Electronic Supplementary Information (ESI)

A naphthaoxazole based highly sensitive cell permeable ratiometric chemodosimeter for hydrazine †

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EXPERIMENTAL

1.1 Apparatus:

The IR Spectra for the probe **P1** was recorded on Perkin Elmer - FTIR Spectrophotometer while ^{1}H NMR and ^{13}C NMR spectra for the same were recorded on a JEOL AL 300 FT NMR Spectrometer. Mass spectrometric analysis was carried out on Bruker amaZon SL spectrometer using ultrascan mode (Bruker Daltonics, Bremen, Germany). Electronic spectra were recorded at room temperature (298 K) on a UV spectrophotometer (Model Name: UV-1800, Manufacturer: SHIMADZU CORPORATION, ANALYTICAL & MEASURING INSTRUMENTS DIVISION) with quartz cuvette (path length=1 cm). Emission spectra were recorded on Fluorolog R-3 spectrofluorometer (Model Name: FL3-11, Manufacturer: JY HORIBA Scientific).

1.2 Materials:

All reagents for synthesis were purchased from Sigma-Aldrich and were used without further purification.

1.3 General Methods:

All titration experiments were carried at room temperature. All the cations were used as their chloride salts while anions were used as their tetrabutyl-ammonium (TBA) salts. The ^{1}H NMR spectra were recorded by using tetramethylsilane (TMS) as an internal reference standard. For the ^{1}H NMR titration spectra of **P1**, 5×10^{-3} M solutions were prepared in DMSO-d_6 while the stock solution of hydrazine was prepared in DMSO-d_6. For UV-visible/fluorescence titration experiments, the solutions of cations were prepared in aqueous medium. Tetrabutyl Ammonium (TBA) salts of anions were used and their solutions were prepared in DMSO. Due to insufficient

solubility of **P1** in pure water its stock solution of 1×10^{-3} M was prepared in DMSO which was used for fluorescence titration experiment in pure PBS buffer (10 mM, pH=7.4) at 1μM concentration through dilution.

1.4 X-ray diffraction studies:

Single crystals of the receptor **P1** were grown by slow evaporation of saturated solution of receptor **P1** in DMF: MeOH (1:1 v/v) over a period of few weeks. The single crystal X-ray diffraction measurements were carried out on an Oxford Diffraction Xcalibur system with a Ruby CCD detector as well as on a Bruker SMART APEX CCD diffractometer using graphite-monochromated MoKa radiation (k = 0.71073 Å). All the determinations of unit cell and intensity data were performed with graphite-monochromated Mo-Kα radiation (λ=0.71073 A°). Data for P1 was collected at liquid nitrogen temperature. The structures were solved by direct methods, using Fourier techniques and refined by full-matrix least-squares on F2 using the SHELXTL-97 program package. Crystal data and details of the structure determination for receptor **P1** are summarized in **Table 1**. CCDC **1453378** (**P1**), contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the **Cambridge Crystallographic Data Centre** via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

1.5 Cell Imaging Studies:

(a) MTT assay: Viability of cells was determined through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. Hela cells were seeded (1 × 104 cells/well) in a 96-well plate and incubated for 24 h at 37°C and 5% CO_2. After 24 h of incubation culture was replaced with media containing P1 and exposed to varying concentrations for 24 h. Negative control (only media) was also kept for comparison. After required period of incubation, cells were washed with 1X PBS and then 10μl of MTT solution (HiMedia) (5 mg ml-1 stock prepared in 1X PBS) in 100 μl of medium were added in culture and incubated for 3h at 37°C as a result formazan crystals were formed which was dissolved in 100 μl dimethyl sulfoxide(DMSO) for 15 min for further incubation at 37°C which develops color that was measured by a micro plate reader (Bio-RAD 680, USA) at 570 nm. All experiments were performed in triplicate, result showing cell viability was presented through a graph.

(b) Cellular imaging methodology

For the cell imaging study 60-80 % confluent cells were used for experiment. Trypsinised 10^5 HeLa cells were seeded in six well plate having cover slip in each well and allowed to grow in complete media (DMEM with 10% FBS and 1X antibiotic) overnight in a 5% CO_2 incubator at 37˚C. After 24h of incubation cells were washed with 1X PBS, then probe P1 was added in media and maintained at 10 μM concentration in solution and incubated for 30 min in 5% CO_2 incubator at 37˚C followed by addition of 50 μM concentration of Hydrazine (N_2H_4) for 2 h. The confocal projection images in a panel show only P1 and P1 in the presence of hydrazine (P1+ hydrazine). For cell imaging study LSM510-Meta software was used.

1.6 Determination of Quantum yield (Φ)

For measurement of the quantum yields of **P1** and **P1-N_2H_4** we recorded the absorbance of the compounds in pure PBS buffer solution. The emission spectra were recorded using the maximal excitation wavelengths, and the integrated areas of the fluorescence-corrected spectra were measured. The quantum yields were then calculated by comparison with quinine sulphate (0.1M H_2SO_4, Φs = 0.58 in water) as reference using the following equation:

$$\Phi_x = \Phi_s \times (I_x/I_s) \times (A_s/A_x) \times (n_x/n_s)^2$$

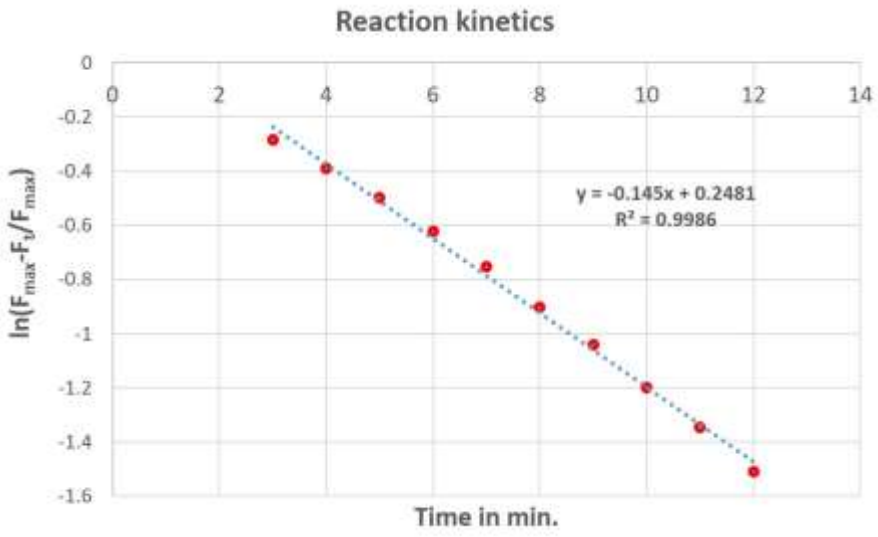
Where, x & s indicate the unknown and standard solution respectively, Φ is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance and n is the refractive index of the solvent.

1.7 Reaction Kinetic study

The reaction rate constant of P1 (1.0 μM) with hydrazine (300 equiv.) was estimated assuming a pseudo first- order kinetic. The reaction was monitored at 400 nm at room temperature. The rate constant was determined according to the following equation:

$$\ln [(F_{max} - F_t)/F_{max}] = -k't$$

where F_t and F_{max} are the fluorescence intensities at time t, and the time after completion, respectively. The constant k' obtained from figure given below.



1.8 Determination of detection limit:

The detection limit was calculated using UV-visible/fluorescence titration data according to the IUPAC definition **[1]**. The detection limit of **P1** towards hydrazine was determined from a plot of fluorescence intensity (at 400 nm) as a function of the concentration of the added hydrazine. To determine the S/N ratio, the fluorescence intensity of **P1** in absence of any analyte was measured by 10 times and the standard deviation of blank measurements was determined. The detection was calculated as three times the standard deviation from the blank measurement (in the absence of analyte) divided by the slope of calibration plot between analyte concentration and fluorescence intensity.

Ref [1]: (*a*) IUPAC, *Spectrochim. Acta Part B*, 1978, 33, p. 242; (*b*) USEPA, Appendix B to Part 136-Definition and Procedure for the Determination of the Method Detection Limit- Revision 1.11, Federal Register 49 (209), 43430, October 26, 1984. Also referred to as "40 CFR Part136.

Electronic Supplementary Information (ESI)

Table and Contents

Figure S1: IR spectrum of **3**:

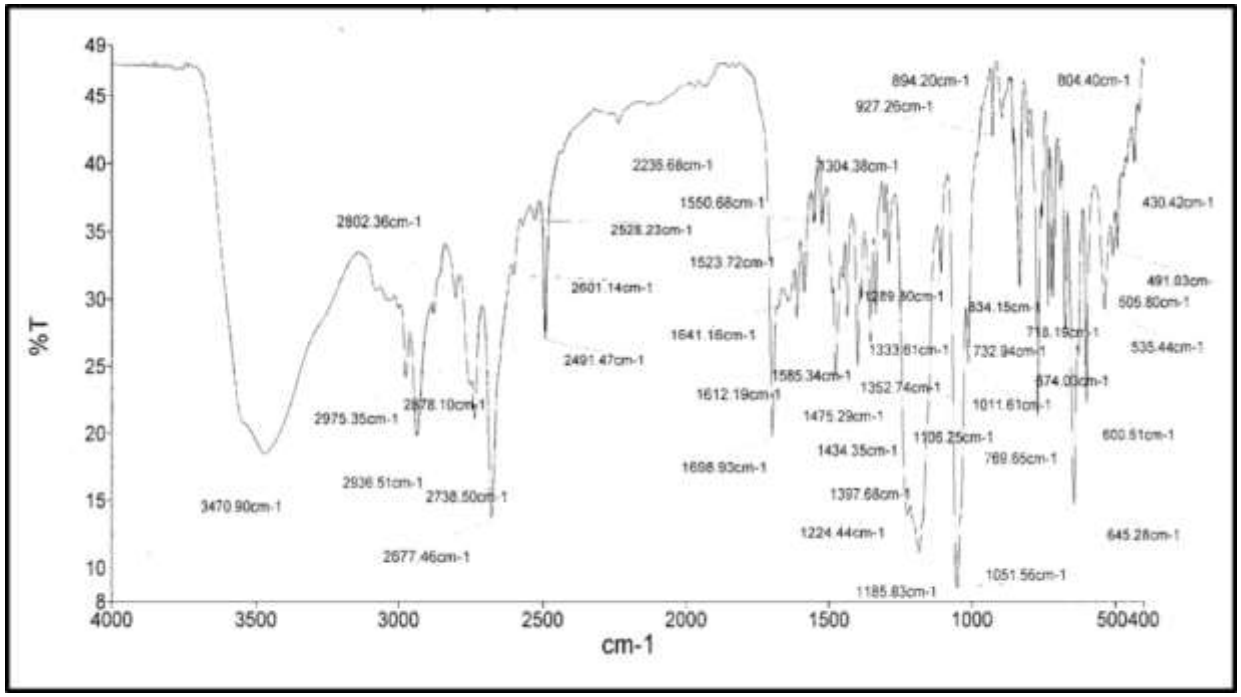


Figure S2: ^{1}H NMR spectrum of **3**:

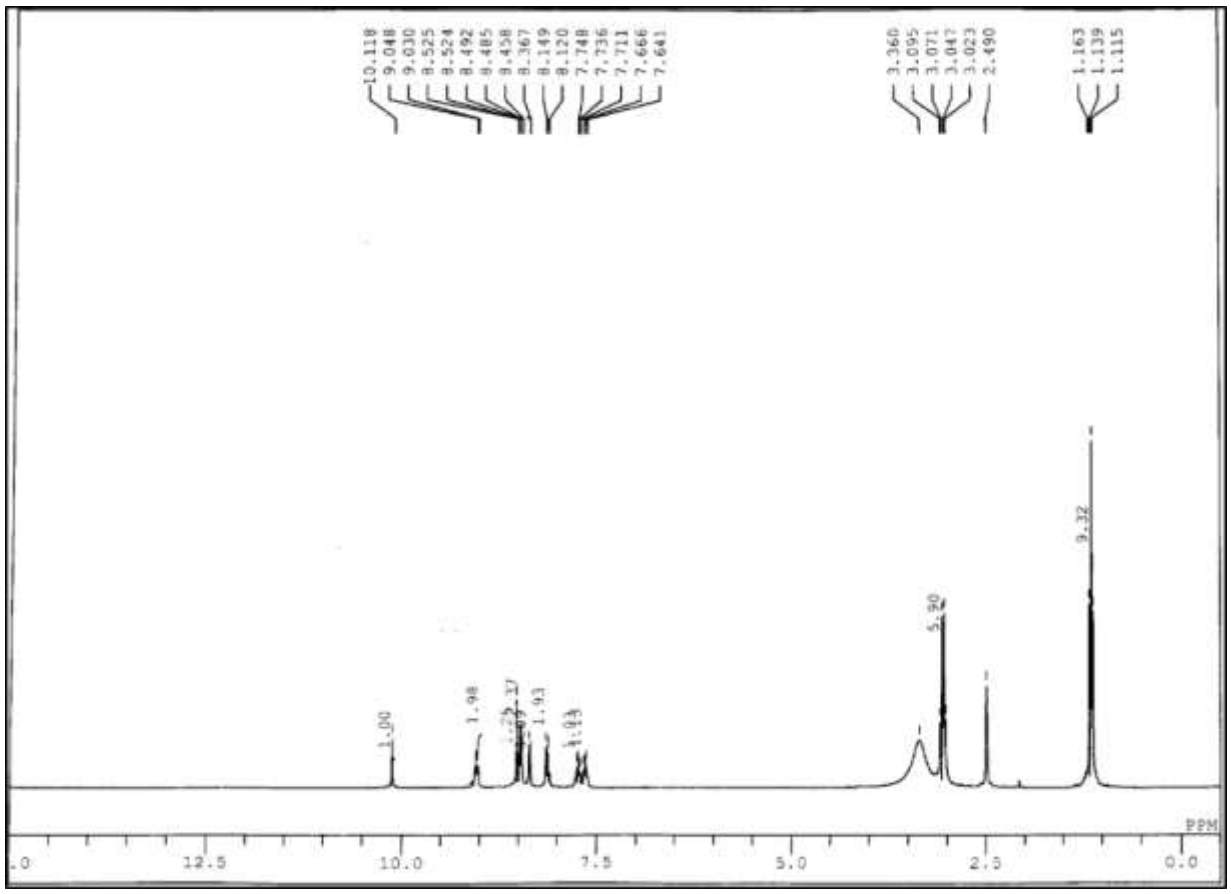


Figure S3: ^{13}C NMR spectrum of **3**:

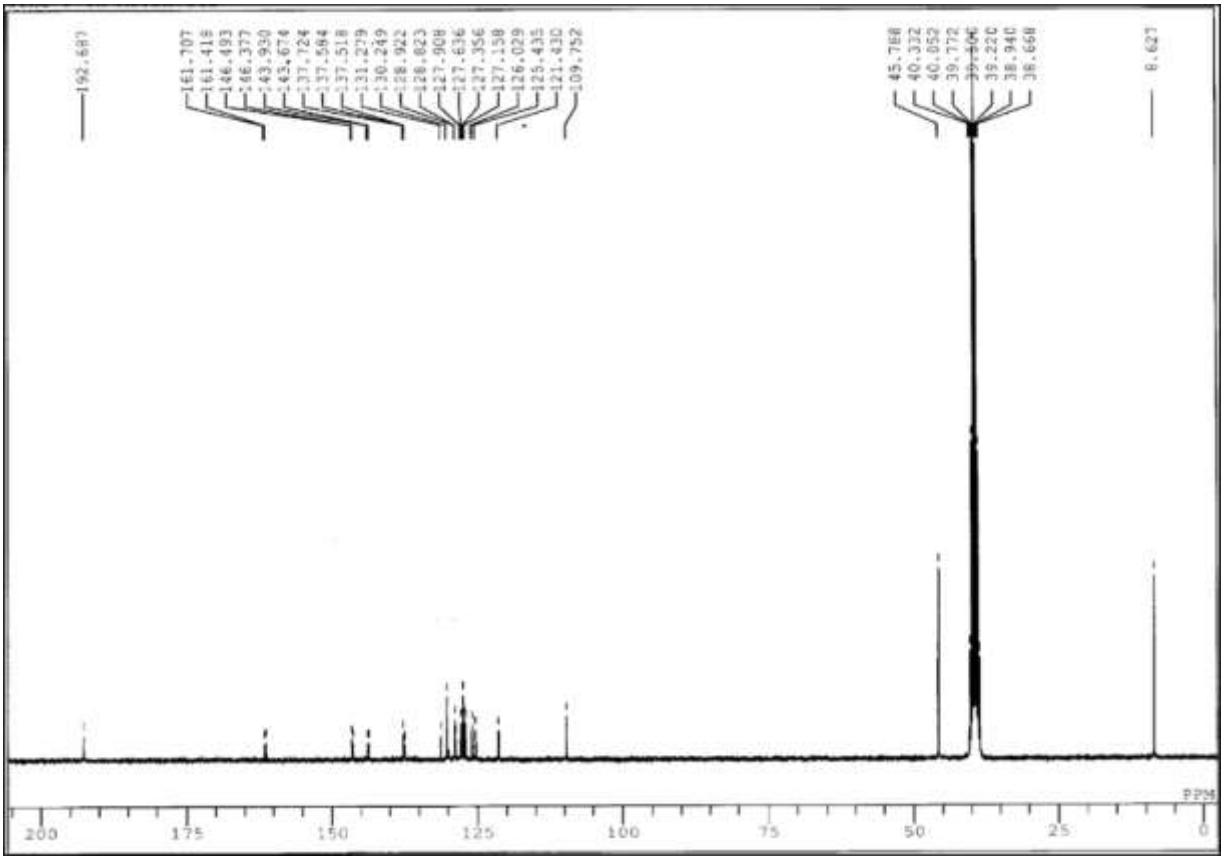


Figure S4: IR spectrum of **P1**:

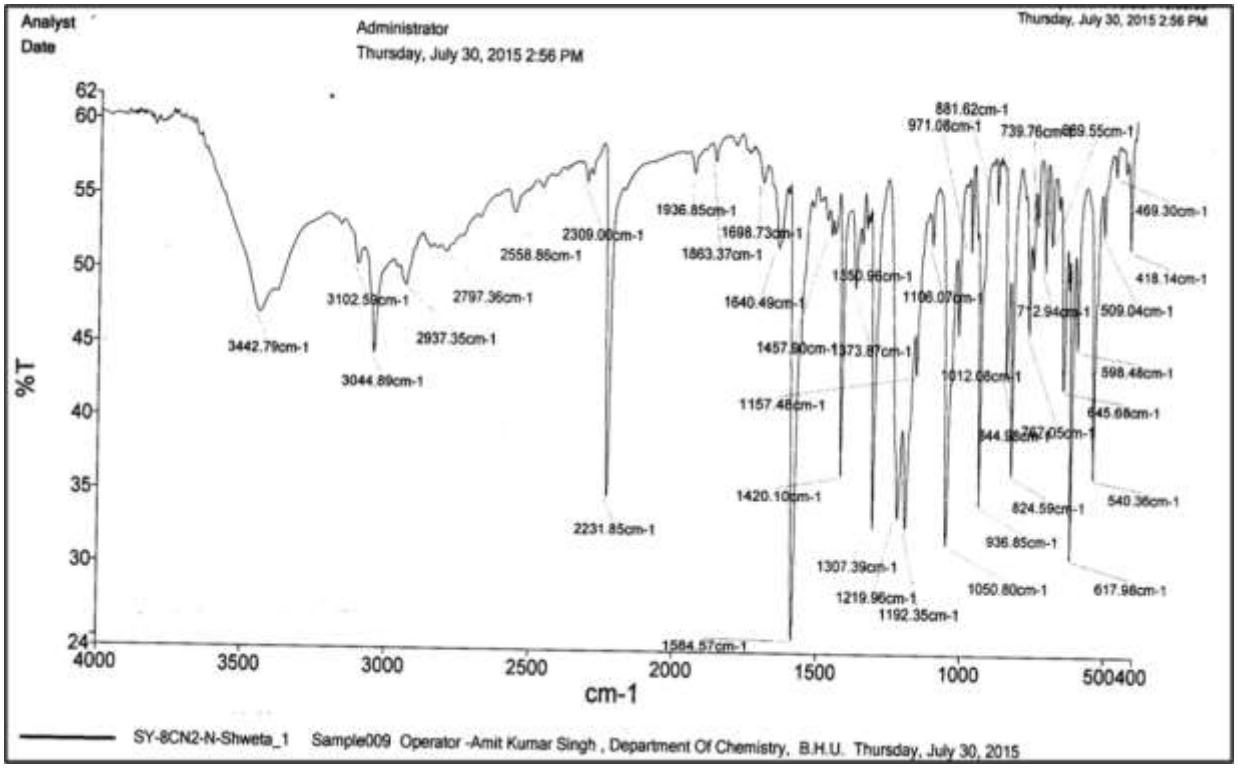


Figure S5: ^{1}H NMR spectrum of **P1**

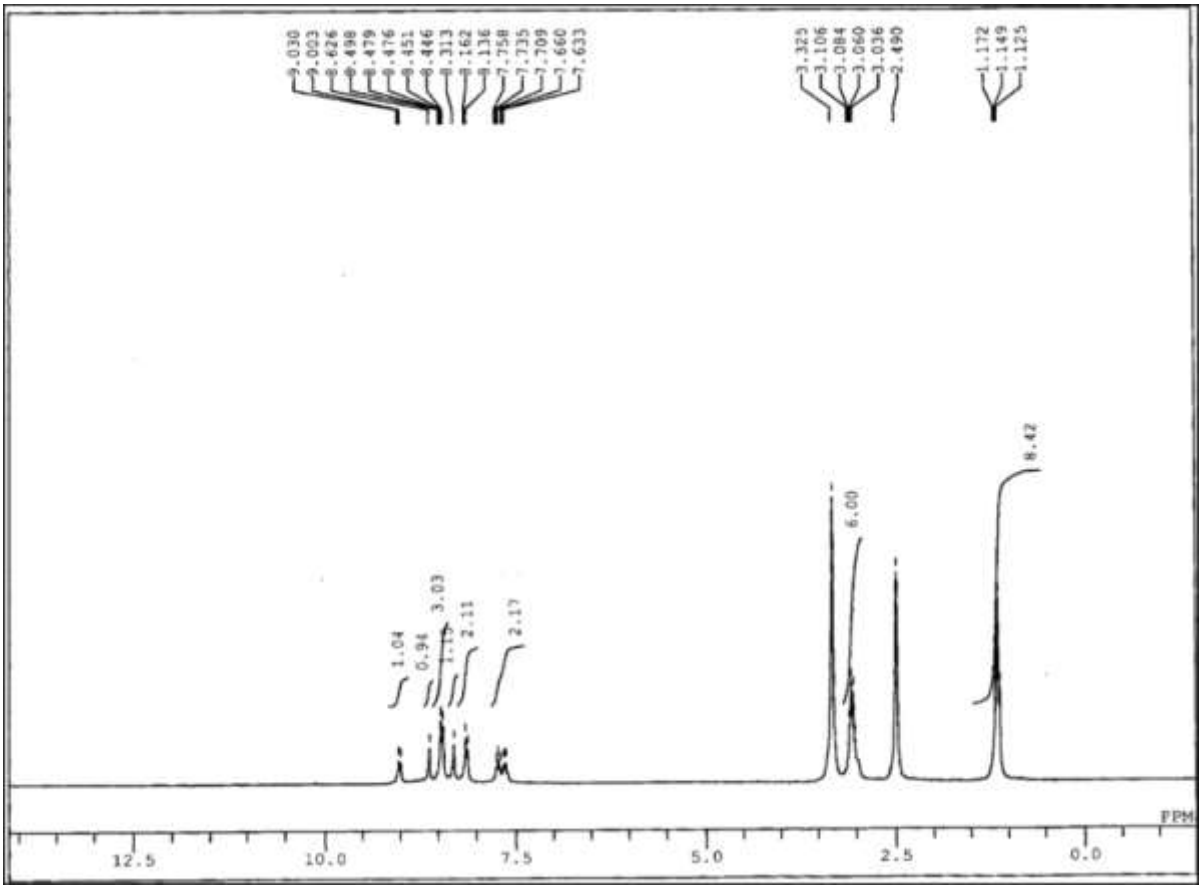


Figure S6: ^{13}C NMR spectrum of **P1**

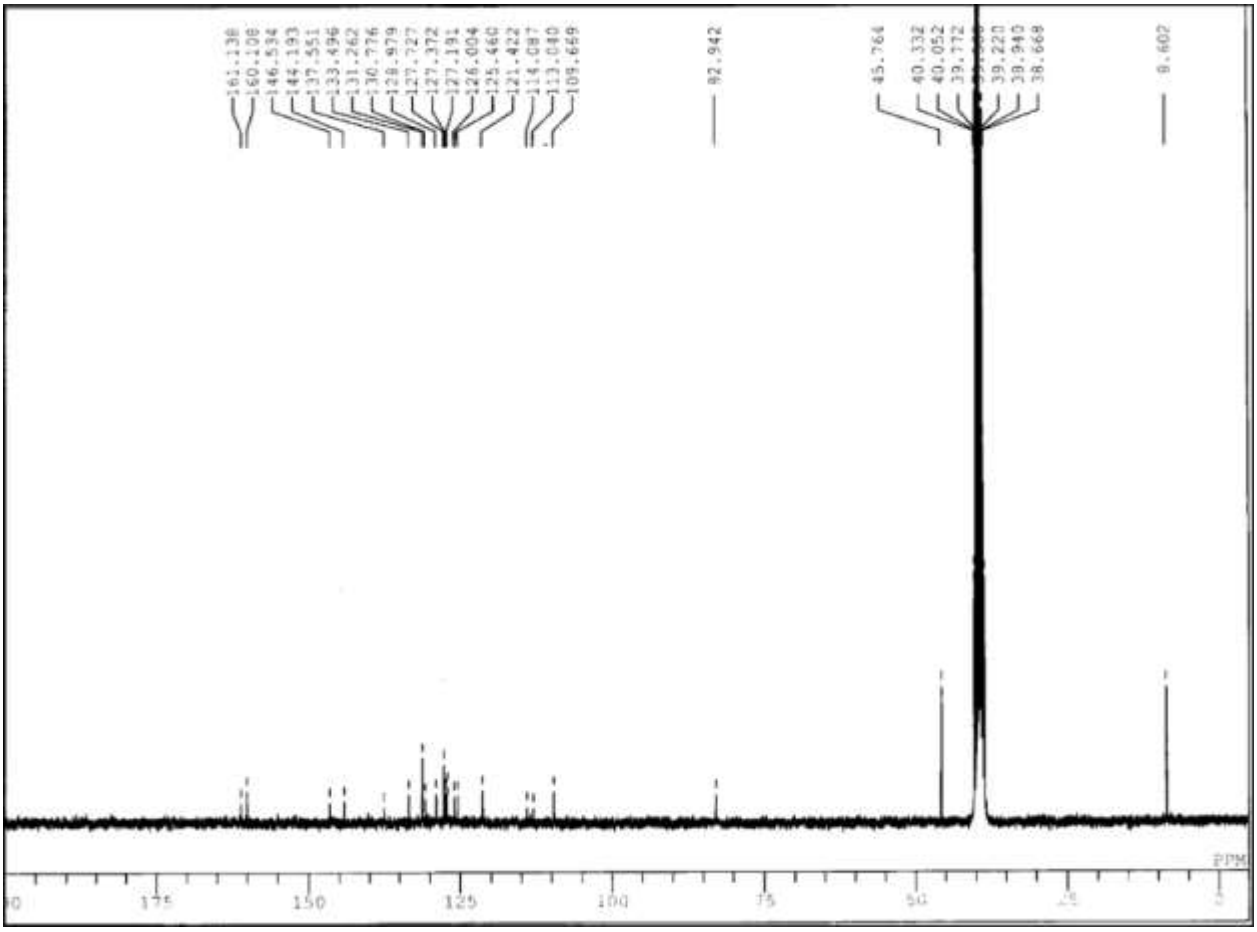


Figure S7: ESI-MS spectrum of **P1**

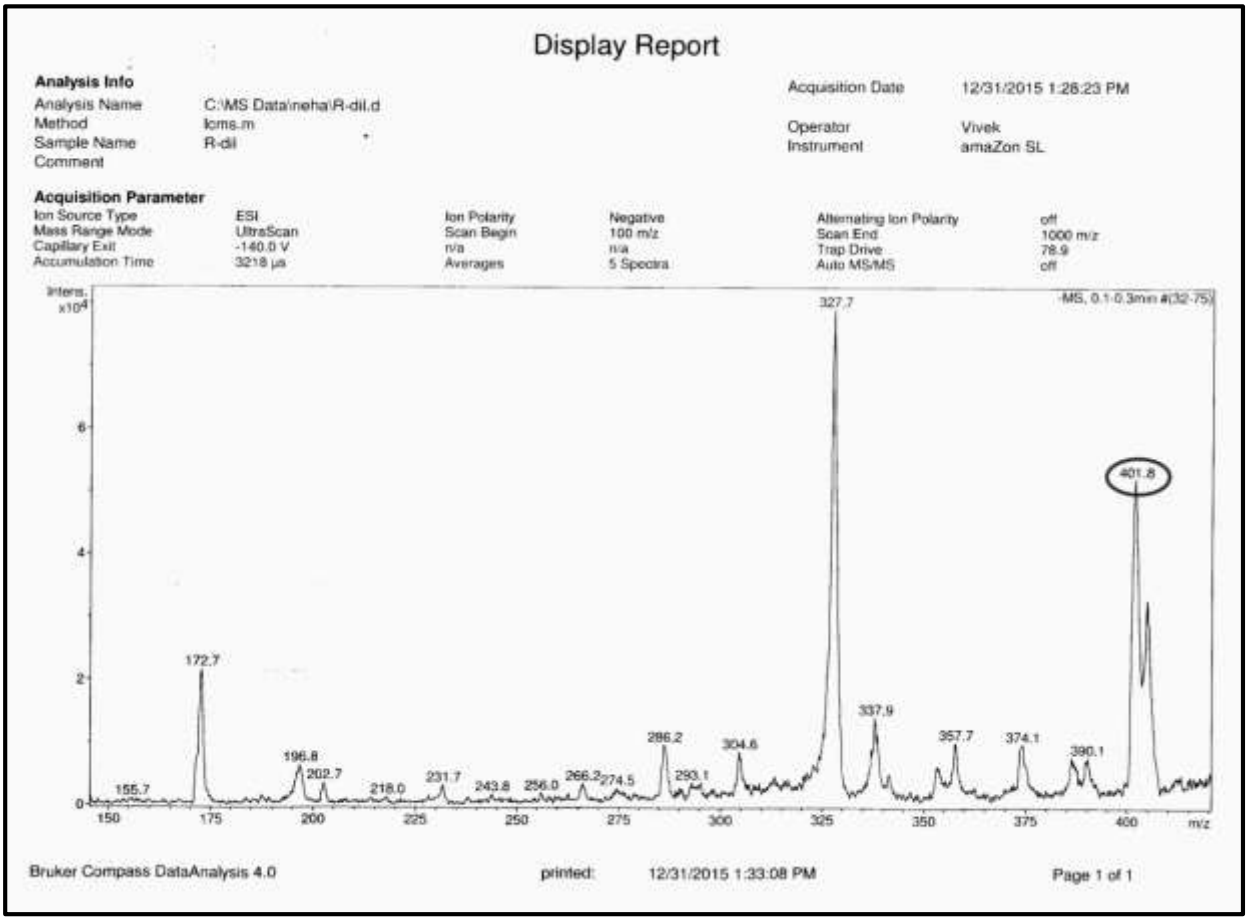


Figure S8: IR spectrum of **P1-N_2H_4**

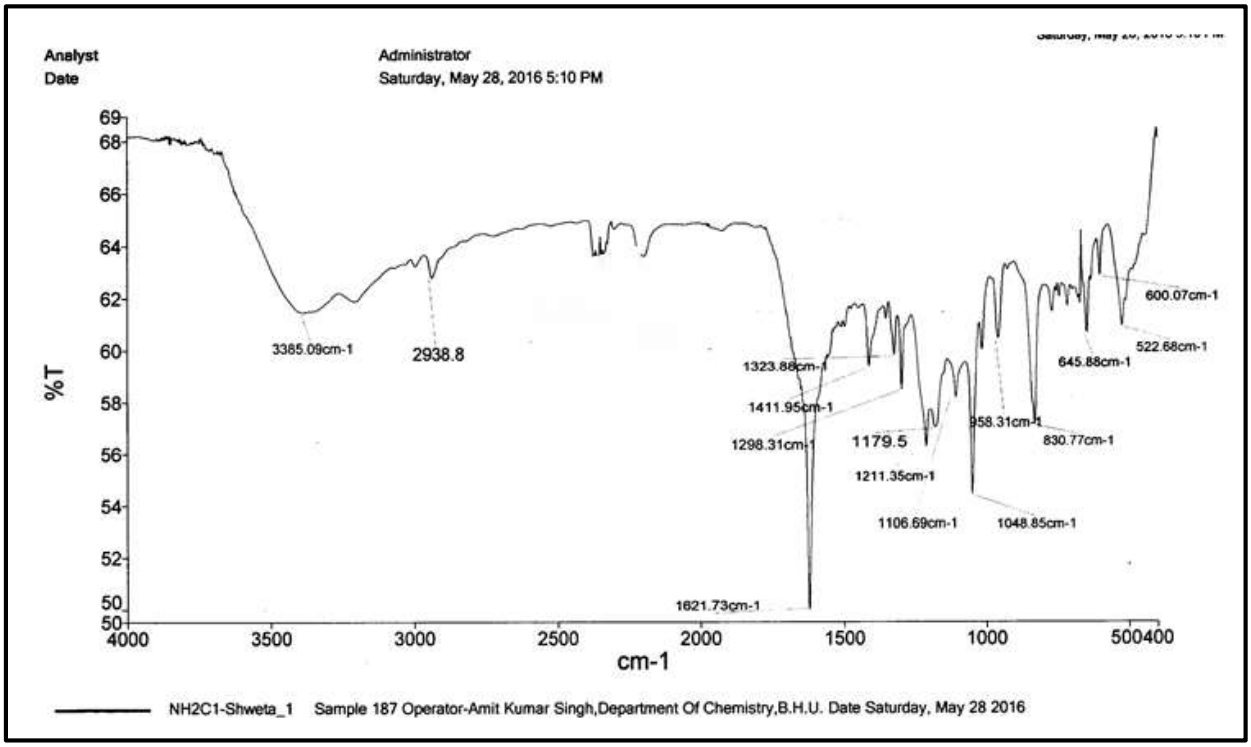


Figure S9: ^{1}H NMR spectrum of **P1-N_2H_4**

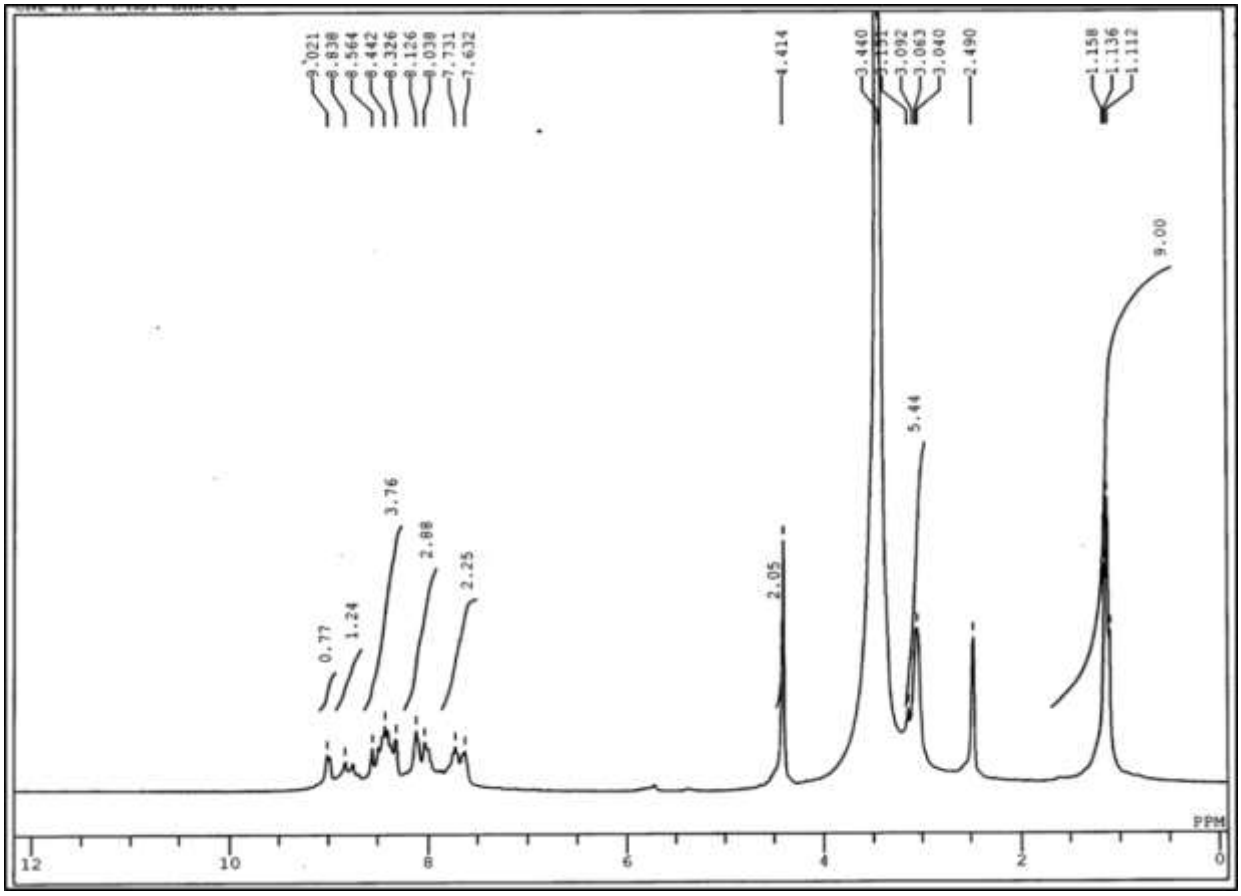


