Supporting Information

For

Reaction-based ratiometric fluorescent probe for selective recognition of sulfide anions with a large stokes shift through switching on ESIPT

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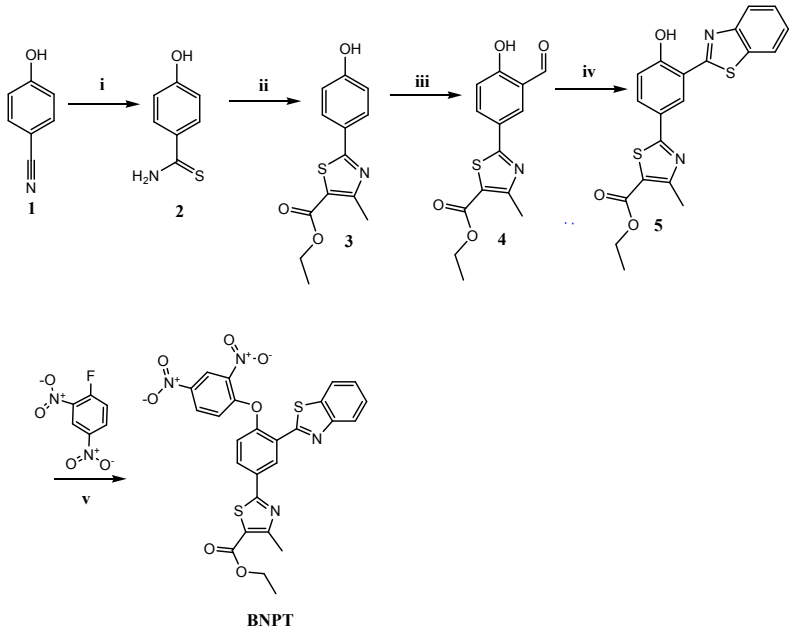
Synthetic Scheme:



Reagents & conditions: (i) P_2S_5, Ethanol, 70 ^oC, 12h (ii) 2-chloroacetoacetic acid ethyl ester, Isopropanol, 85 ^oC, 2h (iii) HMTA, ACOH, 90 ^oC, 12h, 20% HCl (iv) 2-aminothiophenol, Ethanol cat. $KHSO_4$, 12h (v) 2,4-Dinitro fluoro benzene, K_2CO_3,DMF, 80 ^oC, 7h.

