Synthesis, Characterization, Evaluation & Complexation of sensors containing hydrazones

A portion of this chapter has been published as given below:
Hydrazones\textsuperscript{1} symbolize a group of compounds that has importance in organic synthesis,\textsuperscript{2} medicinal chemistry\textsuperscript{3} and supramolecular chemistry.\textsuperscript{4} The azomethine group (C=N-N) of hydrazones has been used in various fields due to its diverse nature. The latter is attributed to the nucleophilic imine nitrogen; the imine carbon which has electrophilic as well as nucleophilic character; the amine group which is reactive as well as acidic in many cases and the possibility of configurational isomerism.\textsuperscript{1} The first three characteristics of the hydrazones have been exploited in forming highly selective and sensitive cation/anion sensors, as reviewed in chapter I. For e.g. both monopodal (21) and triphenylamine based tripodal hydrazone (22) containing probes have been used for fluorogenic sensing of Cu\textsuperscript{2+} with high selectivity even at 1\(\mu\)M level in CH\textsubscript{3}CN. Tweezer-type hydrazone 87 has been developed as a “turn-on” Zn\textsuperscript{2+} sensor, by taking advantage of the restriction of the C=N bond rotation. As for anion sensing, the imine C of hydrazones is highly susceptible to the nucleophilic attack by CN\textsuperscript{−} ion making them good candidates for effective chemodosimetric probes for CN\textsuperscript{−} ions, as seen for the monopodal designs 150, 151, 153, 154. For F\textsuperscript{−} ion sensing, the acidic character of NH proton of hydrazones facilitates anion-sensor interactions, especially in the presence of an electron withdrawing −NO\textsubscript{2} group which further increases its acidity (for e.g. 149, 152, 155-158, chapter I). The addition of F\textsuperscript{−} ions causes charge transfer transitions between the anion-H-bonded NH/OH units and the electron-deficient NO\textsubscript{2} moiety, leading to optical responses.

From literature’s point of view the reports on use of hydrazone moiety as sensors, are rather scarce and the reported designs are mostly monopodal with one hydrazone unit in it. Also there is no example reported so far which is based on a mesitylene anchor. Hence, presently following two types of hydrazone group based, dipodal sensors are presented (Scheme 2.1)

(i) Benzene based, isomeric hydrazone Schiff bases (1), (2) and (3) with hydroxyl groups as binding and signaling unit.

(ii) Mesitylene based, isomeric hydrazone Schiff bases (5), (7) and (9) with nitro groups as signalling unit.

In the dipodal (1-3) sensors two imine groups along with two hydroxyl groups create a pre-organized chelating environment for Al(III) ions and turn out to be highly selective and sensitive fluorogenic sensors for it. On the other hand, highly acidic,
mesitylene based designs (5, 7, 9) proved to be very selective for highly basic CN$^-$ ions in aqueous medium. Designs (1-3) are very similar to the already reported design (159, chapter 1) which responds to CH$_3$CO$_2^-$, H$_2$PO$_4^-$ and F$^-$ ions equally well in organic and aqueous media and is not selective.

The compounds have been prepared by Schiff base condensation reactions as per the scheme 2.1. The characterization of these molecules has been done by $^1$H, $^{13}$C, IR, ESI/MS and X-ray analysis. The recognition/sensing behaviour of benzene based sensors for cations has been evaluated by using naked-eye, UV-vis, fluorescence and NMR spectral techniques in solution at 25 0°C. Their practical applications have been checked through “dip-stick” experiments and through bio-imaging in cells. Mesitylene based sensors showed selective sensing behaviour for CN$^-$ ion in aqueous medium and their sensing properties have been evaluated by naked-eye, UV-vis and NMR spectral techniques in solution at 25 0°C.

2.1 Reaction Methodologies used for the synthesis of compounds/sensors
Scheme 2.1
2.2 Experimental

General information
All the commercially available chemicals were purchased from Aldrich and used without further purification. The solvents used in the reactions/titrations were dried by standard methods. TLC was performed on glass sheets pre-coated with silica gel. The elemental analyses (C, H and N) were performed on a Perkin–Elmer model 2400 CHN analyzer and HRMS spectra wherever reported, were recorded on a Bruker’s microTOF-QII spectrophotometer. The $^1$H and $^{13}$C NMR spectra were carried out in DMSO-$d_6$ with TMS as an internal reference, on a JEOL-FT NMR-300 MHz spectrophotometer and/or Bruker Topspin-FT NMR-500 MHz. The infrared spectra (KBr pellet) were recorded using Perkin-Elmer FT-IR C92035 spectrophotometer in the range 400–4000 cm$^{-1}$. The electronic absorption spectra were recorded on a Shimadzu Pharmaspec UV-1700 UV–vis spectrophotometer with a quartz cuvette (path length, 1cm). For consistency in the recordings, cell holder of the spectrophotometer was thermostatted at 25 °C. The fluorescence spectra were recorded on a Varian fluorospectrophotometer. HRMS spectra were recorded on a Bruker’s microTOF-QII spectrophotometer. Confocal microscopy imaging was performed on NIKON A1R confocal laser scanning microscope using diode laser excitation at 405 nm. Imaging was performed using Plan Apo 60X oil immersion objective lens.

X-ray measurement and structure determination
The crystals of compound (1) were grown by slow evaporation from a mixture of N,N-Dimethylformamide and ethanol and those of (4) and (8) were grown from chloroform. X-ray data were collected on a Bruker’s Apex-II CCD diffractometer using Mo Kα ($\lambda=0.71069\text{Å}$) at room temperature. The data were corrected for Lorentz and polarization effects and empirical absorption corrections were applied using SADABS from Bruker. The important crystal and refinement parameters are given in Table 2.1. The structures was solved by direct methods using SIR-92$^{5a}$ and refined by full-matrix least squares refinement methods based on $F^2$, using SHELX-97.$^{5b}$ The hydrogens of the -OH and –NH groups in (1) were located from the difference Fourier synthesis and were refined isotropically with Uiso values 1.2 times that of their carrier oxygen atoms, with restraints on the bond distances. All non-
hydrogen atoms in all three compounds were refined anisotropically. All other hydrogen atoms were fixed geometrically with their $U_{iso}$ values 1.2 times of the phenylene carbons. All calculations were performed using Wingx$^c$ package.

**UV-vis and Fluorescence studies for cation recognition**

Molecular interaction of (1), (2) and (3) with 19 different metal nitrates under study were investigated using UV–vis spectroscopy at $10^{-5}$ M and Fluorescence spectroscopy at $2.5 \times 10^{-6}$ M in HEPES buffer (pH 7.4, containing 30% DMSO as a co-solvent). Stock solutions of (1), (2) and (3) ($10^{-3}$M) and of metal nitrates ($10^{-1} - 10^{-3}$ M) were prepared in DMSO and distilled water respectively. Selectivity tests were performed by $2.5 \times 10^{-6}$ M of all the sensors and 10 equivalents of Al(III) in the presence of 50 equivalents of other interfering metal ions. The binding stoichiometry of (1-3) - Al(III) complexes were determined by the method of continuous variation (Job’s plot). Ten solutions were made by varying L/M ratio and keeping the total concentration of all the sensors and cationic guest constant ($2.5 \times 10^{-5}$ M) with continuous variation of mole fraction of Al(III). The results indicate the formation of complexes with stoichiometric ratio of 1:1. Stability constant was determined by using Benesi-Hildebrand plot in each case. Fluorescence quantum yield, $\Phi_f$ of (1), (2) and (3) was determined at room temperature by using optically matching solutions of 9,10-diphenylanthracene ($\Phi_f = 0.90$) in ethanol as the standard at an excitation wavelength of 325, 340 and 358 nm respectively. The quantum yield was calculated by using eqn. (1), in which $\Phi_{fs}$ is the radiative quantum yield of the sample, $\Phi_{fr}$ is the radiative quantum yield of reference, $A_s$ and $A_r$ symbolize the absorbance of the sample and the reference, respectively, $D_s$ and $D_r$ symbolize areas of emission for the sample and reference, $L_s$ and $L_r$ denote the lengths of the absorption cells,

$$\Phi_{fs} = \Phi_{fr} \times \frac{1 - 10^{-Ays/Ls}}{1 - 10^{-Ar/Lr}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r} \quad \ldots 1$$

$N_s$ and $N_r$ symbolize the refractive indices of the sample and reference solutions (pure solvents were assumed).

**UV-vis studies for anion recognition**

Molecular interaction of (5), (7) and (9) with various anionic species under study were investigated using UV–vis spectroscopy at $10^{-5}$ M in DMSO and in HEPES buffer (pH 7.4, containing 25% DMSO as a co-solvent). Stock solutions of (5), (7) and (9) ($10^{-3}$M) and of tetrabutylammonium anions ($10^{-1} - 10^{-3}$ M) were prepared in DMSO. Stock
solutions of sodium salts of anions for aqueous medium studies (10^{-1} - 10^{-3} \text{ M}) were prepared in double distilled water. Selectivity tests were performed by 10^{-5} \text{ M} of all these sensors and 10 equivalents of sodium cyanide in the presence of 100 equivalents of other interfering anions. Stoichiometry of the interaction of (5), (7) and (9) with CN^- ions was determined by Job’s plot and binding constants by using Benesi-Hildebrand plot.

**Cell Imaging Studies**
Both the tested cell lines (HeLa and C6 glioma) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml penicillin, 100 µg/ml streptomycin and 100 µg/ml gentamycin. The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. The day before treatment, a total of 2 × 10^5 cells were seeded on 11 mm glass coverslips into each well of a 24-well plate and these were grown for 24 hours (till 60-70% confluence) and treatment was carried out in triplicates in FBS and antibiotic free media. HeLa and C6 glioma cells were incubated with three different ligands i.e., (1), (2) and (3) (10µM) (each in triplicates) at 37 °C with 5% CO_2 for 30 min. followed by three times wash with 1X phosphate buffered saline (PBS) (pH = 7.4) and the treatment with Al(III) (10 µM and 50 µM conc. each in three replicates) for another 30 min. by incubating the cells at same conditions. The cells were then washed three times with 1X PBS, fixed in ice cold 4% paraformaldehyde, washed again three times with 1X PBS and mounted on glass slides. To investigate the cytotoxicity of (1), (2) and (3), MTT [3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide] assay with HeLa cell lines as well as C6 glioma cells was performed to determine the effect of (1), (2) and (3) on the cell proliferation.

### 2.3 Synthesis and Characterization of compounds/sensors

**Synthesis of compounds/sensors**
The compounds (1), (2) and (3) were produced by Schiff base condensation reaction of isopthalohydrazide with 2,3-/2,4-/2,5-dihydroxybenzaldehyde in ethanol at room temperature in the presence of a catalytic amount of Zn(ClO_4)_2. The compounds (4), (6) and (8) were synthesized by the reaction of 2,4-bis(bromomethyl)-1,3,5-trimethylbenzene with 2-/3-/4-hydroxybenzaldehyde under nitrogen in dry acetonitrile. The compound (8) was already reported in the literature, but in the
present work, this compound was prepared in a modified manner (vide infra). Further, these compounds (4), (6) and (8) were used for Schiff base condensation reaction with 2,4-dinitrophenylhydrazine in acetonitrile to construct compounds (5), (7) and (9) respectively.

