Concentration dependent auto-relay-recognition by same analyte: Dual fluorescence switch-on by hydrogen sulfide via Michael addition followed by reduction and staining bio-activity

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Calculation of the detection limit:

The detection limit (DL) of **PND** in emission spectra for H_2S was determined from the following equation[1]:

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 the graph Fig.S2(a), we get slope = 32.252, and Sb1 value is 12.494.

Thus using the formula we have detected the blue fluorescence of **PND** using mininum 0.77 μM H_2S.

From the graph Fig.S2(b), we get slope = 38.27325, and Sb1 value is 49.659.

Thus using the formula we have detected the green fluorescence of **PND** using mininum 13.19 μM H_2S.

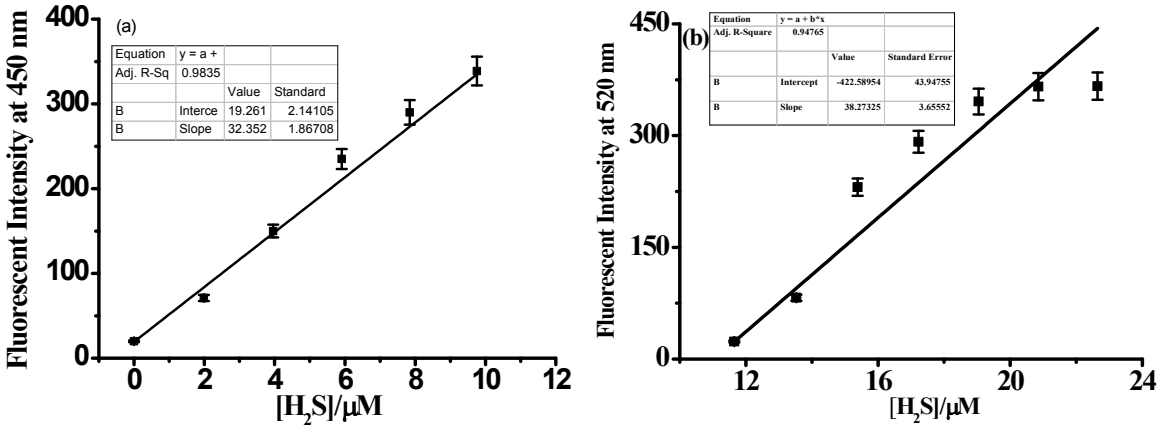


Figure S_1 (a) Changes of emission intensity of **PND** (c = 2 x10^{-5}M) as a function of [H_2S](c = 4x10^{-4}M) at 450 nm. **(b)** Changes of emission intensity of **PND** (c = 2 x10^{-5}M) as a function of [H_2S](c = 4x10^{-4}M) at 520 nm.

pH Titration:

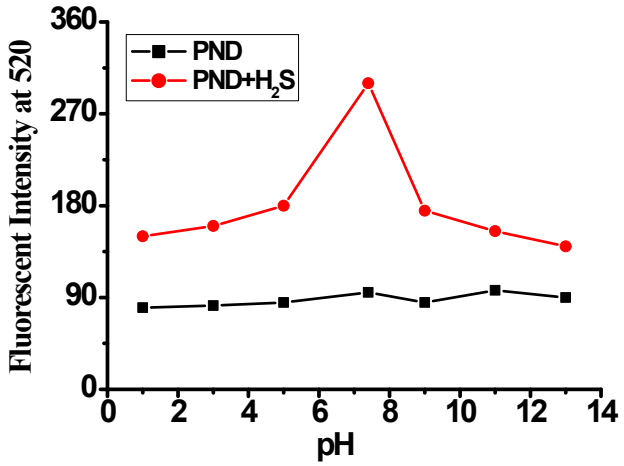


Figure S_2: The effect of pH on the fluorescence intensity changes of **PND** (c = 2×10^{-5} M) in presence and absence of H_2S(c = 4×10^{-4}M).

MTT assay:

Cytotoxicity of a **PND** was measured by MTT assay. MCF-7 cells were seeded in 96-well tissue culture plate. After 24 hrs of cell seeding, **PND** ($5x10^{-5}$M) mixed with different concentrations of H_2S ($5x10^{-5}$ to 4 $x10^{-4}$M) were added to the culture medium. Cells were incubated for 24 hrs at 37^0C and then subjected to tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay (1). The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by live cells, and forms a purple color after dissolved in DMSO and intensity of purple color is proportionate with number of live cells which will be quantified by optical density measured in UV VIS spectrophotometer. The color changes were measured using a ELISA reader (Robonik, Readwell touch ELISA PLATE analyzer, India). The rate of survival of cells were calculated by using the

following formulae: Cell viability (%) = (1-ODA$_1$/OD A $_o$)/100, where Ao = Absorbency of control cells and A_1=Absorbency of treated cells.

