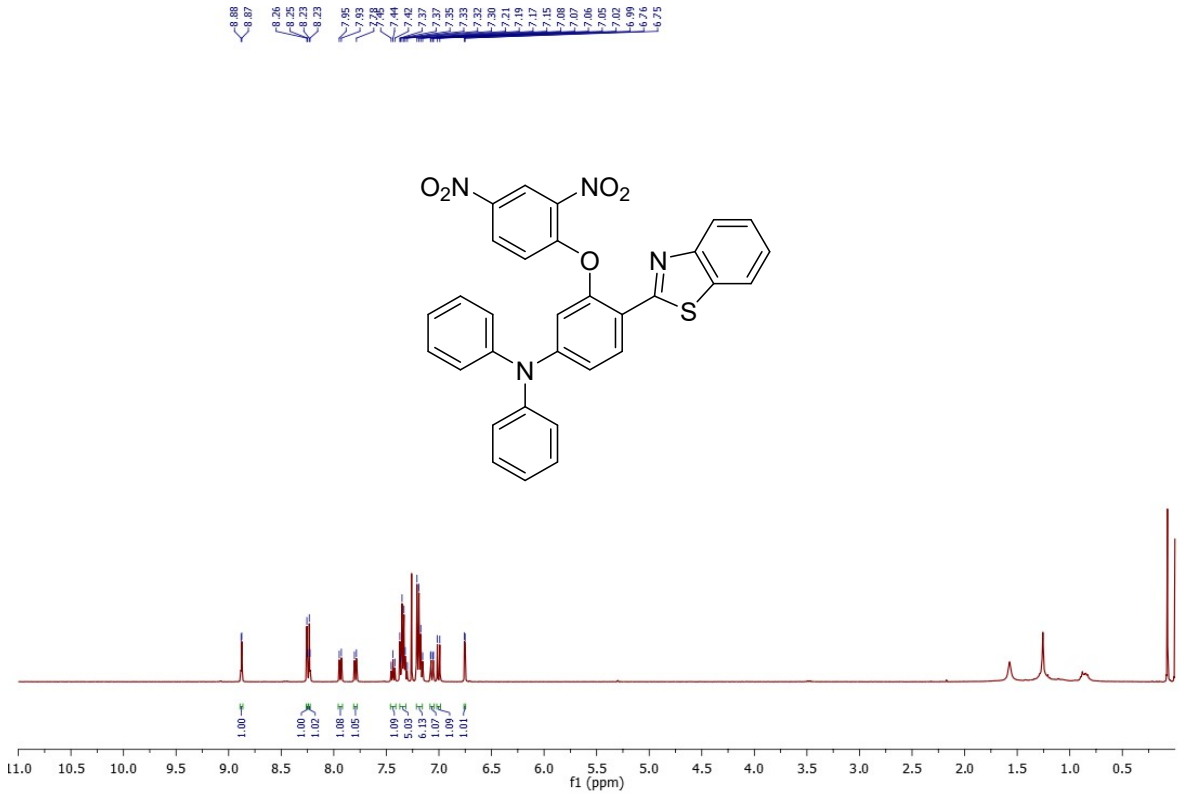


Figure S10. ^{1}H NMR spectrum of the compound **TPB-NO$_2$** in $CDCl_3$

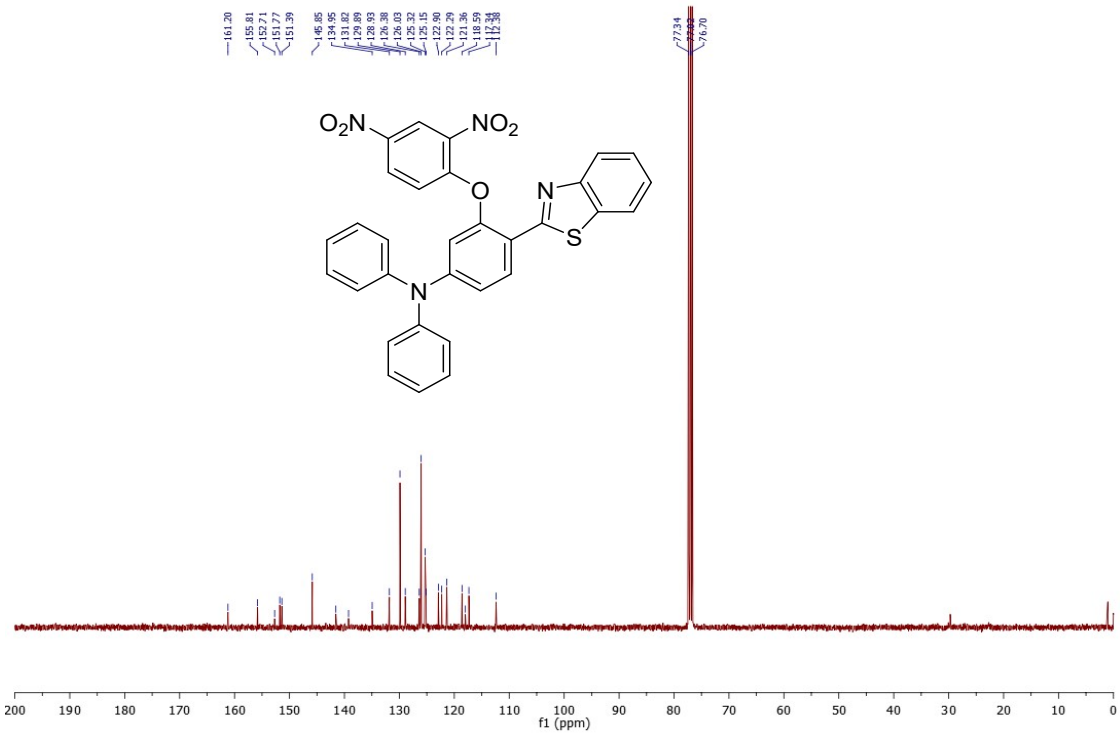


Figure S11. ^{13}C NMR spectrum of the compound **TPB-NO$_2$** in $CDCl_3$

11. ESI-MS Spectra

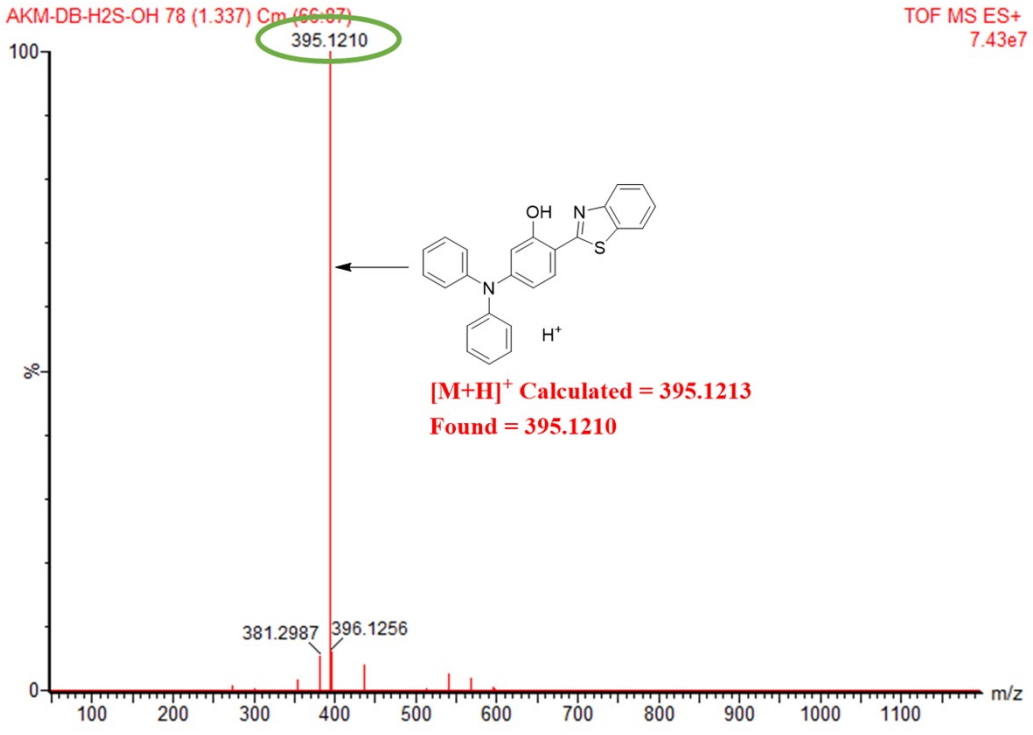


