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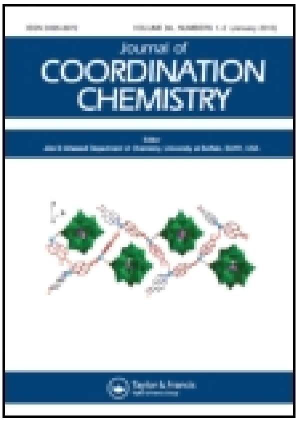

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Synthesis and studies of selective chemosensors for anions and cations by azo-containing salicylaldimine-based receptors

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Synthesis and studies of selective chemosensors for anions and cations by azo-containing salicylaldimine-based receptors

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Three new chromogenic receptors containing electron-withdrawing groups appended to the azophenol moiety were synthesized, characterized, and their chromogenic behavior toward various anions and cations investigated. These chemosensors show visual changes towards biologically relevant anions like F^- and OH^-, and also towards various cations such as Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+}. Yet, other anions such as Cl^-, Br^-, and NO_3^- could not cause any color change. The sensing action was further confirmed by UV–Vis titration. ^{1}H-NMR experiments were carried out to explore the nature of interaction between receptors with F^- and OH^-. The binding constants (K_a) of the host–guest complexes formed were determined.

Keywords: Chemosensor; Transition metal azo complexes; Receptors

1. Introduction

Considerable attention has been focused on recognition and sensing of anions and cations since these play important roles in biology, industry, and catalysis [1]. Generally, pyrrole, urea/thiourea, amine, or phenol act as binding sites for anions [2–4] and cations [5–8]. In particular, sensing of fluoride has attracted growing attention due to unique properties compared to its congeners as a result of high electronegativity, smaller size, and high basicity. Among anions, fluoride is of particular interest owing to its established role in treatment of osteoporosis and analysis of drinking water [9, 10]. Currently available methods for detection of fluoride include spectrophotometry, electrochemistry, and ion chromatography [11–13]. From the pharmacological and environmental importance of fluoride, there is need to develop selective and sensitive methods for fluoride detection in the environment that is not easily served by conventional ion selective electrodes. In this context, colorimetric chemosensors are of particular interest due to their simplicity. Colorimetric chemosensors allow "naked-eye" detection of anions without need to resort to spectroscopic instrumentation [14]. Most colorimetric chemosensors only respond to anions or cations separately [15–18]. Development of chemosensors for detection of transition metal cations is active due to potential applications as diagnostic tools in medical, physiological, and environmental applications [19]; transition metal ions are present as essential elements in biological systems [20].

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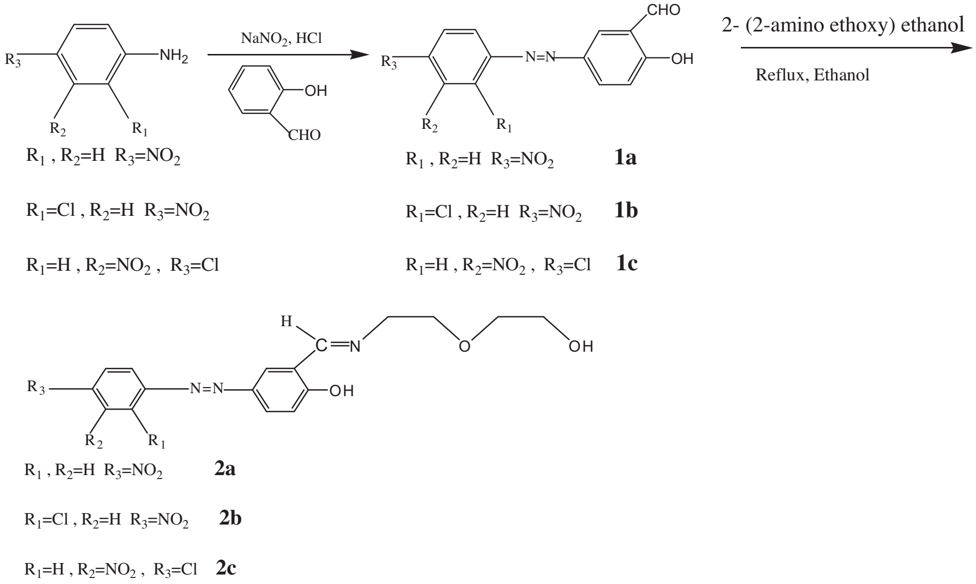
We report for the first time the colorimetric studies of cation and anion sensing by chromogenic receptors **2a**–**2c** possessing a phenolic OH, which binds F^- and OH^- via H–bond interaction or deprotonation, with an electron-withdrawing nitro which acts as a chromogenic signaling unit. The nature of these Schiff bases was altered by the position of nitro in the receptors, which was able to tune anion recognition selectivity. These receptors are selective sensors for Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} in DMSO (Scheme 1).