Characterization of compounds/sensors

All the compounds were characterized by various spectroscopic techniques such as $^1$H NMR, $^{13}$C NMR, IR, elemental analyses, ESI/MS (Figure 2.8 - Figure 2.25) and single crystal X-ray diffraction (in some cases). In the case of compound (9), due to poor solubility $^{13}$C NMR could not be taken. The elemental analysis (CHN data) and mass spectra were in accordance with the molecular formulae.

**Compound (1).** 200 mg (1.02 mmol) of isopthalohydrazide was dissolved in 10 ml of ethanol, to which was added 284 mg (2.05 mmol) of 2,3-dihydroxybenzaldehyde in 10 ml of ethanol along with 2-3 mg of zinc perchlorate. The color of the solution changed immediately to turbid yellow and precipitates separated out within ten minutes. These precipitates were filtered, washed with methanol and dried under vacuum for 24 hours to get light yellow solid. Yield 90%; mp = 238-240 °C; $^1$H NMR (300 MHz, DMSO-d$_6$, $\delta$): 6.78 (t, 2H, Ar, J = 7.8 Hz), 6.89 (d, 2H, Ar, J = 6.6 Hz), 7.03 (d, 2H, Ar, J = 6.6 Hz), 7.76 (t, 1H, Ar, J = 7.5 Hz), 8.19 (d, 2H, Ar, J = 7.8 Hz), 8.56 (s, 1H, Ar), 8.67 (s, 2H, -CH=N), 9.31 (s, 2H, -OH); 11.09 (s, 2H, -OH); 12.32 (s, 2H, -NH); $^{13}$C NMR (75 MHz, DMSO-d$_6$, $\delta$): 117.8 (Ar), 119.2 (Ar), 119.6 (Ar), 120.3 (Ar), 127.4 (Ar), 129.4 (Ar), 131.4 (Ar), 133.6 (Ar), 146.0 (CH=N), 146.5 (-C-OH), 149.6 (-C-OH), 162.6 (-C=O); FTIR (KBr, cm$^{-1}$): 3299, 3090, 1647, 1609; Elemental analysis calculated for C$_{22}$H$_{18}$N$_4$O$_6$: C, 60.83; H, 4.18; N, 12.90%. Found: C, 61.34; H, 4.20; N, 11.32%; HRMS m/z: 435.1284 [M+1]$^+$ ion (calc. 435.1299).

**Compound (2).** Same procedure as for (1) except that 2,4-dihydroxybenzaldehyde was used in place of 2,3-dihydroxybenzaldehyde. The color of the solution changed immediately to light orange and precipitates separated out within half an hour. These precipitates were filtered, washed with methanol and dried under vacuum for 24 hours to get yellowish brown solid. Yield 80%; mp = 298-300 °C; $^1$H NMR (300 MHz, DMSO-d$_6$, $\delta$): 6.33 (s, 2H, Ar), 6.36 (d, 2H, Ar, J = 8.4 Hz), 7.33 (d, 2H, Ar, J = 8.1
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75 Hz), 7.69 (t, 1H, Ar, J = 8.1 Hz), 8.12 (d, 2H, Ar, J = 7.8 Hz), 8.47 (s, 1H, Ar), 8.54 (s, 2H, -CH=N), 9.95 (s, 2H, -OH), 11.40 (s, 2H, -OH), 12.05 (s, 2H, -NH); \(^{13}\text{C NMR} (75 \text{ MHz, DMSO-d}_6, \delta): 102.6 (\text{Ar}), 107.7 (\text{Ar}), 110.5 (\text{Ar}), 126.7 (\text{Ar}), 128.8 (\text{Ar}), 130.6 (\text{Ar}), 131.3 (\text{Ar}), 133.3 (\text{Ar}), 149.5 (\text{CH=N}), 159.4 (-\text{C-OH}), 160.8 (-\text{C-OH}) 161.9 (-\text{C=O}); \text{FTIR (KBr, cm}^{-1}): 3349, 3128, 1660, 1607; \text{Elemental analysis calculated for C}_{22}\text{H}_{18}\text{N}_4\text{O}_6: C, 60.83; H, 4.18; N, 12.90%. Found: C, 60.86; H, 4.16; N, 12.87%; \text{HRMS m/z: 435.1284 [M+1]^+ (calc. 435.1299).}

\textbf{Compound (3).} Same procedure as for (1) except that 2,5-dihydroxybenzaldehyde was used in place of 2,3-dihydroxybenzaldehyde. The color of the solution changed immediately to brownish orange and precipitates separated out within an hour. These precipitates were filtered, washed with methanol and dried under vacuum for 24 hours to get reddish brown solid. Yield 50%; mp = 300-302 °C; \(^1\text{H NMR} (300 \text{ MHz, DMSO-d}_6, \delta): 6.75 (4H, s, Ar), 7.01 (2H, s, Ar), 7.71 (1H, t, Ar, J = 7.8 Hz), 8.13 (2H, d, Ar, J = 8.1 Hz), 8.50 (1H, s, Ar), 8.61 (2H, s, -CH=N), 8.96 (2H, s, -OH), 10.31 (2H, s, -OH), 12.13 (2H, s, -NH); \(^{13}\text{C NMR} (75 \text{ MHz, DMSO-d}_6, \delta): 113.6 (\text{Ar}), 117.2 (\text{Ar}), 119.1 (\text{Ar}), 127.0 (\text{Ar}), 128.9 (\text{Ar}), 130.9 (\text{Ar}), 133.4 (\text{Ar}), 147.8 (\text{CH=N}), 149.9 (-\text{C-OH}), 150.3 (-\text{C-OH}), 162.3 (-\text{C=O}); \text{IR (KBr, cm}^{-1}): 3385, 3204, 1625, 1580; \text{Elemental analysis calculated for C}_{22}\text{H}_{18}\text{N}_4\text{O}_6: C, 60.83; H, 4.18; N, 12.90%. Found: C, 60.38; H, 4.19; N, 12.11%; \text{HRMS m/z: 435.1348 [M+1]^+ (calc. 435.1299).}

\textbf{Compound (4).} Dipodal aldehyde 4 was prepared by taking 1g of K\(_2\text{CO}_3\) in dry acetonitrile alongwith 244 mg of 2-hydroxybenzaldehyde (2 mmol). The reaction mixture was refluxed for 30 minutes and then 306 mg of 2,4-bis(bromomethyl)-1,3,5-trimethylbenzene (1 mmol) was added slowly. The reaction mixture was refluxed for another 24 hours and the progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was filtered and the filtrate was concentrated. Again filtered off the precipitate, washed with methanol and dried. The crude product was recrystallized from CHCl\(_3\)-CH\(_3\)OH mixture to get pure white material. Yield 77%; mp = 122-124 °C; \(^1\text{H NMR} (300 \text{ MHz, DMSO-d}_6, \delta): 2.67 (s, 9H, -CH\(_3\)), 5.38 (s, 4H, -CH\(_2\)), 7.12 (s,
1H, -Ar), 7.29 (t, 2H, -Ar, J = 7.1 Hz), 7.67 (d, 2H, -Ar, J = 4.8 Hz), 7.88 (d, 4H, -Ar, J = 7.2 Hz), 10.37 (s, 2H, -CHO); \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\), δ): 15.3 (-CH\(_3\)), 19.5 (-CH\(_3\)), 66.7 (-CH\(_2\)), 114.2 (-Ar), 121.1 (-Ar), 124.6 (-Ar), 127.8 (-Ar), 130.2 (-Ar), 130.7 (-Ar), 136.6 (-Ar), 138.6 (-Ar), 138.9 (-Ar), 161.3 (-Ar), 189.2 (-CHO); FTIR (KBr, cm\(^{-1}\)) 1701, 1600, 1461, 1213, 982, 761; 13C NMR (75 MHz, DMSO-d\(_6\), δ): 19.3 (-CH\(_3\)), 15.2 (-CH\(_3\)), 66.2 (-CH\(_2\)), 113.1 (-Ar), 116.7 (-Ar), 120.9 (-Ar), 122.3 (-Ar), 122.7 (-Ar), 129.1 (-Ar), 129.3 (-Ar), 129.4 (-Ar), 130.0 (-Ar), 130.9 (-Ar), 131.9 (-Ar), 136.5 (-Ar), 138.5 (-Ar), 138.7 (-Ar), 144.4 (CH=NO), 145.0 (-Ar), 157.6 (-Ar); FTIR (KBr, cm\(^{-1}\)) 3293, 1622, 1510, 1337, 1143; Elemental analysis calculated for C\(_{25}\)H\(_{24}\)O\(_4\): C, 77.30; H, 6.23 Found: C, 77.01; H, 6.20%. HRMS m/z: 411.1447 [M+Na]\(^+\) ion (calc. 411.1572).

**Compound (5).** 0.20 g (0.515 mmol) of compound 4 was dissolved in 10 ml dry acetonitrile, to which a solution of 0.204 g (1.03 mmol) of 2,4-dinitrophenylhydrazine in 90 ml of acetonitrile was added. The color of the solution changed immediately to dark orange and precipitates separated out in quantitative yield. These precipitates were filtered, washed with methanol and dried. The crude product obtained was recrystallized from CHCl\(_3\)-CH\(_3\)OH mixture to get orange color solid. Yield 90%. mp = 140 - 142°C; \(^1\)H NMR (300 MHz, DMSO-d\(_6\), δ): 2.37 (s, 6H, -CH\(_3\)), 2.45 (s, 3H, -CH\(_3\)), 5.22 (s, 4H, -CH\(_2\)), 7.07 (d, 2H, -Ar, J = 5.1), 7.11 (s, 1H, -Ar), 7.41 (d, 2H, -Ar, J = 7.2), 7.50 (t, 2H, -Ar, J = 6.7), 7.90 (m, 4H, -Ar), 8.20 (d, 2H, -Ar, J = 9.3), 8.73 (s, 2H, -CH=N, 2H, -Ar), 11.66 (s, 2H, -NH); \(^{13}\)C NMR (75 MHz, DMSO-d\(_6\), δ): 19.3 (-CH\(_3\)), 15.2 (-CH\(_3\)), 66.2 (-CH\(_2\)), 113.1 (-Ar), 116.7 (-Ar), 120.9 (-Ar), 122.3 (-Ar), 122.7 (-Ar), 129.1 (-Ar), 129.3 (-Ar), 129.4 (-Ar), 130.0 (-Ar), 130.9 (-Ar), 131.9 (-Ar), 136.5 (-Ar), 138.5 (-Ar), 138.7 (-Ar), 144.4 (CH=NO), 145.0 (-Ar), 157.6 (-Ar); FTIR (KBr, cm\(^{-1}\)) 3293, 1622, 1510, 1337, 1143; Elemental analysis calculated for C\(_{37}\)H\(_{32}\)N\(_8\)O\(_{10}\): C, 59.36; H, 4.31; N, 14.97%. Found: C, 59.14; H, 4.02; N, 14.92%; HRMS m/z: 771.2124 [M+Na]\(^+\) ion (calc. 771.2139).

