Electronic Supplementary Information(ESI†)

Reaction-Based Bi-signaling Chemodosimeter Probe for Selective Detection of Hydrogen Sulfide and Cellular Studies

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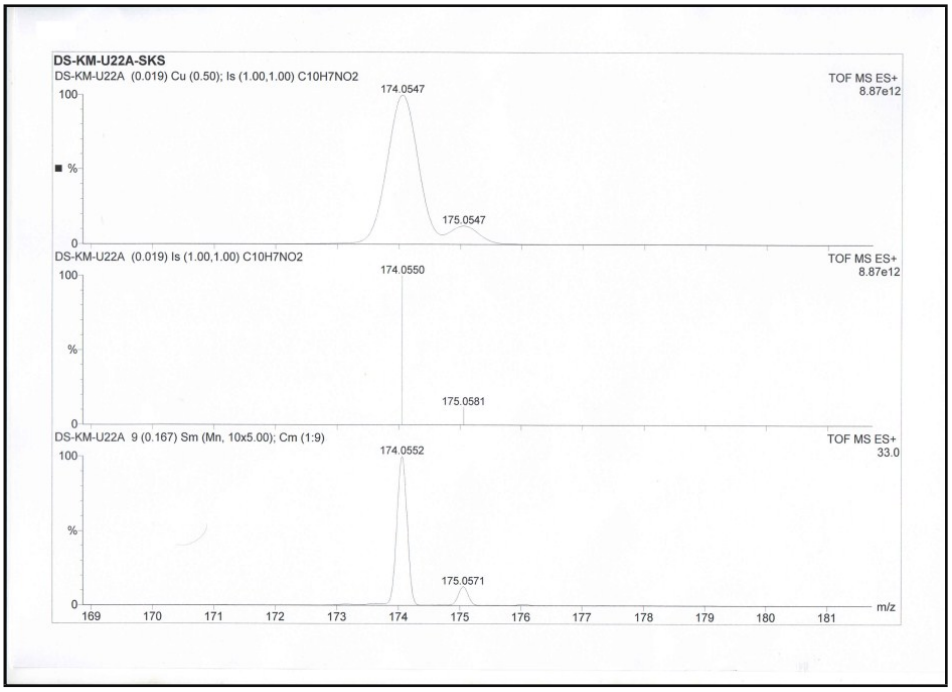