Scheme 1. Syntheses of receptors (**2a**–**2c**).

2. Experimental

2.1. *Physical measurements*

C, H, and N analyses were undertaken using an elemental analysis system Gmb H Vario EL III. Electronic spectra of receptors in DMSO were recorded on a Perkin-Elmer Lambda 15 spectrophotometer. FT-IR spectra of the compounds as KBr disks were obtained from 4000 to 300 cm^{-1} with a Galaxy series FT-IR 5000 spectrophotometer. Melting points of all newly prepared compounds were determined on an Electrothermal 9200 apparatus. ^{1}H-NMR spectroscopy was performed using a Bruker 300 MHz spectrometer.

2.2. *Materials*

All anions as tetrabutylammonium salts were purchased from Fluka and used without purification. All solvents were of reagent grade quality, purchased from Merck Chemical Company and used as received. The Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} were added as chloride solutions in DMSO.

2.3. *Synthesis*

Azo dyes (**1a**–**1c**) were synthesized according to the well-known literature procedure [21].

2.3.1. Synthesis of the Schiff bases. A mixture of 0.02 M of 2-(2-aminoethoxy) ethanol and 0.02 M of the related azo dye (**1a**–**1c**) was dissolved in 50 mL absolute ethanol with a few drops of glacial acetic acid as a catalyst. The resulting mixture was allowed to stir magnetically under reflux for 1–2 h. The product was filtered under vacuum and washed with a small amount of heated ethanol.

Synthesis of 1-{3-[(1-ethoxy-2-hydroxyethyl-imino)methyl]-4-hydroxyphenylazo}-4-nitro benzene (2a). Brownish red, yield: 63%, m.p.: 174 °C. Anal. Calcd for $C_{17}H_{18}N_4O_5 \cdot 0.5$-$CH_3OH$: C, 56.17; H, 5.34; N, 14.96. Found: C, 56.70; H, 6.17; N, 15.22%. FT-IR (KBr, cm^{-1}): 3110–3650 (–OH group), 3105 (C–H, aromatic), 2874 (C–H, aliphatic), 1651 (C=N), 1614 (C=C, aromatic), 1421 (N=N), 1271 (C–O, phenolic) cm^{-1}. UV–Vis (DMSO, nm): $\lambda_{max} = 260$ (14,150), 407 (16,125), 449 (16,775) nm. ^{1}H-NMR ($CDCl_3$): $\delta = 10$–11 (1H, s), 8.46 (1H, s), 8.39 (2H, d, $J = 9.0$ Hz), 8.03 (2H, dd, $J = 9$ Hz), 7.99 (2H, d, $J = 7$ Hz), 7.97 (1H, d, $J = 2.4$ Hz) 7.0 (1H, d, $J = 9.03$ Hz), 3.87 (4H, m, $J = 3.6$ Hz), 3.77 (2H, t, $J = 3.3$ Hz), 3.64 (2H, t, $J = 4.02$ Hz) ppm.