Figure S10: ^{13}CNMR spectrum of **P1-N_2H_4**

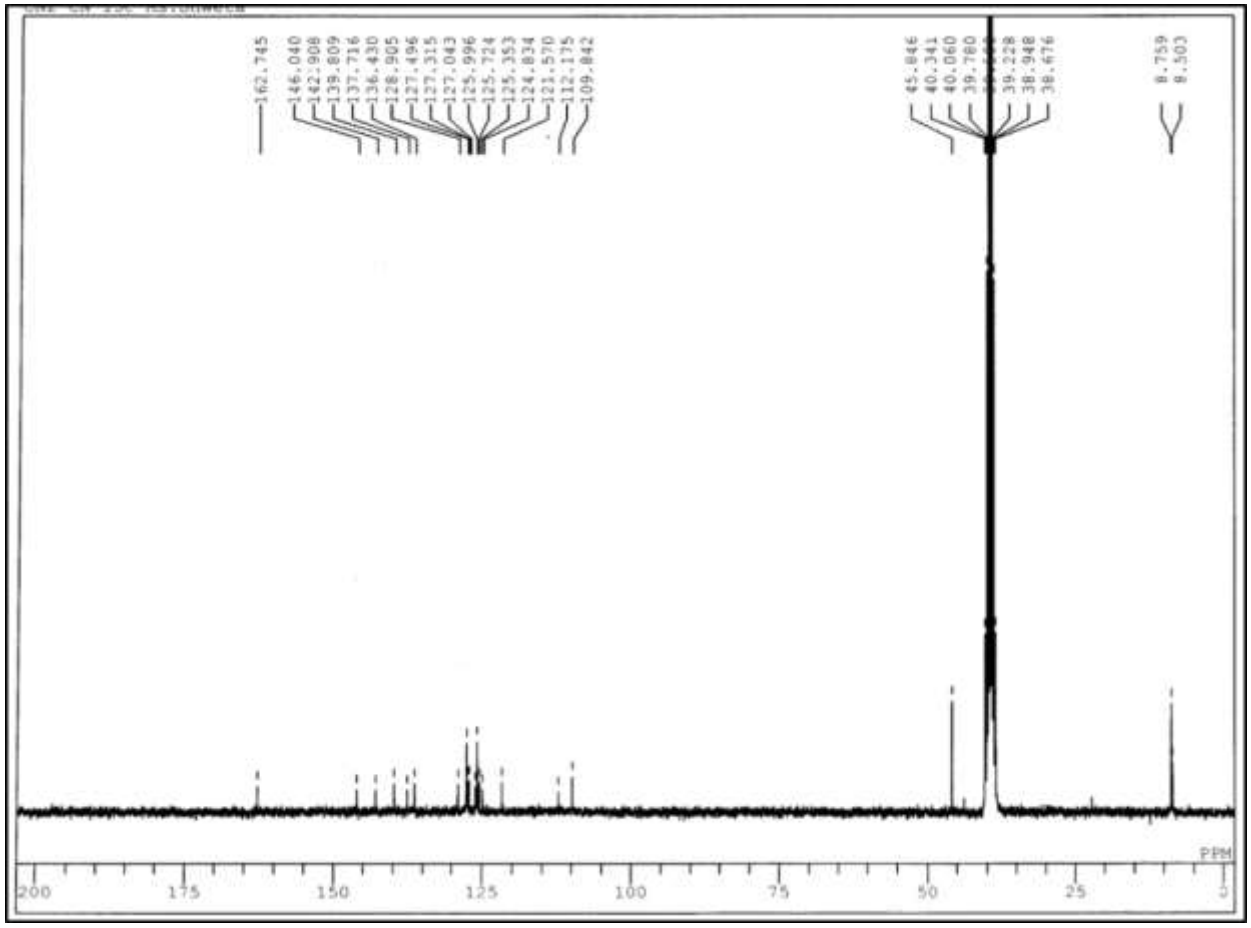


Figure S11: ESI-MS spectrum of **P1-N_2H_4**

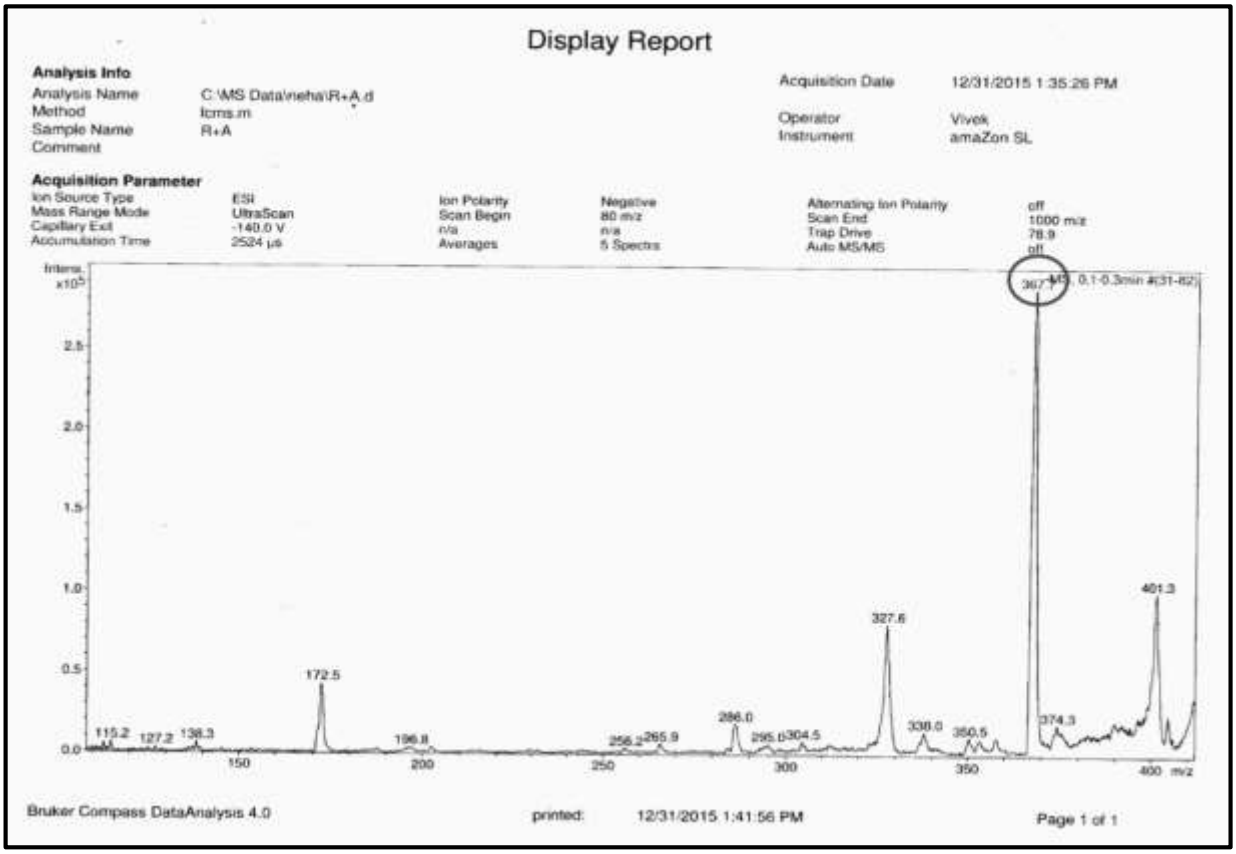


Figure S12: UV-Vis response of **P1** with various amines. R-**P1**, 1-hydrazine, 2-aniline, 3-4-nitrophenylaniline, 4-ammonia, 5-hydroxylamine, 6-phenylhydrazine, 7-pyridine, 8-ethylamine, 9-triethylamine, 10-methylamine, 11-urea, 12-ethylenediamine, 13-cysteine, 14-benzylamine, 15-thiourea, 16-2,4-dinitrophenylhydrazine, 17-butylamine:

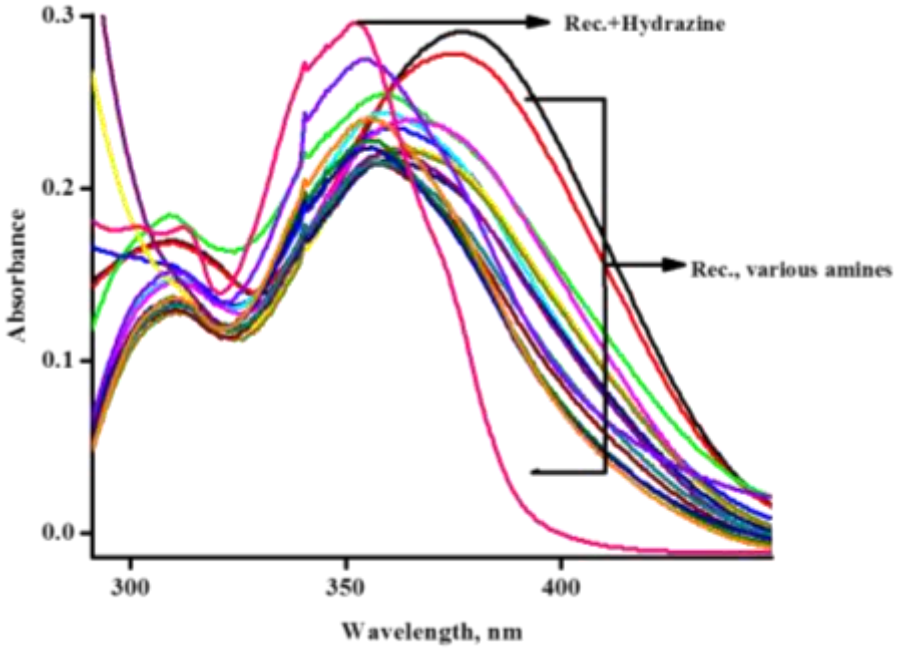


Figure S13: UV-Vis response of **P1** with various cations and anions. R-P1,1. Hydrazine, 2-Al^{3+}, 3-Cr^{3+}, 4-Mn^{2+}, 5-Fe^{3+}, 6-Co^{2+}, 7-Ni^{2+}, 8-Cu^{2+}, 9-Zn^{2+}, 10-Cd^{2+}, 11-Hg^{2+}, 12-Pb^{2+}, 13-$S_2O_3^{2-}$, 14-HSO_3^-, 15-HSO_4^- , 16-HPO_4^{2-}, 17-$H_2PO_4^-$, 18-PO_4^{2-}, 19-BzO^-, 20-SO_3^{2-}, 21-S^{2-}, 22-F^-, 23-Cl^-, 24- Br^-, 25-I^-, 26-Aco^-, 27-PPi, 28-ClO_4^-, 29-BF_4^-:

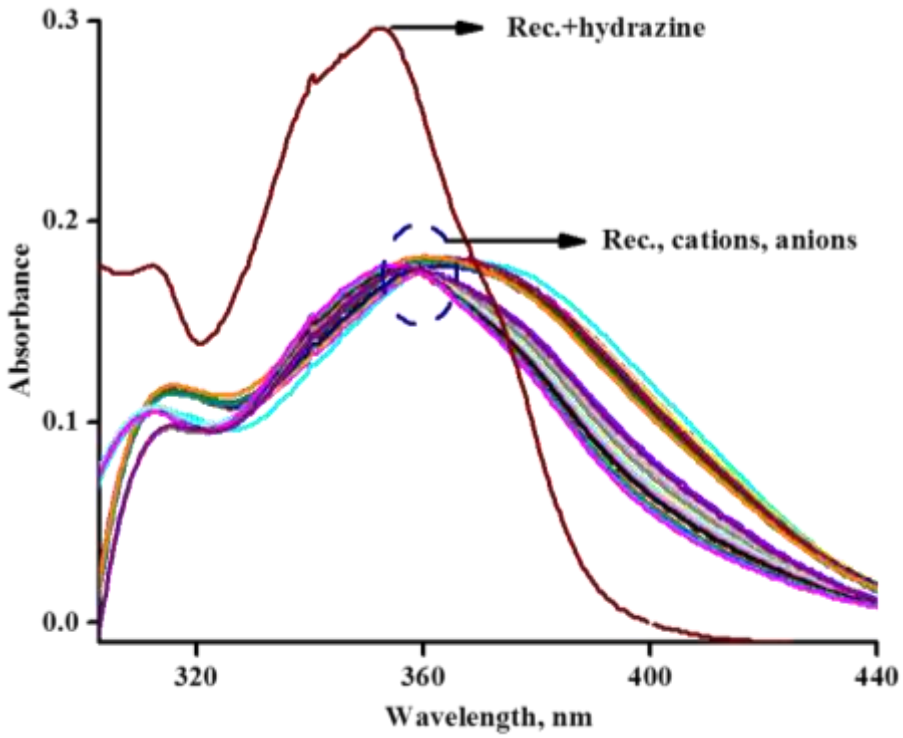


Figure S14: Absorbance ratio response in the form of bar graph representation and naked eye visual response of **P1** with various amines. R-**P1**, 1-hydrazine, 2-aniline, 3-4-nitrophenylaniline, 4-ammonia, 5-hydroxylamine, 6-phenylhydrazine, 7-pyridine, 8-ethylamine, 9-triethylamine, 10-methylamine, 11-urea, 12-ethylenediamine, 13-cysteine, 14-benzylamine, 15-thiourea, 16-2,4-dinitrophenylhydrazine, 17-butylamine, 18- Semicarbazide (SC), 19- Thiocarbohydrazide (TCH), 20- Carbohydrazide (CH), 21-$KMnO_4$, 22- $K_2Cr_2O_7$, 23-H_2O_2

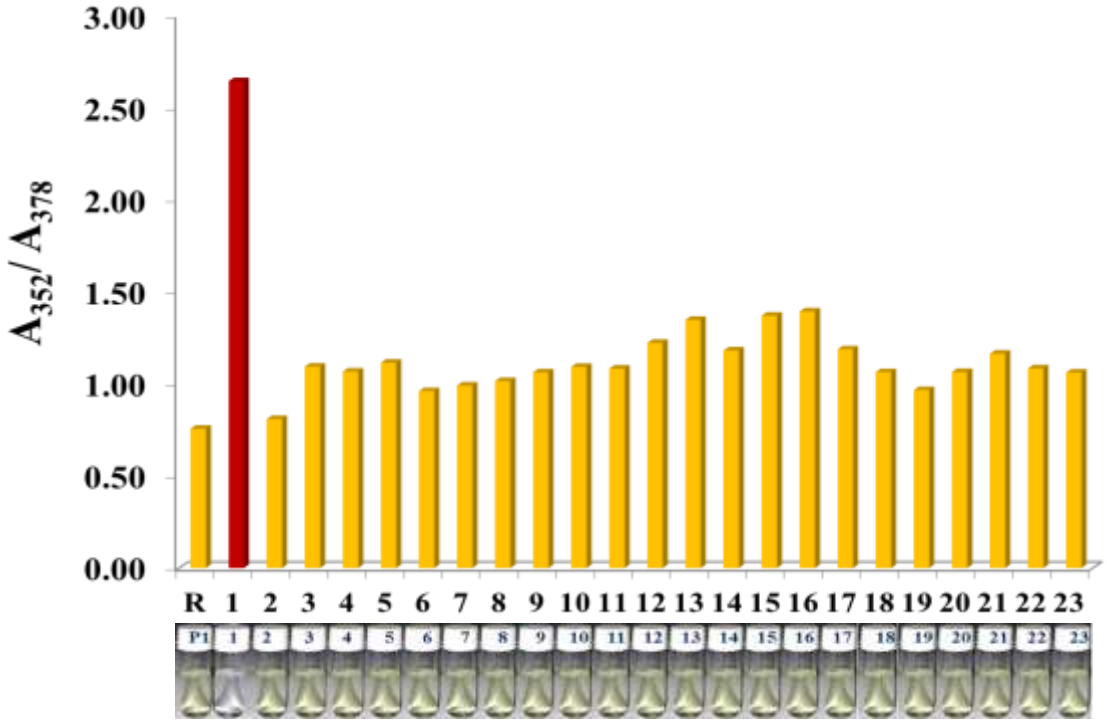


Figure S15: Absorbance ratio response in the form of bar graph representation and naked eye visual response of **P1** with various cations and anions. R-P1, 1-Hydrazine, 2-Al^{3+} , 3-Cr^{3+} ,4-Mn^{2+} , 5-Fe^{3+} ,6-Co^{2+}, 7-Ni^{2+}, 8-Cu^{2+}, 9-Zn^{2+}, 10-Cd^{2+}, 11-Hg^{2+}, 12-Pb^{2+}, 13-$S_2O_3^{2-}$, 14-HSO_3^- ,15-HSO_4^- ,16-HPO_4^{2-} ,17-$H_2PO_4^-$, 18-PO_4^{3-} , 19-BzO^- , 20-SO_3^{2-} , 21-S^{2-} , 22-F^-, 23-Cl^-, 24- Br^-, 25-I^-, 26-Aco^-, 27-PPi, 28-ClO_4^-, 29-BF_4^-:

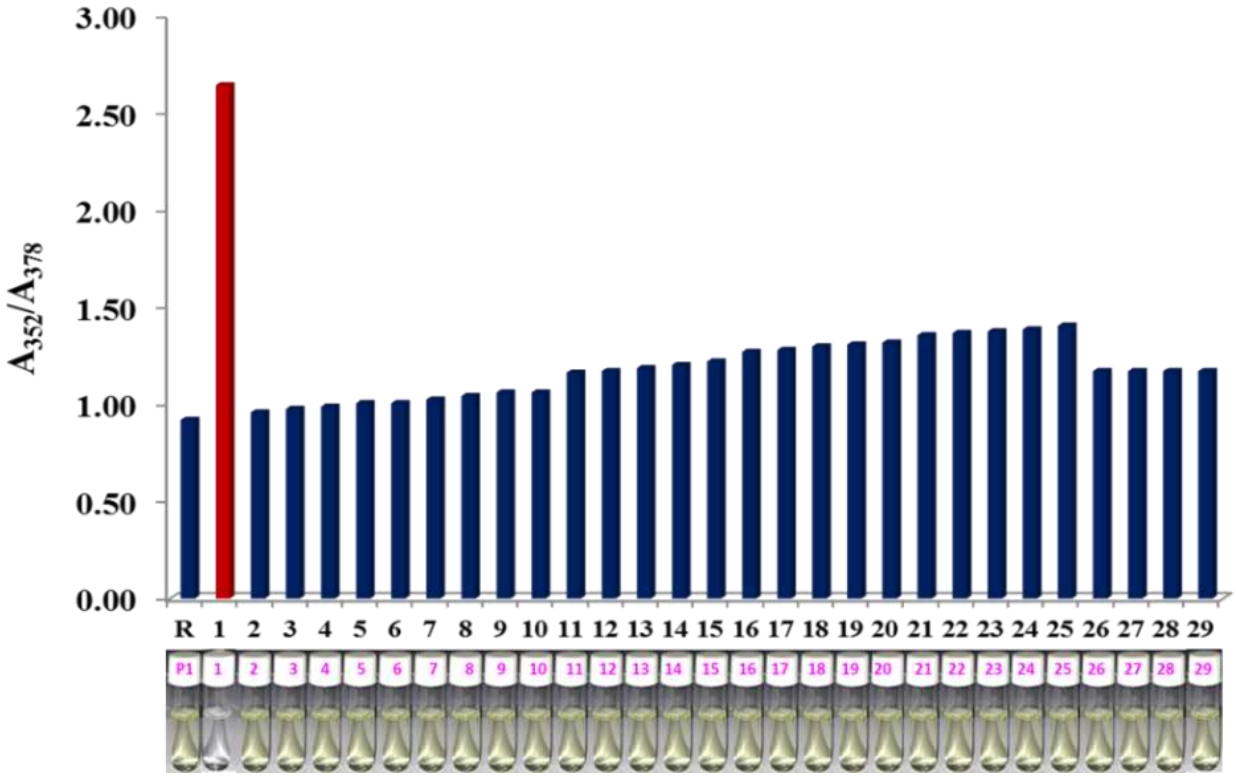


Figure S16: Detection limit and calibration curve of probe **P1** with hydrazine from UV-Vis. titration data