Figure S1. ESI-MS mass spectrum of **Compound 2**

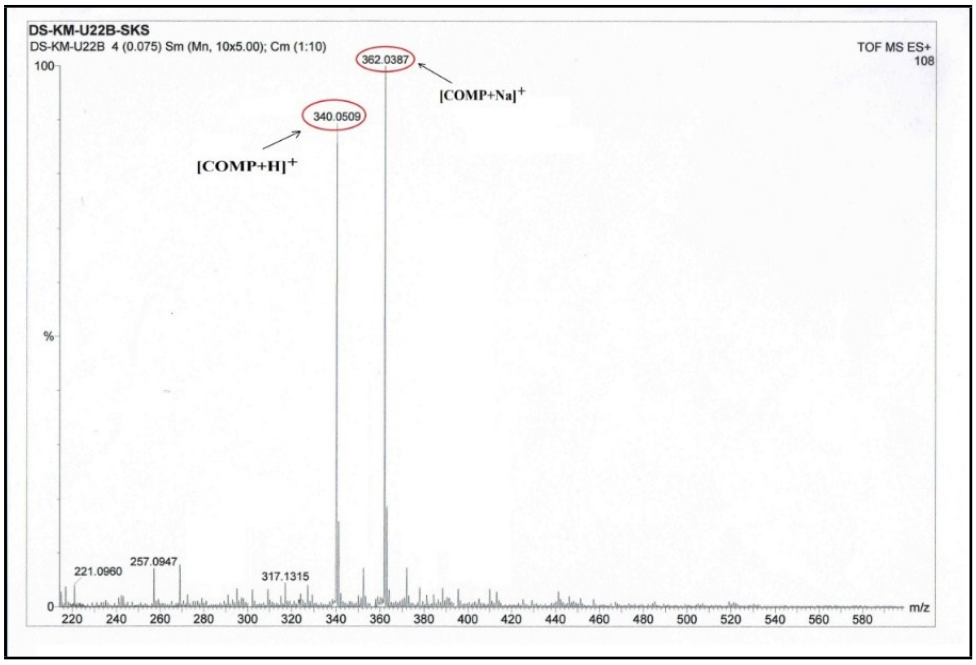


Figure S2. ESI-MS mass spectrum of Compound **3**

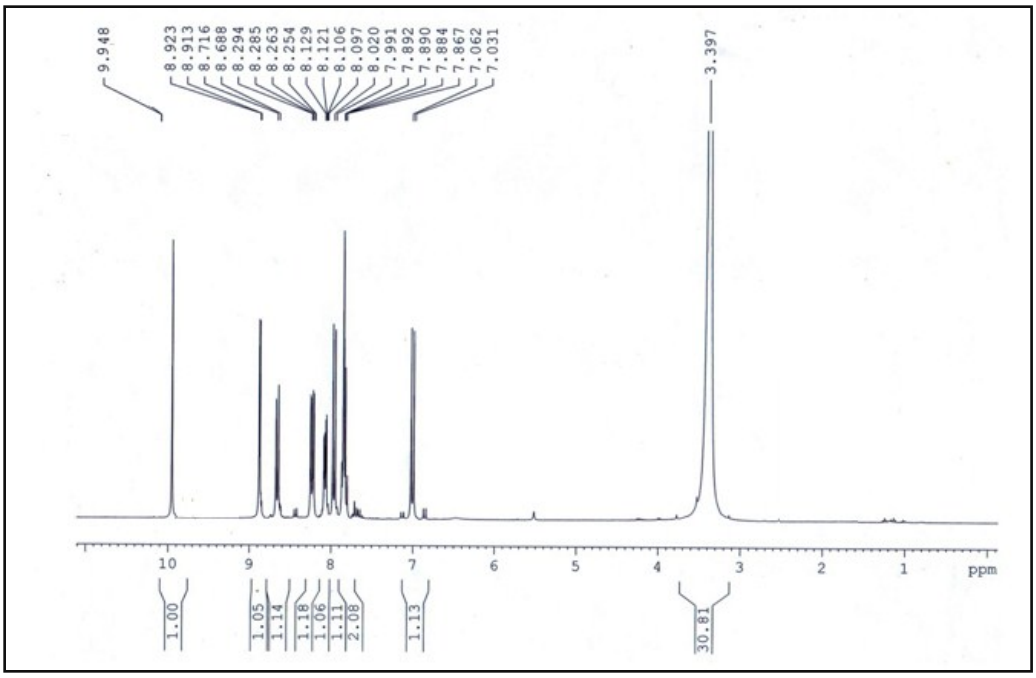


Figure S3: [1]H NMR of compound Compound **3** inDMSO-d6

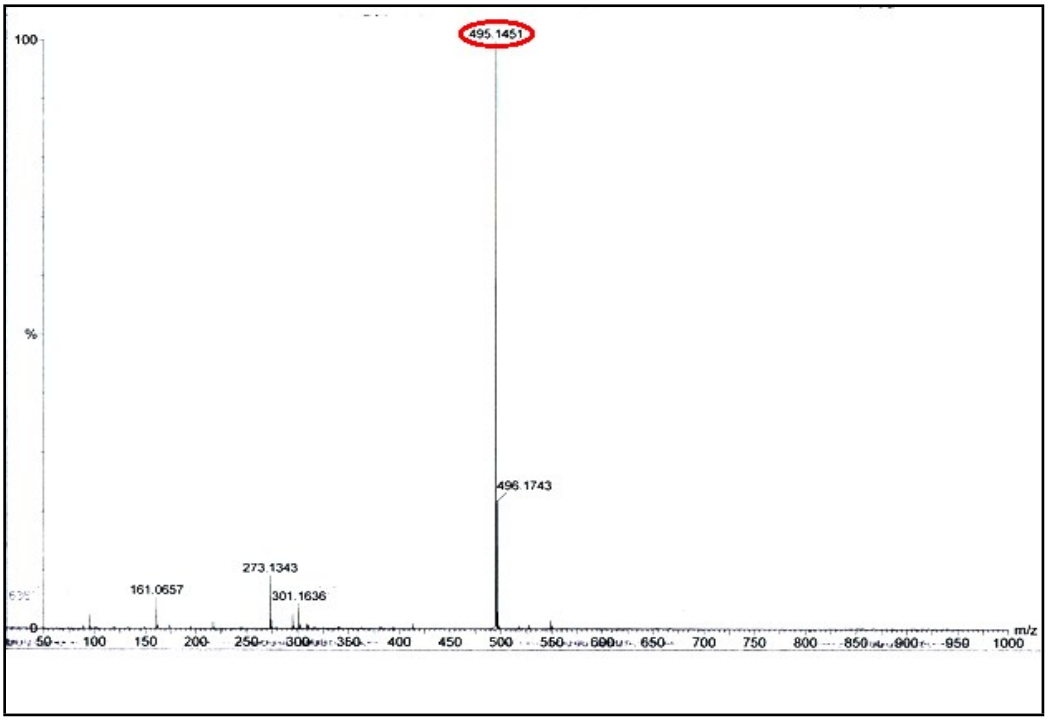