Synthesis of 1-{3-[(1-ethoxy-2-hydroxethyl-imino)methyl]-4-hydroxyphenylazo}-2-chloro-4-nitrobenzene (2b). Dark brown, yield: 46%, m.p.:185 °C. Anal. Calcd for $C_{17}H_{17}N_4O_5Cl \cdot H_2O$: C, 49.71; H, 4.62; N, 13.63. Found: C, 50.40; H, 4.60; N, 13.87%. FT-IR (KBr, cm^{-1}): 3120–3700 (–OH group), 3115 (C–H, aromatic), 2889 (C–H, aliphatic), 1651 (C=N), 1614 (C=C, aromatic), 1408 (N=N), 1269 (C–O, phenolic) cm^{-1}. UV–Vis (DMSO, nm): $\lambda_{max} = 260$ (13,525), 407 (14,575), 469 (17,375) nm. ^{1}H-NMR ($CDCl_3$): $\delta = 10.2$ (1H, s), 8.44 (1H, s), 8.42 (1H, d, $J = 2.2$ Hz), 8.1 (1H, dd, $J = 8.7$, 2.2 Hz), 8.0 (1H, dd, $J = 9.0$ Hz), 7.9 (1H, d, $J = 2.0$ Hz), 7.7 (1H, d, $J = 8.8$ Hz), 7.0 (1H, d, $J = 9.0$ Hz), 3.86 (4H, m, $J = 4.8$ Hz), 3.78 (2H, t, $J = 4.5$ Hz), 3.65 (2H, t, $J = 4.2$ Hz) ppm.

Synthesis of 1-{3-[(1-ethoxy-2-hydroxyethyl-imino)methyl]-4-hydroxyphenylazo}-4-chloro-3-nitrobenzene (2c). Light brown, yield: 32%, m.p.: 223 °C. Anal. Calcd for $C_{17}H_{17}N_4O_5Cl \cdot$-$CH_3OH$: C, 50.90; H, 4.94; N, 13.18. Found: C, 50.40; H, 3.69; N, 13.89%. FT-IR (KBr, cm^{-1}): 3300–3700 (–OH group), 3200 (C–H, aromatic), 2918 (C–H, aliphatic), 1647 (C=N), 1616 (C=C, aromatic), 1406(N=N), 1288 (C–O, phenolic) cm^{-1}. UV–Vis (DMSO, nm): $\lambda_{max} = 262$ (17,600), 387 (19,575), 430 (18,875) nm. ^{1}H-NMR ($CDCl_3$): $\delta = 10$ (1H, s),8.47 (1H, s), 8.36 (1H, d, $J = 2.2$ Hz), 8.06 (1H, dd, $J = 17.4$, 2.2 Hz), 8.02 (1H, d, $J = 2.3$ Hz), 7.9 (1H, d, $J = 2.3$ Hz), 7.7 (1H, d, $J = 3.6$ Hz), 7.0 (1H, d, $J = 9$ Hz), 3.8 (4H, m, $J = 3.3$ Hz), 3.7 (2H, t, $J = 4.5$ Hz), 3.6 (2H, t, 3.9 Hz) ppm.

3. Results and discussion

3.1. *Anion sensing*

Recognition properties of **2a**–**2c** towards different anions were studied by methods such as naked-eye examination, the ^{1}H-NMR titration, and UV–visible titration.

3.1.1. Colorimetric investigations. In the naked-eye experiments, receptors **2a**–**2c** showed dramatic color changes in the presence of two equivalents of tetrabutylammonium fluoride (5×10^{-4} M in DMSO). Receptor **2a** displays noticeable color change from light pale orange to violet and **2b** and **2c** gave dramatic color changes from yellow to blue and orange, respectively. In addition, **2a**–**2c** could sense OH^- (see Supplementary Material, figure S The receptors **2a**–**2c** did not show color change for Cl^-, Br^-, and $NO_3{}^-$. The receptors bound anions through hydrogen bonds to form stable complexes and the electron density in the supramolecular system was increased to enhance the charge-transfer interactions between the electron-rich and electron-deficient moieties, resulting in a visible color change [22]. Restoration of the original spectrum of the sensor system from this receptor F^- adduct upon addition of a trace amount of water/methanol suggests that complexations between F^- and OH^- with **2a**–**2c** are reversible in nature [23].