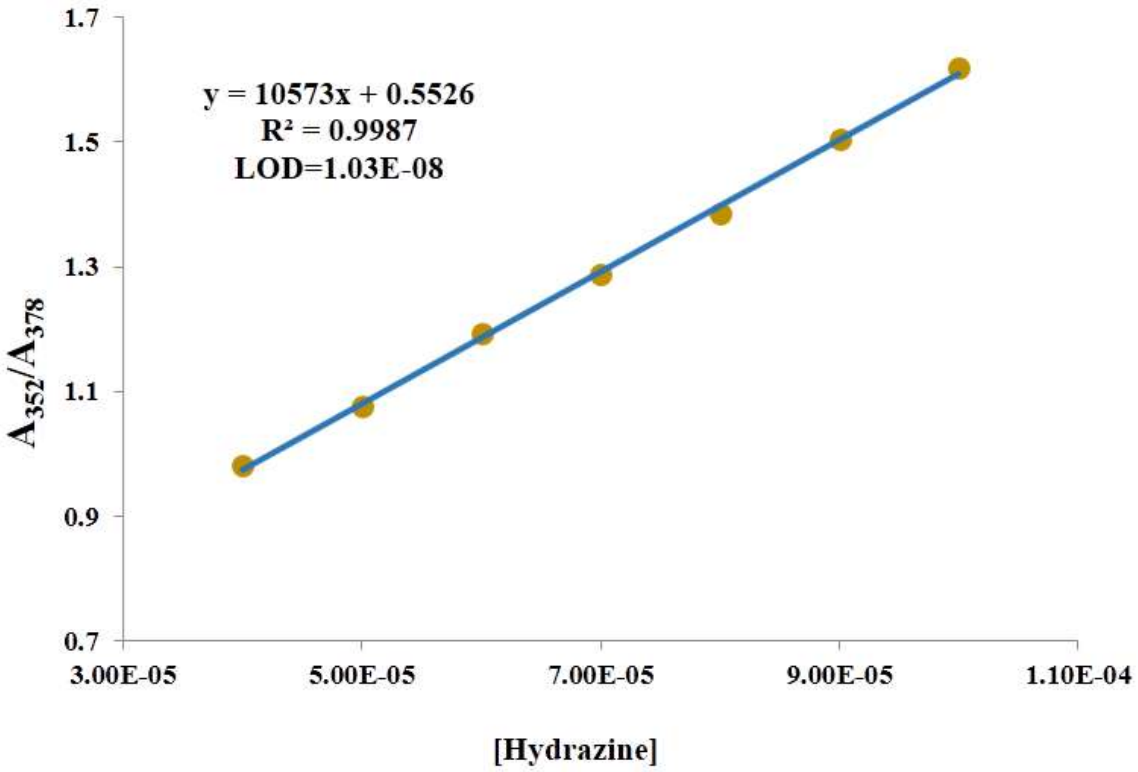


Figure S17: The visual response of **P1** in presence of various cations and anions under UV light;

P1, 1-Hydrazine, 2-Al^{3+}, 3-Cr^{3+}, 4-Mn^{2+}, 5-Fe^{3+}, 6-Co^{2+}, 7-Ni^{2+}, 8-Cu^{2+}, 9-Zn^{2+}, 10-Cd^{2+}, 11-Hg^{2+}, 12-Pb^{2+}, 13-$S_2O_3^{2-}$, 14-HSO_3^-, 15-HSO_4^-,16-HPO_4^{2-},17-$H_2PO_4^-$, 18-PO_4^{3-}, 19-BzO^-, 20-SO_3^{2-}, 21-S^{2-}, 22-F^-, 23-Cl^-, 24- Br^-, 25-I^-, 26-Aco^-, 27-PPi, 28-ClO_4^-, 29-BF_4^-

Figure S18: The visual response of **P1** in presence of various amines under UV light; **P1**, **1**-hydrazine, **2**-aniline, **3**-4-nitrophenylaniline, **4**-ammonia, **5**-hydroxylamine, **6**-phenylhydrazine, **7**-pyridine, **8**-ethylamine, **9**-triethylamine, **10**-methylamine, **11**-urea, **12**-ethylenediamine, **13**-cysteine, **14**-benzylamine, 1**5**-thiourea, **16**-2,4-dinitrophenylhydrazine, **17**-butylamine, **18**- Semicarbazide (SC), **19**-Thiocarbohydrazide (TCH), **20**- Carbohydrazide (CH), **21**- $KMnO_4$, **22**- $K_2Cr_2O_7$, **23**-H_2O_2

Figure S19: Fluorescence ratio response in the form of bar graph showing competition experiment representation of **P1** with various cations and anions with hydrazine added. R-**P1**, 1-Hydrazine, 2-Al^{3+}, 3-Cr^{3+}, 4-Mn^{2+}, 5-Fe^{3+}, 6-Co^{2+}, 7-Ni^{2+}, 8-Cu^{2+}, 9-Zn^{2+}, 10-Cd^{2+}, 11-Hg^{2+}, 12-Pb^{2+}, 13-$S_2O_3^{2-}$, 14-HSO_3^-, 15-HSO_4^-,16-HPO_4^{2-},17-$H_2PO_4^-$, 18-PO_4^{3-}, 19-BzO^-, 20-SO_3^{2-}, 21-S^{2-}, 22-F^-, 23-Cl^-, 24- Br^-, 25-I^-, 26-Aco^-, 27-PPi, 28-ClO_4^-, 29-BF_4^-:

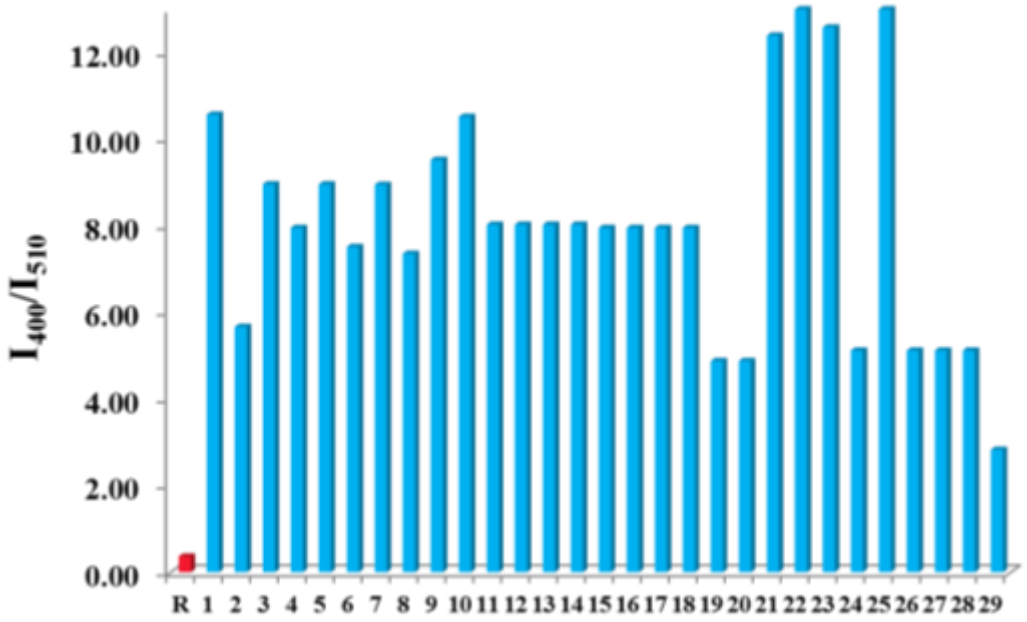


Figure S20: Fluorescence ratio response in the form of bar graph showing competition experiment representation of **P1** various amines with hydrazine added. R-**P1**, 1-hydrazine, 2-aniline, 3-4-nitrophenylaniline, 4-ammonia, 5-hydroxylamine, 6-phenylhydrazine, 7-pyridine, 8-ethylamine, 9-triethylamine, 10-methylamine, 11-urea, 12-ethylenediamine, 13-cysteine, 14-benzylamine, 15-thiourea, 16-2,4-dinitrophenylhydrazine, 17-butylamine, 18- Semicarbazide (SC), 19- Thiocarbohydrazide (TCH), 20- Carbohydrazide (CH), 21- $KMnO_4$, 22- $K_2Cr_2O_7$, 23-H_2O_2