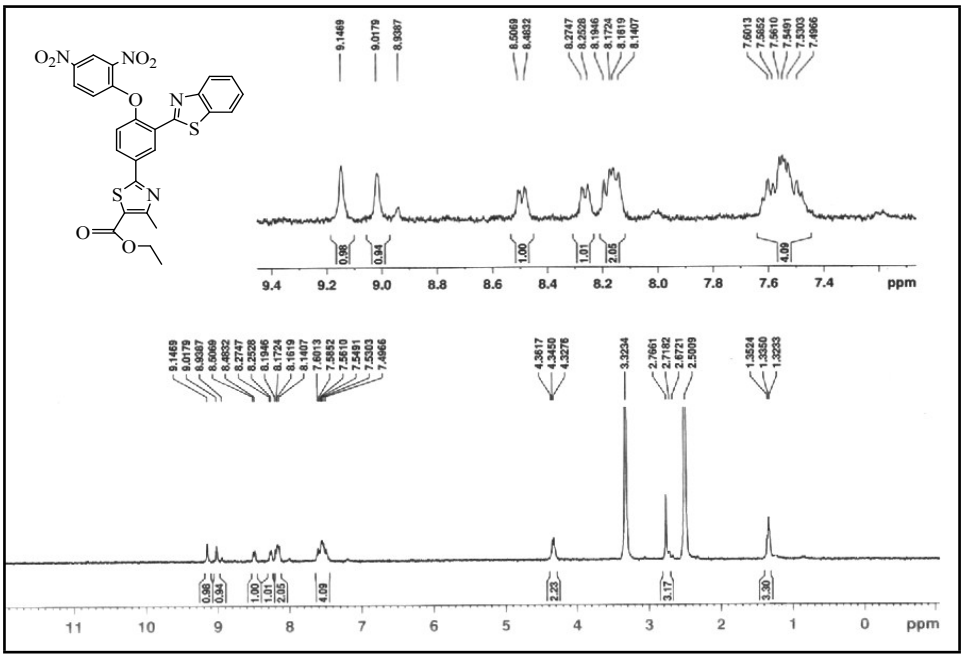


Fig. S1 ^1H NMR spectra recorded for **BNPT** in d_6-DMSO

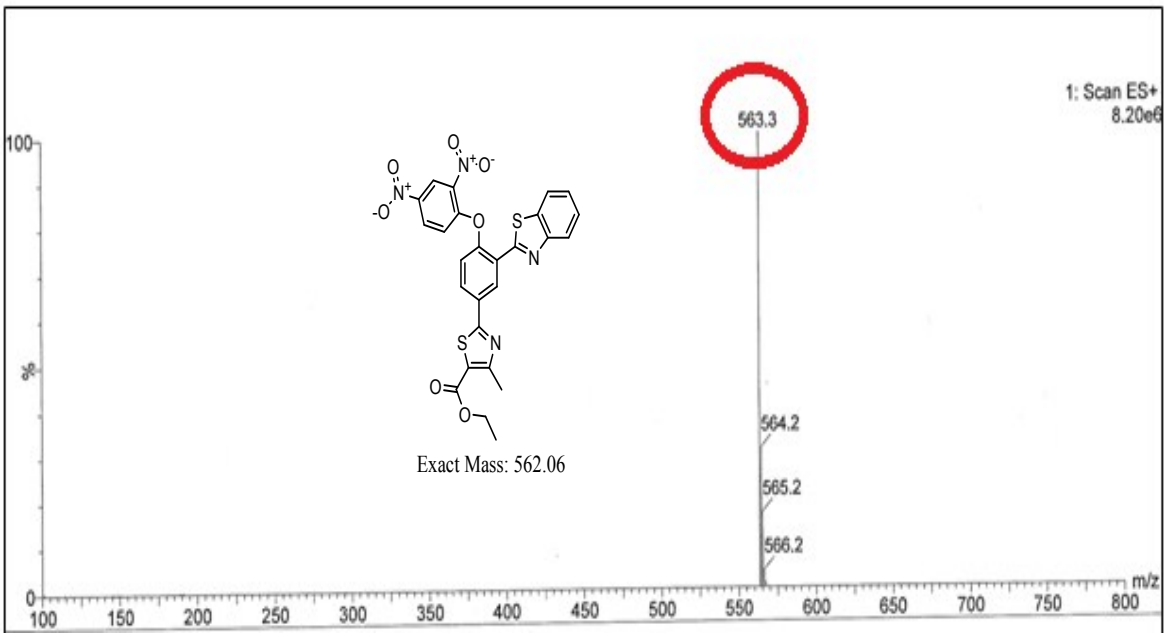


Fig. S2 Mass spectrum recorded for **BNPT** ($C_{26}H_{18}N_4O_7S_2$), corresponding to $[M+H]^+$ = 563.3