Electronic spectra of the receptors showed three transitions in DMSO. The first band at 260 nm was assigned to excitation of the π-electrons of the aromatic system. The second band observed at 387 and 407 nm could be due to the transition between the π-orbital localized on the central bond of the azomethine group (CH = N). The third band located at 449, 469, and 430 nm is due to an intramolecular charge transfer (CT) transition within the whole molecule. This important band in salicylaldimine compounds indicates the presence of a strong intramolecular hydrogen bond between hydroxyl and azomethine nitrogen that causes planarity and facilitates CT bands which are more sensitive to solvent changes than bands resulting from local transitions [24, 25].

3.1.2. ^{1}H-NMR spectral studies. We investigated the interaction of receptors with anions through ^{1}H-NMR studies. In ^{1}H-NMR spectra of the receptors, singlet resonances in the 10–10.3 ppm region can be attributed to protons of phenol. All three receptors exhibit singlets at 8.36–8.46 ppm, which were attributed to imine protons. ^{1}H-NMR spectra were recorded by treating the receptor with F^- and OH^-. The peak at 10.3 ppm corresponding to proton of phenolic OH disappeared on addition of one equivalent of F^- or OH^-. This demonstrates the interaction of anion through the phenolic OH is more effective than interaction through the alcoholic OH groups present in the receptor. Deprotonation of the receptor can occur and we observed such deprotonation with formation of $HF_2{}^-$ in ^{1}H-NMR spectra of **2b** as a new signal at 16 ppm [figure 1(a)]. A similar case can be observed for addition of OH^- as shown in figure 1(b) [26]. The interesting part of the NMR studies is in figure 1(B) trace C. There is a large displacement in the signals. Further ^{1}H-NMR studies are in progress to understand this behavior.

3.1.3. UV–Vis spectroscopic studies. The sensing behavior of **2a**–**2c** towards F^-, Cl^-, Br^-, $NO_3{}^-$, and OH^- was also investigated by UV–Vis spectroscopy. UV–Vis titration was carried out by incremental addition of tetrabutylammonium salts to **2a**–**2c** and the spectra are shown in figure 2. When increasing the concentration of F^- in **2a**–**2c**, a new red shifted absorption at 565, 583, and 503 nm was gradually enhanced, while the intensity of absorptions at 449, 469, and 387 nm decreased correspondingly. Clear isosbestic points were observed at 492, 524, and 403 nm for **2a**–**2c**, respectively. The appearance of a single isosbestic point indicates the presence of only two species, neutral host and its anion in solution. Figure 2(d) shows the gradual decrease and

