# Electronic Supporting Information

## A new calix[4]arene based molecular probe for selective and sensitive detection of $CN^-$ ion in aqueous medium

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## 1. Experimental

### 1.1 Materials and Methods

2,4-dinitrophenyl hydrazine, *p*-hydroxyphenol, hexamethylenetetramine, ethylbromoacetate, trifluoroacetic acid as well as all anion salts were purchased from Sigma-Aldrich Chemicals Pvt Ltd and Merck India. Silica gel G used for Thin Layer Chromatography was purchased from Merck India. Deionized, triple distilled water and spectroscopic grade solvents were used in spectroscopic studies. The absorption spectra were recorded at room temperature on a Perkin Elmer Lambda 35 spectrophotometer using a quartz cuvette (path length = 1cm). FT–IR spectra (KBr pellets) were recorded on a Perkin Elmer spectrometer. $^1$H NMR spectra (chemical shifts in δ ppm) were recorded on a Bruker FT-NMR (300 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. The association constant for a 2:1 stoichiometry of molecular receptor and cyanide ions in aqueous medium was estimated by modified Benesi-Hildebrandmethods using equation (1)

$$4/(I - I_o) = 4/(I - I_f) + 4/K\,(I - I_f)\,[M]^2 \qquad (1)$$

Where $K$ is the association constant,**M** is concentration of $CN^-$, $I$is absorbance of free receptor **4**, $I_o$ is the observed absorbance of a complex, **4-CN**$^-$and $I_f$ is the absorbance at saturation point.

### 1.2 Preparation of Test Paper Strip:

Test paper strips (*Whatman filter paper Grade 1*) were prepared (0.4 x 2.5 $cm^2$) by treating the *Whatman filter paper Grade 1* with a solution of **4** in chloroform (2 mg/ml) followed by drying in air.

### 1.3 Synthesis of compound 4

#### 1.3.1 Synthesis of 1

The suspension of *p*-tertiarybutylphenol (25 g) in formaldehyde (35%, 17 ml), NaOH (0.3 g) was added and heated at 110-115 $^oC$ for 2 h. The reaction mixture was initially clear but become a yellow colored spongy solid. To this spongy solid, hot diphenyl ether (200 ml) was added and heated at 220-230 $^oC$ for 4 h under $N_2$ atmosphere. After completion of reaction, ethyl acetate was added to the orange colored solution, the off white precipitate was

appeared. Filtered and washed with ethyl acetate and dried in air to get half white solid which was crystalised from toluene. $R_f$= 0.9 (EtOAc:Hexane, 2:8 v/v); $^1$H NMR ($CDCl_3$) δ (ppm): 10.34 (s, 4H, -OH), 7.06 (s, 8H, phenyl), 4.23 (d, 4H, *J* = 7.2 Hz), 3.52 (d, 4H, *J* = 8.1 Hz), 1.22 (s, 36H, t-Bu).

**1.3.2 Synthesis of 2**

To the mixture of **1** (1.94 g, 3 mmol) and $K_2CO_3$ (1.18 g, 9 mmol) in anhydrous acetonitrile (MeCN), α-bromoethylacetate (1.34 g, 7.5 mmol) was added. The reaction mixture was refluxed overnight under nitrogen atmosphere. After completion of the reaction (as monitored on TLC), the reaction mixture was filtered andfiltrate was evaporated to dryness under reduced pressure.The chloroform (25 ml) was added to thick oil followed by washing with cold water (3 x 10 ml). Organic phase was dried over anhy. $Na_2SO_4$, solvent was evaporated to get compound **2**. Recrystalised from chloroform and methanol to get off white colored solid. Yield ~ 76% yield. $R_f$= 0.65 (EtOAc:Hexane, 2:8 v/v); $^1$H NMR ($CDCl_3$) δ (ppm): 6.97 (s, 2H OH), 6.95 (s, 4H, phenyl), 6.73 (s, 4H, phenyl), 4.73 (s, 4H, CO*CH*$_2$), 4.35 (d, 4H, *J* = 13.5 Hz), 4.19 (m, 4H, -*CH*$_2$CH$_3$), 3.43 (d, 4H, *J* = 13.8 Hz), 1.24 (s, 18H, t-Bu), 1.29 (m, 6H, -CH$_2$*CH*$_3$) 0.90 (s, 18H, t-Bu).

**1.3.3 Synthesis of 3**

To a solution of **2** (1 g, 1.2 mmol) in trifluoroacetic acid (100 ml), hexamethylenetetraamine (6.72 g, 48 mmol) was added and refluxed for 2 days. After completion of the reaction (as monitored by TLC), cold water was added to obtain a dirty white solid,which was washed with water and dried in air to get **3** in 85% yield. $R_f$= 0.4 (EtOAc:Hexane, 2:8, v/v); $^1$H NMR ($CDCl_3$) δ (ppm): 9.77 (s, 2H, CHO), 8.54 (s, 2H, OH), 7.60 (s, 4H), 6.91 (s, 4H), 4.74 (s, 4H), 4.49 (d, 4H, *J* = 13.2 Hz), 4.37 (m, 4H), 3.50 (d, 4H, *J* = 13.2 Hz), 1.37 (t, 6H, *J* = 7.2, 6.9 Hz), 1.06 (s, 18H).

**1.3.4 Synthesis of phenolic hydrazone model compound**

A model compound incorporating both the hydrazone and the phenolic functionalities has been synthesized by the method similar to the one used for preparing **4**. The model compound was obtained as a brownish orange solid in 85% yield; M.p. >280 $^o$C; $^1$H NMR (DMSO-$d_6$) δ (ppm): 11.52 (s, 1H, NH), 10.1 (s, 1H, OH), 8.86 (s, 1H, DNP), 8.59 (s, 1H, *CH=N*), 8.33 (d, 1H, *J* = 9.0 Hz, DNP), 8.07 (d, 1H, *J* = 9.6 Hz, DNP), 7.65 (d, 2H, *J* = 8.7 Hz), 6.88 (d, 2H, *J* = 8.7 Hz).

**1.4 Absorption Spectra**

Absorption spectra of upper rim substituted calix[4]arene di-nitrophenylhydrazone **4** was recorded in aqueous-MeCN (50%, v/v)solution. This compound shows absorption band at 407 nm in aqueous-MeCN (50%, v/v) and were recorded in the region between 275-700 nm, the band at 407 nm indicated n-$\pi^*$ transition of **4**.

**1.5 Ion Binding Studies**

Stock solutions of **4** (1 x $10^{-3}$ M) and anions ($F^-$, $Cl^-$, $Br^-$, $I^-$, $AcO^-$, $CN^-$, $HSO_4^-$, $SCN^-$ and $H_2PO_4^-$as their tetrabutylammonium salts) (1 x $10^{-1}$ M) were prepared in freshly purified acetonitrile. The stock solution of **4**was diluted to 10 μM and all the spectroscopic studies were performed at 10 μM concentration. 2μL (10 equiv) of stock solution of each anion was added to 2ml solution of **4** (10 μM) for interaction and interfernce studies. For titration studies, aliquotes of 2μl (0.1 equiv) of $CN^-$ (1 mM) were added to **4** (10 μM) and the changes in the absorption spectra were measured.

**1.6 Response time**:

The response time of **4**was monitored by adding 2 equiv of $CN^-$ to a solution of **4** (10 μM) in aqueous-MeCN (50%, v/v) and recording the changes in the absorption spectra at regular intervals of time. The plot of absorbance *vs* time at 407 and 473 nm indicated that absorbance reached a maximum within 20 sec.