**Compound (6).** Same procedure was used as for 4 except that 2mmol of 3-hydroxybenzaldehyde was used instead of 2-hydroxybenzaldehyde. Yield 85%; mp = 194-200 °C; \(^1\)H NMR (300 MHz, DMSO-d\(_6\), δ):
2.33 (s, 9H, -CH₃), 5.15 (s, 4H, -CH₂), 7.02 (s, 1H, -Ar), 7.39 (s, 2H, -Ar), 7.57 (d, 2H, -Ar, J = 10.8), 10.00 (s, 2H, -CHO); ¹³C NMR (75 MHz, DMSO-d₆, δ): 15.1 (-CH₃), 19.5 (-CH₃), 65.0 (-CH₂), 113.8 (-Ar), 121.7 (-Ar), 130.1 (-Ar), 130.6 (-Ar), 130.9 (-Ar), 138.3 (-Ar), 138.7 (-Ar), 193.3 (-CHO); FTIR (KBr, cm⁻¹) 1701, 1591, 1443, 1250; Elemental analysis calculated for C₂₅H₂₄O₄: C, 77.30; H, 6.23 Found: C, 77.12; H, 6.04%. HRMS m/z: 411.1532 [M+Na]⁺ ion (calc. 411.1572).

**Compound (7).** Same procedure as for 5 except that dipodal aldehyde used was compound 6 instead of compound 4. Yield 80%. Orange solid. mp = 240-242 °C. ¹H NMR (300 MHz, DMSO-d₆, δ): 2.37 (s, 6H, -CH₃), 2.38 (s, 3H, -CH₃), 5.16 (s, 4H, -CH₂), 7.04 (s, 2H, -Ar), 7.19 (d, 2H, -Ar, J = 8.1), 7.36 (d, 2H, -Ar, J = 7.2), 7.43 (d, 2H, -Ar, J = 7.8), 7.48 (s, 2H, -Ar), 8.13 (d, 2H, J = 9.6), 8.34 (d, 2H, -Ar, J = 9.6), 8.67 (s, 2H, -CH=N), 8.85 (s, 2H, -Ar), 11.67 (s, 2H, -NH); ¹³C NMR (75 MHz, DMSO-d₆, δ): 15.4 (-CH₃), 19.6 (-CH₃), 65.1 (-CH₂), 112.8 (-Ar), 114.1 (-Ar), 117.4 (-Ar), 122.0 (-Ar), 123.1 (-Ar), 130.0 (-Ar), 130.1 (-Ar), 130.3 (-Ar), 130.6 (-Ar), 131.4 (-Ar), 135.6 (-Ar), 137.6 (-Ar), 138.6 (-Ar), 139.0 (-Ar), 145.0 (CH=N), 149.5 (-Ar), 159.7 (-Ar); FTIR (KBr, cm⁻¹) 3277, 1610, 1583, 1518, 1417, 1251, 1140; Elemental analysis calculated for C₃₇H₃₂N₈O₁₀: C, 59.36; H, 4.31; N, 14.97%. Found: C, 59.20; H, 4.09; N, 14.86%. HRMS m/z: 771.2028 [M+Na]⁺ ion (calc. 771.2139).

**Compound (8).** Same procedure as for 4 except that 2mmol of 4-hydroxybenzaldehyde was used instead of 2-hydroxybenzaldehyde. Yield 56%; mp = 136-138 °C; ¹H NMR (300 MHz, DMSO-d₆, δ): 2.31 (s, 3H, -CH₃), 2.33 (s, 6H, -CH₃), 5.20 (s, 4H, -CH₂), 7.04 (s, 1H, -Ar), 7.26 (dd, 4H, -Ar, J = 8.55), 7.91 (dd, 4H, -Ar, J = 8.55), 9.89 (s, 2H, -CHO); ¹³C NMR (75 MHz, DMSO-d₆, δ): 15.1 (-CH₃), 19.5 (-CH₃), 66.2 (-CH₂), 115.2 (-Ar), 129.9 (-Ar), 130.1 (-Ar), 130.6 (-Ar), 132.0 (-Ar), 138.5 (-Ar), 138.7 (-Ar), 164.0 (-Ar), 191.5 (-CHO); FTIR (KBr, cm⁻¹) 1701, 1609, 1517, 1241; Elemental analysis calculated for C₂₅H₂₄O₄: C, 77.30; H, 6.23 Found: C, 77.26; H, 6.21%. HRMS m/z: 389.1738 [M+1]⁺ ion (calc. 389.1675).
**Compound (9).** Same procedure as for 5 except that dipodal aldehyde used was compound 8 instead of compound 4. Yield = 65%. mp = 160 - 162 °C. $^1$H NMR (300 MHz, DMSO-d$_6$, δ): 2.34 (s, 9H, -CH$_3$), 5.15 (s, 4H, -CH$_2$), 7.03 (s, 2H, -Ar), 7.18 (d, 4H, -Ar, J = 8.4), 7.78 (d, 4H, -Ar, J = 8.4), 8.09 (d, 2H, -Ar, J = 9.3), 8.36 (dd, 2H, -Ar, J = 9.3), 8.67 (s, 2H, -CH=N), 8.87 (s, 2H, -Ar), 11.62 (s, 2H, -NH); FTIR (KBr, cm$^{-1}$) 3295, 1618, 1498, 1425, 1332, 1241, 1151; Elemental analysis calculated for C$_{37}$H$_{32}$N$_8$O$_{10}$: C, 59.36; H, 4.31; N, 14.97%. Found: C, 59.30; H, 4.18; N, 14.88%. HRMS m/z: 749.2233 [M+1]$^+$ ion (calc. 749.2241).

**X-ray Crystal structure studies**

Table 2.1 shows the crystallographic data and refinement parameters for (1), (4) and (8). The solid state structure of (1) is shown in Figure 2.1.

**Figure 2.1.** ORTEP diagram of the (1) at 50% probability showing the labeling scheme. Inset: Showing dihedral angle between two arms of (1).

The two arms of (1) are twisted with respect to each and the central phenyl anchor, making dihedral angles of 16.9(1) and 31.4(1)$^\circ$ with the anchor, respectively and 46.5(1)$^\circ$ between each other and they lie on opposite sides of the central phenyl ring (Inset, Figure 2.1). There is strong intramolecular H-bonding (Figure 2.1) between the
imine nitrogens N2 and N4 and the ortho- hydroxyl groups (O2-H2A···N2 2.678(4) 
and O5 -H5A···N4 2.717(4) Å), respectively. The meta- hydroxyl group O3 at one 
end of the dipodal molecule is engaged in four intermolecular H-bonding interactions.
It accepts one bond from the imine nitrogen N3 and hydroxyl oxygen O5 whereas it 
donates two H-bonds to imine N4 and carbonyl O4. The latter three H-bonds help 
forming a zig-zag chain parallel to the b axis in a head to tail manner.

Figure 2.2. H-bonding interactions in (1) resulting in the formation of undulating 
tapes of molecules shown (a) down a axis (b) down the b axis.

The O6···O1 intermolecular H-bonding interactions between the hydroxyl group and 
the carbonyl oxygen (Table 2.2) on the other end help in the formation of another zig-
zag chain parallel to the previous one (Figure 2.2), thus forming H-bonded undulating 
tapes, growing the crystal structure in the bc plane. The N3···O3 interactions result in 
similar but centrosymmetric tapes and the overall crystal structure ends up as double 
helical H-bonded structure as shown down the b axis (Figure 2.3).

The X-ray crystal structure of (8) is shown in Figure 2.5. The benaldehyde rings on 
the arms make dihedral angles of 74.7(1) and 84.3(1)° with respect to the central ring, 
signifying that both of them are almost perpendicular to the central ring. The C7-O1 
and C15-O3 bonds are pointing above and below the plane of the anchor ring. The 
two benaldehyde rings are in turn perpendicular to each other (dihedral angle 
89.6(1)°, Figure 2.6). This gives a ‘V’ shaped conformation to the molecule. There are 
strong π···π interactions between the benaldehyde rings (centroid to centroid 
3.684(1) Å) forming a linear chain running diagonally in the ac plane which is further 
augmented by C7-H7B···O4i and C24-H24C···O4i H-bonding interactions.
Figure 2.3. The H-bonded 3D, double helical tapes in the crystal structure of (1), shown down the b axis.

The X-ray crystal structure of (4) is shown in Figure 2.4. The two fold axis passes through phenyl carbons C10 and C13 and the methyl C11, thus the asymmetric unit contains half the molecule. The C9-C8-O2-C3 torsion angle is 177.9(1). The benzaldehyde ring of the arm makes a dihedral angle of 74.76(5) with respect to the central ring.

Figure 2.4. ORTEP diagram of the (4) at 50% probability showing the labeling scheme.
Figure 2.5. ORTEP diagram of the (8) at 50% probability showing the labeling scheme.

Figure 2.6. Showing 'V' shaped conformation in (8).

Figure 2.7. Showing $\pi\cdots\pi$ and other C-H$\cdots$O interactions helping to form a 2D arrangement in (8).
These chains are linked to each other due to C22-H22···O2\textsuperscript{iii} and C14-H14···O4\textsuperscript{iii} interactions forming a 2D crystal structures in the \textit{ac} plane (Figure 2.7). These planes are then joined to each other due to C21···O4\textsuperscript{vi}, C22···O1\textsuperscript{vi} and C23···O2\textsuperscript{vii} interactions between phenylene and methyl carbons and the carbonyl oxygens (Table 2.3).

**Table 2.1** Crystal data and structure refinement parameters for (1), (4) and (8)

<table>
<thead>
<tr>
<th>Identification code</th>
<th>(1)</th>
<th>(4)</th>
<th>(8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C\textsubscript{22}H\textsubscript{18}N\textsubscript{4}O\textsubscript{6}</td>
<td>C\textsubscript{25}H\textsubscript{21}O\textsubscript{4}</td>
<td>C\textsubscript{25}H\textsubscript{24}O\textsubscript{4}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>434.41</td>
<td>385.42</td>
<td>388.42</td>
</tr>
<tr>
<td>Temperature</td>
<td>295(2) K</td>
<td>296(2) K</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
<td>0.71073 Å</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Tetragonal</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P 2\textsubscript{1}/c</td>
<td>P 4\textsubscript{2}2\textsubscript{1}2\textsubscript{1}</td>
<td>P 2\textsubscript{1}/n</td>
</tr>
</tbody>
</table>
| Unit cell dimensions| a = 10.393(3) Å \ 
|                    | b = 26.134(7) Å \ 
|                    | c = 7.765(2) Å \ 
|                    | α= 90° \ 
|                    | β= 109.958(13)° \ 
|                    | γ = 90° \ 
| Volume              | 1983.1(10) Å\textsuperscript{3} | 2129.7(13) Å\textsuperscript{3} | 1977.6(5) Å\textsuperscript{3} |
| Z                   | 4 | 4 | 4 |
| Density (calculated)| 1.455 Mg/m\textsuperscript{3} | 1.202 Mg/m\textsuperscript{3} | 1.305 Mg/m\textsuperscript{3} |
| Absorption coefficient| 0.108 mm\textsuperscript{-1} | 0.081 mm\textsuperscript{-1} | 0.087 mm\textsuperscript{-1} |
| F(000)              | 904 | 812 | 824 |
| Crystal size        | 0.21 x 0.17 x 0.10 mm\textsuperscript{3} | 0.12 x 0.09 x 0.07 mm\textsuperscript{3} | 0.13 x 0.11 x 0.08 mm\textsuperscript{3} |
| Theta range for data collection | 1.558 to 25.90° | 2.18 to 33.52° | 1.86 to 27.61° |
| Index ranges        | -12<=h<=12, \ 
|                    | -31<=k<=31, \ 
|                    | -9<=l<=7, \ 
| Reflections collected | 15046 | 33998 | 17564 |
| Independent reflections | 3788 [R(int) = 0.0891] | 4159[R(int) = 0.0456] | 4575[R(int) = 0.0587] |
| Absorption Correction | Semi-empirical from equivalents |
| Max. and min. transmission | 0.745 and 0.671 | 0.7466 and 0.6533 | 0.7456 and 0.6730 |
| Refinement method   | Full-matrix least-squares on F\textsuperscript{2} |
| Data / restraints / parameters | 3788 / 6 / 308 | 4159 /0 /135 | 4575 / 1 / 271 |
| Goodness-of-fit on F\textsuperscript{2} | 0.967 | 1.035 | 1.023 |
| Final R indices     | R1 = 0.0540, \ wR2 = 0.1149 | R1 = 0.0564, \ wR2 = 0.1462 | R1 = 0.0606, \ wR2 = 0.1565 |
| [I>2sigma(I)]       | 1.023 |
| R indices (all data) | R1 = 0.1368, \ wR2 = 0.1501 | R1 = 0.0913, \ wR2 = 0.1753 | R1 = 0.0985, \ wR2 = 0.1810 |
| Largest diff. peak and hole | 0.180 and -0.185 e. Å\textsuperscript{-3} | 0.207 and -0.192 e. Å\textsuperscript{-3} | 0.695 and -0.314 e. Å\textsuperscript{-3} |
Table 2.2  Showing important hydrogen bonds in (I) (Å, °)