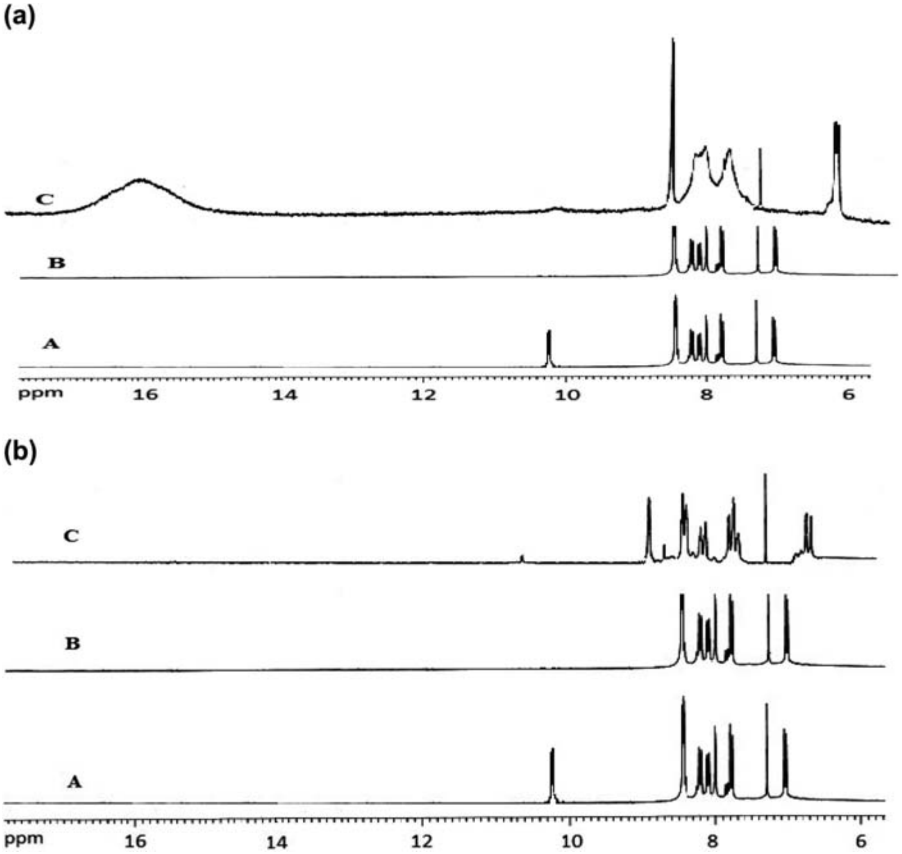


Figure 1. (a) Partial ^{1}H-NMR (300 MHz) spectra of **2b** in $CDCl_3$, (A) in the absence, (B) presence of 1.0 equiv., and (C) presence of 3.0 equiv. of TBAF. (b) Partial ^{1}H-NMR (300 MHz) spectra of **2b** in $CDCl_3$, (A) in the absence, (B) presence of 1.0 equiv., and (C) presence of 3.0 equiv. of TBAOH.

increase in the concentration of these two species with increase in equivalents of tetrabutylammonium fluoride, respectively. Similar changes could be observed during the addition of OH^- (see Supplementary Material, figure S2). The negative charge brought about by anion-induced deprotonation increases the dipole moment and stabilizes the excited state, causing a red shift of **2a**–**2c**. These results demonstrate that complex-formation of **2a**–**2e** with F^- and OH^- takes place via hydrogen bonding electrostatic interactions (table 1). No change was observed with Cl^-, Br^-, and NO_3^- in DMSO [figure 2(e)] (see Supplementary Material, figures S3–S5).

The chromogenic diazo unit is introduced at the 5-position of salicylaldehyde to achieve direct resonance between them [27–33]. As shown in figure 3, the electron-withdrawing nitro group at the end increases not only the degree of π-conjugation but also the hydrogen bond donor ability of the phenolic OH. The strong hydrogen bonding to or deprotonation/protonation of the phenolic moiety might modulate the electronic properties of the chromophore and give rise to large color changes [25].