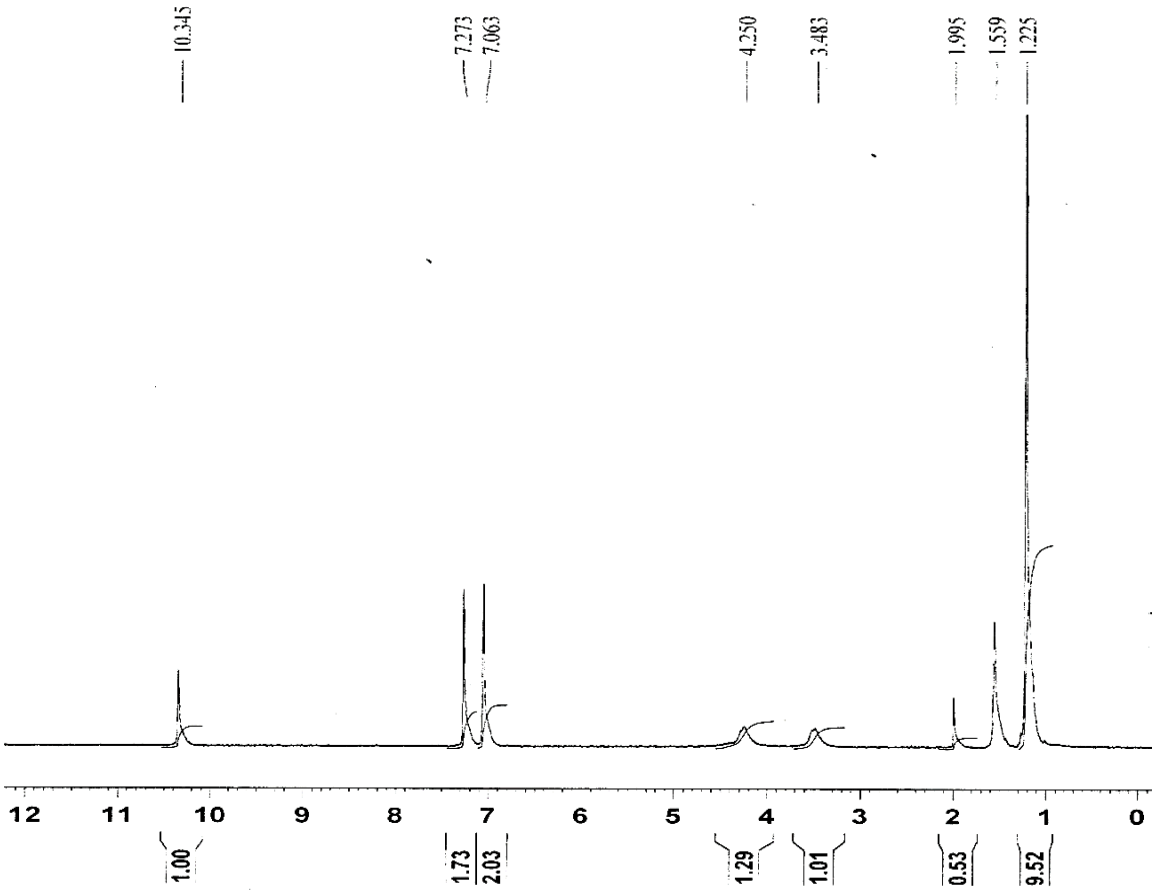


**Figure S1:** $^1$H NMR spectrum of **1** in $CDCl_3$.

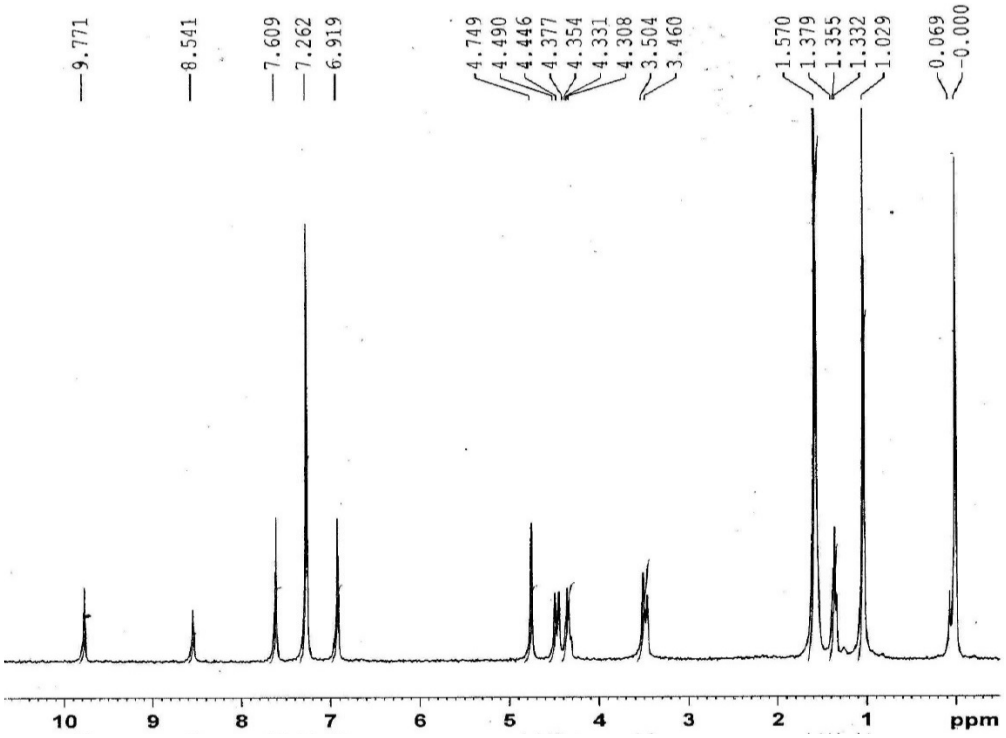


**Figure S2:** $^1$H NMR spectrum of **3** in $CDCl_3$.

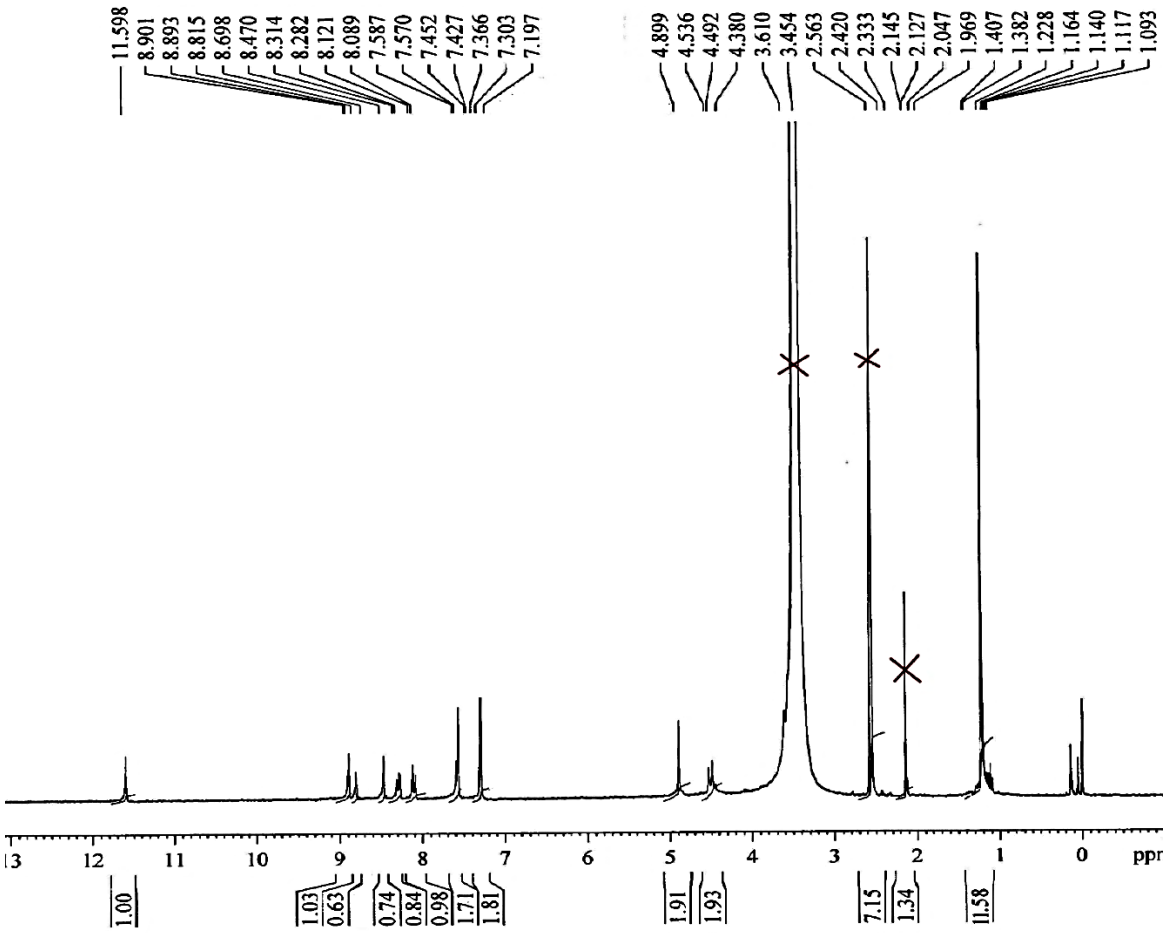


**Figure S3:** $^1$H NMR spectrum of **4** in DMSO-$d_6$.

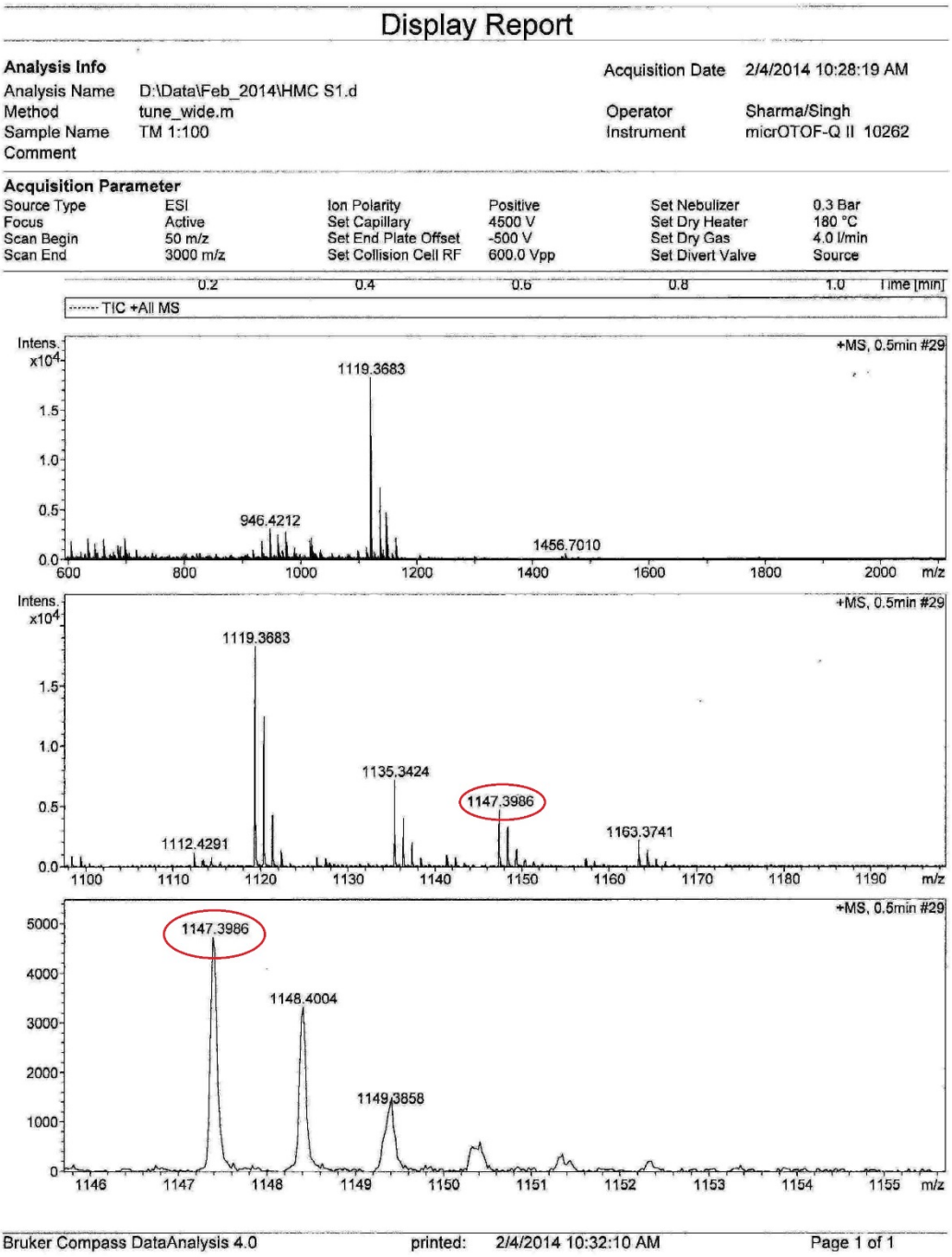


**Figure S4:** HRMS spectrum of **4**.

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*A new calix[4]arene based molecular probe for selective and sensitive detection of $CN^-$ ion in aqueous medium, H. M. Chawla, M.Shahid, D. Black and N. Kumar; New J. Chem.*