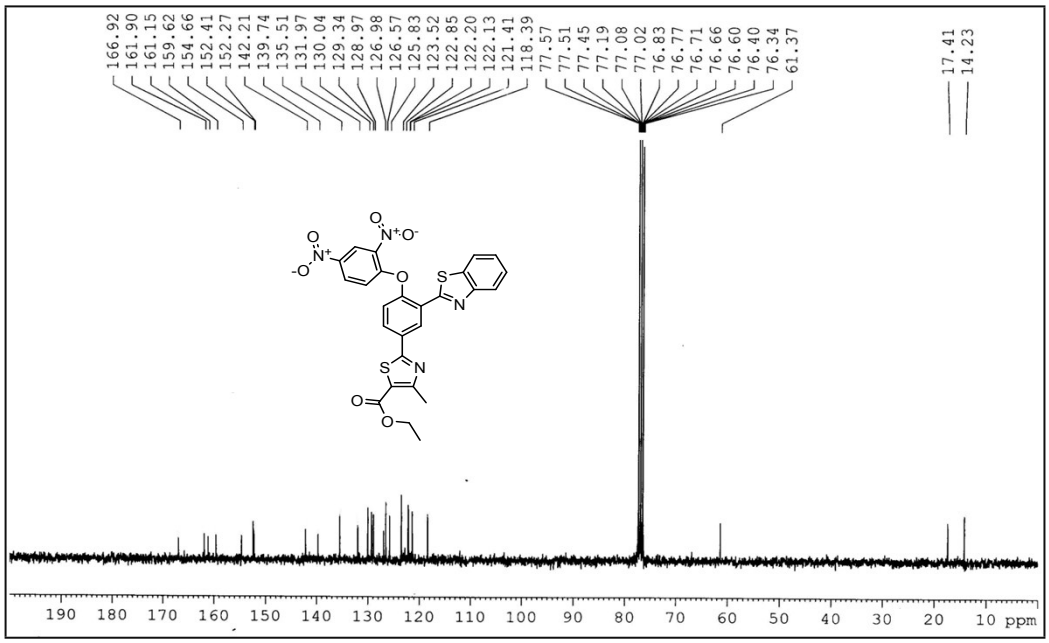


Fig. S3 ^{13}C NMR spectra recorded for **BNPT** in $CDCl_3$

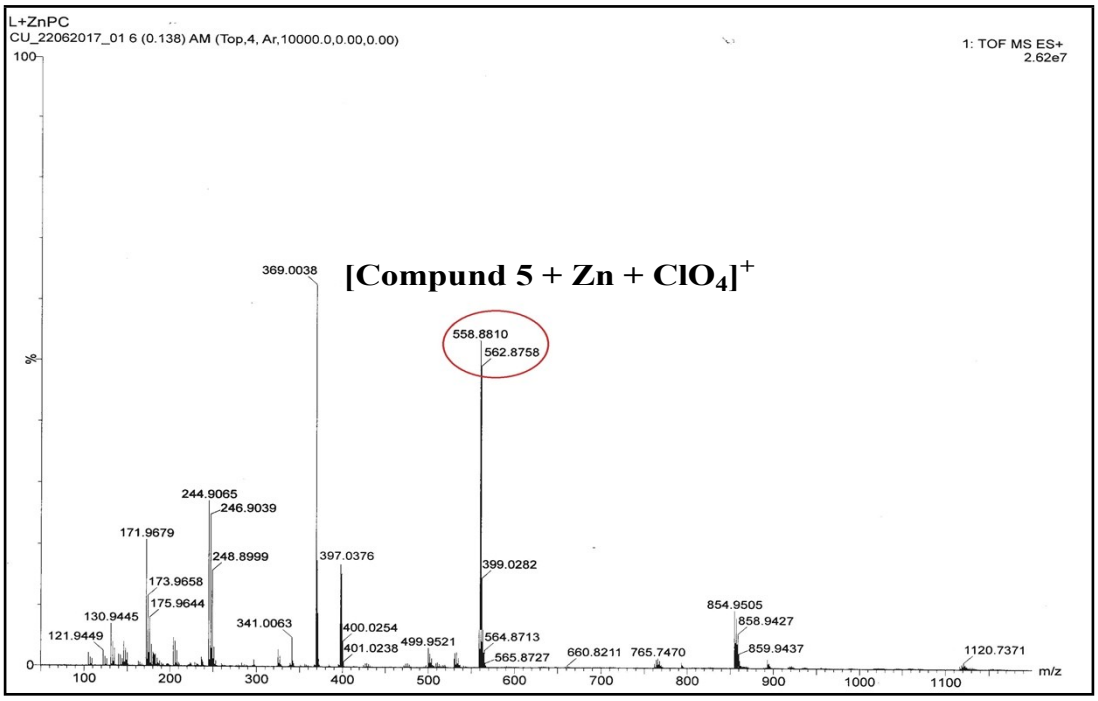


Fig. S4 HR-MS Spectrum recorded for **[Zn-5]** in methanol-water (1;1) solution.

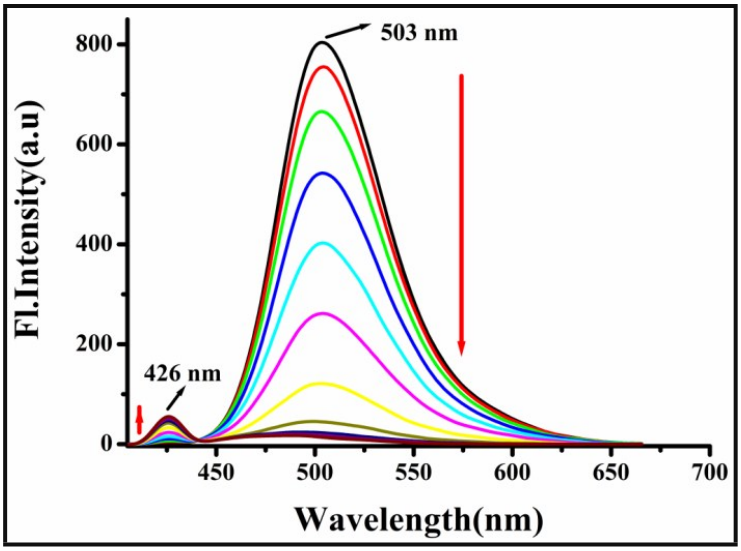


Fig. S5 Fluorescence emission spectra (λ_{ex} = 347 nm) of the reaction product of **BNPT** ($1x10^{-5}$M) and H_2S (compound 5 formed in-situ) in presence of increasing amount of Zinc ion ($1x10^{-4}$M) in aqueous acetonitrile ($CH_3CN:H_2O$ = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4) at room temperature