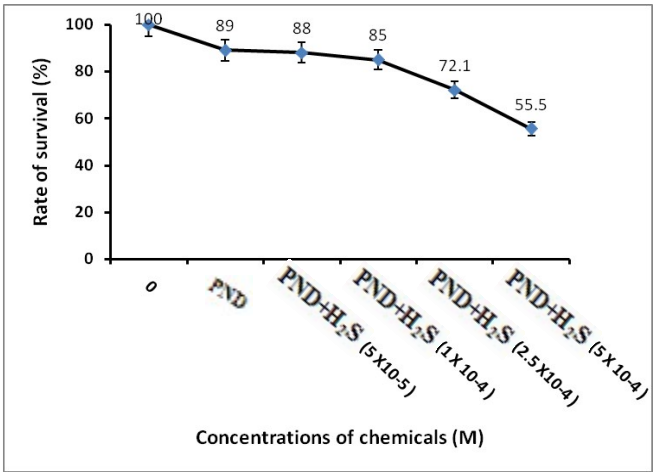


Figure S_3: The rate of survival of cells with different concentrations of H_2S added to **PND** - incubated cell. The results are derived from three different experiments (SD<5).

The results of cytotoxicity indicate that with increasing concentrations of H_2S, rate of cell death were also increased indicating that H_2S generated after reaction with **PND** and H_2S is relatively toxic for MCF-7 cell line. The rate of survival at $5x10^{-5}$M **PND** is 89% i,e **PND** alone can cause 11% cell death. When H_2S is added at $5x10^{-4}$M concentration, in combination of **PND** ($5x10^{-5}$M), the cell death increases upto 34%. Therefore, the photograph was taken at lower concentration of H_2S ($2x10^{-4}$M).

Kinetics study:

The changes of emission intensity of PND(c =2x10^{-5}M) at different time interval by addition of H_2S(c =4x10^{-4}) and calculation of first order rate constant:

Fig.S_4 (a) represents the changes of emission intensity at wavelength 450 nm at different time interval by addition of H_2S. **Inset:** From the time vs. emission intensity plot (Fig.S_4(a)) at fixed wavelength at 450 nm by using first order rate equation we get the rate constant K=slope x 2.303= 0.009 x 2.303=2.07 x 10^{-2} Sec^{-1}.

Fig.S_4 (b) represents the changes of emission intensity at wavelength 520 nm at different time interval by addition of H_2S. **Inset:** From the time vs. emission intensity plot (Fig.S_4(b)) at fixed wavelength at 520 nm by using first order rate equation we get the rate constant K=slope x 2.303= 0.015 x 2.303=3.45 x 10^{-2} Sec^{-1}.