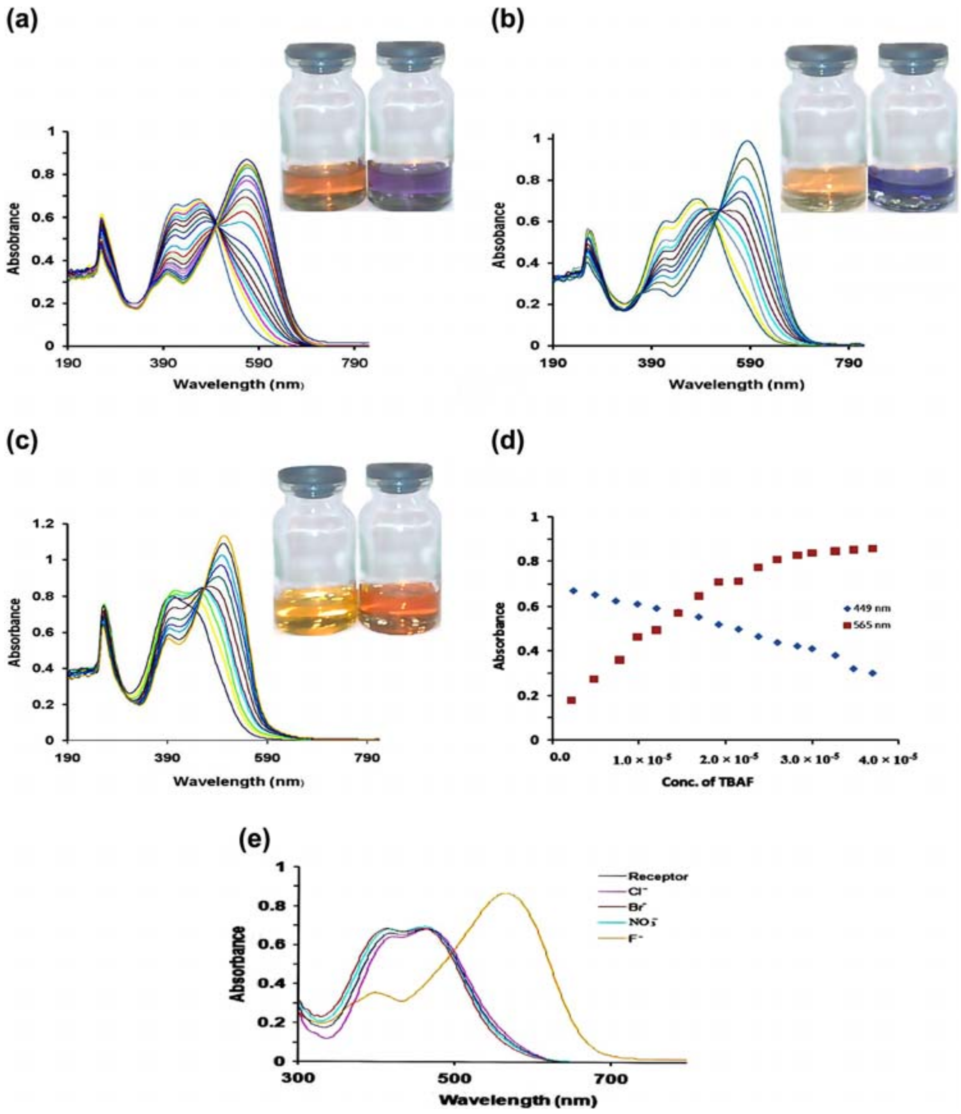


Figure 2. (a–c) UV–Vis spectra of **2a**–**2c** in the course of titration of DMSO solutions of **2a**–**2c** (4×10^{-5} M) with a standard solution of TBAF (c ≈ 5×10^{-4} M) at 25 °C; inset shows the visual color change on addition of F^-. (d) Plot of **2a** absorption vs. concentration of F^- showing decrease and increase in absorption of the 449 nm and 565 nm bands, respectively. (e) UV–Vis absorption spectra of **2a** upon addition of a particular anion salt.

Table 1. Data obtained from the UV–Vis spectra upon titration of **2a**–**2c** with n-$Bu_4N^+F^-$ in DMSO.

	Receptor, λ_{max} (nm)	Complex, λ_{max} (nm)	Bathochromic shift, $\Delta\lambda_{max}$ (nm)	Isosbestic point (nm)	K_a (M^{-1})	Detection limit (M)
2a	449	565	114	492	8.616×10^3	1.64×10^{-6}
2b	469	583	117	524	3.7×10^3	2.19×10^{-6}
2c	387	503	98	403	1.262×10^3	2.55×10^{-6}

3.2. *Calculation of binding constant*

3.2.1. UV–Vis titration. The binding constants of **2a**–**2c** were calculated from the fluoride-induced absorption changes using equation (1) [34],

$$\frac{b}{\Delta A} = \frac{1}{S_t K_a \Delta\varepsilon} \times \frac{1}{[L]} + \frac{1}{S_t \Delta\varepsilon} \tag{1}$$

where ΔA is the change in absorbance, b is path length, S_t is total concentration of substrate, K_a is association constant, and $\Delta\varepsilon$ is the change in molar absorption coefficient. A plot can be made of $1/\Delta A$ as a function of $1/[L]$. The binding constant can be obtained by the ratio of intercept and slope (see Supplementary Material, figures S6 and S7). The detection limits of the sensors toward F^- and OH^- were obtained from UV–Vis titration. The result can also be calculated from the fluoride quantities added, which made the initial changes in absorbance of the sensors during UV–Vis titration [35]. The binding constants and detection limits are summarized in table 1.