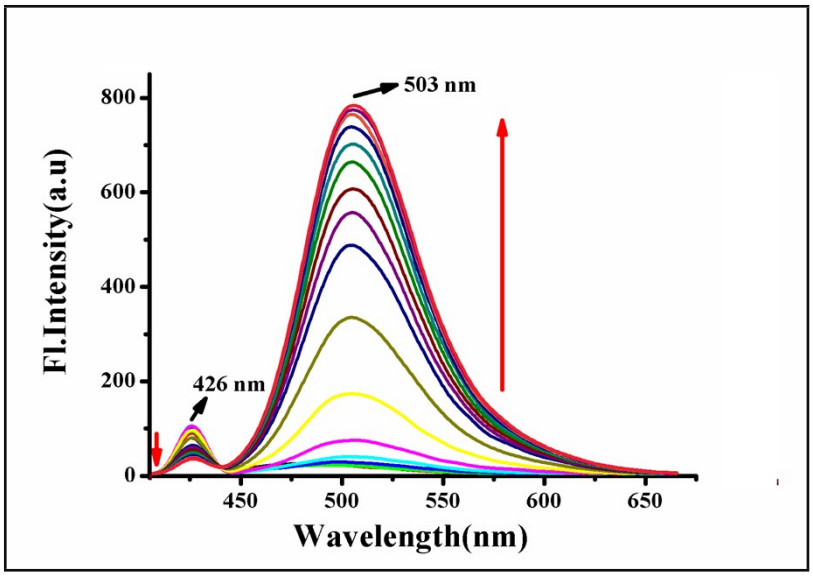


Fig. S6 Change in fluorescence emission upon addition of increasing concentration of H_2S ($1x10^{-4}$M) in aqueous acetonitrile ($CH_3CN:H_2O$ = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4) at room temperature to the Compound 5-Zn^{2+} ensemble.

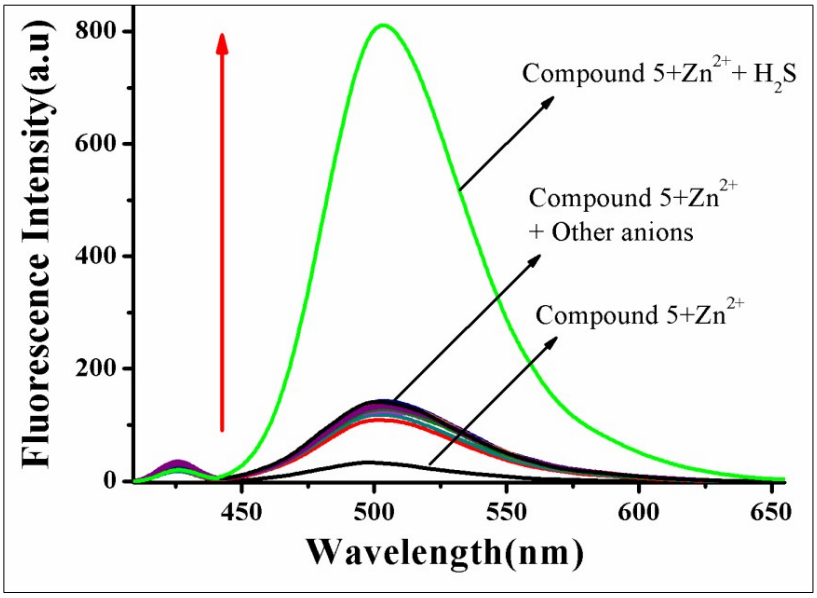


Fig. S7 Change in fluorescence emission of Compound 5-Zn2+ ensemble (10µM) in presence of various anions (50µM) and H2S (50µM) in aqueous acetonitrile (CH3CN:H2O = 3:2 v/v, 10 mM HEPES bu□er, pH = 7.4).