<table>
<thead>
<tr>
<th></th>
<th>X···Y</th>
<th>H···Y</th>
<th>∠X-H···Y</th>
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</thead>
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<td>1.96(3)</td>
<td>145</td>
</tr>
<tr>
<td>O5-H5A···N4</td>
<td>2.717(4)</td>
<td>2.03(3)</td>
<td>139</td>
</tr>
<tr>
<td>N1-H1B···O5</td>
<td>3.193(4)</td>
<td>2.26(2)</td>
<td>175</td>
</tr>
<tr>
<td>N1-H1B···O6</td>
<td>3.157(4)</td>
<td>2.67(3)</td>
<td>113</td>
</tr>
<tr>
<td>N3-H3B···O3</td>
<td>2.876(4)</td>
<td>2.36(3)</td>
<td>113</td>
</tr>
<tr>
<td>N3-H3B···O2</td>
<td>3.398(4)</td>
<td>2.46(3)</td>
<td>172</td>
</tr>
<tr>
<td>O2-H2A···O6</td>
<td>3.152(4)</td>
<td>2.63(3)</td>
<td>122</td>
</tr>
<tr>
<td>O3-H3A···O4</td>
<td>2.655(3)</td>
<td>1.84(3)</td>
<td>161</td>
</tr>
<tr>
<td>O3-H3A···N4</td>
<td>2.960(4)</td>
<td>2.76(3)</td>
<td>95</td>
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<tr>
<td>O5-H5A···O3</td>
<td>2.801(5)</td>
<td>2.18(4)</td>
<td>131</td>
</tr>
<tr>
<td>O6-H6A···N2</td>
<td>3.122(1)</td>
<td>2.76(3)</td>
<td>107</td>
</tr>
<tr>
<td>O6-H6A···O1</td>
<td>2.668(4)</td>
<td>1.86(4)</td>
<td>161</td>
</tr>
</tbody>
</table>

(i)  x,-y+1/2+1,+z+1/2  (ii) -x+1,-y+1,-z+1
(iii) -x+1,+y-1/2,-z+1/2+1  (iv) -x+1,+y-1/2,-z+1/2+1
(v)  -x+1,+y+1/2,-z+1/2+1  (vi) -x+1,+y+1/2,-z+1/2
Table 2.3 Showing important H-bonding interactions in (8) (Å, °)

<table>
<thead>
<tr>
<th>X···Y</th>
<th>H···Y</th>
<th>∠X-H···Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>C7-H7B···O4\textsuperscript{i}</td>
<td>3.376(3)</td>
<td>2.66</td>
</tr>
<tr>
<td>C24-H24C···O4\textsuperscript{i}</td>
<td>3.619(4)</td>
<td>2.85</td>
</tr>
<tr>
<td>C9-H9···O4\textsuperscript{ii}</td>
<td>3.784(3)</td>
<td>2.87</td>
</tr>
<tr>
<td>C14-H14···O4\textsuperscript{iii}</td>
<td>3.002(3)</td>
<td>2.82</td>
</tr>
<tr>
<td>C15-H15B···O2\textsuperscript{iv}</td>
<td>3.705(3)</td>
<td>2.86</td>
</tr>
<tr>
<td>C21-H21···O4\textsuperscript{v}</td>
<td>3.413(3)</td>
<td>2.51</td>
</tr>
<tr>
<td>C22-H22···O1\textsuperscript{vi}</td>
<td>3.712(3)</td>
<td>2.72</td>
</tr>
<tr>
<td>C23-H23A···O2\textsuperscript{vii}</td>
<td>3.356(3)</td>
<td>2.47</td>
</tr>
</tbody>
</table>

\textsuperscript{(i)} x-1/2,-y+1/2,+z+1/2 \quad \textsuperscript{(ii)} -x+1/2,+y+1/2,-z+1/2+1

\textsuperscript{(iii)} x-1/2,-y-1/2,+z+1/2 \quad \textsuperscript{(iv)} x+1/2,-y+1/2,+z-1/2

\textsuperscript{(v)} -x+1/2+1,+y+1/2,-z+1/2+1 \quad \textsuperscript{(vi)} -x+1/2,+y-1/2,-z+1/2+1

\textsuperscript{(vii)} -x,-y,-z+2
Spectral Characterization

Figure 2.8. (a) $^1$H NMR of (1) (b) $^{13}$C NMR of (1).

Figure 2.9. (a) IR of (1) (b) ESI-MS of (1).

Figure 2.10. (a) $^1$H NMR of (2) (b) $^{13}$C NMR of (2).
Figure 2.11. (a) IR of (2) (b) ESI-MS of (2).

Figure 2.12. (a) $^1$H NMR of (3) (b) $^{13}$C NMR of (3).

Figure 2.13. (a) IR of (3) (b) ESI-MS of (3).
Figure 2.14. (a) $^1$H NMR of (4) (b) $^{13}$C NMR of (4).

Figure 2.15. (a) IR of (4) (b) ESI-MS of (4).

Figure 2.16. (a) $^1$H NMR of (5) (b) $^{13}$C NMR of (5).
Figure 2.17. (a) IR of (5) (b) ESI-MS of (5).

Figure 2.18. (a) $^1$H NMR of (6) (b) $^{13}$C NMR of (6).

Figure 2.19. (a) IR of (6) (b) ESI-MS of (6).
Figure 2.20. (a) $^1$H NMR of (7) (b) $^{13}$C NMR of (7).

Figure 2.21. (a) IR of (7) (b) ESI-MS of (7).

Figure 2.22. (a) $^1$H NMR of (8) (b) $^{13}$C NMR of (8).
Figure 2.23. (a) IR of (8) (b) ESI-MS of (8).

Figure 2.24. $^1$H NMR of (9).

Figure 2.25. (a) IR of (9) (b) ESI-MS of (9).
2.4 Cation recognition studies of benzene anchored Schiff bases containing hydrazone (-C=N-NH-) and hydroxyl (-OH) groups

The construction of chromo-fluorogenic systems with a proper co-ordination environment for selective and sensitive sensing is a subject of colossal interest. In the present work, three hydrazones as positional isomers have been developed which contain a combination of –CONH, -CH=N and –OH groups and then evaluate their cooperative effect on cation sensing. Since Al(III) is a hard acid accordingly it prefers systems containing hard base sites as O and N, hence, (1-3) provide an ideal co-ordination environment for selective and sensitive detection of Al(III) in HEPES buffer (pH 7.4, containing 30% DMSO as a co-solvent). Devoid of any conventional fluorophore, these sensors have detection limits in nanomolar scale with high quantum yields and naked eye sensing of Al(III). Moreover, these probes have been demonstrated to enable the Al(III) detection in live human HeLa cells and rat C6 glioma cells using a confocal microscope. In addition, (1-3) find practical application in the form of ‘dip-sticks’ which can provide instant detection of Al(III) and at the same time in the estimation of Al(III) in live HeLa cells and C6 glioma cells using confocal microscope. The cation sensing properties of (1), (2) and (3) have been studied by naked-eye, UV-Vis, Fluorescence and \(^1\)H NMR studies. In the literature, there are very few chemosensors which comply with all four desirable features in a chemical sensor at the same time i.e. working in aqueous systems, low detection limit, high selectivity and applicability in living systems. Sensors (1), (2) and (3) reported here meet these criterions hence provide high selectivity with low detection limit in aqueous medium and have good response in biological systems.

2.4.1 \(^1\)H NMR comparison of sensors (1), (2) and (3)

NMR spectra of these sensors showed chemical shift illustrating ortho-para effect as the chemical shifts of two –OH groups in the three isomers follow a trend since using the same concentration (5mM). The α and β protons appear more downfield shifted in the meta isomer (2) (Figure 2.26) which may be attributed to the –ortho, -para directing effect of the –OH groups, which causes shielding effect on the C atoms bearing them. The aromatic protons ‘i’ of meta isomer is also much downfield shifted than ‘j’ and ‘l’ as they are shielded due to the presence of partial negative charge on them, owing to the +R effect of –OH groups. These effects are in accordance with the data from the studies of emission spectra of these compounds (vide infra).
2.4.2 Chromogenic spectral response of sensors

Figure 2.26. $^1H$ NMR of (A) (1) (B) (2) (C) (3) at 5mM concentration of each sensor.

Figure 2.27. Changes in UV-vis spectra of (10 μM) solutions of (a) (1) (b) (2) (c) (3), upon the addition of various metal ions (100 μM) in HEPES buffer. Inset: Response of Al(III), Cu(II) and Fe(III) towards (1), (2) and (3), respectively.
The photophysical properties of sensors (1-3) were studied by using UV-vis and fluorescence spectroscopy. The absorption spectra exhibit maxima centered at $\lambda_{\text{max.}}$ 318 nm ($\epsilon_{\text{max}} = 3.90 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), $\lambda_{\text{max.}}$ 340 nm ($\epsilon_{\text{max}} = 3.09 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and at $\lambda_{\text{max.}}$ 358 nm ($\epsilon_{\text{max}} = 1.37 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) for (1-3), respectively which have been designated as internal charge transfer (ICT) bands involving imine and hydroxyl group. The UV-vis spectral response of sensors, (1), (2) and (3) towards various metal ions under study revealed significant bathochromic shifts for Cu(II), Fe(III) and Al(III). From Figure 2.27, it is clear that with Cu(II) new bands are formed at $\lambda_{\text{max.}}$ 410 nm for (1), at 384 and 403 for (2) and at 430 nm for (3). On the other hand, interaction of (1) and (2) with Fe(III) caused only a very small shift in band wavelength whereas in case of (3) a new band is formed at 430 nm. With the addition of Al(III), new bands are formed in UV-vis spectra at $\lambda_{\text{max.}}$ 403 nm for (1), at 384 and 403 for (2) and at 430 nm for (3), with isobestic points at 328 nm, 358 nm and at 310, 351 and 387 nm, respectively. Hence, chromogenic spectral response of (1), (2) and (3) towards these metal ions under study lacked selectivity.