Figure S4. ESI-MS mass spectrum of **DPQI**

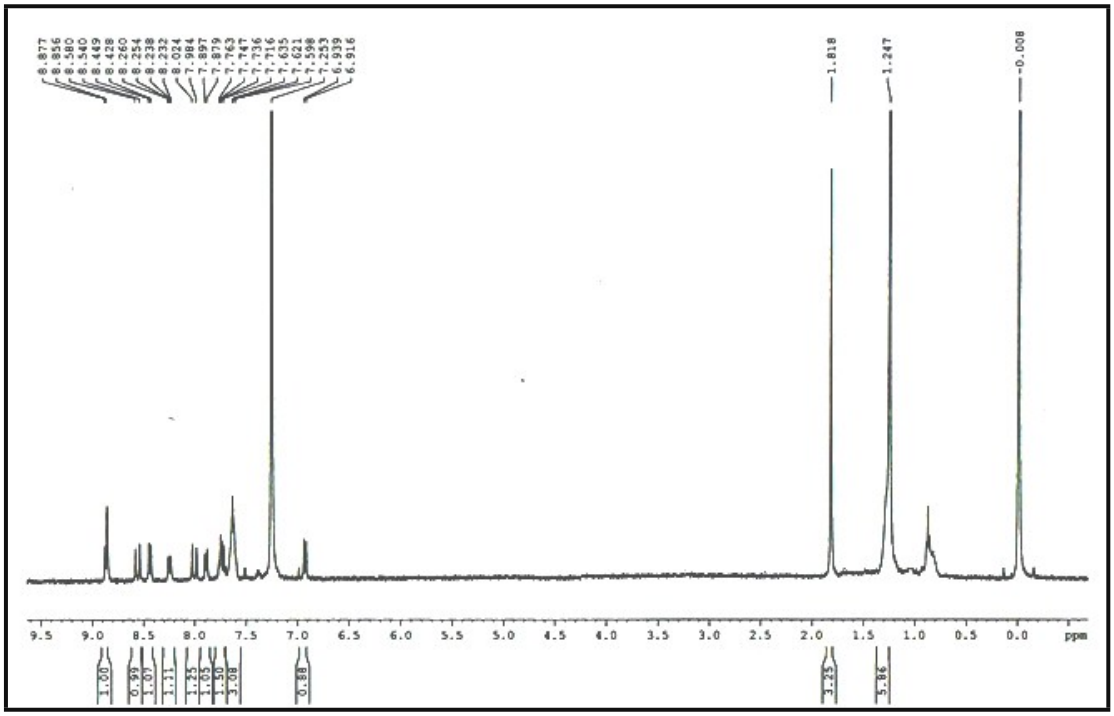


Figure S5: ^{1}H NMR of **DPQI** in $CDCl_3$

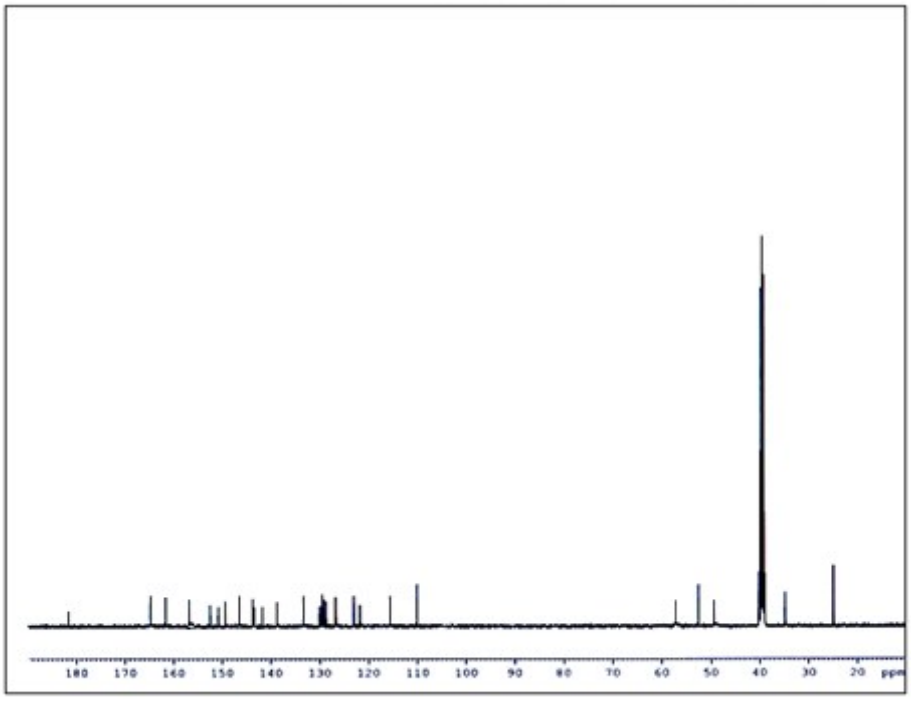


Figure S6: ^{13}C NMR of **DPQI**

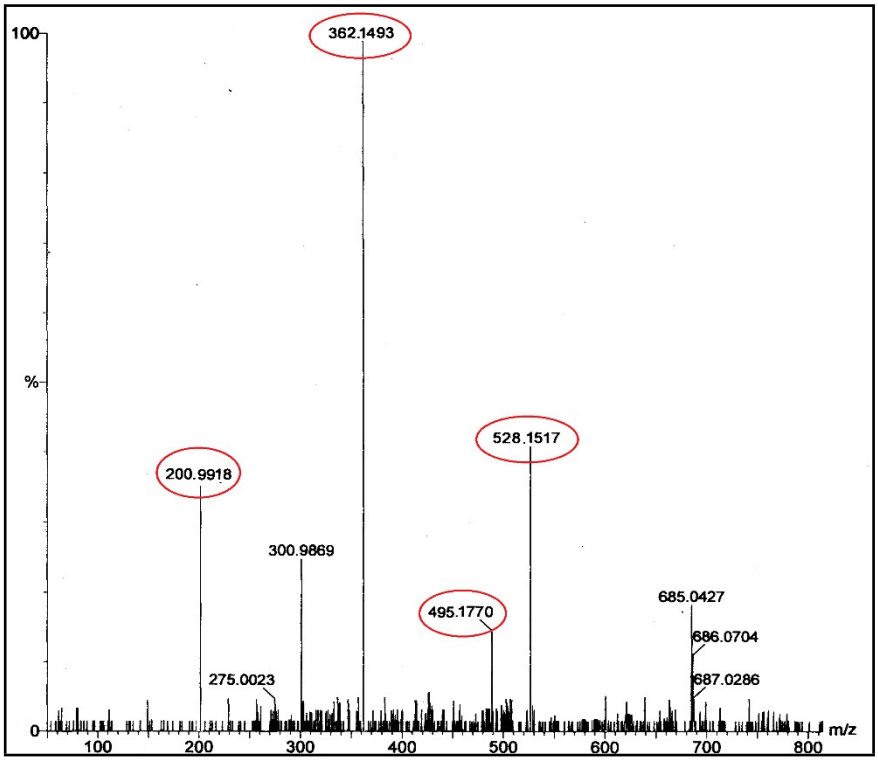


Figure S7.HR-MS (partial) of sample withdrawn from assay system.