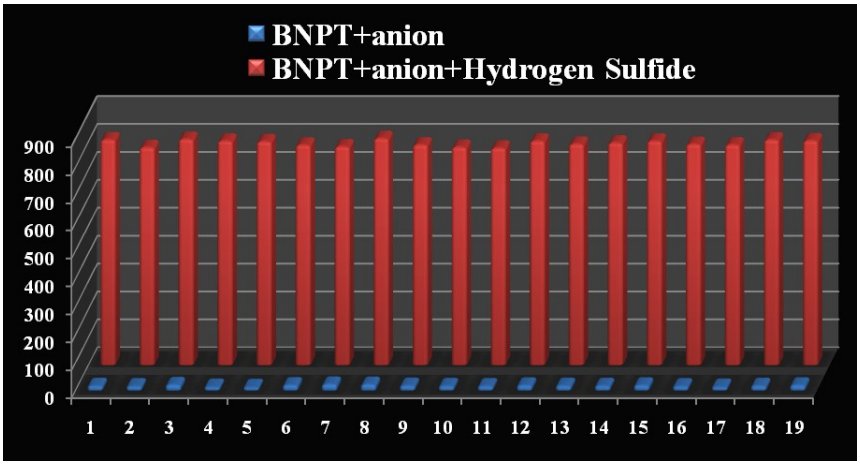


Fig. S8 Fluorescence responses of BNPT (10 μM) to various competitive species(N_3^-, AcO^-, SCN^-, PO_4^{3-}, $H_2PO_4^-$, HCO_3^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, F^-, Cl^-,Br^-,I^-, ,HSO_4^-, SO_3^{2-} ,$S_2O_3^{2-}$,Cys,Hcy and GSH) in aqueous acetonitrile (CH_3CN:H_2O = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4). The blue bars represent the emission changes of BNPT in the presence of above anions (all are 50 μM). The red bars represent the changes of the emission that occurs upon the subsequent addition of 20 μM of H_2S to the above solution. The intensities were recorded at 503 nm.

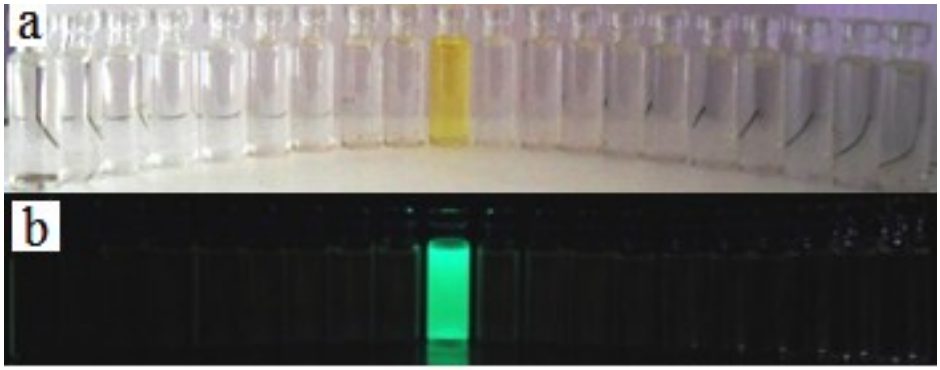


Fig. S9 Visual color change (a) under daylight (b) handheld UV-lamp of probe BNPT (10μM) in presence of various anions (50μM) in aqueous acetonitrile (CH_3CN:H_2O = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4) at room temperature. (from left to right): N_3^-, AcO^-, SCN^-, PO_4^{3-}, $H_2PO_4^-$, HCO_3^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, H_2S, F^-,Cl^-,Br^-,I^-, ,HSO_4^-, SO_3^{2-} ,$S_2O_3^{2-}$,Cys,Hcy and GSH

Effect of pH.

The effect of pH on the photophysical properties of **BNPT** was investigated by the fluorescence spectroscopy (Fig.4). A series of buffers with pH values ranging from 2 to 12 was prepared by mixing sodium hydroxide solution and hydrochloric acid in HEPES buffer. Thus, we proceeded to investigate the effect of pH on the fluorescence intensity of the probe **BNPT** in the absence or presence of S^{2-}. The results showed that fluorescence intensity (I_{503} nm) of **BNPT** is maximum at pH ~ 7.0 with S^{2-}, **BNPT** is stable in the pH range 1 to 10. Thus, considering the environmental and biological applications, we employed the near neutral pH (pH = 7.4) for the detection of S^{2-}.

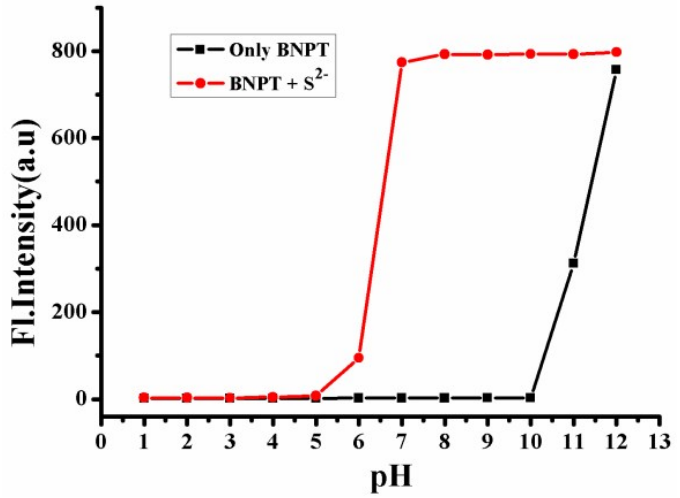


Fig. S10 pH dependent fluorescent spectra of **BNPT** in absence (black line) and in presence (red line) of S^{2-}.