2.4.3 Fluorogenic spectral response of sensors

![Fluorogenic spectral response of sensors](image)

**Figure 2.28.** Changes in fluorescence spectra of (1) (2.5 µM) upon the addition of 10 equivalents of metal nitrates. Inset. Changes in color of sensor solution with Al(III) under UV lamp.

Fluorescence characteristics of (1), (2) and (3) (2.5 µM) were investigated in HEPES buffer (pH 7.4, containing 30% DMSO as a co-solvent) (Figure 2.28 and 2.29). (1) and (2) exhibit very weak fluorescence at $\lambda_{\text{em}}$ 508 nm and $\lambda_{\text{em}}$ 528 nm (excitation at
325 nm and 358 nm, respectively). Such fluorescence response is known to be due to the well-known ESIPT phenomenon in the case of Schiff bases.\textsuperscript{10} Whereas (2) shows a much weaker emission band at $\lambda_{\text{em}}$ 455 nm (excitation at 340 nm) which may be due to the PET phenomenon due to availability of lone pairs of N of the $–$C=N group and O of OH group.\textsuperscript{11}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.29.png}
\caption{Changes in fluorescence spectra of (a) (2) (b) (3) (2.5 µM) upon the addition of 10 equivalents of metal nitrates under study. Inset. Color changes in sensor solutions with Al(III) under UV lamp.}
\end{figure}

This difference in their emission behaviour may again be attributed to the ortho-para directing capability of the $–$OH groups which places partial negative charges on the $–$OH bearing carbons in the case of ortho and para isomers, making them more susceptible to quinone forms, facilitating the transfer of proton. These effects are corroborated by the observed chemical shift values of $\alpha$- and $\beta$-OH and the aromatic protons (\textit{vide supra}). All three isomers form a rigid chelated system with Al(III) due to coordination through OH and imine (CH=N) groups and consequently exhibit strong fluorescence enhancement due to (CHEF) mechanism. (1) exhibits strong fluorescence enhancement ($\Phi = 0.63$) upon addition of Al(III) centered at 508 nm ($\lambda_{\text{ex}}$ 325 nm) (Figure 2.28) accompanied with a green fluorescence (Inset, Figure 2.28). Similarly, upon Al(III) addition to (2) remarkable fluorescence enhancement (Figure 2.29a) at $\lambda_{\text{em}}$ 455 nm ($\lambda_{\text{ex}}$ 340 nm) was observed with quantum yield\textsuperscript{12} ($\Phi = 0.7$) giving blue fluorescence (Inset, Figure 2.29a). (3), when excited at $\lambda_{\text{ex}}$ 358 nm after Al(III) addition fluorescence enhancement (Figure 2.29b) was observed at 528 nm with quantum yield at ($\Phi = 0.77$) yielding green fluorescence (Inset, Figure 2.29b).

For all the sensors, the addition of Li(I), Na(I), K(I), Ag(I), Mg(II), Ca(II), Cd(II), Ba(II), Sr(II), showed no significant change whereas Bi(III), Zn(II) caused very little enhancement and Cu(II), Cr(III), Co(II), Ni(II), Hg(II), Pb(II), Fe(III) cause
quenching up to various extents, which may be attributed to the energy or charge transfer interactions of these metal ions\textsuperscript{11d,13} with sensors. Due to significant and selective fluorogenic response of sensors (1), (2) and (3) towards Al(III) as compared to Cu(II) and Fe(III), all the further studies were carried out with Al(III) only. Selectivity of sensors (1), (2) and (3) showed no change, when other aluminium salts such as Al\(_2\)(SO\(_4\))\(_3\) and AlCl\(_3\) are used (Figure 2.30). Job’s plot\textsuperscript{14} obtained from emission data showed 1:1 stoichiometry for all the Al(III) complexes of (1), (2) and (3) (Figure 2.31). This complex formation was further supported by ESI/MS, peaks at m/z 459.0798 \([\text{M} - 2\text{H} + \text{Al(III)}]^+\) (calc. 459.0885), 495.0797 \([(\text{M}-2\text{H})+\text{Al(III)} + 2\text{H}_2\text{O}]^+\) (calc. 495.1097), 537.0963 \([(\text{M}-2\text{H})+\text{Al(III)}+\text{NO}_3^-+\text{H}_2\text{O}-2]^+\) (calc. 537.0713), 615.1080 \([(\text{M}-2\text{H})+\text{Al(III)}+\text{NO}_3^-+\text{H}_2\text{O}+\text{DMSO}-2])^+\) (calc. 615.0852) for (1). Similarly peaks at m/z 459.0888, 537.1035, 615.1165 for (2) and at m/z 459.0457, 537.1008, 615.0988 for (3) were observed (Figure 2.39, 2.41 and 2.43). The occurrence of peak at 537.07 in all three cases indicates the formation of the proposed \([\text{C}_{22}\text{H}_{18}\text{AlN}_5\text{O}_{10}]-2\) complex (Figure 2.32).

![Figure 2.30](image)

**Figure 2.30.** The fluorescence responses of (a) (1) (2.5 µM) (b) (2) (2.5 µM) (c) (3) (2.5 µM) towards different Al(III) salts (10 equivalents).

![Figure 2.31](image)

**Figure 2.31.** Showing Job’s plot for stoichiometry of complex of (a) (1) (b) (2) (c) (3) with Al(III).
Titration of (1), (2) and (3) with Al(III) (Figure 2.33) was followed by fluorescence to determine binding constants, $5.57 \times 10^5 \text{ M}^{-1}$, $2.5 \times 10^6 \text{ M}^{-1}$ and $7.0 \times 10^4 \text{ M}^{-1}$ respectively, employing Benesi-Hildebrand plot\(^\text{15}\) (Figure 2.34 (a), (b) and (c)).

**Figure 2.32.** Proposed structure of Al(III) complex of (1), (2) and (3).

**Figure 2.33.** Changes in fluorescence spectra of (1), (2) and (3) (2.5 µM) upon gradual addition of Al(III).

**Figure 2.34.** Benesi-Hildebrand plot for stability constant of (1), (2) and (3) with Al(III) from fluorescence data.
Figure 2.35. Fluorescence intensity of (1), (2) and (3) for different concentrations of Al (III) normalized between the maximum emission and the minimum emission (0.0 µM Al (III)) intensity.

The metal complexation is confirmed by the free energy values of the complexation processes such as \(-32.75\) KJ mol\(^{-1}\), \(-36.50\) KJ mol\(^{-1}\) and \(-27.64\) KJ mol\(^{-1}\) for (1), (2) and (3) respectively, obtained using equation \(\Delta G = -2.303 RT \log K_a\). Respective detection limits of these sensors for Al(III) calculated according to the literature\(^{16}\) were found to be \(8.91 \times 10^{-9}\) M, \(14.1 \times 10^{-9}\) M and \(19.95 \times 10^{-9}\) M, which are quite low to detect the submicromolar concentration of Al(III) (Figure 2.35 (a), (b) and (c)). Competitive selectivity of (1), (2) and (3) for Al(III) (Figure 2.36 (a), (b) and (c)) was determined by fluorescence titration with Al(III) in the presence of other metal ions under study, which revealed that Al(III) can be detected in the presence of other competitive metal ions. For this experiment, (1), (2) and (3) were treated with 10 equivalent of Al(III) in the presence of 50 equivalents of other metal ions under study. There is no significant interference for the detection of Al(III) in the presence of other metal ions except in case of Cu(II) and Fe(III) which showed some quenching of fluorescence but enhancement is still very prominent.

Figure 2.36. Competitive Selectivity of (1), (2) and (3) (2.5µM) towards Al(III) (10 equivalents) in the presence of other metal ions (50 equivalents) under study.
2.4.4 $^1$H NMR titration experiments of sensors (1), (2) and (3) with Al(III)

In order to investigate the mode of binding sensor to Al(III) ions, NMR titrations were carried out in DMSO-d$_6$, (Figure 2.37). In all the three cases, signals of $\alpha$-OH protons disappear as a result of deprotonation and complexation but those of $\beta$-OH, imine and $\–$NH group sustain. The chemical shift values of $\beta$–OH protons show low frequency shifts in (1) and (2) ($\Delta\delta$ 0.042 and 0.712) and a high frequency shift in (3) ($\Delta\delta$ 0.267) which may be due to different kinds and extents of H-bonding interactions. The complexation was confirmed by isolating the solid complexes by reacting (1), (2) and (3) with Al(NO$_3$)$_3$·9H$_2$O in ethanol and characterizing them by NMR, IR and mass spectroscopy (Figure 2.38 – Figure 2.43).

![Figure 2.37. Changes in partial $^1$H NMR of (1), (2) and (3) (5mM) upon the addition of Al(III) in DMSO-d$_6$.](image)

**Synthesis and Characterization of 1 – Al(III) complex.** To a 5 ml suspension of (1) (0.05 g, 1.15 mmol) in ethanol, a 10 ml solution of Al(NO$_3$)$_3$·9H$_2$O (0.086 g, 2.30 mmol) in distilled water was added drop wise over 15 minutes and then stirred it for half an hour. After stirring, concentrate the reaction mixture and placed it in an ice
bath. Collected the dark brown precipitates on Buchner funnel. mp = 300-302 °C. $^1$H NMR (300 MHz, DMSO-d$_6$, δ): 6.74 (t, 2H, Ar, J = 7.5 Hz), 6.86 (d, 2H, Ar, J = 7.5 Hz), 6.98 (d, 2H, Ar, J = 7.8 Hz), 7.72 (t, 1H, Ar, J = 7.5 Hz), 8.14 (d, 2H, Ar, J = 7.8 Hz), 8.51 (s, 1H, Ar), 8.63 (s, 2H, -CH=N), 9.27 (s, 2H, -OH), 12.30 (s, 2H, -NH); IR (KBr, cm$^{-1}$): 3242 (OH), 3064 (NH), 1660 (C=O), 1614 (C=N), 1385 (-NO$_3$); HRMS m/z: 459.0798 [(M - 2H) + Al(III)]$^+$ (calc. 459.0885), 495.0797 [(M-2H)+Al(III) + 2H$_2$O]$^+$ (calc. 495.1097), 537.0963 [((M-2H)+Al(III)+NO$_3$+H$_2$O)-2]$^+$ (calc. 537.0713), 615.1080 [((M-2H)+Al(III)+NO$_3$+H$_2$O+DMSO)-2)]$^+$ (calc. 615.0852).

![Figure 2.38](image1.png)  
(a) $^1$H NMR (b) IR of (1)-Al(III).

![Figure 2.39](image2.png)  
ESI-MS of (1)-Al(III).