Figure S12. HRMS spectra of the compound **TPB-OH**

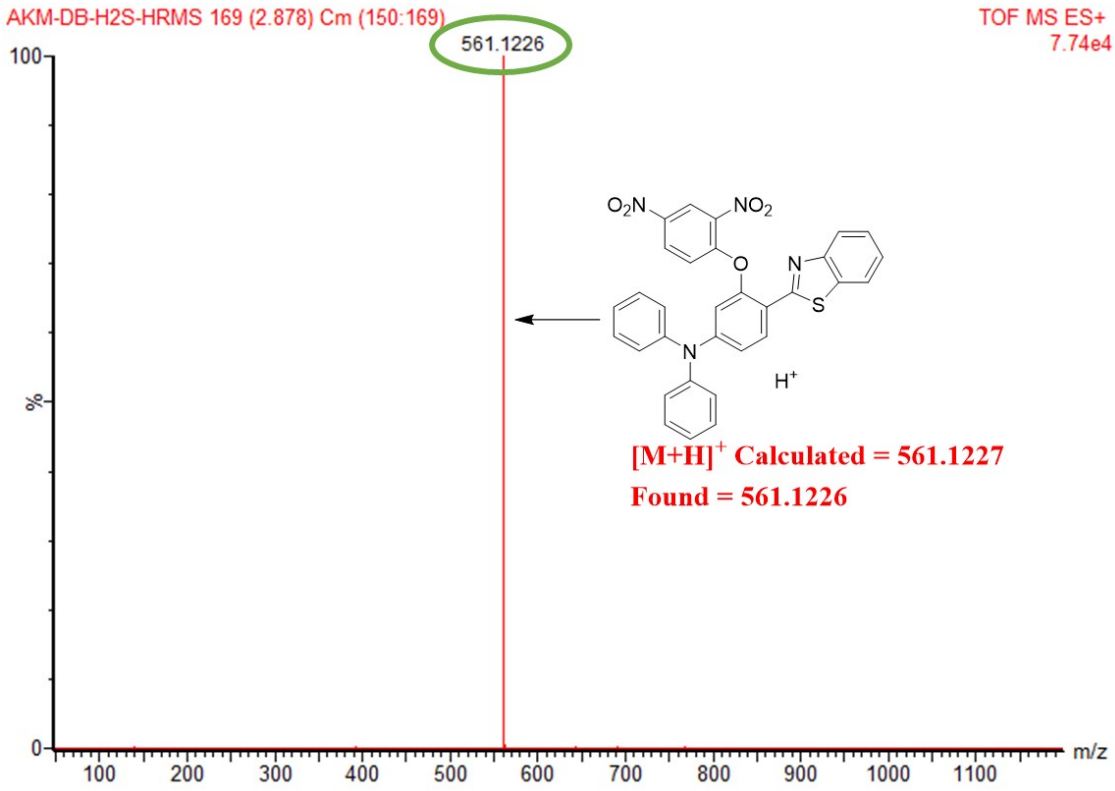


Figure S13. HRMS spectra of the compound **TPB-NO_2**

12. Table S3 Environmental water sample study by using probe TPB-NO_2

Water sample	H_2S spiked (μM)	Found (μM)	% Recovery	RSD (%)
Tap water	2.0	1.92 ± 0.09	96.0	4.69
	4.0	3.40 ± 0.22	85.0	6.47
	6.0	5.82 ± 0.15	97.0	2.57
	8.0	8.02 ± 0.35	100.25	4.36
Ganges River water	2.0	1.97 ± 0.15	98.50	7.61
	4.0	4.05 ± 0.25	101.25	6.17
	6.0	5.92 ± 0.20	98.66	3.38
	8.0	8.07 ± 0.35	100.87	4.34
Lake Water	2.0	1.88 ± 0.17	94.0	9.04
	4.0	3.97 ± 0.32	99.25	8.06
	6.0	6.08 ± 0.42	101.33	6.90
	8.0	8.06 ± 0.43	100.75	5.33