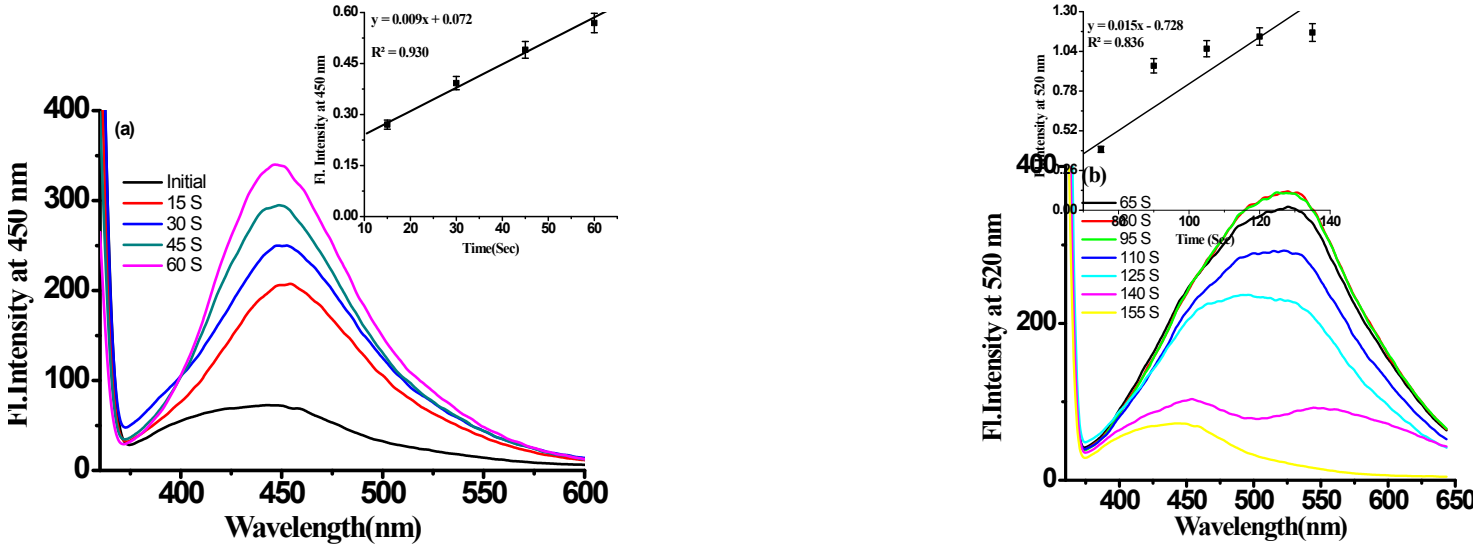


Figure S_4 (a): The changes of emission spectra at 450 nm wavelength at different time intervals of **PND** in presence of H_2S in CH_3CN: HEPES buffer solution CH_3CN-HEPES buffer (50/50, v/v, 25°C) at pH 7.4. **Left inset:** Changes of emission intensity at different time intervals ('S' denotes Second). **Right inset:** The first order rate equation by using Time vs. fluorescent intensity plot at 450 nm. **(b)** The changes of emission spectra at different time intervals of **PND** in presence of H_2S in CH_3CN: HEPES buffer solution CH_3CN-HEPES buffer (50/50, v/v, 25°C) at pH 7.4. **Left inset:** Changes of emission intensity at different time intervals ('S' denotes Second). **Right inset:** The first order rate equation by using Time vs. fluorescent intensity plot at 520 nm.

Fluorescence spectra of PNDH (PND+H_2S) with external oxidizing agent OCl^- (Reversibility test).

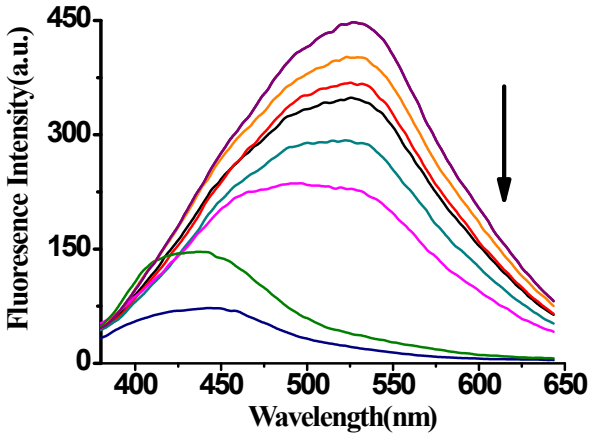


Figure S_5. Fluorescence spectra of **PNDH (PND**+H_2S**)** (c = 2x10^{-5}M) with OCl^- (c = 4x10^{-4}M) in CH_3CN: HEPES buffer solution (50:50, v:v) at pH 7.4.