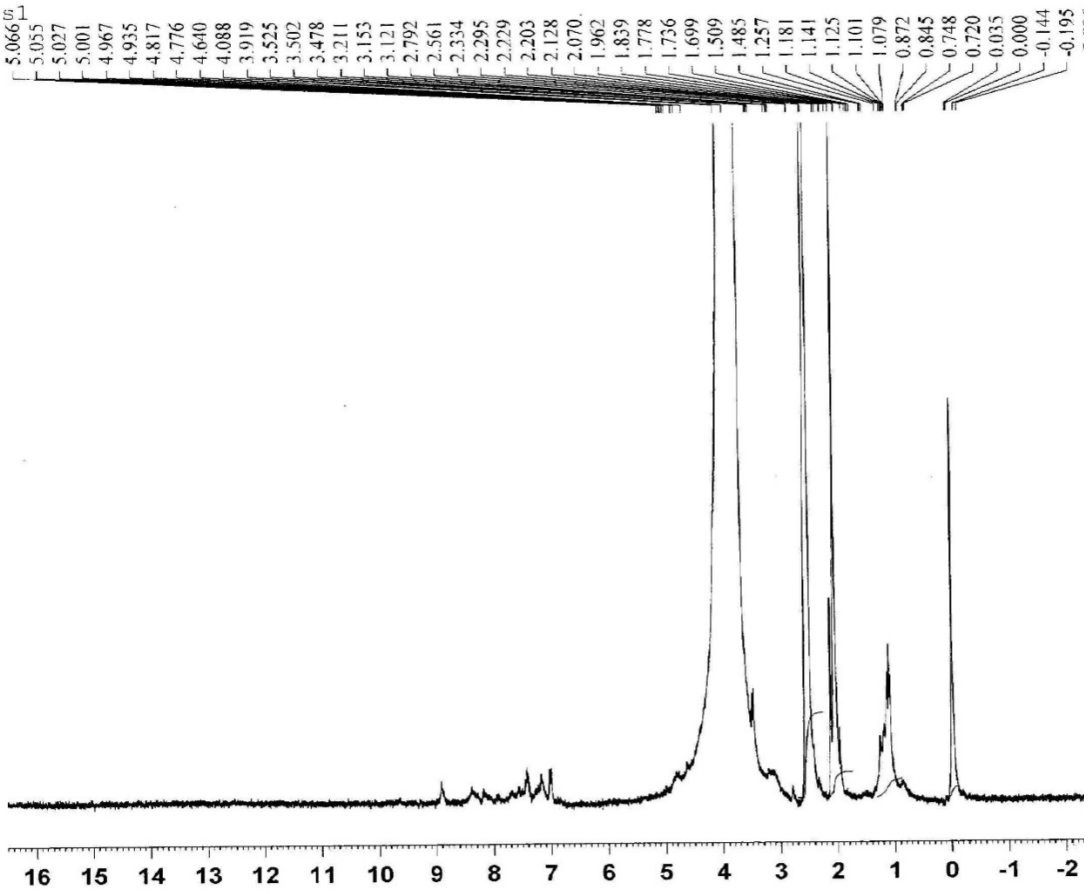



**Figure S5:** $D_2O$ exchanged $^1H$ NMR spectrum of **4**.

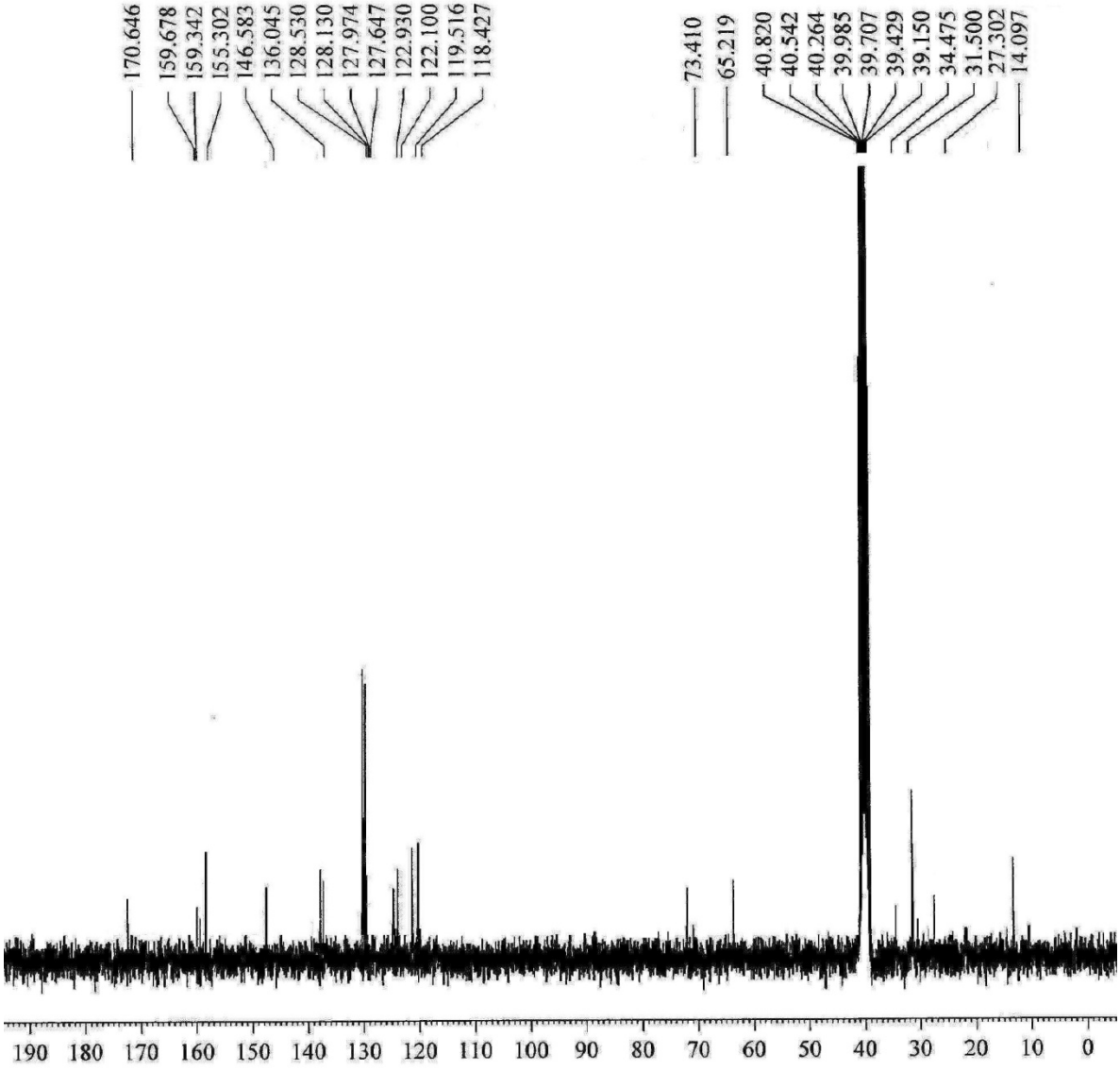



**Figure S6:** $^{13}$C NMR spectrum of **4** in DMSO-$d_6$.

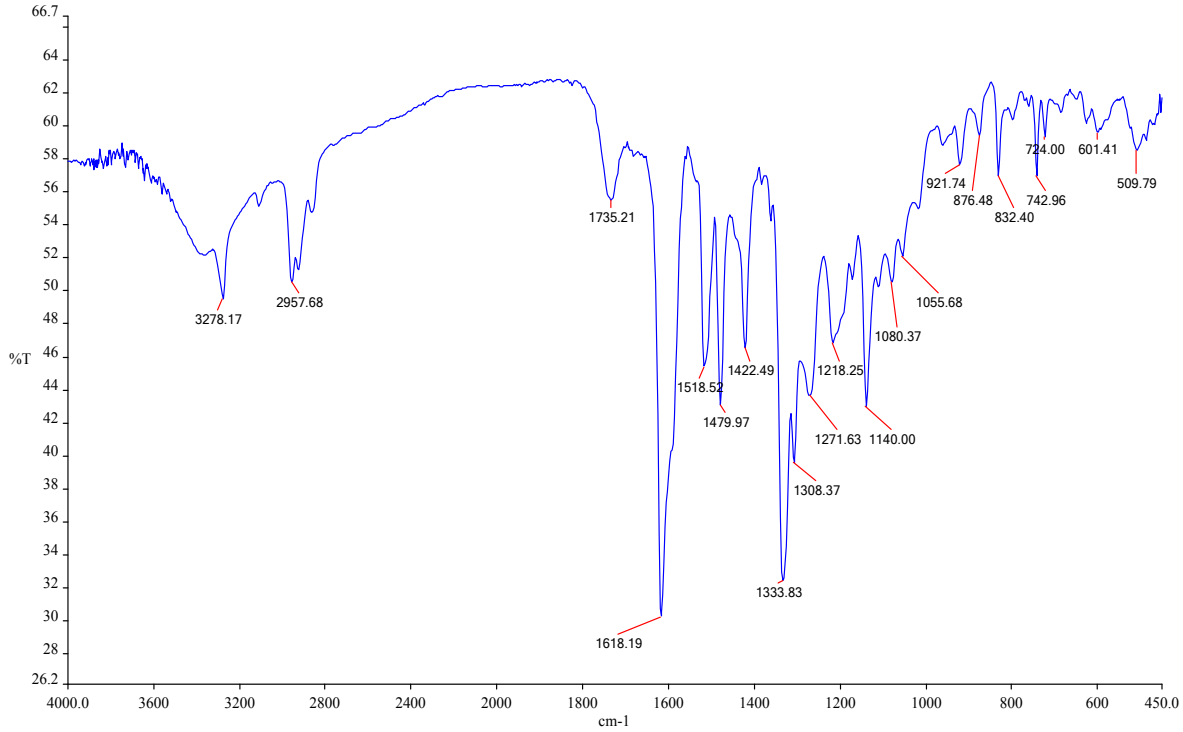



**Figure S7:** FT-IR spectrum of **4**.

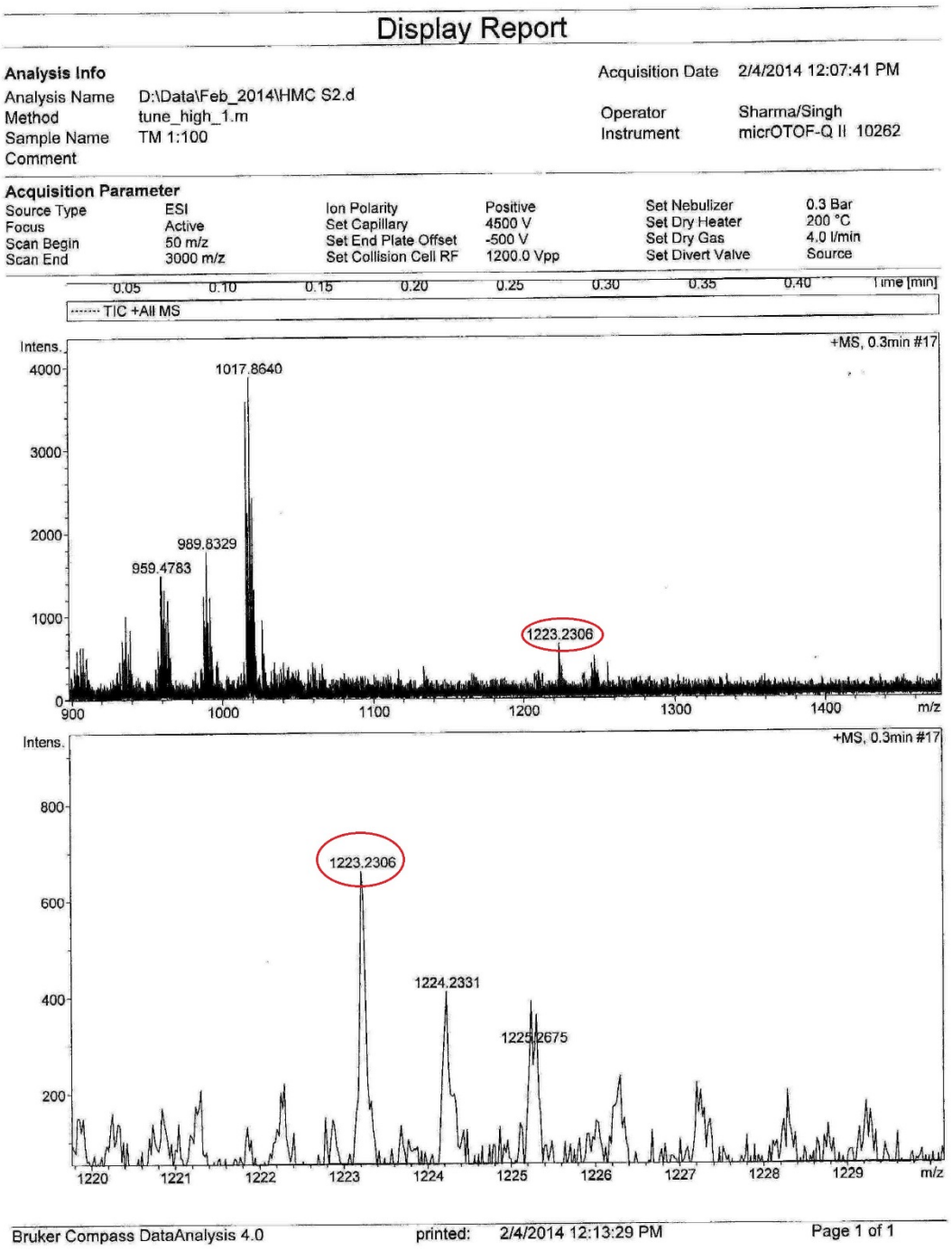