13. Selectivity toward H_2S over various analyte

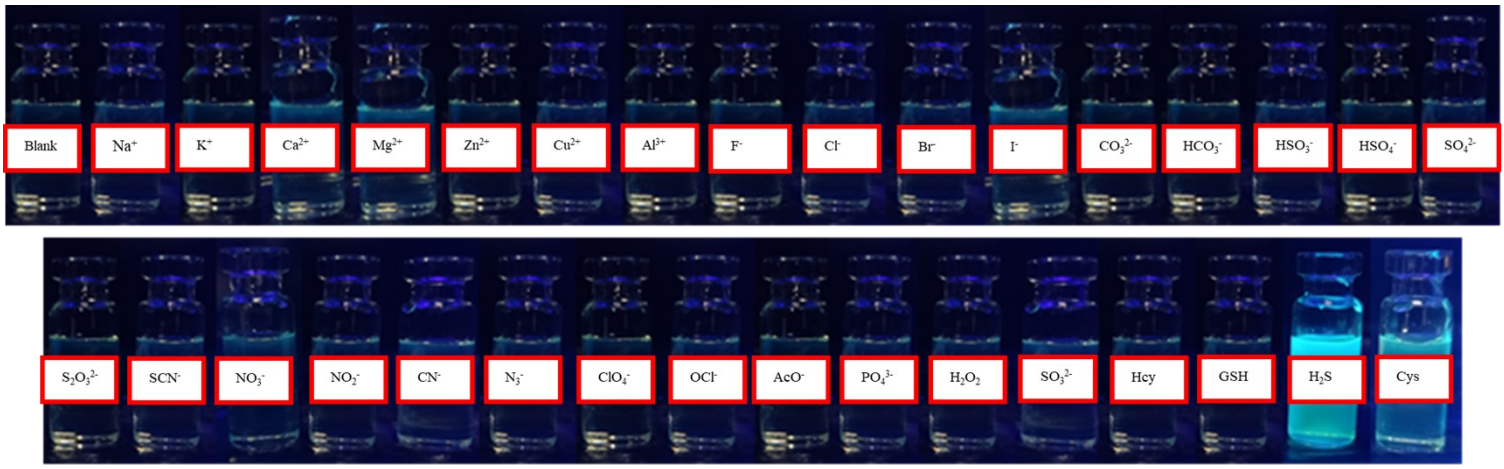
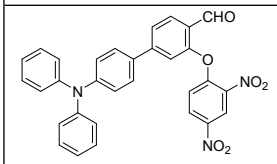
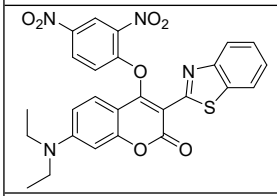
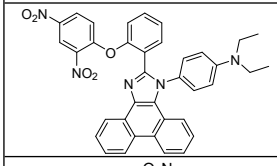
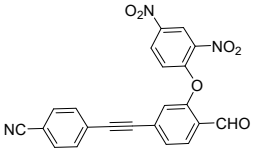
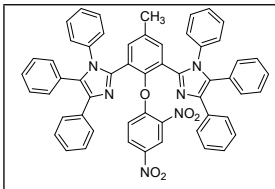
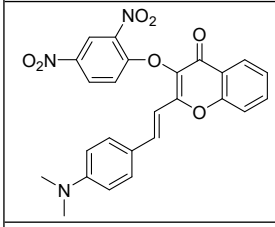
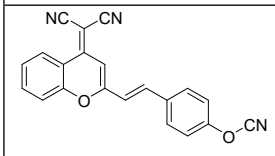
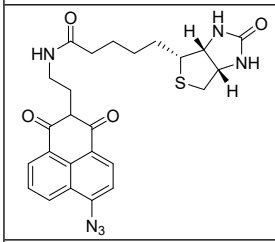
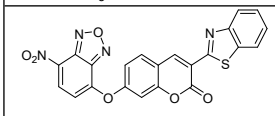
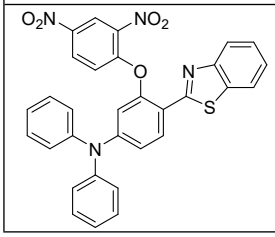


Figure S14. Fluorescence color change of the probe **TPB-NO_2** in DMSO/H_2O solution (1:1, v/v; 10 mM HEPES buffer, pH 7.4) upon the addition of various analytes under a UV lamp.

14. Table S4 Comparison table of important parameters between reported H_2S probes with our probe TPB-NO_2

Structure of the probe	Solvent system	Mode of sensing	LOD	Response time	Applications Water sample/TLC strip/Food sample	Cell imaging	Cytotoxicity assay Human cancer cell line/human epithelial cell line	Ref
	THF/H_2O (2/8, v/v, HEPES 20 mM, pH = 7.12)	FL Turn-on/AIE	7.63 µM	20 min	Yes/No/No	Yes	Yes/No	6
	PBS buffer (10 mM, containing 50% DMSO, pH = 6).	FL Turn-on	90 nM	-	Yes/No/No	Yes	Yes/No	7
	PBS (20 mM, pH = 7.4, 10% DMSO, v/v)	FL Turn-on/ PET	1.11 µM	4.5 min	No/No/No	Yes	Yes/No	8
	PBS buffer (10% EtOH, pH=7.4)	FL Turn-on/ PET and ESIPT	0.61 µM	9 min	No/No/No	Yes	Yes/No	9