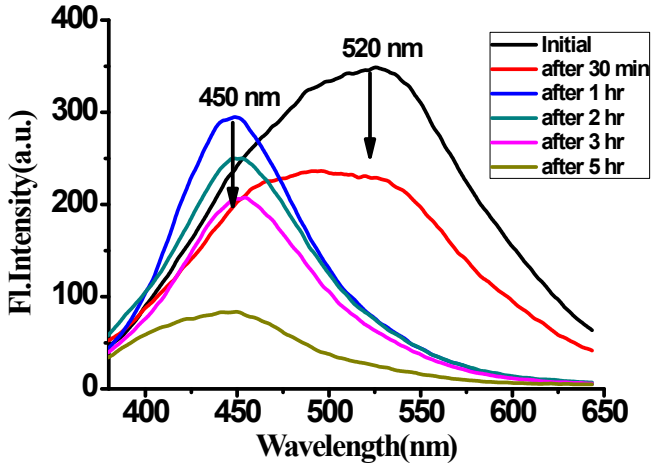
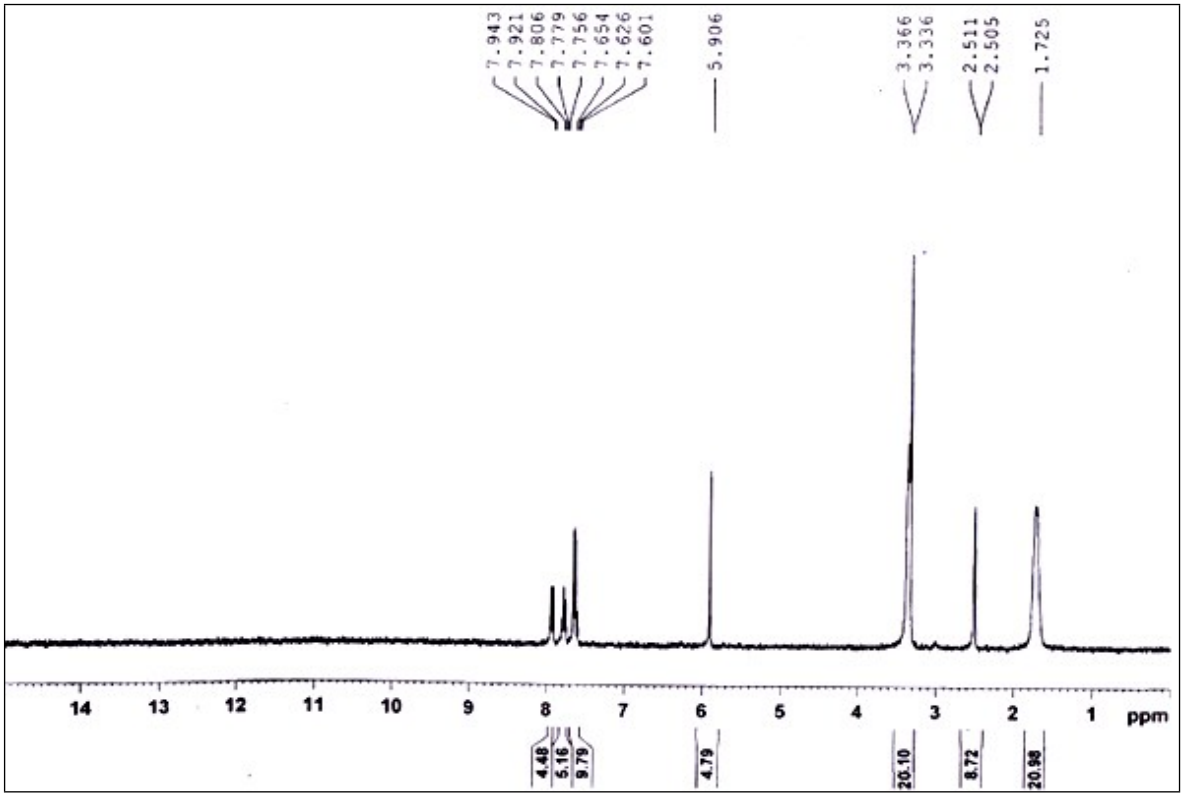
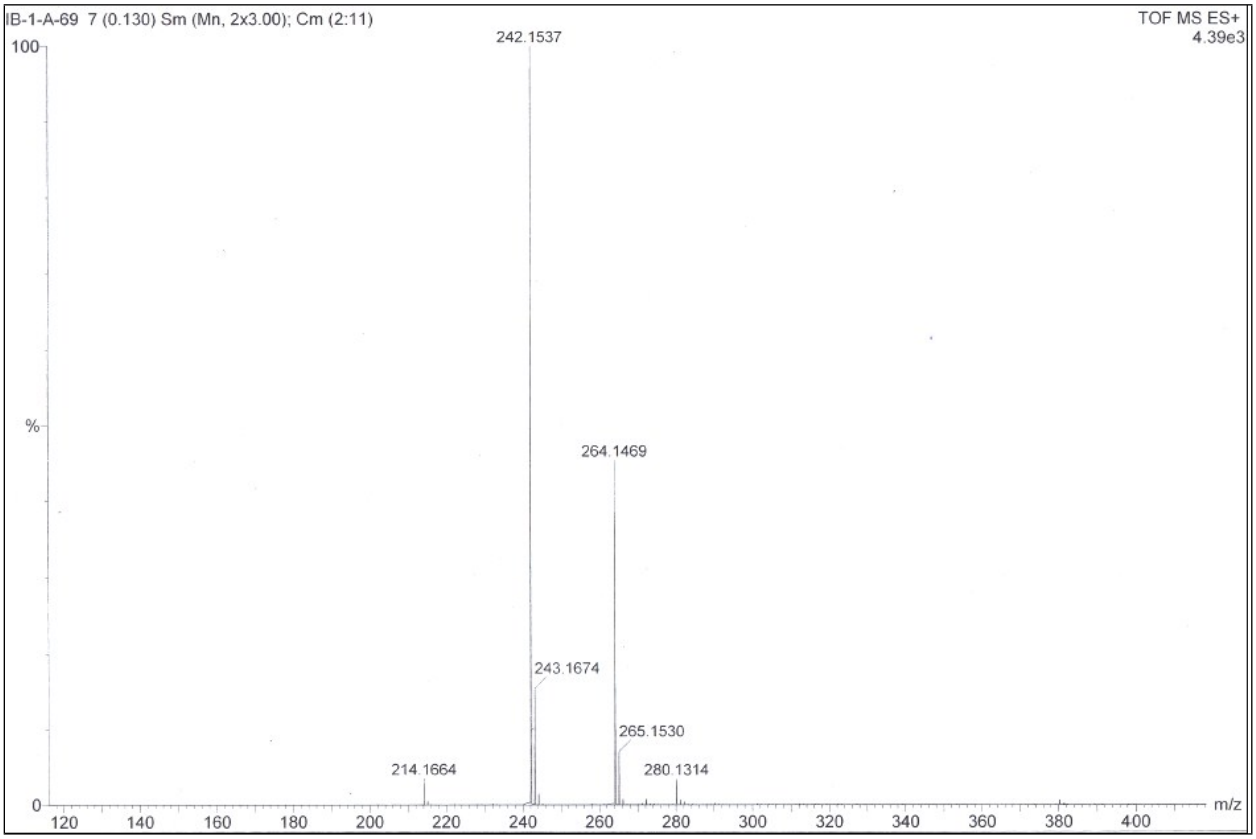


Figure S_6. Fluorescence intensity changes of the resulting solution of **PNDH (**PND+H_2S**)** (c = 2x10^{-5}M) on staying the solution in open air at different time intervals in CH_3CN: HEPES buffer solution (50:50, v:v) at pH 7.4.