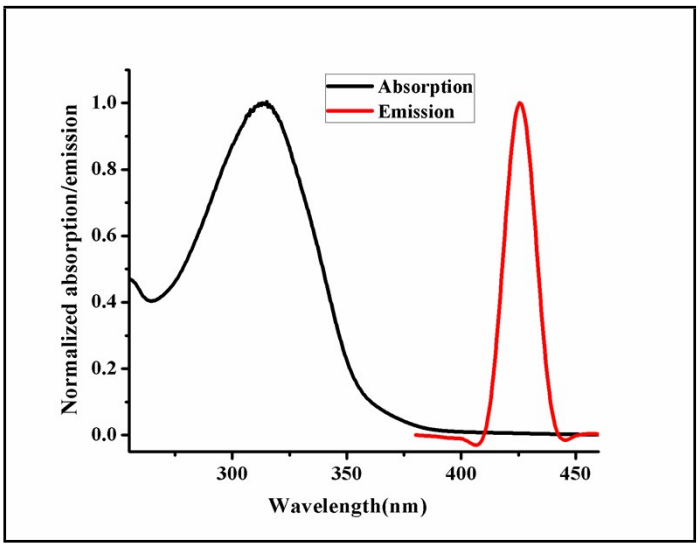


Fig. S11 Normalized UV-vis and fluorescence spectra of **BNPT** in aqueous acetonitrile ($CH_3CN:H_2O$ = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4) at room temperature. The excitation wavelength λex for fluorescence measurement was set at 426 nm.

Job's Plot:

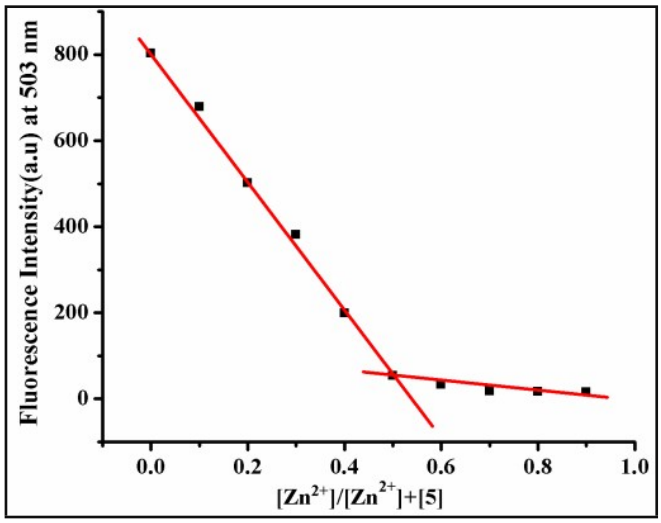


Fig. S12 Job plot of a 1:1 complex of **compound 5** and Zn^{2+}

Calculation of Detection limit (For BNPT):

The detection limit (DL) of **BNPT** for hydrazine were determined from the following equation:
DL = K* Sb1/S
Where K = 2 or 3 (we take 2 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.
From graph Sb1=2.00142, S=1.28077E8. DL= 3.13 x 10^{-8}M = 31.3 nM