	HEPES buffer (10mM containing 1% DMSO, pH=7.4)	FL Turn-on/ ESIPT	0.13 nM	16 min	Yes/Yes (Filter Paper)/No	Yes	Yes/No	10
	PBS buffer	FL Turn-on/ PET	90 nM	30 min	No/No/No	Yes	No/No	11
	PBS buffer (1% DMSO)	FL Turn-on	0.28 μM	5 min	Yes/No/No	No	No/No	12
	PBS (20 mM, pH =7.4, 5% MeOH)	FL Turn-on	71 nM	25 min	No/No/No	Yes	Yes/No	13
	HEPES buffer solution (20 mM, pH 7.4, 10% CH_3CN)	FL Turn-on	0.12 μM	2 min	No/No/No	Yes	Yes/No	14
	DMSO/H_2O solution (1:1, v/v; 10 mM HEPES buffer, pH 7.4)	FL Turn-on/Colorimetric/ PET	9.81 nM	< 2 min	Yes/Yes/Yes	Yes	Yes/Yes	This work

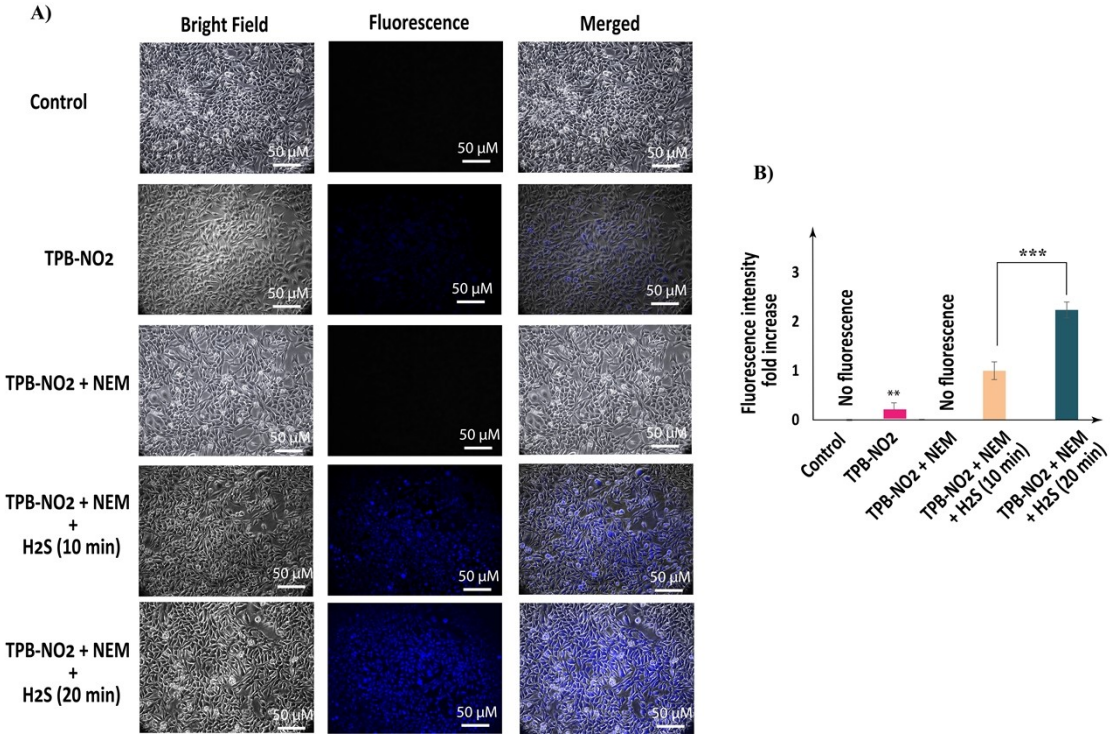


Fig S15: A) Fluorescence Microscopic images and B) fold increase in fluorescence intensity of untreated MDA-MB 231 cells (Control), cells treated with only 10 µM of ligand TPB-NO_2, NEM pre-treated cells treated with 10 µM of ligand TPB-$NO_2$and NEM+ ligand TPB-NO_2(10 µM)in the presence of H_2S (10 µM) together after 10 min and 20 min incubation periods under bright, fluorescence and merge field.

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