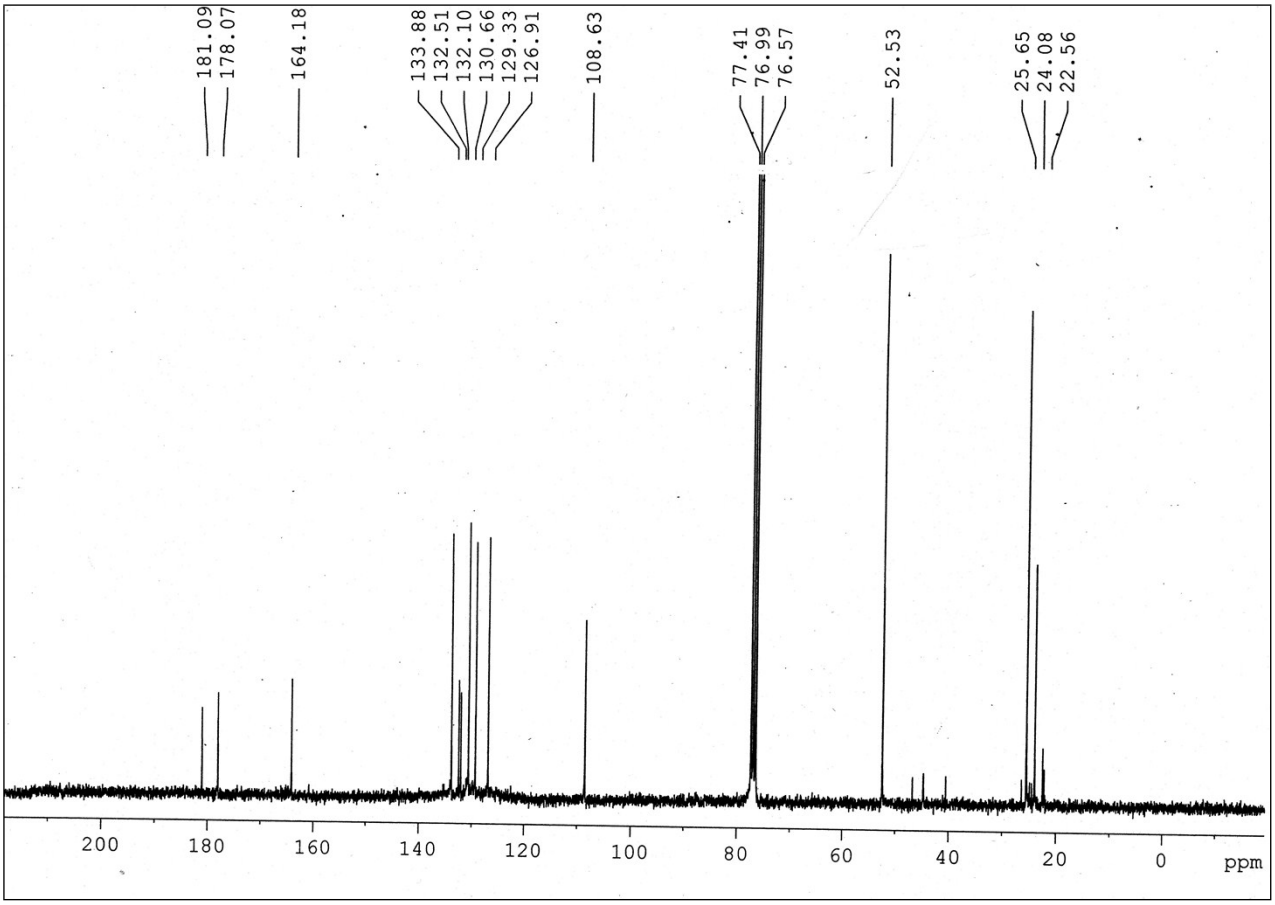
^{1}H NMR spectra (S_7) of PND:



Mass spectra (S_8) of PND:

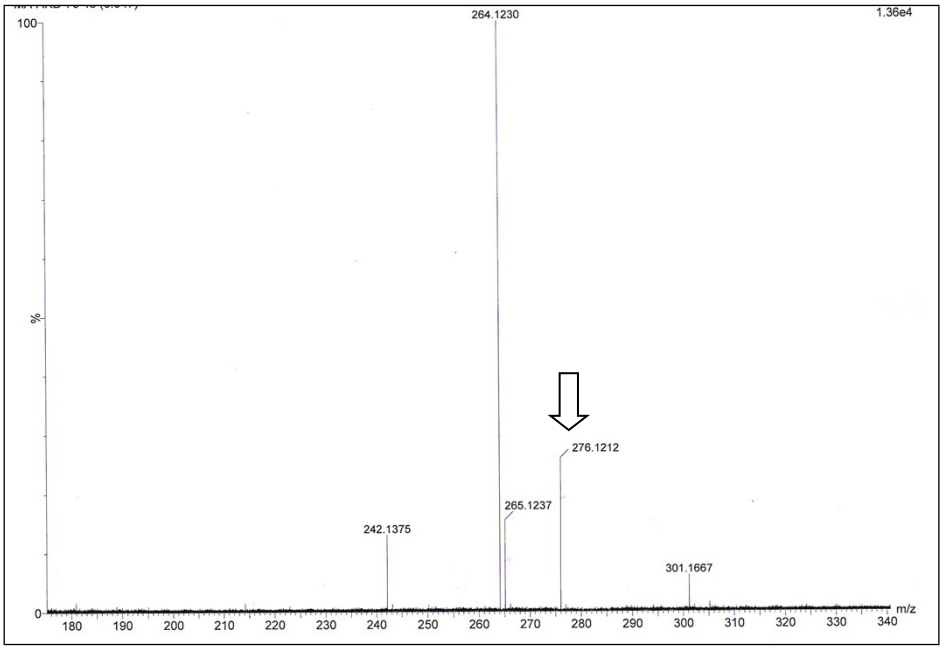


^{13}C NMR spectra (S_9) of PND:



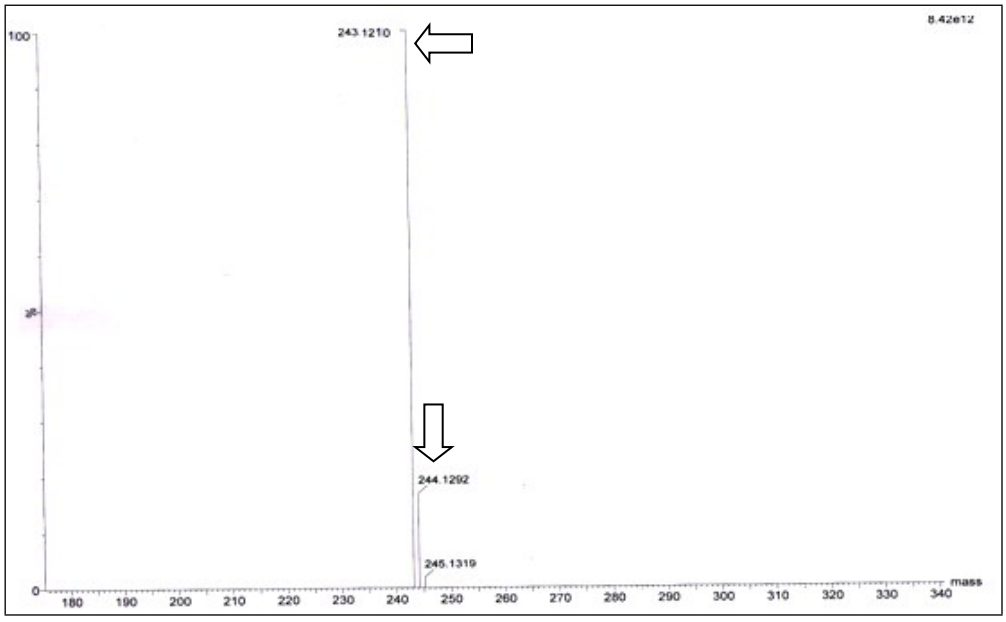
HRMS spectra (S_{10}) of PND+ H_2S (Lower concentration):

HRMS (ESI-TOF): (m/z, %): M+ Calculated for $C_{15}H_{17}NO_2S$ (PNDI) is 275.098. Observed: 276.1212 $(M+H)^+$.



HRMS spectra (S_{11}) of PND+ H_2S (Higher concentration):

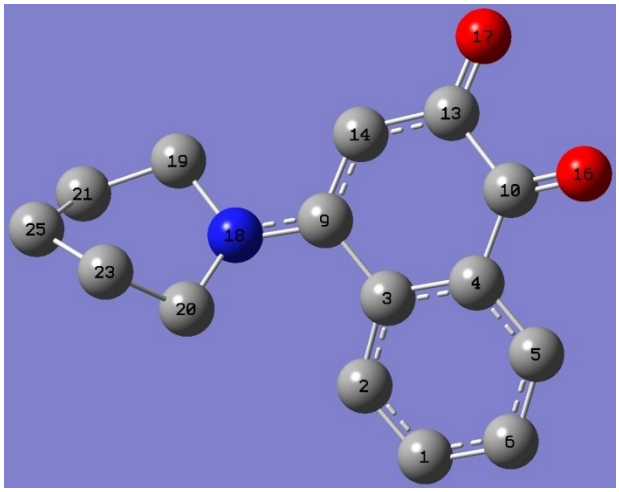
HRMS (ESI-TOF): (m/z, %): M+ Calculated for $C_{15}H_{17}NO_2$ (PNDH) is 243.1259. Observed: 243.1210 $(M)^+$ and 244.1292$(M+H)^+$.