**Figure S8:** ESI-MS spectrum of **4-$CN^-$ adduct**.

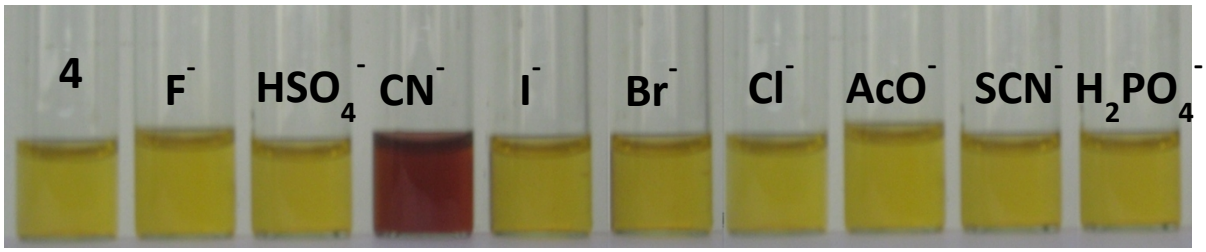



**Figure S9:** Change in color of **4** upon interaction with different anions in aqueous-MeCN (50%, v/v).

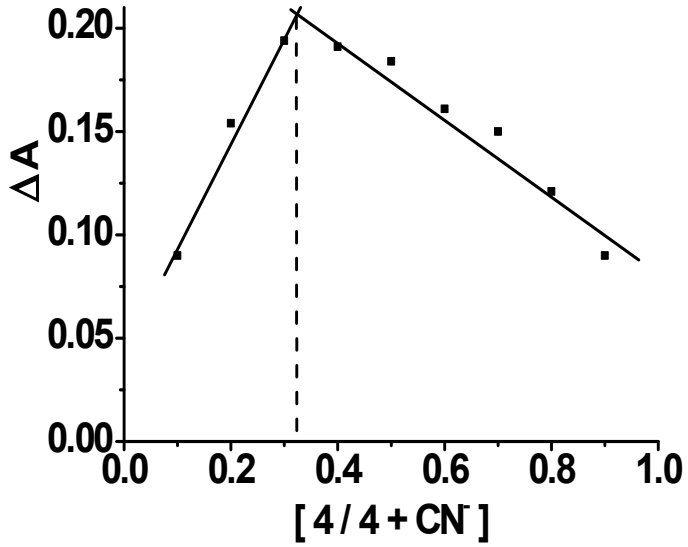
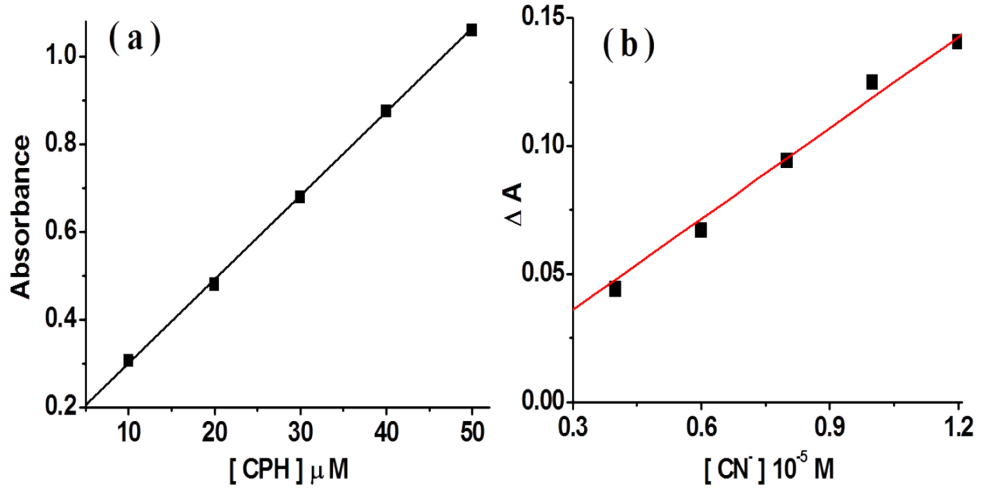