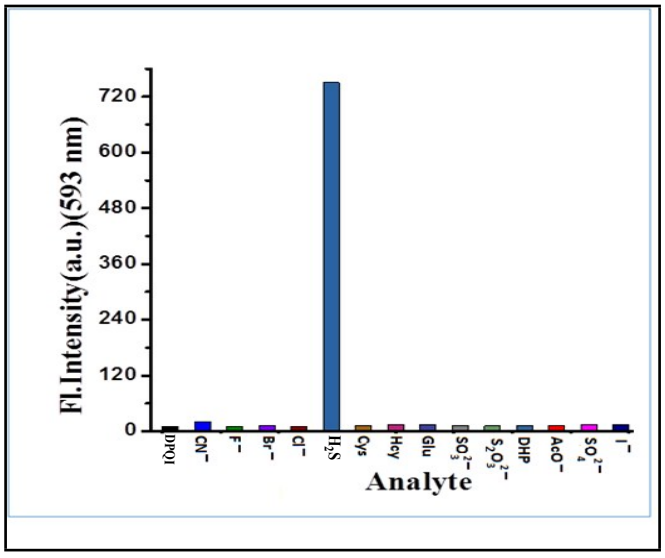


Figure S8. Comparative fluorescence spectra of **DPQI** (at 593 nm)(1.0 x10^{-5} M) in the presence of tetrabutyl ammonium and sodium salts of different analytes (10.0 x10^{-4} M) (CN^-,F^-, Cl^-, Br^-, I^-, $S_2O_3^{2-}$, SO_4^{2-}, AcO^-, and $H_2PO_4^-$) and Cys, Hcy, GSH in DMSO-H_2O(1:2 V/V; pH 7.4).

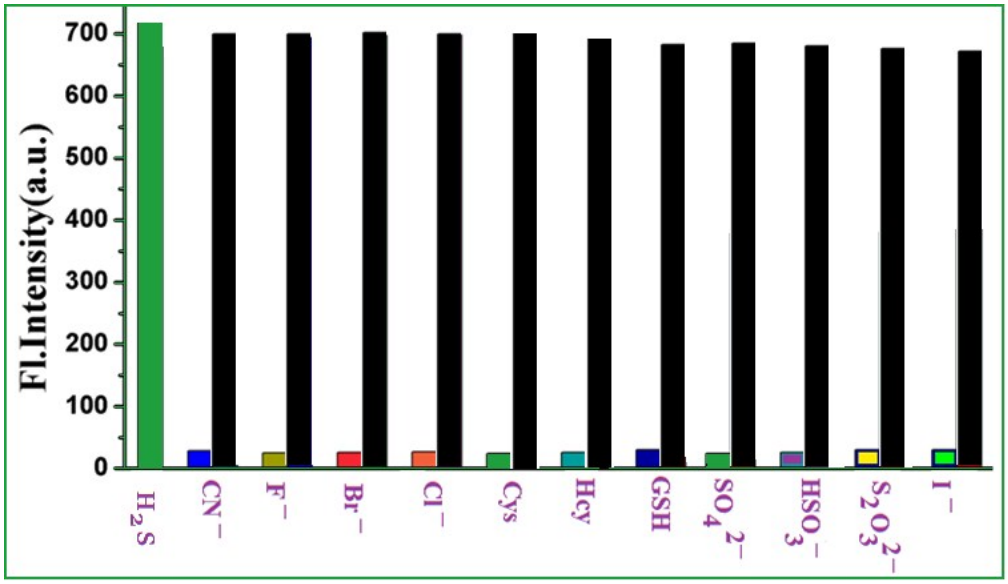


Figure S9.Change in fluorescence spectra of **DPQI** (at 593 nm) (c= 1× 10^{-5} M) to 100 eqv. addition of H_2S[green bigbar]and 100 eqv. of other analytes[small bars] and to the mixture of 100 eqv. of other analytes 100 eqv. addition of H_2S[big black bars].

Calculation of Detection limit:

The detection limit (DL) of**DPQI**for H_2S 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= 1.08x10^7, S=18.89. DL= 3.5µM

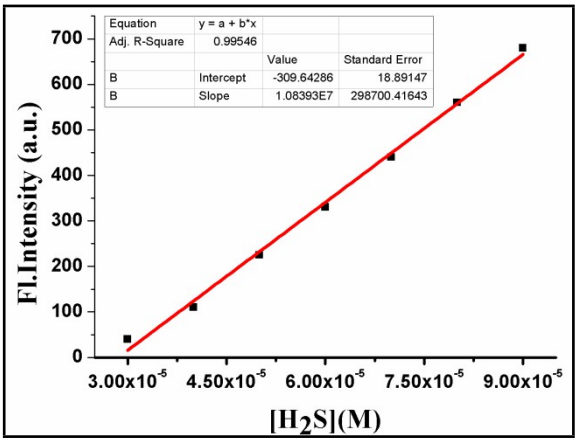


Figure S10: Calibration curve for Fluorescence titration of **DPQI** at 593 nm(Ex@500nm) with H_2S.