Theoretical Study:

- All the compound was fully optimized B3LYP hybrid functional and 6-31g (d, p) basis set. All the computational studies were done in water as the solvent medium using Polarizable Continuum Model (PCM). The calculation of absorption is performed using Time Dependent Density Functional Theory (TD-DFT).
- Cartesian Coordinates 1. R (in water Phase).

B3LYP optimized structure



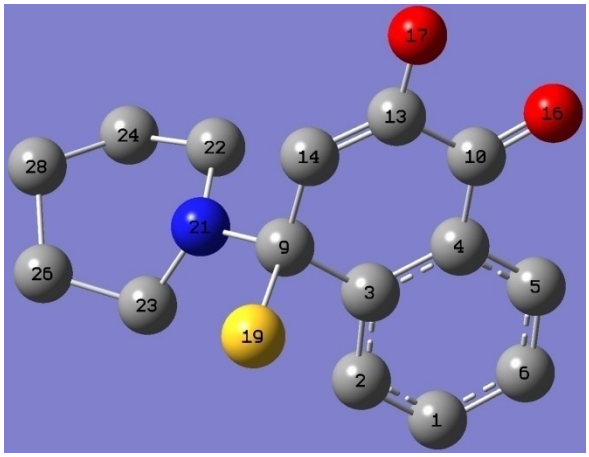
Energy (B3LYP/6-31G (d,p)) = -785.845587949 a.u.

ATOM	Coordinates (Angstroms) x	y	z
C	-1.56108700	2.96579200	-0.52304200
C	-0.55541000	1.99654200	-0.56212500
C	-0.81184700	0.67079000	-0.18500500
C	-2.14516700	0.32690200	0.14367400
C	-3.14146200	1.30628000	0.19730000
C	-2.85248200	2.63208300	-0.11700000
H	-1.32718900	3.98358500	-0.81870100

H	0.42528900	2.28576000	-0.91370200
C	0.23304700	-0.39669300	-0.22420600
C	-2.54103300	-1.09290700	0.31322600
H	-4.14763000	0.99776800	0.46013200
H	-3.63001800	3.38789200	-0.07715600
C	-1.51109700	-2.16167300	-0.09639000
C	-0.16681400	-1.72420800	-0.30807900
H	0.56070600	-2.51731200	-0.41591300
O	-3.64644100	-1.42784500	0.71629100
O	-1.87591200	-3.34458800	-0.13557400
N	1.55372400	-0.05445700	-0.25766800
C	2.54384700	-1.08703600	-0.62950800
C	2.10891800	1.11939700	0.46455300
C	3.92950600	-0.47128100	-0.81699300
H	2.20526900	-1.55971200	-1.55585000
C	3.29120800	0.69642700	1.34265600
H	1.32439100	1.55428700	1.08118400
C	4.45739000	0.14252300	0.49583200
H	4.60572000	-1.24776300	-1.18667800
H	2.94143700	-0.05701400	2.05713700
H	5.00251700	-0.61140400	1.07317000
H	2.58558900	-1.87084100	0.14098400
H	3.87227800	0.29160000	-1.60149700
H	5.17203400	0.93879600	0.26356700
H	3.60924000	1.56279800	1.93114800
H	2.43801600	1.88458700	-0.24925100

Cartesian Coordinates 2. Intermediate (in water Phase).

B3LYP optimized structure



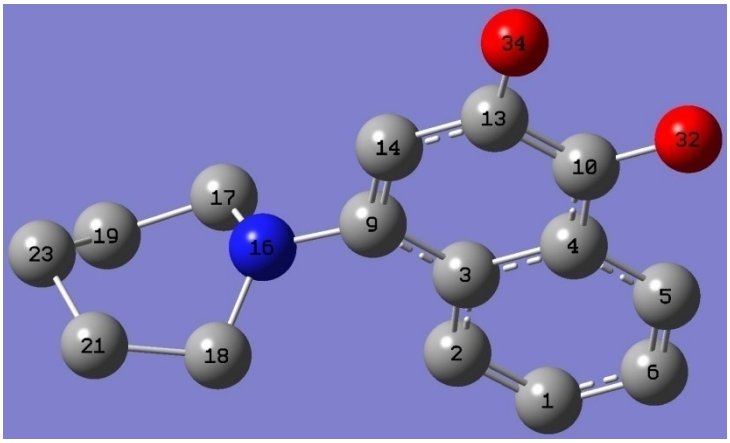
Energy (B3LYP/6-31G(d,p)) = -1185.22866253 a.u.