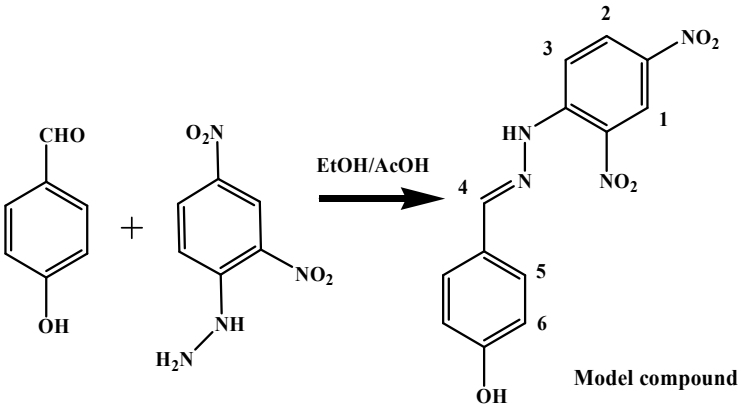

**Figure S10:** Job's plot of **4** with $CN^-$ ions.




**FigureS11:** (a) Calibration curve for **4** and (b) calibration sensitivity of $CN^-$ ion in aqueous-MeCN (50%, v/v).

### 1.7 Studies on interaction of different anions with phenoic hydrazone model compound:

The suggested model compound has been synthesized by using a method similar to the one used for the synthesis of **4.** Itssensing ability was analyzed in aqueous-MeCN solution (1:1, MeCN:$H_2O$). It was determined that the model compound exhibited an absorption maxima at 398 nm. Upon interaction with different anions, the 398 nm band disappeared with the generation of new band at 431 nm and two isosbestic points at 347 and 417 nm. Job's plot analysis revealed its stoichiometery as 1:1 with an association constant, *K*assoc = 1.15 x $10^3$ $M^{-1}$(Figure S12). This$K_{assoc}$ is very less for model compound as compare to that obtained for **4.** The color change is also not very prominent in the model compound in comparision to **4.** Thus**4** is unambigously a more efficient molecular receptor for cyanide than the synthesized Model compound.




**Scheme S1:** Synthesis of phenolic hydazone model compound.

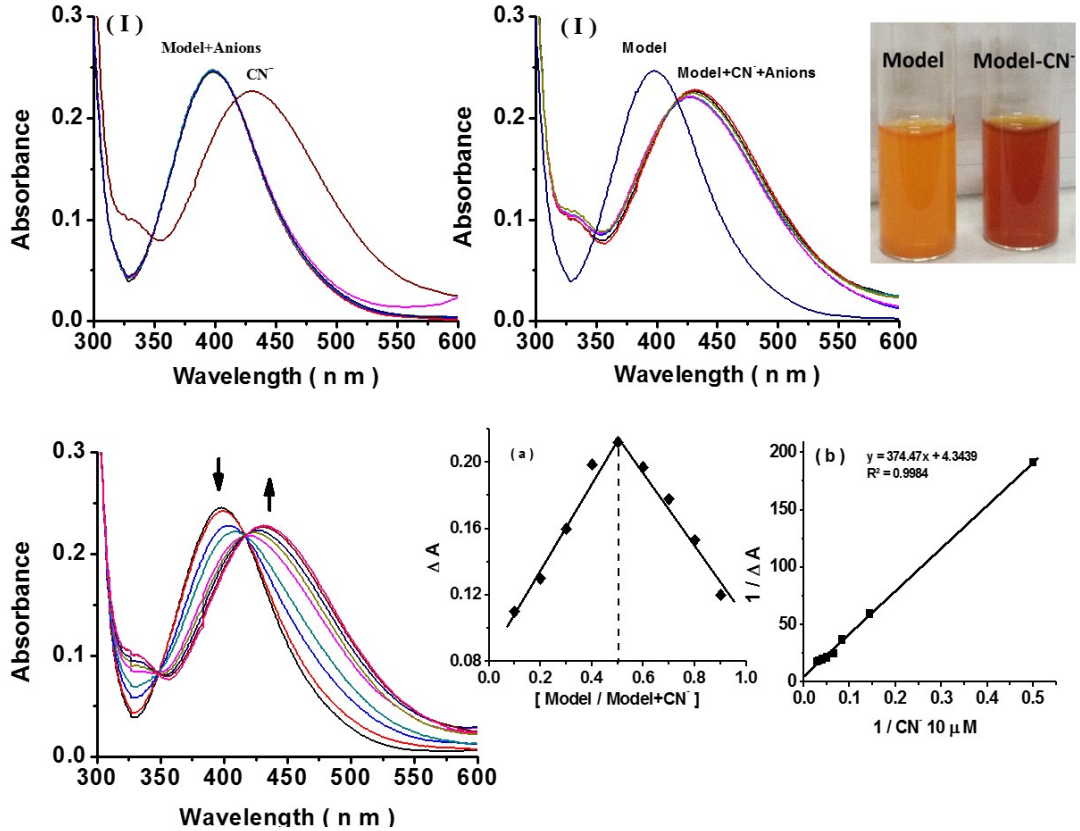



**Figure S12:**(I) Change in the absorption spectra of phenolic hydrazone model compound (10 μM) on addition of various anions (50 equiv) in aqueous-MeCN (50%, v/v) and (II) Studies on interference of different ions when interacted with the model compound and change in color of the solution. Absorption titration spectrum of model compound. Inset shows (a) Job's plot, (b) B-H plot.