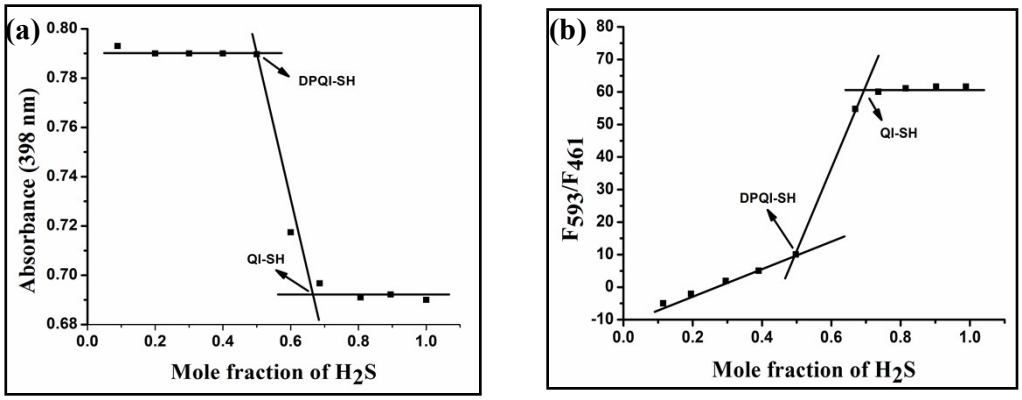


Figure S11: Job's plot [(a) is by absorbance and (b) is by fluorescence method] of sensor **DPQI** (1×10^{-5} M) with H_2S (1×10^{-5} M) in DMSO–H_2O (1:2 v/v; 20 mM, HEPES buffer, pH = 7.4) by UV spectroscopic method, that indicates 1:2 stoichiometry.

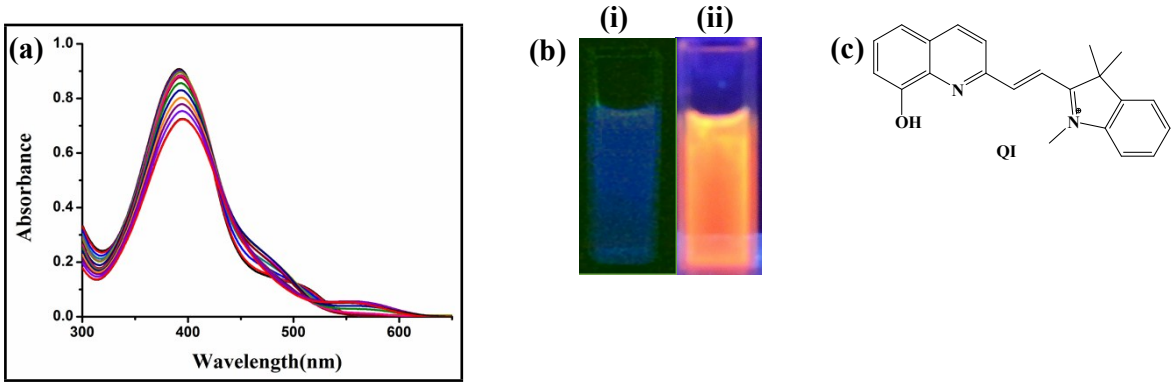


Figure S12: (a)Absorption spectra of **DPQI** (1.0 μM, 20 mM HEPES buffer PH 7.4, DMSO-H_2O; 1:2; v/v) upon gradual addition of Na_2S (0–100 equiv.). (b) (i) Fluorescence color of DPQI and (ii) fluorescence color of DPQI in presence of 100 equiv. of H_2S. (c) Structure of Quinoline-Indole (QI) required for DFT calculation to know the fluorescence behavior of the probe DPQI.

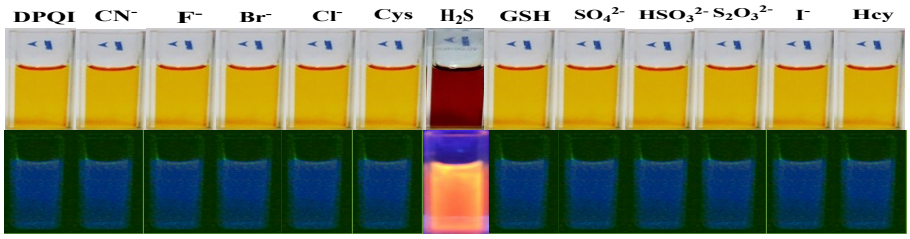


Figure S13: The visible color (top) and fluorescence changes (bottom) of receptor **DPQI** in aq. DMSO (DMSO: H_2O = 1:2 v/v, 20 mM HEPES buffer, pH = 7.4) upon addition of various analytes.

Kinetic Study:

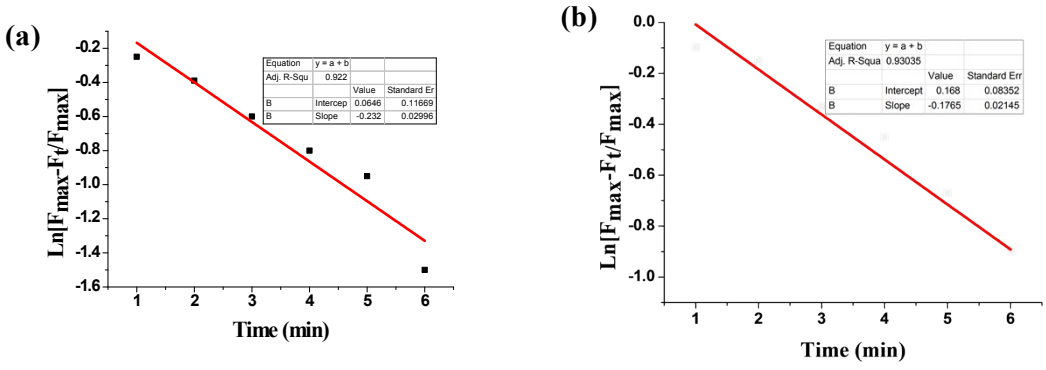
The solution phase chemodosimetric reactions of **DPQI** in DMSO were followed by measuring the fluorescence spectra after mixing **DPQI** and Na_2S in a cubic 4-sided quartz cell of 3 ml. The reaction was carried out at 35°c under the excess amount of H_2S (25-100 eqv.) (initial concentration [**DQPI**] << [H_2S]) and the reaction was expected to reach 100% conversion. Separate solutions of different concentrations of **DPQI** and Na_2S in DMSO were prepared and mixed to investigate the kinetics. The excitation wavelength was 500 nm and in all cases the concentration was low enough to maintain a UV absorption that was < 0.1. The rate of the reaction was determined by fitting the fluorescence intensities of the samples to the Pseudo-First Order Equation (1):

$$Ln(F_{max} - F_t)/F_{max} = - k^{/}t \quad \text{.....(1)}$$

Where F(t) and F(max) are the fluorescence intensities at the monitoring wavelengths at times t and the maxima values which are the last fluorescence intensities when **DPQI** reached the conversion of 100%. The $k^{/}$is the apparent rate constant. Figure S14a is the pseudo first order plot of **DPQI** with 100 equiv. of H_2S. The negative slope of the plot shows the apparent rate constant = **0.232 min^{-1}.** The apparent rate constant, $k^{/}$, contains the concentration of H_2S as a constant and is related to the second-order rate constant, k_2 ($M^{-1}min^{-1}$), by equation (S2):

$$k^{/} = k_2[H_2S] \qquad \text{(S2)}$$

The second-order rate constant for this reaction is thus the slope of a linear plot of k'
Versus the concentration of H_2S (Fig. S15): k_2 = **775.44** $M^{-1}min^{-1}$



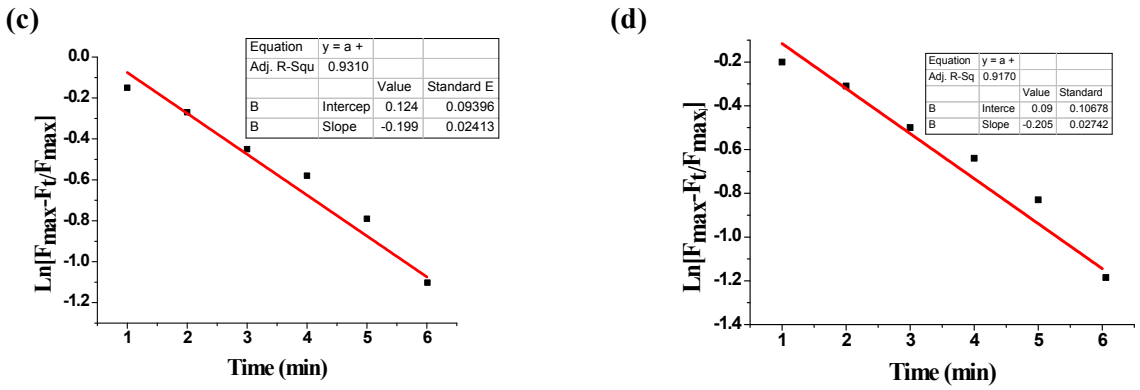


Figure S14: (a) Pseudo first-order kinetic plot of reaction of **DPQI** (10μM) with H_2S (100 equiv.) in DMSO. Slope = -0.23 min^{-1}. (b) Kinetic plot of **DPQI** with 25 equiv. H_2S, (c) Kinetic plot of **DPQI** with 50 equiv. H_2S(d) Kinetic plot of **DPQI** with 80 equiv. H_2S.

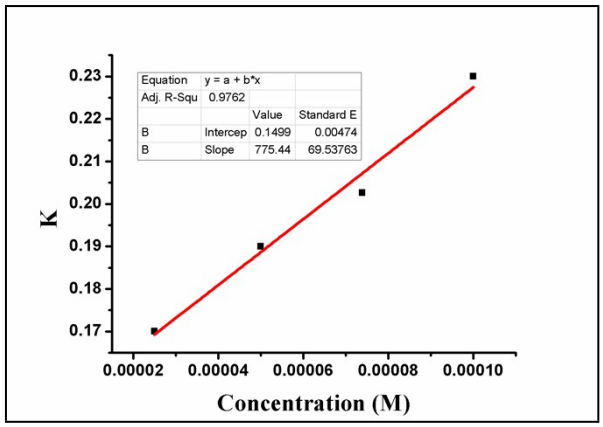


Figure S15: Plot of the observed k versus the concentration of H_2S for the pseudo first-order reaction of **DPQI** (10μM) with varying concentration of H_2S (25-150 eq). Slope = 775.44 $M^{-1}min^{-1}$.

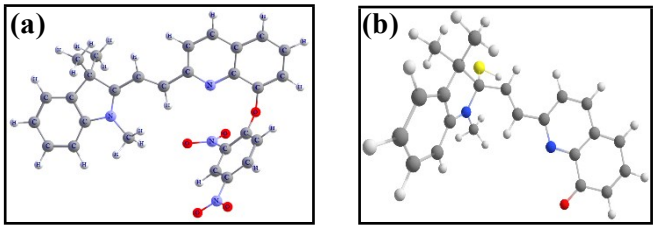


Figure S16: The energy optimized structures of (a) **DPQI** and (b) **QI-SH**.