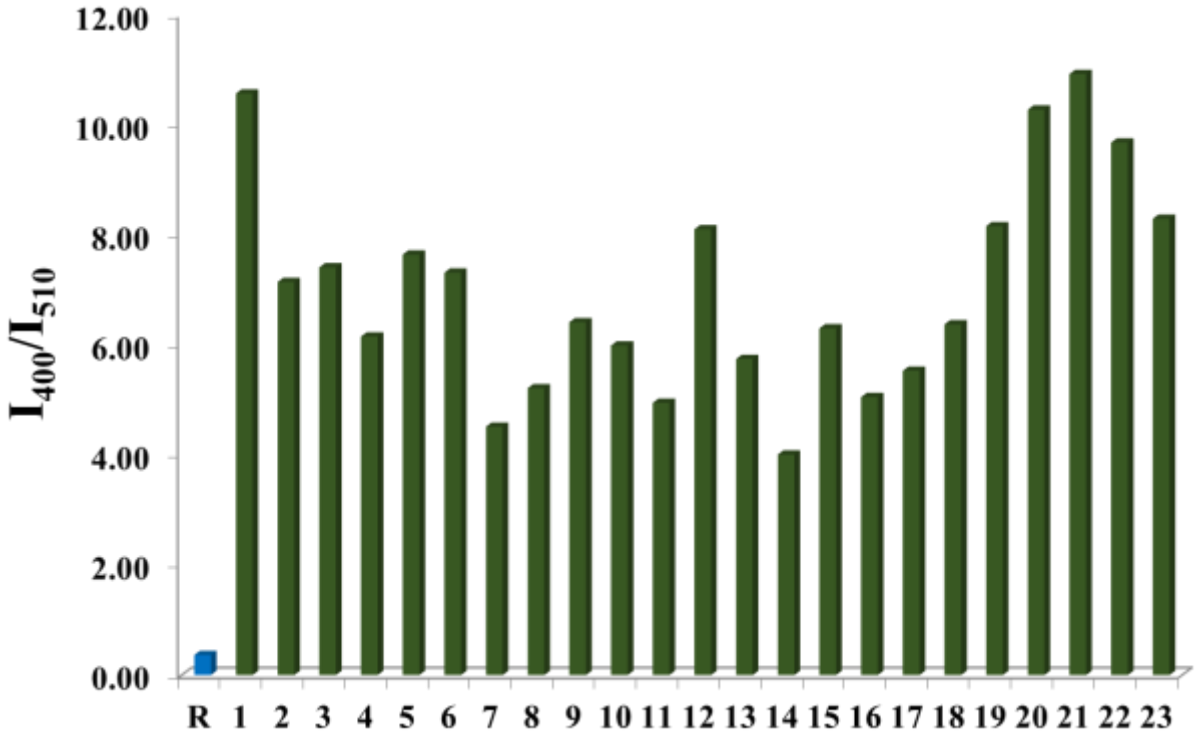


Figure S21: The variation in fluorescence intensity in **P1** with the change in pH in the presence of hydrazine

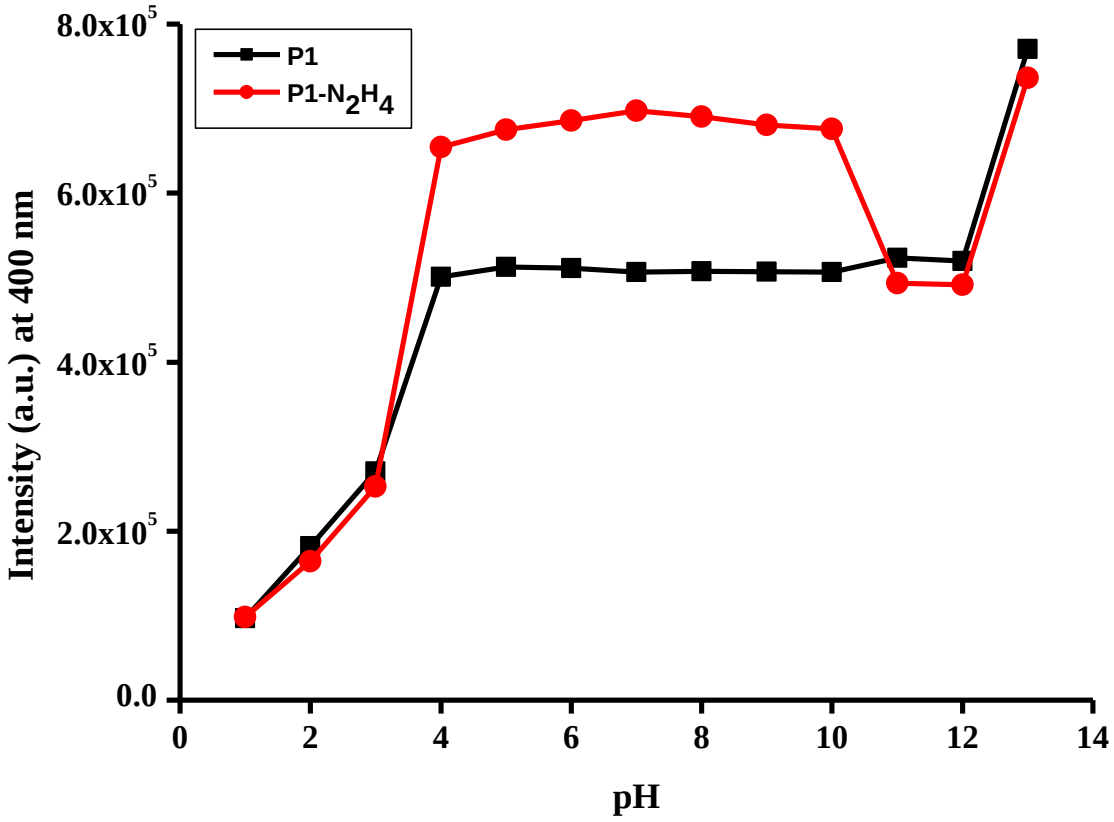


Figure S22: Cell viability of **P1** at different concentration in 24 hrs.

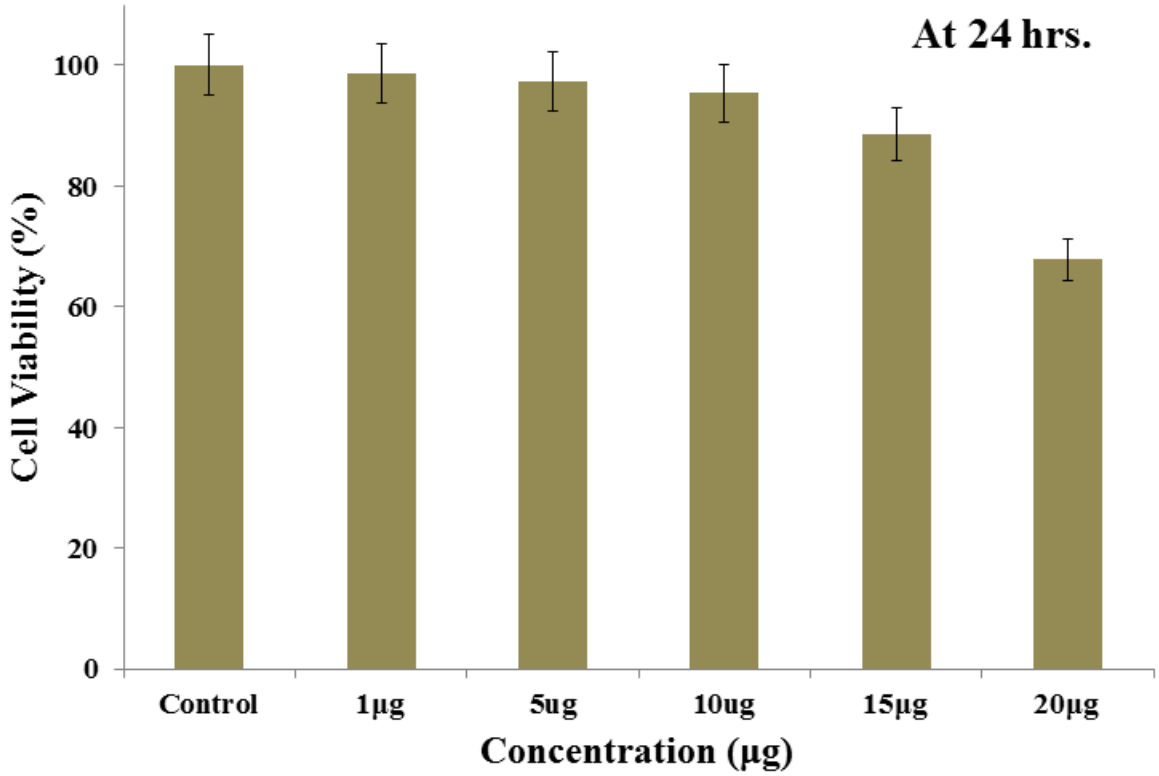


Table 1 Crystal data of **P1**

Identification code	**P1**
CCDC number	1453378
Empirical formula	$C_{48}H_{36}N_7O_8S_2$
Formula weight	902.96
Temperature/K	100
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	a = 6.817(5) Å alpha = 103.091(5) deg. b = 18.957(5) Å, beta=90.526(5) deg. c = 20.898(5) Å, gamma =94.227(5) deg.
Volume	$2622(2)A^3$
Absorption coefficient	0.155 mm^{-1}
F(000)	938.0
Crystal size	0.3 × 0.2 × 0.1mm
Theta range for data collection	5.994 to 56.766 deg.
Limiting indices	$-9 \le h \le 9, -25 \le k \le 25, -27 \le l \le 27$
Reflections collected / unique	51549 / 12607 [R_{int} = 0.0901]
Completeness to theta = 28.383	98.0 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	12607/0/589
Goodness-of-fit on F^2	0.984
Final R indices [I>2sigma(I)]	R1 = 0.0881, wR2 = 0.2092
R indices (all data)	R1 = 0.1450, wR2 = 0.2315
Largest diff. peak and hole	2.04/-0.44 $e.A^{-3}$