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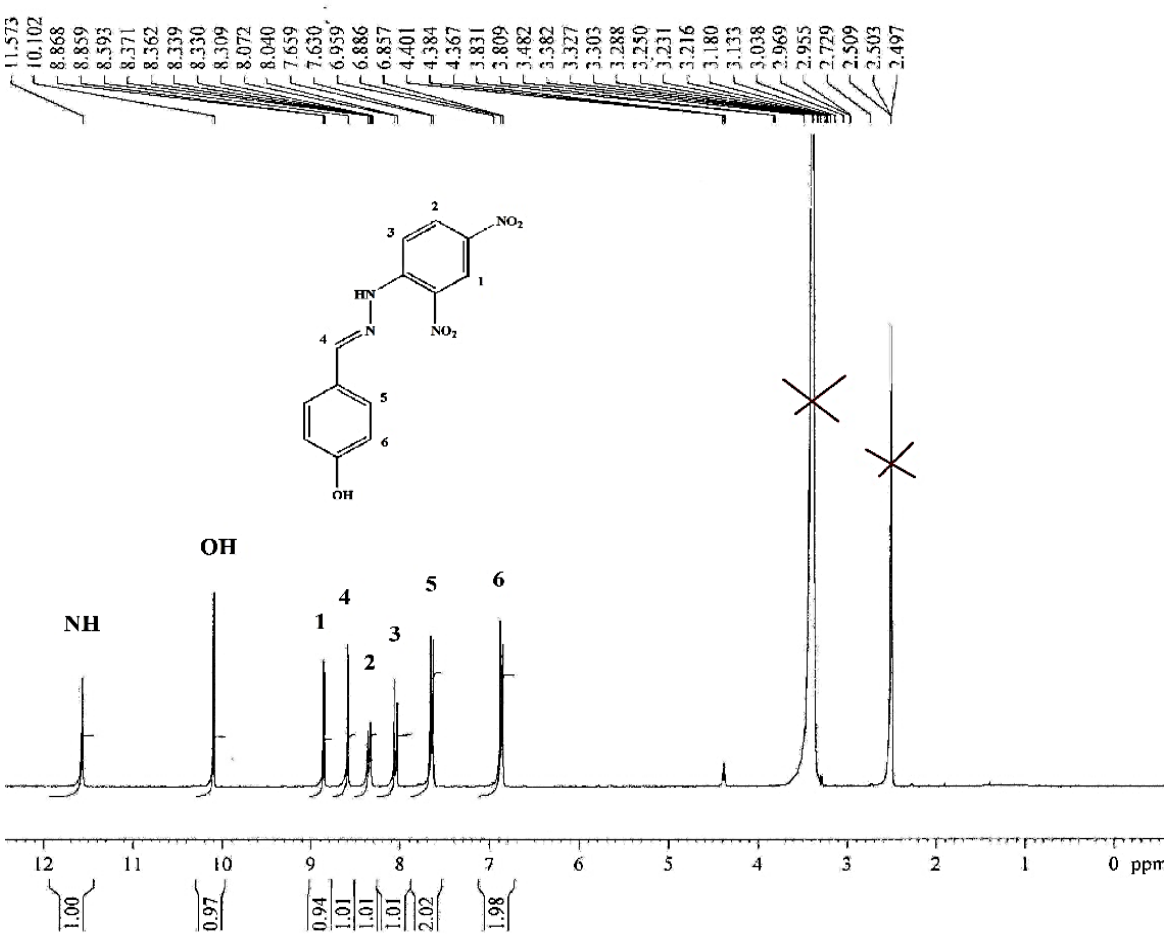


**Figure S13:**$^1$H NMR spectrum of **Model** compound in DMSO-$d_6$.

### 1.8 Studies on Interaction of 4 with different ions in MeCN:

It is note worthy that **4** (10 μM) in MeCN solution could detect both $F^-$ and $CN^-$ ions with the change of color of solution from light orange to violet and red-wine respectively (Figure S14). The extent of binding efficiency could be evaluated by titration experiments with $F^-$it showed the disappearence of 398 nm band with the development of a new band at 484 nm. Association constant for 1:2 stoichiometry of **4** and $F^-$ revealed a $K_{assoc}$ as 5.52 x $10^9$ $M^{-2}$ (Figure S15). This differential behavior of **4** in aqueous and MeCN can be attributed to differential labilities of *NH* proton in solution.

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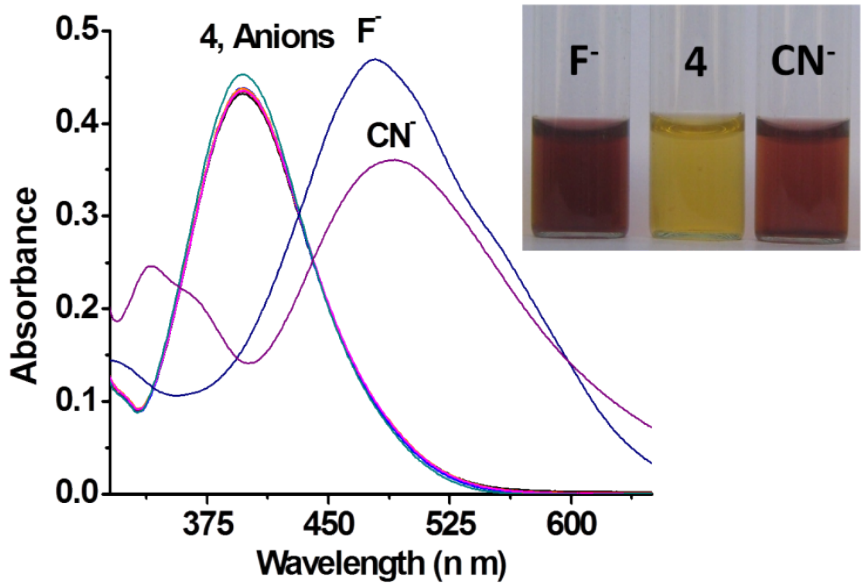
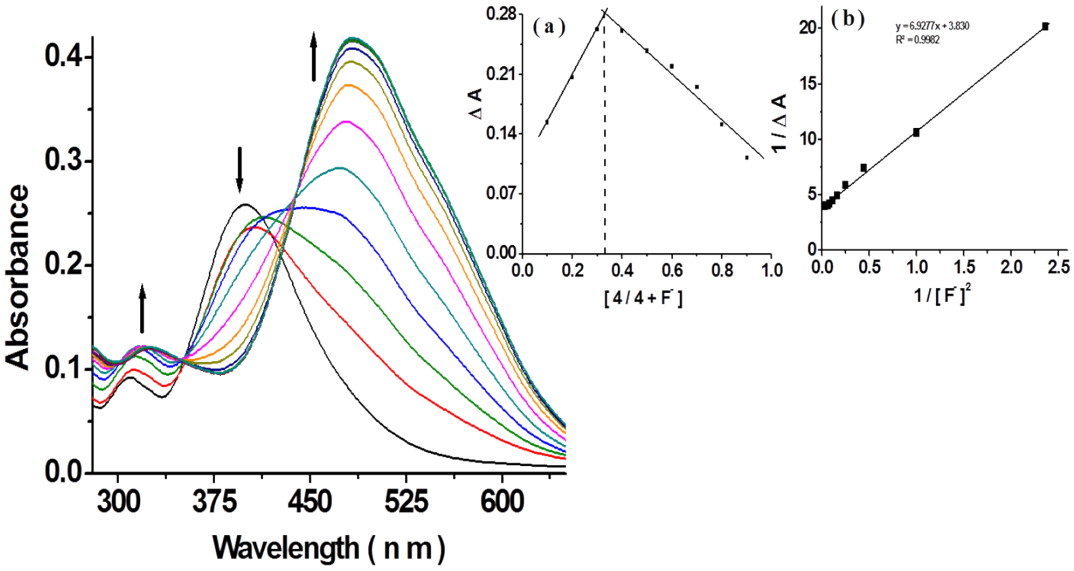



**Figure S14:**Change in absorption spectra of **4** (10 μM) upon addition of various anions (5 equiv) in MeCN. Inset shows the change in color of solution upon addition of $F^-$ and $CN^-$ ions.




**FigureS15:** Absorption titration spectrum of **4** (10 μM) upon addition of $F^-$ ions (0 – 5 equiv) in MeCN. Inset: (a) Jobs Plot and (b) B-H plot of **4** for $F^-$.