**Synthesis and Characterization of (2) – Al(III) complex.** Same procedure was used as for the synthesis of (1) – Al(III) complex except that (2) was used instead of sensor
(1). mp = 300-302 °C; \(^1\)H NMR (300 MHz, DMSO-d\(_6\), \(\delta\)): 6.33 (s, 2H, Ar), 6.35 (d, 2H, Ar, \(J = 8.4\) Hz), 7.33 (d, 2H, Ar, \(J = 8.9\) Hz), 7.69 (t, 1H, Ar, \(J = 8.1\) Hz), 8.10 (d, 2H, Ar, \(J = 8.1\) Hz), 8.45 (s, 1H, Ar), 8.54 (s, 2H, -CH=N), 9.91 (s, 2H, -OH), 12.05 (s, 2H, -NH); IR (KBr, cm\(^{-1}\)): 3391 (OH), 3206 (NH), 1639 (C=O), 1618 (C=N), 1385 (-NO\(_3\)); HRMS m/z: 459.0888 [(M - 2H) + Al(III)]\(^+\) (calc. 459.0885), 537.1035 [((M-2H)+Al(III)+NO\(_3\)+H\(_2\)O)-2]\(^+\) (calc. 537.0713), 615.1165 [((M-2H)+Al(III)+NO\(_3\)+H\(_2\)O+DMSO)-2]\(^+\) (calc. 615.0852).

Figure 2.40. (a) \(^1\)H NMR (b) IR of (2) -Al(III).

Figure 2.41. ESI-MS of (2) -Al(III).
Synthesis and Characterization of (3) – Al(III) complex. Same procedure was used as for the synthesis of (1) – Al(III) complex except that (3) was used instead of sensor (1). mp = 300-302 ºC; \( ^1H \) NMR (300 MHz, DMSO-d_6, \( \delta \)): 6.75 (s, 4H, Ar); 7.00 (s, 2H, Ar); 7.71 (t, 1H, Ar, J = 7.8 Hz); 8.13 (d, 2H, Ar, J = 8.1 Hz); 8.49 (s, 1H, Ar); 8.61 (s, 2H, -CH=N); 10.17 (s, 2H, -OH); 12.13 (s, 2H, -NH); IR (KBr, cm\(^{-1}\)): 3408 (OH), 3250 (NH), 1666 (C=O), 1590 (C=N), 1385 (-NO_3); HRMS m/z, (Fig. S33): HRMS m/z: 459.0457 \([(M-2H) + Al(III)]^+\) (calc. 459.0885), 537.1008 \([(M-2H)+Al(III)+NO_3^-+H_2O)-2]^-\) (calc. 537.0713), 615.0988 \([(M-2H)+Al(III)+NO_3^-+H_2O+DMSO)-2)]^-\) (calc. 615.0852).

![Figure 2.42.](image1.png) Figure 2.42. (a) \(^1H\) NMR (b) IR of (3)-Al(III).

![Figure 2.43.](image2.png) Figure 2.43. ESI-MS of (3)-Al(III).

Further, in order to check the practical applicability of (1), (2) and (3), we prepared dip sticks by coating paper strips with DMSO-H_2O solution of sensors. After drying, these strips were dipped in the solution (5 µM) of Al(NO_3)_3 in distilled water, dried and observed under UV lamp. Similar color changes were observed in the solid state (Figure 2.44) as earlier in the solution state. The different fluorescent colours obtained
not only detect the presence of Al(III) but also distinguish between three positional isomers.

Figure 2.44. Fluorescent color changes with dip sticks formed from (1), (2) and (3) (2.5µM) in DMSO-H₂O upon treatment with 5 µM of Al(III). Left – Before and Right – After Al(III) treatment.

2.4.5 Bioimaging of Al(III) in live cells by sensors (1), (2) and (3)

For the investigation of biological applications of the sensors (1-3), cell imaging studies have been performed with both human cervical cancer cell line (HeLa cells) (Figure 2.45) as well glial cells of the rat brain (C6 glioma cells) (Figure 2.46). Both the HeLa and C6 glioma cells themselves and after incubation with Al(III) (10µM and 50µM) did not exhibit any fluorescence (Figure 2.45 (a), (b), (c) and 2.46 (a), (b), (c), respectively). Further, HeLa and C6 glioma cells were incubated with 10µM of (1), (2) and (3) for 30 min at 37 °C. When imaged after incubation, HeLa as well as C6 glioma cells showed no fluorescence for (1) (Figure 2.45(d), 2.46(d)) and (2) (Figure 2.45(g), 2.46(g)) but faint green fluorescence for 3 (Figure 2.45(j) and 2.46(j)) was observed in both HeLa and C6 glioma cells. The excitation laser used for (1), (2) and (3) was 405 nm. Once the treated cells (HeLa and C6 glioma) were incubated with Al(III) (10µM) for another 30 min at 37 °C, bright green fluorescence was observed in both the types of cells for (1) (Figure 2.45(e), 2.46(e)) which increased significantly with 50 µM Al(III) (Figure 2.45(f) and 2.46(f)). Similarly, green fluorescence of (3) got enhanced when incubated with Al(III) (10µM) (Figure 2.45(k), 2.46(k)) and fluorescence got further enhanced significantly with 50µM Al(III) (Figure 2.45(l), 2.46(l)). For (2), blue fluorescence was observed with 10µM Al(III) treatment (Figure 2.45(h), 2.46(h)) which also increased considerably with 50 µM Al(III) (Figure 2.45(i) and 2.46(i)) in tested both the cell lines. The fluorescence was observed in the perinuclear region as well as cytosol, hence, it indicates that
Chemosensors (1), (2) and (3) are permeable to HeLa and C6 glioma cells and can be used for detecting Al(III) in the live cells. The excitation laser used for (1), (2) and (3) was 405 nm. Once the treated cells (HeLa and C6 glioma) were incubated with Al(III) (10µM) for another 30 min at 37 °C, bright green fluorescence was observed in both the types of cells for (1) (Figure 2.45(e), 2.46(e)) which increased significantly with 50 µM Al(III) (Figure 2.45(f) and 2.46(f)).

**Figure 2.45.** Images of HeLa cells (a) brightfield image of HeLa cells (b) fluorescence image of HeLa cells incubated with Al(III) (10 µM) for 30 min.(c) fluorescence image of HeLa cells incubated with Al(III) (50 µM) for 30 min. (d) fluorescence image of HeLa cells incubated with (1) (10µM) for 30 min. and further incubation with (e) 10 µM Al(III) (f) 50 µM Al(III) (g) fluorescence image of HeLa cells incubated with (2) (10µM) for 30 min. and further incubation with (h) 10 µM Al(III) (i) 50 µM Al(III) (j) fluorescence image of HeLa cells incubated with (3) (10µM) for 30 min. and further incubation with (k) 10 µM Al(III) (l) 50 µM Al(III).
**Figure 2.46.** Images of C6 glioma cells (a) brightfield image of C6 glioma cells. (b) fluorescence image of C6 glioma cell incubated with Al(III) (10 µM) for 30 min. (c) fluorescence image of C6 glioma cells incubated with Al(III) (50 µM) for 30 min. (d) fluorescence image of C6 glioma cells incubated with (1) (10µM) for 30 min. and further incubation with (e) 10 µM Al(III) (f) 50 µM Al(III) (g) fluorescence image of C6 glioma cells incubated with (2) (10µM) for 30 min. and further incubation with (h) 10 µM Al(III) (i) 50 µM Al(III) (j) fluorescence image of C6 glioma cells incubated with (3) (10µM) for 30 min. and further incubation with (k) 10 µM Al(III) (l) 50 µM Al(III).

Similarly, green fluorescence of (3) got enhanced when incubated with Al(III) (10µM) (Fig. 2.45(k), 2.46(k)) and fluorescence got further enhanced significantly with 50µM Al(III) (Figure 2.45(l), 2.46(l)). For (2), blue fluorescence was observed with 10µM Al(III) treatment (Figure 2.45(h), 2.46(h)) which also increased considerably with 50 µM Al(III) (Figure 2.45(i) and 2.46(i)) in tested both the cell lines. The fluorescence was observed in the perinuclear region as well as cytosol, hence, it indicates that chemosensors (1), (2) and (3) are permeable to HeLa and C6.
glioma cells and can be used for detecting Al(III) in the live cells. To investigate the cytotoxicity of (1), (2) and (3), MTT assay with HeLa cells as well as C6 glioma cells was performed. No significant differences in the proliferation of the HeLa and C6 glioma cells were observed in the absence or presence of 10µM of chemosensors (1), and (3) (80-90% cell viability with chemosensor (1) and 90-94% cell viability with (3) for both the tested cell lines (Figure 2.47). However, with chemosensor (2) HeLa cells showed nearly 27% cell survival while C6 glioma cells had more than 50% (53%) cell viability, indicating chemosensor (2) to be toxic for the cells. With Al(III) (50 µM) alone there was not much effect on cell viability (90% cell viability) with both the tested cell lines. Again addition of 10 µM or 50 µM of Al(III) in the presence of chemosensors (1) and (3) (10µM), did not show any significant effect but (2) showed considerable effect on the cell viability of both the cell types. These data show that chemosensors (1) and (3) have very low cytotoxicity while (2) is substantially cytotoxic.

Figure 2.47. Cell viability values (%) estimated by an MTT proliferation test with HeLa and C6 glioma cells at 37°C for (1), (2) and (3). Blue bars represent the results with HeLa Cells and red bars represent the results with C6 cells.
2.5 Anion recognition studies of mesitylene anchored Schiff bases containing hydrazone (-C=\text{N}-N\text{H}-) and nitro (-NO\text{2}) groups

Currently, the development of colorimetric sensors for anions especially for cyanide has enticed much attention, because the color change can be observed easily by naked eyes and no special equipment is required.\textsuperscript{17} Then again, ratiometric chromogenic sensors permit signal rationing by performing the measurement of absorption intensities at two different wavelengths and thus increase the dynamic range.\textsuperscript{18} The sensors that can work in aqueous phase are required more frequently due to their use in biological and environmental systems.\textsuperscript{19} Hence, construction of ratiometric colorimetric anion sensors that can work in both organic as well as in aqueous phase is the need of the hour. In literature,\textsuperscript{20} the colorimetric anion sensors generally used groups such as amide, urea, thiourea and pyrrole etc., but very few selective anion sensors have been synthesized which rely on the use of hydrazone group.\textsuperscript{21} The sensing mechanism involved in the probes with above-mentioned functional groups depends upon the acidity of the protons of –NH groups, which can be regulated by introducing electron-withdrawing groups. Hence, designing of probes to detect CN\textsuperscript{-}, which can operate through deprotonation of the –NH proton of hydrazone group with enhanced acidic strength by incorporation of electron withdrawing –NO\text{2} groups is a good synthetic approach. In light of the above facts, three new hydrazones (5), (7) and (9) with nitro groups as signaling units have been constructed, characterized by various spectroscopic techniques and evaluated as sensitive and selective cyanide sensors in aqueous phase, working via frozen proton transfer mechanism.

The presence of electron withdrawing –NO\text{2} groups increases the Bronsted acidity of –NH proton of the hydrazone group of (5), (7) and (9), favors the deprotonation and leads to larger bathochromic shift and naked-eye detection. The anion binding studies
of (5), (7) and (9) have been performed by visual, UV-vis and $^1$H NMR titration experiments.