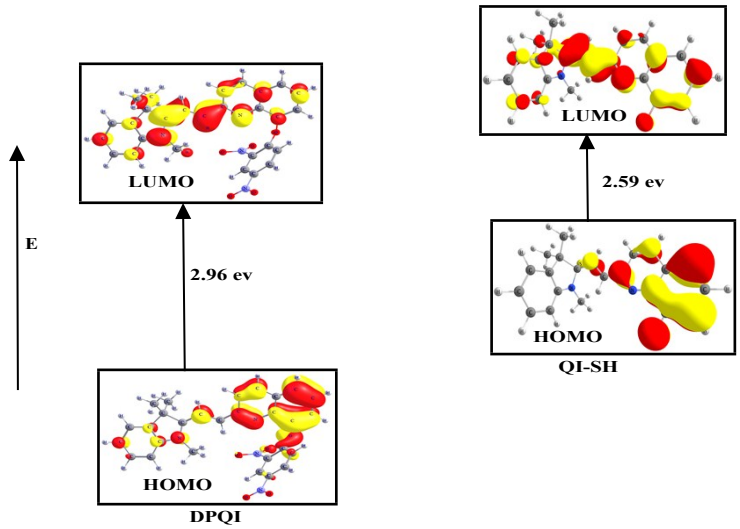


Figure S17: HOMO-LUMO distribution and energy difference of **DPQI** and **QI-SH**.

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) %
DPQI	$S_0 \rightarrow S_1$	2.5540 eV 495.45 nm	0.1833	H → L	93.3
	$S_0 \rightarrow S_2$	2.9837 eV 415.54 nm	0.6022	H-1 → L	86.1
	$S_0 \rightarrow S_{23}$	4.4035 eV 281.56 nm	0.1559	H-2 → L+2	53.4
	$S_0 \rightarrow S_{33}$	4.8286 eV 256.77 nm	0.2141	H-14 → L H-3 → L+2 H-1 → L+3	68.2
QI-SH	$S_0 \rightarrow S_1$	2.2867 eV 542.20 nm	0.2349	H → L	96.7

[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)**
DPQI	-0.34444	-0.23556	0.10888	2.962799	68.3
QI-SH	-0.04069	0.05442	0.09511	2.588095	59.7

Computational details:
Geometries have been optimized using the B3LYP/6-31+G(d,p) level of theory 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.

Water specimens: Different water specimens that we have collected from different areas of West Bengal, India, are given below.

Specimen 1: Water of River Ganga (West Bengal, India).

Specimen 2: Tap water of IIEST, Shipur campus (Howrah, West Bengal, India).

Specimen 3: Water of Hooghly River (East Midnapur, West Bengal, India).

Specimen 4: Shalimar hand pump water (Howrah, West Bengal, India).

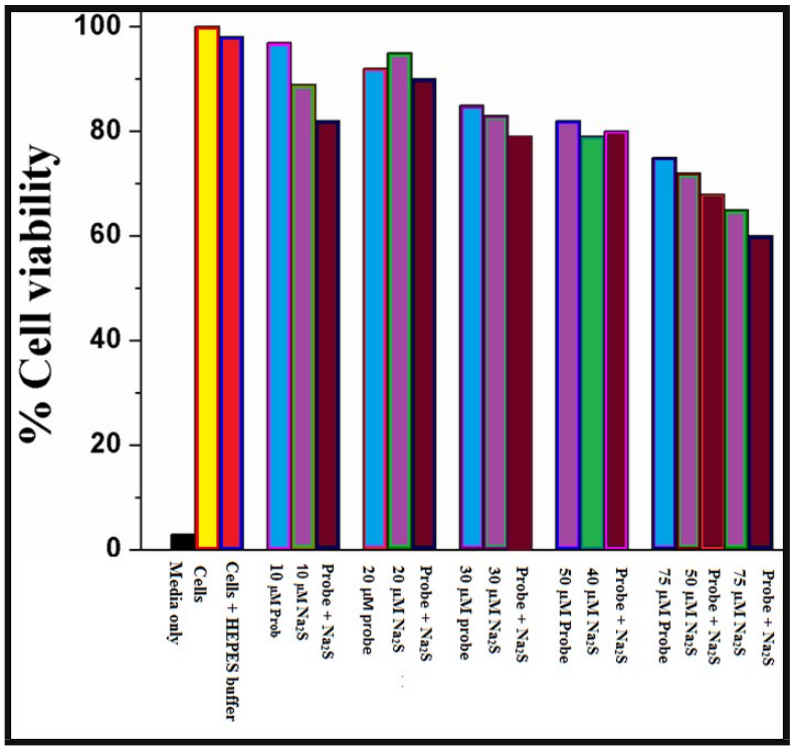


Figure S18: MTT assay to determine the cytotoxic effect of **DPQI** and Na_2S complex on Vero cell.