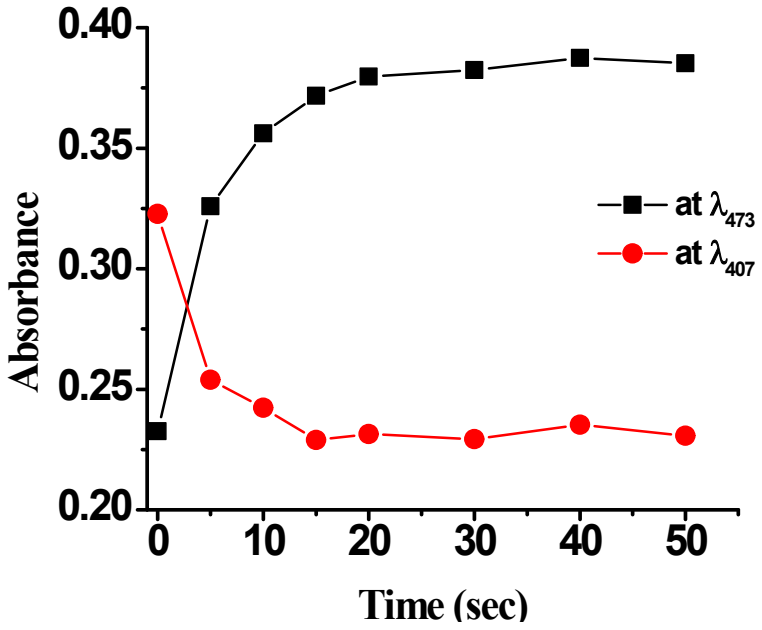



**Figure S16:**Response time curve evalauted through UV-Vis absorption response of **4** (10 μM) upon addition of 2 equiv of $CN^-$ in aqueous-MeCN (50%, v/v).

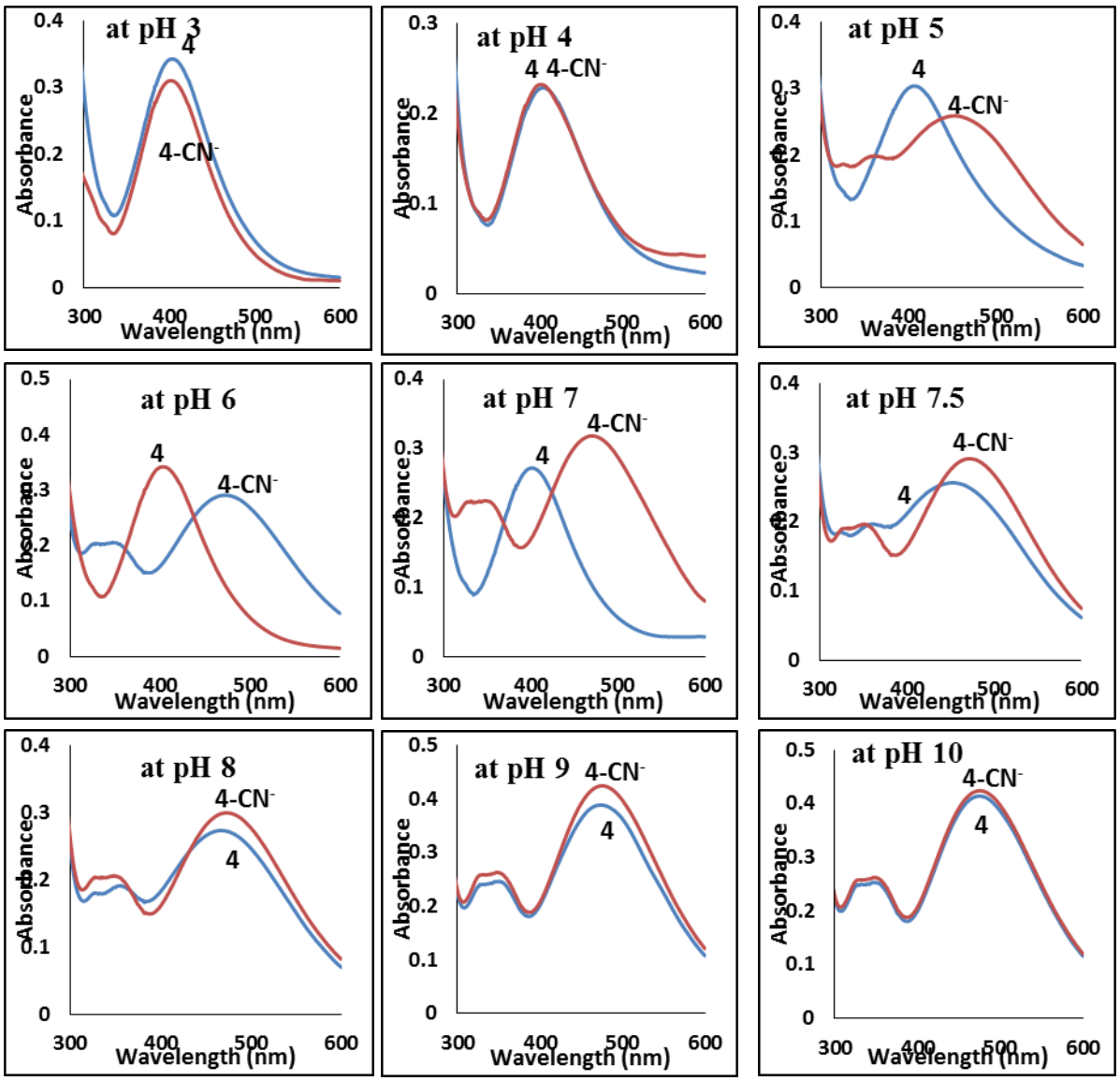
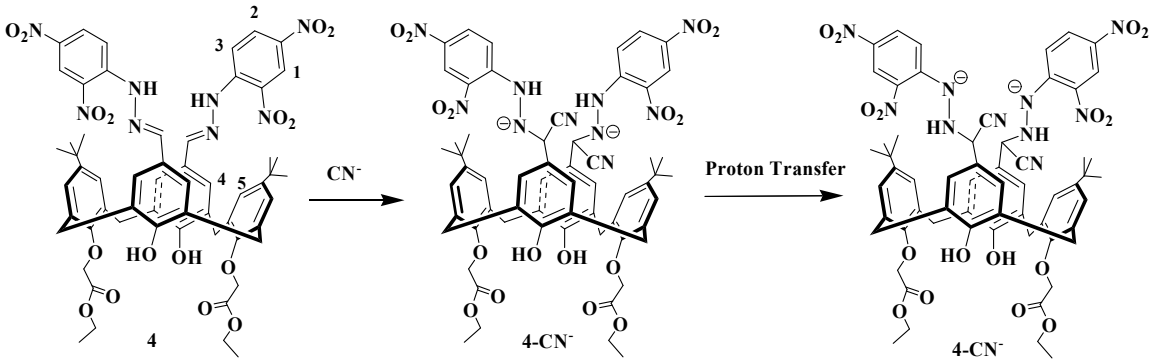



**Figure S17:**Studies on interaction of **4** with cyanide at different pHs.




**Scheme S2:** Plausible mechanism of sensing $CN^-$ by **4**.

**Table:**Change in chemical shifts in**4** upon interaction with $CN^-$.

| ***Protons*** | **$CN^-$ (0.0 equiv) $\delta$ ppm** | **$CN^-$ (0.5 equiv) $\delta$ ppm** | **$CN^-$ (2.0 equiv) $\delta$ ppm** |
|---|---|---|---|
| ***NH*** | 11.59 | 11.43 | ---- |
| ***New signal (NH')*** | ---- | 8.91 | 8.92 |
| ***H1*** | 8.90 | 8.81 | 8.71 |
| ***OH*** | 8.81 | 8.35 | 8.12 |
| ***-HC=N-*** | 8.47 | 8.41 | ---- |
| ***H2*** | 8.31 | 8.16 | 8.04 |
| ***H3*** | 8.12 | 7.97 | 7.80 |
| ***H4*** | 7.58 | 7.26 | 7.01 |
| ***H5*** | 7.26 | 7.26 | 7.22 |
| ***$COCH_2$*** | 4.89 | 4.68 | 4.40 |
| ***HV'*** | ---- | ---- | 4.40 |
| ***$CH_2$*** | 4.53 | 4.68 | 4.78 |

**Reference:**

1. H. A. Benesi and J.H. Hildebrand, *J. Am. Chem. Soc*., 1949, **71,** 2703.