2.5.1 Colorimetric and UV-vis spectral response of sensors (5), (7) and (9)

2.5.1.1 Response in organic media

The anion binding ability of (5), (7) and (9) (10µM) in DMSO was studied by the changes in their absorption spectra measured upon addition of various tetrabutylammonium anions, TBAX (where X = F$^-$, Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, CN$^-$, ClO$_4^-$, CH$_3$CO$_2^-$, HSO$_4^-$ and H$_2$PO$_4^-$ (100 µM). Free sensors (5), (7) and (9) exhibit their main absorption bands centered at $\lambda_{\text{max}}$ 403 nm ($\epsilon_{\text{max}} = 3.4 \times 10^4$ M$^{-1}$ cm$^{-1}$), $\lambda_{\text{max}}$ 398 nm ($\epsilon_{\text{max}} = 3.4 \times 10^4$ M$^{-1}$ cm$^{-1}$) and at $\lambda_{\text{max}}$ 408 nm ($\epsilon_{\text{max}} = 3.4 \times 10^4$ M$^{-1}$ cm$^{-1}$) respectively. These bands may be assigned to intraligand or internal charge transfer (ICT) between nitrogen of -NH of hydrazone group and electron-deficient 2,4-dinitrophenyl group. Upon addition of 10 equivalents of the various tetrabutylammonium anions (TBAX) under study to the solution of sensor (5) in DMSO, significant changes were observed with CN$^-$, F$^-$, CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$ only (Figure 2.48 (a) and (b)) along with the visual color change from pale yellow to pink (Figure 2.49). However, the remaining anions (NO$_3^-$, Cl$^-$, Br$^-$, I$^-$, ClO$_4^-$, H$_2$PO$_4^-$ and HSO$_4^-$) did not show any noticeable spectral or visual change. The formation of clear isobestic point at $\lambda$ 443 nm indicates the formation of stable complex or new species with distinctive spectroscopic features as a result of interaction between sensor and analyte. As (5) responded to CN$^-$, F$^-$, CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$ ions in organic media, therefore to have a better vision of the recognition phenomenon, UV-vis titrations were performed for all four anions. On gradual addition of aliquots of CN$^-$, F$^-$, CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$ ions to 10 µM solution of (5) in DMSO, a significant spectral and visual color change (light yellow to dark pink) was observed for F$^-$ and CN$^-$ ions with disappearance of intraligand charge transfer (ICT) band at $\lambda_{\text{max}}$ 403 nm and appearance of a new band at $\lambda_{\text{max}}$ 504 nm ($\epsilon_{\text{max}} = 4.3 \times 10^4$ M$^{-1}$ cm$^{-1}$), whereas CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$ ions showed a weak color change (light yellow to light orange). It was noted that positional isomers (7) and (9) also showed similar spectral response and visual color changes for CN$^-$, F$^-$, CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$. With (7), these anions cause bathochromic shift of 102 nm from $\lambda_{\text{max}}$ 398 nm to $\lambda_{\text{max}}$ 500 nm ($\epsilon_{\text{max}} = 3.8 \times 10^4$ M$^{-1}$ cm$^{-1}$) [Figure 2.50 (a), (b) and Figure 2.51] whereas with (9) a
spectral shift of 83 nm from $\lambda_{\text{max}}$ 408 nm to $\lambda_{\text{max}}$ 491 nm ($\epsilon_{\text{max}} = 2.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) is observed [Figure 2.52 (a), (b) and Figure 2.53].

**Figure 2.48.** (a) Changes in UV-vis spectra (b) $(A/A_0)$ of (5) (10 µM) upon addition of 10 equivalents of various tetrabutylammonium anions in DMSO.

**Figure 2.49.** Visual color changes in (5) in DMSO with various tetrabutylammonium anions.

**Figure 2.50.** (a) Changes in UV-vis spectra (b) $(A/A_0)$ of (7) (10 µM) upon addition of 10 equivalents of various tetrabutylammonium anions in DMSO.
Figure 2.51. Visual color changes in (7) in DMSO with various tetrabutylammonium anions.

Figure 2.52. (a) Changes in UV-vis spectra (b) (A/A₀) of (9) (10 µM) upon addition of 10 equivalents of various tetrabutylammonium anions in DMSO.

Figure 2.53. Visual color changes in (9) in DMSO with various tetrabutylammonium anions.

The binding constants for the interactions of (5), (7) and (9) with CN⁻, F⁻, CH₃CO₂⁻ and C₆H₅CO₂⁻ were calculated by using Benesi-Hildebrand plot (Table 2.4). The optimal stoichiometry for all cases was found to be 1:1 according to Job’s plot studies.

Table 2.4. Binding Constants for various tetrabutylammonium anions towards (5), (7) and (9) in DMSO.

<table>
<thead>
<tr>
<th>Sensors in DMSO</th>
<th>Binding Constants (M⁻¹) for Tetrabutylammonium salts in DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN⁻</td>
</tr>
<tr>
<td>(5)</td>
<td>2.46 × 10⁴</td>
</tr>
<tr>
<td>(7)</td>
<td>3.92 × 10⁴</td>
</tr>
<tr>
<td>(9)</td>
<td>8.04 × 10⁴</td>
</tr>
</tbody>
</table>
2.5.1.2 Response in aqueous media

Sensors, (5), (7) and (9) have limited solubility in water, therefore absorption studies were performed in mixed solvent system such as DMSO:HEPES (1:1, v/v). Spectral and visual changes of (5), (7) and (9) were tested with aqueous solutions of sodium salts of anions such as CH$_3$CO$_2^-$, Br$^-$, C$_6$H$_5$CO$_2^-$, Cl$^-$, CN$^-$, F$^-$, I$^-$, H$_2$PO$_4^-$, NO$_3^-$, ClO$_4^-$ and HSO$_4^-$. Interestingly, in aqueous media, these sensors responded selectively to CN$^-$ ion only with a large bathochromic shift and a color change from very pale yellow to dark pink (Figure 2.54, 2.56, 2.58 and 2.60) whereas, they become insensitive to F$^-$, CH$_3$CO$_2^-$ and C$_6$H$_5$CO$_2^-$ as evident from their absorption spectra (Figure 2.55(a), (b), 2.57(a), (b) and 2.59(a), (b)). On the other hand, no significant spectral or visual response was observed with other anions as Br$^-$, Cl$^-$, I$^-$, H$_2$PO$_4^-$, NO$_3^-$, ClO$_4^-$ and HSO$_4^-$. Consequently, due to highly selective and immediate response of sensors towards CN$^-$ only, all further studies in aqueous medium were carried out with CN$^-$ only.

*Figure 2.54.* Visual color changes in (5), (7) and (9) in semi aqueous medium with sodium cyanide.

Upon CN$^-$ addition to the aqueous solution of (5), the intraligand charge transfer band$^{22,23}$ at $\lambda_{\text{max}}$ 403 nm ($\epsilon_{\text{max}} = 3.5 \times 10^4$ M$^{-1}$ cm$^{-1}$) disappears and a new band at $\lambda_{\text{max}}$ 504 nm ($\epsilon_{\text{max}} = 4.8 \times 10^4$ M$^{-1}$ cm$^{-1}$) appears (Figure 2.55) associated with increase in extent of charge transfer from anionic hydrazide part (generated by deprotonation) of (5) to electron-withdrawing moiety of (5).$^{24,25b}$ This observation was further supported by the upward shift of aromatic protons of (5,7,9) shown in $^1$H NMR titration experiments (vide infra), due to increased electron density caused by deprotonation of $\text{--NH}$ protons, when titrated with tetrabutylammonium cyanide in DMSO-d$_6$. 


Figure 2.55. (a) Changes in UV-vis spectra (b) (A/A₀) of (5) (10 µM) upon addition of 10 equivalents of various sodium salts of anions in DMSO:HEPES. A and A₀ are absorbance in the absence and presence of anions.

Figure 2.56. Visual color changes in (5) in semi aqueous medium with various sodium salts of anions.

Figure 2.57. (a) Changes in UV-vis spectra (b) (A/A₀) of (7) (10 µM) upon addition of 10 equivalents of various sodium salts of anions in DMSO:HEPES.

Figure 2.58. Visual color changes in (7) in semi aqueous medium with various sodium salts of anions.
Figure 2.59. (a) Changes in UV-vis spectra (b) \( (A/A_0) \) of (9) (10 µM) upon addition of 10 equivalents of various sodium salts of anions in DMSO:HEPES.

Figure 2.60. Visual color changes in (9) in semiaqueous medium with various sodium salts of anions.

Sensor (7) (Figure 2.57, 2.58) and (9) (Figure 2.59, 2.60), when titrated with various anions under study as their sodium salts showed selective response towards CN\(^-\) ion only. To study the binding characteristics of (5), (7) and (9) towards CN\(^-\) ions in aqueous medium, titration experiments were carried out by progressive addition of aqueous solution of NaCN to the semi aqueous solutions of sensors. These titrations revealed that all three sensors elicit, a ratiometric spectral response towards CN\(^-\) ions through simultaneous increase and decrease at two different wavelengths (Figure 2.61, 2.62 and 2.63) and hence provide built-in correction for environmental effects.\(^{18,25}\)
Figure 2.61. (a) Showing changes in UV-vis of (5) (10 μM) in DMSO-HEPES with gradual addition of NaCN (b) CN⁻ titration profile at 403 nm and 504 nm.

Figure 2.62. (a) Showing changes in UV-vis of (7) (10 μM) in DMSO-HEPES with gradual addition of NaCN (b) CN⁻ titration profile at 398 nm and 500 nm.

Figure 2.63. (a) Showing changes in UV-vis of (9) (10 μM) in DMSO-HEPES with gradual addition of NaCN (b) CN⁻ titration profile at 408 nm and 491 nm.

Figure 2.64. Job’s Plot (a) for (5) (b) for (7) and (c) for (9) with CN⁻ ions.

The optimal stoichiometry was determined as 1:1 according to Job’s plot studies (Figure 2.64). Various photophysical parameters are tabulated as Table 2.5.
Table 2.5. Changes in spectroscopic properties of (5), (7) and (9) on interaction with aqueous solution of NaCN.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>$\lambda_{\text{max}}$(nm), $\epsilon_{\text{max}}$ (M$^{-1}$ cm$^{-1}$) before CN$^-$ addition</th>
<th>$\lambda_{\text{max}}$(nm), $\epsilon_{\text{max}}$ (M$^{-1}$ cm$^{-1}$) after CN$^-$ addition</th>
<th>$\Delta\lambda_{\text{max}}$ (nm)</th>
<th>Isobestic point (nm)</th>
<th>Binding Constant (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5)</td>
<td>403, 3.5 $\times$ 10$^4$</td>
<td>504, 4.8 $\times$ 10$^4$</td>
<td>101</td>
<td>444</td>
<td>2.17 $\times$ 10$^4$</td>
</tr>
<tr>
<td>(7)</td>
<td>398, 2.68 $\times$ 10$^4$</td>
<td>500, 3.2 $\times$ 10$^4$</td>
<td>102</td>
<td>430</td>
<td>4.42 $\times$ 10$^4$</td>
</tr>
<tr>
<td>(9)</td>
<td>408, 2.5 $\times$ 10$^4$</td>
<td>491, 2.2 $\times$ 10$^4$</td>
<td>83</td>
<td>440</td>
<td>1.71 $\times$ 10$^4$</td>
</tr>
</tbody>
</table>

The sensors (5), (7) and (9), have some structural similarity with two sensors (i) and (ii), recently reported by Gupta and coworkers.$^{26}$ These sensors, (i) and (ii) have been synthesized by Schiff base condensation of 1,7-Bis(2-formyl phenyl)-1,4,7-trioxahexane and 2,4-dinitrophenylhydrazine. The spectral titrations of (i) and (ii), in DMSO/H$_2$O (95%), showed visual and spectral response towards CN$^-$ as well as towards CH$_3$CO$_2^-$ ions with 1:2 stoichiometry and hence lack selective detection of CN$^-$ in aqueous solution. Whereas, sensors (5), (7) and (9) showed high selectivity towards CN$^-$ ions even in aqueous medium.