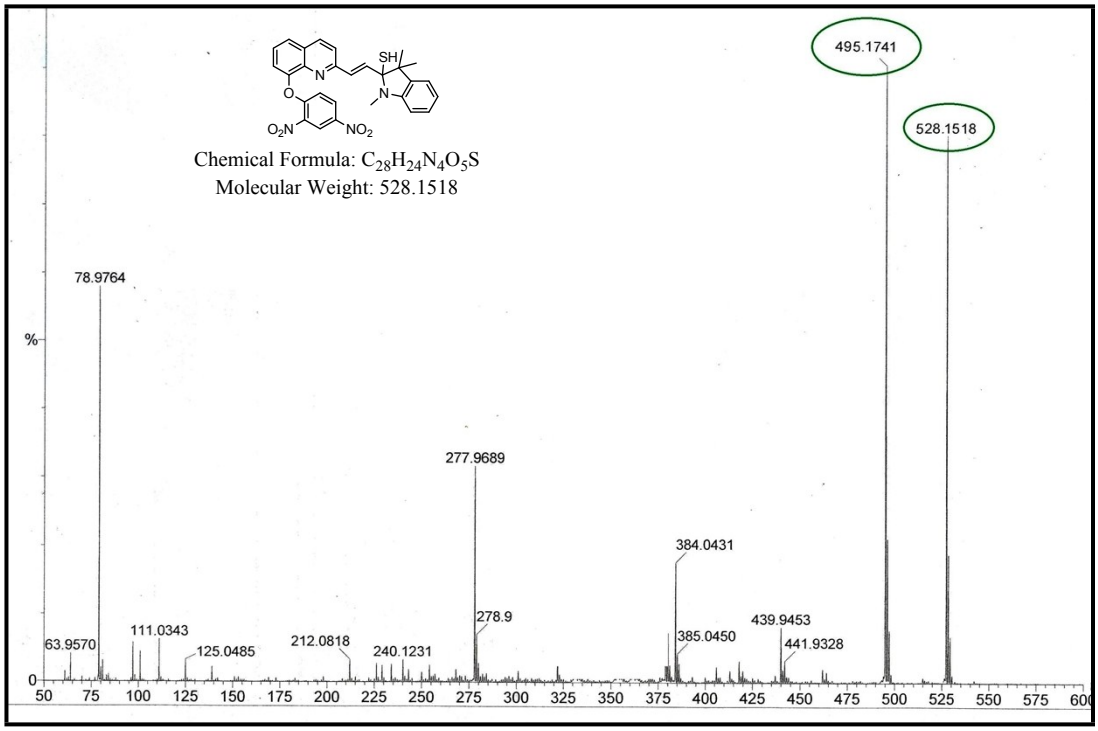


Figure S19: HR-MS of **DPQI** treated with 0.5 equiv. of H_2S.

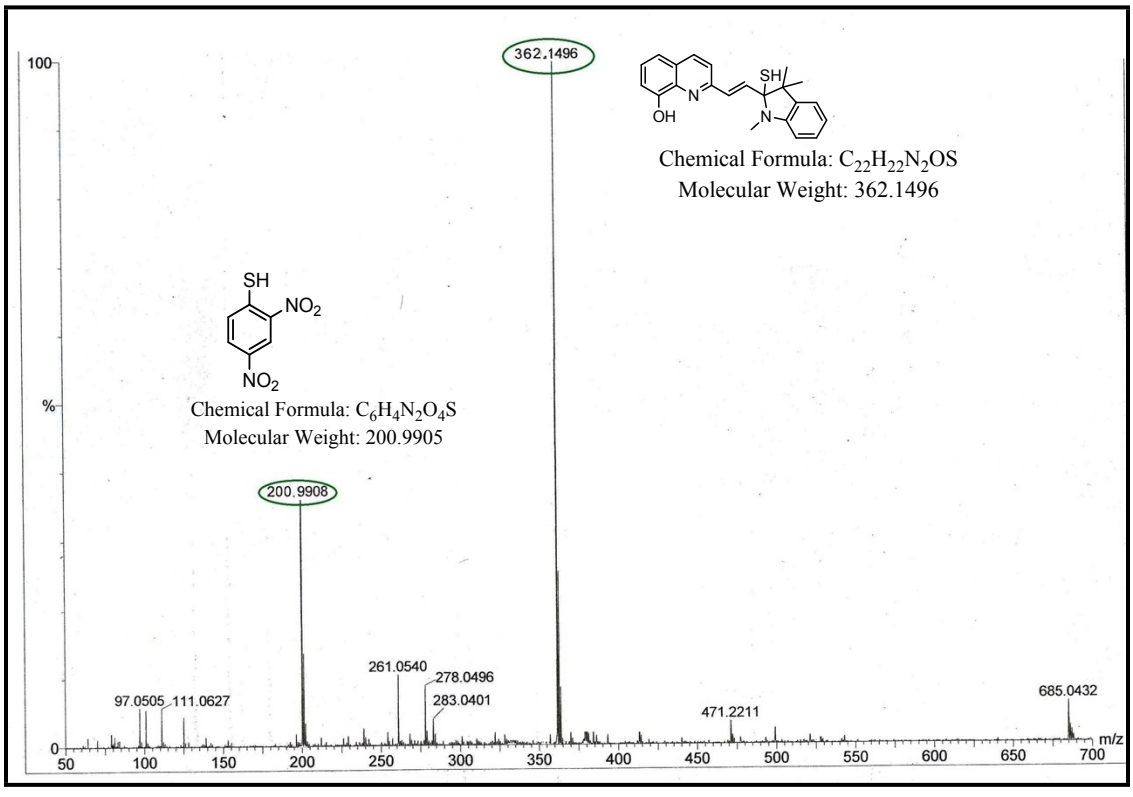


Figure 20: HR-MS of **DPQI** treated with excess of H_2S