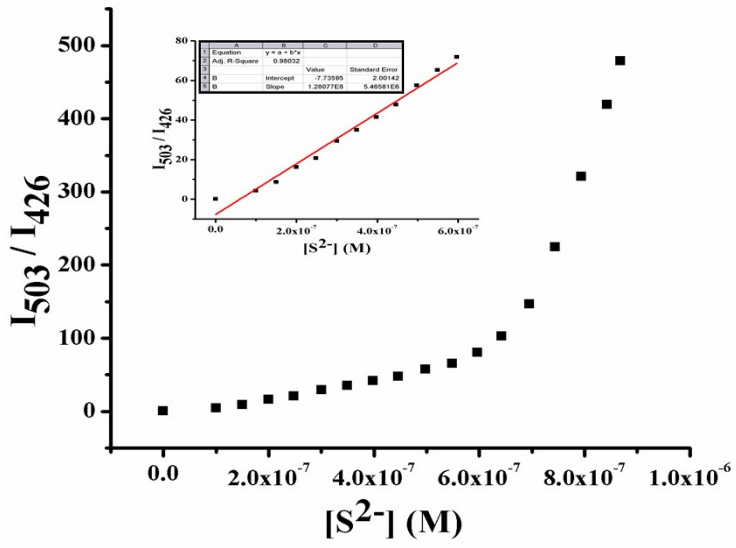


Fig. S13 (a) Changes in fluorescence intensity ratio of probe **BNPT** (10μM) upon addition of increasing concentrations of S^{2-} in in aqueous acetonitrile ($CH_3CN:H_2O$ = 3:2 v/v, 10 mM HEPES buffer, pH = 7.4) at room temperature at 503 nm. **Inset**: a linear calibration curve between the fluorescent intensity at 503 nm and the concentration of S^{2-} in the range of 0 to 0.6 μM. Each spectrum was collected at 5 min after S^{2-} addition. λex = 347 nm

Binding constant:

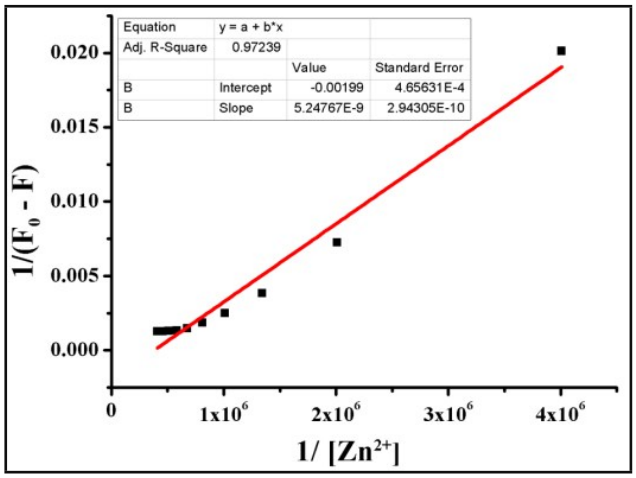


Fig. S14 Benesi−Hildebrand plot for Compound 5 and Zn^{2+} to calculate the binding constant ($Ka=4.0 \times 10^5\ M^{-1}$)

Cytotoxic effect on Cells.

The cytotoxic effects of the receptor and sulphide were determined by MTT assay following the manufacturer protocol (Himedia). Briefly, Human adult dermal fibroblast (HADF, Himedia Laboratories, India) cells were seeded onto 96-well plates (approximately 103 cells/well) and incubated for overnight. The media was removed and fresh serum free media was added. Receptor at 10-4 M concentration in DMEM were added to the cells and incubated for 1 h. 10-2 M sulphide was added followed by 1 h incubated. A control (only media) was included in the study. Growth media was removed, and fresh serum free DMEM containing (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) solution was added. The plate was incubated for 4 h at 37°C. Subsequently, the supernatant was removed, the solubilization solution was added to solubilize formazan. Absorbance was measured in a UV-Vis micro plate reader (Thermo Fischer Scientific) at 570 nm and 670 nm as a reference wave length.

Cell viability:

After the observation of cell uptake of the **BNPT**, further we investigated the cytotoxicity of the **BNPT** through cell viability assay. The standard MTT assays suggested low cytotoxicity of the Receptor to HADF cells (Figure S14). HADF cells showed more than 77% viable after treatment with **BNPT**; however, followed by sulfide treatment, cell viability was reduced to about 55%. These results clearly indicated that either Receptor or both Receptor and sulfide have less cytotoxic effect to the live cells.

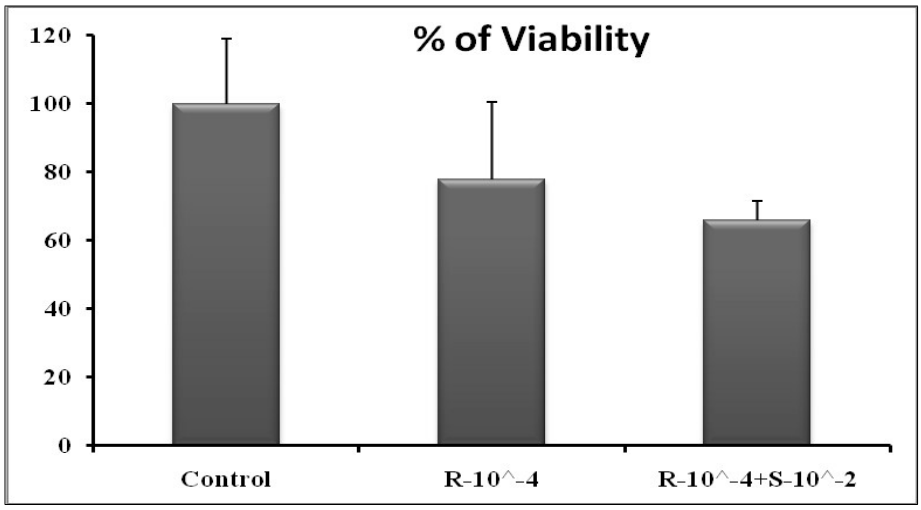


Fig. S15 Cell viability assay of HADF cells to observe the effect of cytotoxicity of **BNPT** and both **BNPT** and S^{2-}.