ATOM	Coordinates (Angstroms) x	y	z
C	-1.42078400	3.11339600	-0.24067900
C	-0.51743800	2.18049900	0.26808300
C	-0.78277100	0.80508100	0.20479800
C	-2.00903200	0.39639200	-0.36538300
C	-2.91312000	1.34077200	-0.87731900
C	-2.62078200	2.69667500	-0.82453000
H	-1.18608100	4.17133200	-0.17579400
H	0.39401600	2.53556700	0.73071800
C	0.24143300	-0.22204300	0.71871400
C	-2.39423700	-1.02524600	-0.39353400
H	-3.84343900	0.98124900	-1.30346500
H	-3.32088900	3.42496300	-1.22074700
C	-1.45547800	-2.00483200	0.18396300
C	-0.25597200	-1.64030600	0.66721600
H	0.43234400	-2.39400800	1.03700900
O	-3.46066900	-1.44463400	-0.86054200

O	-1.89062900	-3.28753500	0.16057000
H	-2.77066300	-3.24233000	-0.26514100
S	0.41959800	0.17372500	2.56919000
H	1.31684200	-0.80094300	2.82486400
N	1.56072900	-0.20017100	0.06881400
C	1.52822400	-0.69865600	-1.32704400
C	2.42643800	0.97990200	0.19353000
C	2.90122200	-0.52725600	-2.00938600
H	0.75528800	-0.18685000	-1.92471800
C	3.89778200	0.54093200	0.03640600
H	2.27900800	1.43002000	1.17592500
C	4.03319700	-0.61256300	-0.98228200
H	3.00627700	-1.29198300	-2.78597400
H	4.29704500	0.23000200	1.00789700
H	3.97567000	-1.57709900	-0.46473600
H	1.26199100	-1.75953600	-1.30051700
H	2.95029800	0.44201100	-2.52009300
H	5.00983500	-0.57799900	-1.47640600
H	4.48399200	1.40926400	-0.28683500
H	2.17808000	1.74879000	-0.55626300

Cartesian Coordinates 3. P (in water Phase).

B3LYP optimized structure



Energy (B3LYP/6-31G (d,p)) = -787.054929946a.u.

ATOM	Coordinates (Angstroms)		
	x	y	z
C	-1.14068400	3.10216300	0.02945700
C	-0.23848100	2.06129700	0.01057300
C	-0.66932000	0.70469200	0.00776600
C	-2.08574300	0.45162200	0.01562600
C	-2.99395300	1.54606100	0.03955200
C	-2.53257800	2.84264100	0.04745100
H	-0.78353400	4.12772200	0.02982700
H	0.82339100	2.27351200	-0.00538500
C	0.24266100	-0.40333600	-0.00371300
C	-2.55390600	-0.88805100	-0.00485600
H	-4.05818400	1.33799300	0.05024800
H	-3.23595400	3.66990500	0.06548300
C	-1.65283000	-1.93879400	-0.02950400
C	-0.26685300	-1.69077400	-0.02448100
H	0.42934200	-2.52496100	-0.03180500
N	1.66493400	-0.25938500	-0.01054900
C	2.27797600	0.11253000	-1.29540100
C	2.32984800	0.29555200	1.17195900
C	3.79544600	0.32106200	-1.12658000

H	1.82748500	1.01962000	-1.73397500
C	3.72910700	-0.34079400	1.31787900
H	1.71365300	0.07253200	2.04720200
C	4.35841700	-0.63227800	-0.06638900
H	4.28697000	0.17480300	-2.09426800
H	3.65707700	-1.27089100	1.89225100
H	4.13505400	-1.66324900	-0.36518400
H	2.09119900	-0.70431000	-2.00511300
H	4.00107400	1.35670700	-0.82851700
H	5.44944200	-0.55075800	-0.01476300
H	4.36736800	0.34036600	1.89374900
H	2.42747200	1.39430300	1.13210500
O	-3.90146100	-1.12105500	-0.00093100
H	-4.03009300	-2.08249600	-0.02013300
O	-2.20391700	-3.19762100	-0.04774700
H	-1.50793100	-3.86725700	-0.08447900

Table S1:

Compound	Energy (au)	Absorption	
		Calculated	Expt.
PND	**-785.845587949**	483.82 nm (f=0.0689)	470 nm
PNDI	**-1185.22866253**	358.8 nm (f=0.085)	380 nm
PNDH	**-787.054929946**	343.3 nm (f=0.0189) 321.47 nm (f=0.092)	380 nm

References:

1. M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem*., 1996, **68**, 1414. (b) W. Lin,; Yuan, L.; Cao, Z.; Feng, Y.; Long, L. *Chem. Eur. J*. 2009, **15**, 5096.(c) Zhu, M.; Yuan, M.; Liu, X.; Xu, J.; Lv, J.; Huang, C.; Liu, H.; Li, Y.; Wang, S.; Zhu, D. *Org. Lett.* 2008, **10**, 1481-1484.