![Image of sensors (i) and (ii)]

The mechanism of interaction of anions with sensors is known to depend upon various factors: (i) intrinsic acidity of sensor (ii) polarity of solvent system used (iii) basicity of anions. (iv) H-bonding ability of anions.$^{27}$ The selectivity behavior of (5), (7) and (9) towards CN$^-$ ions in aqueous medium may be due to difference in the basicity and H-bonding tendency of anions towards protic and aprotic solvents.$^{27}$ Because, as far as the ligand acidity is concerned the electron-withdrawing nature of --NO$_2$ groups makes --NH protons highly acidic and facilitates the deprotonation of all these sensors.
In DMSO, which is a good proton acceptor, deprotonation is caused by all the four F\(^{-}\), CN\(^{-}\), CH\(_3\)CO\(_2\)\(^{-}\) and C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) ions. However a better spectral response of sensors (5), (7) and (9) towards F\(^{-}\) and CN\(^{-}\) in comparison to CH\(_3\)CO\(_2\)\(^{-}\) and C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) ions is in accordance to the order of basicity of these ions in DMSO\(^{27}\) give (pKa values: F\(^{-}\) (15)> CN\(^{-}\) (12.9) >CH\(_3\)CO\(_2\)\(^{-}\) (12.3) > C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) (12.1)). On the other hand, in aqueous medium, the order of basicity\(^{27}\) of these four anions is: CN\(^{-}\) > CH\(_3\)CO\(_2\)\(^{-}\) ~ C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) > F\(^{-}\) which is in order of their pKa values: (9.1) > (4.75) > (4.25) > (3.2). Further, the order of hydration energies\(^{28}\) of these ions, F\(^{-}\)(\(\Delta H_{hyd} = -505\) KJ/mol) > CH\(_3\)CO\(_2\)\(^{-}\) (\(\Delta H_{hyd} = -375\) KJ/mol) ~ C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) > CN\(^{-}\) (\(\Delta H_{hyd} = -67\) KJ/mol) also reflects that F\(^{-}\), CH\(_3\)CO\(_2\)\(^{-}\) and C\(_6\)H\(_5\)CO\(_2\)\(^{-}\) ions lose their basicity in aqueous medium due to very high hydration energies and hence showed no spectral or color change for sensors (5), (7) and (9). But, due to low hydration energy and high basicity of CN\(^{-}\) ion in water, it causes the deprotonation of the –NH protons of hydrazone group and leads to the red shift associated with instant color change from light yellow to pink. It is very important to check the selectivity of sensors towards CN\(^{-}\) over other anions, especially F\(^{-}\) and CH\(_3\)CO\(_2\)\(^{-}\), because many reported cyanide sensors suffer from the deleterious interference of these anions.\(^{24a,26}\)

**Figure 2.65.** Competitive Selectivity of (5), (7) and (9) (10µM) towards CN\(^{-}\) ions (10 equiv.) in the presence of 100 equivalents of other anions under study.

The selectivity was checked by performing competitive experiments in DMSO-HEPES. Figure 2.65 showed the spectral changes when (5,7,9) titrated with CN\(^{-}\) in the presence of 100 equivalents of other anions as their sodium salts such as CH\(_3\)CO\(_2\)\(^{-}\), C\(_6\)H\(_5\)CO\(_2\)\(^{-}\), Br\(^{-}\), Cl\(^{-}\), CN\(^{-}\), F\(^{-}\), I\(^{-}\), NO\(_3\)\(^{-}\), H\(_2\)PO\(_4\)\(^{-}\), ClO\(_4\)\(^{-}\), and HSO\(_4\)\(^{-}\) which revealed that CN\(^{-}\) can be detected in the presence of other competitive anions. Reversible nature of sensors (5), (7) and (9) could be established when addition of few drops of 5mM acetic acid restored the original spectra of (5), (7) and (9) due to protonation of CN\(^{-}\) in preference.\(^{28}\)
2.5.2 $^1$H NMR titration experiments of (5), (7) and (9) towards CN$^-$ ions

In order to investigate the sensing mechanism involved in the interaction of (5), (7) and (9) with CN$^-$ ions, NMR titrations were carried out in DMSO-$d_6$, with increasing volumes of tetrabutylammonium cyanide (Figure 2.67, 2.68 and 2.69). In all three cases, signals due to -NH protons disappear completely indicating that response towards CN$^-$ ions is not through H-bonding but through deprotonation of the -NH groups (Figure 2.66). The negative charge brought about by deprotonation caused by CN$^-$ ions leads to an increase in electron density on the phenyl groups in vicinity, through bond propagation, so the aromatic protons and imine (CH=N) protons experience shielding effect and move upfield (Table 2.6). All these shifts in $^1$H NMR spectra for titrations confirmed the deprotonation mechanism for CN$^-$ sensing by (5), (7) and (9).

![Diagram](image.png)

**Figure 2.66.** Proposed mechanism for the interaction of sensor towards CN$^-$ (showing using example of (5)).

**Table 2.6.** Changes in $^1$H NMR shifts of (5), (7) and (9) on interaction with TBACN in DMSO.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Shift in -CH=N- protons ($\delta$)</th>
<th>Shift in aromatic protons ($\delta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before CN$^-$</td>
<td>After CN$^-$</td>
</tr>
<tr>
<td>(5)</td>
<td>8.733</td>
<td>8.524</td>
</tr>
<tr>
<td>(9)</td>
<td>8.672</td>
<td>8.239</td>
</tr>
</tbody>
</table>
Figure 2.67. (a) partial $^1$H NMR of (5) (5 mM) in DMSO-d$_6$ showing changes on addition of TBACN ions (b) Expanded aromatic region showed upward shifts more clearly.

Figure 2.68. (a) partial $^1$H NMR of (7) (5 mM) in DMSO-d$_6$ showing changes on addition of CN$^-$ ions (b) expanded aromatic region showed upward shifts more clearly.

Figure 2.69. (a) partial $^1$H NMR of (9) (5 mM) in DMSO-d$_6$ showing changes on addition of CN$^-$ ions (b) Expanded aromatic region showed upward shifts more clearly.
Another evidence for deprotonation mechanism of CN\(^{-}\) sensing comes from the comparison of \(^1\)H NMR spectra of (5), (7) and (9) with TBACN and TBAF in DMSO-d\(_6\). Figure 2.70 showed that similar changes were observed in both cases, whereas, the triplet formation in case of TBAF at \(\delta\) 16.180, \(\delta\) 16.166 and \(\delta\) 16.176 for (5), (7) and (9) respectively, confirms the presence of HF\(_2^+\) (Figure 2.70, Inset a, b, c) and hence deprotonation mechanism. Consequently, same deprotonation mechanism could be proposed for CN\(^{-}\) sensing also, both in aqueous and non-aqueous media.

**Figure 2.70.** (a) Partial \(^1\)H NMR of (5), (b) (7), (c) (9) (5 mM) in DMSO-d\(_6\) showing changes on addition of CN\(^{-}\) and F\(^{-}\) ions. Inset. Triplet formation due to HF\(_2^+\).

For many practical purposes, the detection limit is an essential standard in sensing. Based on absorption titration profiles, the detection limits of sensors (5), (7) and (9) were determined towards NaCN as 0.66 ppm, 1.3 ppm and 1.6 ppm, respectively in aqueous medium. These detection limit values lie below the highest allowable level of cyanide in drinking water i.e., 1.9 ppm, suggested by health advisory bodies such as World Health Organisation (WHO),\(^{29}\) which indicates that these sensors may be sensitive enough for potential applications.
In order to establish practical applicability of (5), (7) and (9) for the detection of CN⁻ ion, a thin layer chromatography experiment was performed by dropping semi aqueous solution of sensors on TLC strips which on drying gave a light yellow color circle in all the cases. Thereafter, these strips were treated with the aqueous solutions of various sodium salts of anions under study; significant red color change was observed in case of NaCN only for all sensors. From these results, it is clear that (5), (7) and (9) can be used as practical sensors for CN⁻ ions in aqueous medium. The solid state color changes (Figure 2.71) were similar as earlier in the solution state (Figure 2.54).

Figure 2.71. Photographs of TLC plates showing solid state color changes of (A) (5), (B) (7), (C) (9) (10 μM) when treated with aqueous solutions of various anions (5 μM), where (a) = CH₃CO₂⁻ (b) = C₆H₅CO₂⁻ (c) = Br⁻ (d) = Cl⁻ (e) = CN⁻ (f) = F⁻ (g) = I⁻ (h) = H₂PO₄⁻ (i) = NO₃⁻ (j) = ClO₄⁻ (k) = HSO₄⁻.
Conclusions

- A series of three chromo-fluorogenic sensors (1), (2) and (3) for Al(III) was reported which can detect Al(III) up to nanomolar level, with high quantum yields in aqueous medium. Due to the excellent detection limits, these sensors can be used for the detection of trace quantities of Al(III) in biological and environmental samples.

- UV-vis studies of (1), (2) and (3) reveal that these sensors lacked selectivity in chromogenic behaviour. As all the sensors exhibit significant spectral response towards Cu(II), Fe (III) and Al(III) ions.

- Fluorogenic studies show large enhancement in fluorescence band towards Al(III). The detection mechanism involved is CHEF activation due to the formation of rigid aluminium complexes. At the same time, the addition of few transition elements, such as Cu(II), Cr(III), Co(II), Ni(II), Hg(II), Pb(II), Fe(III) causes quenching up to various extents which may be attributed to the energy or charge transfer interactions of these ions with sensors. This difference in sensing mechanisms provide high selectivity of sensors (1), (2) and (3) towards Al(III) among 19 different metal ion studied presently.

- The differential response of sensors towards Al(III) can be distinguished visually under UV light illumination, as well as by dip stick experiment, even at 5 µM of Al(III) without the aid of any sophisticated instrument.

- The results of cell imaging experiments performed for evaluation of potential applications of sensors in imaging Al^{3+} in HeLa and C6 glioma cells, establish the utility of these sensors for tracking Al(III) in live cells.

- Another series of three mesitylene-based chromogenic sensors for selective and sensitive detection of CN^- in aqueous medium which can
detect CN\(^{-}\) below the highest allowable level of cyanide according to WHO, in drinking water.

- The change of selectivity response of sensors, change from organic to aqueous medium is explained through difference in the basicity and H-bonding tendency of anions towards protic and aprotic solvents.

- The detection mechanism involved is deprotonation of \(-\text{NH}\) protons of hydrazone group of sensors confirmed by \(^1\text{H} \text{ NMR}\) and UV-vis spectroscopy. Additionally, practical usability of these sensors is checked by performing thin layer chromatography experiment.
References