Computational studies:

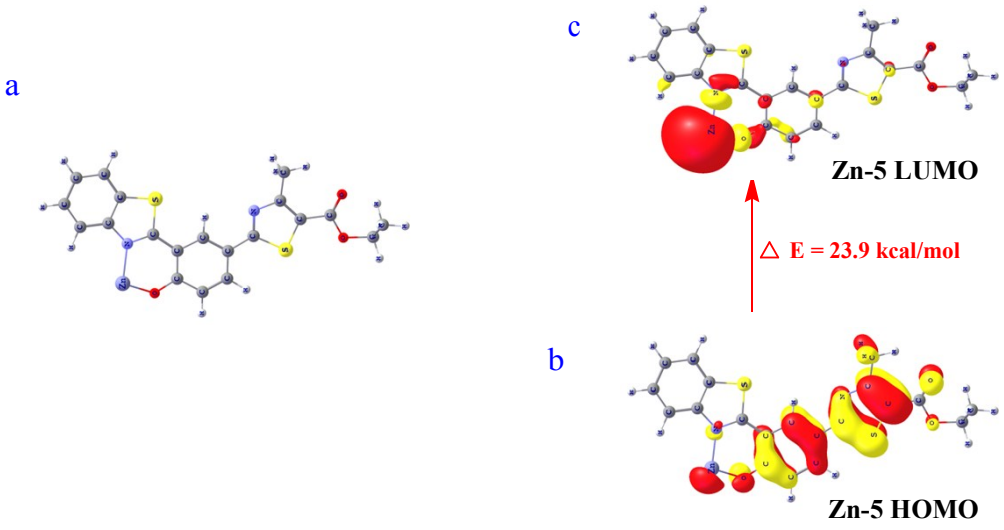


Fig. S16 The optimized configuration of (a) [Zn-5]. HOMO−LUMO energy gaps for respective compounds and interfacial plots of the orbitals: (b) [Zn-5] HOMO (c)) [Zn-5] LUMO

Table S1. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of all the complexes. The data were calculated by TDDFT//B3LYP/6-31+G(*d,p*) based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c]	(composition) %
BNPT	$S_0 \rightarrow S_{13}$	3.8708 eV 320.30 nm	0.3561	H-4 → L H → L +4	51.8
	$S_0 \rightarrow S_{14}$	3.8870 eV 318.97 nm	0.6376	H-1 → L	56.6
Compound 5	$S_0 \rightarrow S_1$	3.3836 eV 366.42 nm	0.2667	H → L H → L+1	95.6
	$S_0 \rightarrow S_2$	3.6226 eV 342.25 nm	0.5833	H → L+1	62.0
	$S_0 \rightarrow S_3$	4.0500 eV 306.14 nm	0.4408	H → L+2	83.0
[Zn-5] complex	$S_0 \rightarrow S_1$	0.8622 eV 1438.03 nm	0.1044	H → L	100
	$S_0 \rightarrow S_{13}$	3.5845 eV 345.89 nm	0.3345	H-2 → L+1 H → L+2	86.4
	$S_0 \rightarrow S_{15}$	3.7452 eV 331.05 nm	0.6086	H-3 → L+1	75.6

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO.

Table S2. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	**E_{HOMO} (a.u)**	**E_{LUMO}(a.u)**	**ΔE(a.u)**	**ΔE(eV)**	**ΔE(kcal/mol)**
BNPT	-0.23717	-0.13141	0.10576	2.877899	68.3
Compound 5	-0.22283	-0.10893	0.06838	1.860729	42.9
[Zn-5] complex	-0.31981	-0.28171	0.0381	1.036762	23.9

Computational details:
Geometries have been optimized using the B3LYP/6-31+G(d,p) level of theory and for Zn Lanl2dz with same ECP is used. The geometries are verified as proper minima by frequency calculations. Time-dependent density functional theory calculation has also been performed at the same level of theory. All calculations have been carried out using Gaussian 09 program.