3.3. *Cation sensing*

We investigated the recognition ability of **2a**–**2c** (2×10^{-5} M in DMSO) by naked-eye colorimetric experiments for Cu^{2+} (2.5×10^{-3} M), Co^{2+} (8×10^{-3} M), Ni^{2+} (3×10^{-2} M), Mn^{2+} (6×10^{-2} M), and Zn^{2+} (6×10^{-2} M) (for color change of **2a**–**2c**, see Supplementary Material, figures S8–S10).

The sensing behavior of **2a**–**2c** towards Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} was monitored by the spectrophotometric method in DMSO. A solution of **2a**–**2c** was titrated with increasing volume of concentrated solution of a given cation. On incremental addition of Cu^{2+} to **2a**, the peak at 260 nm gradually increased with a red shift and a new band arose at 885 nm. On further addition of Co^{2+} to **2a** a new band was formed at 623, 685 nm. On gradual addition of Ni^{2+} to **2a**, a new absorption band at 805 nm appeared. On successive addition of Mn^{2+} and Zn^{2+}, the spectrum of **2a** shifted from 460 to 470 and 447 nm. Similar changes were observed for **2b** and **2c**. The insert plot in the UV–Vis spectrum shows the variation of absorbance at a particular wavelength vs. equivalents of the corresponding ion added (for titration spectrum, see Supplementary Material, figures S11–S13).

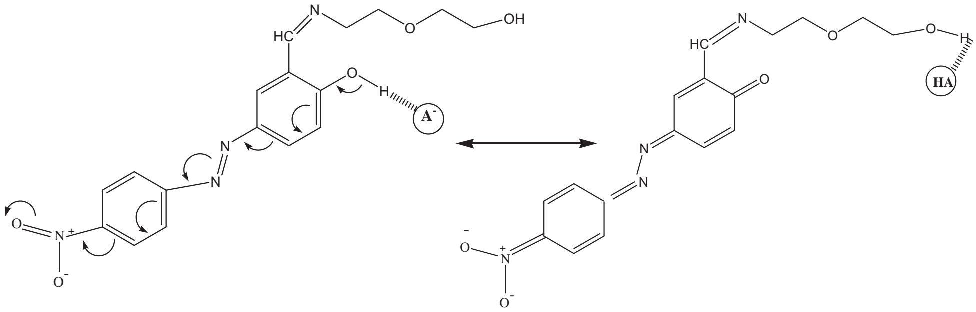


Figure 3. Resonance structures of phenolic OH and chromogenic diazo scaffold.

3.4. *Sensing in the presence of competing ions*

Since **2a**–**2c** sense both transition metal ions and F^- and OH^-, we examined the sensing ability in the presence of competing ions. These experiments were carried out in two different manners. First, the receptors were treated with two equivalents of anions and to this, successive addition of cations (20–200 µl) was done; in another set of experiments, the receptors were first treated with two equivalents of cations and to that 20–200 µl of anion was added incrementally. For this dual-sensing experiment, Cu^{2+} and F^- were chosen. The spectral changes are shown in figures S14–S16 (see Supplementary Material). When **2a** was treated with two equivalents of Cu^{2+}, a new band was observed at 805 nm which might be due to the d–d band of **2a**·Cu^{2+} complex. Addition of F^- (0.5–2 equiv.) ions to the above (2a·Cu^{2+}) solution leads to reduction in intensity of the band at 805 nm, possibly due to formation of a Cu:F ion pair ion in solution. In the second experiment, the titration was carried out in the presence of two equivalents of F^- to which Cu^{2+} was added incrementally. When **2a** was added to two equivalents of F^-, the intensity of the band at 449 nm decreased and a new band at 565 nm indicated formation of 2a·F^- complex. Upon successive addition of Cu^{2+} the band at 565 nm gradually decreased, suggesting sequestering of the F^- to form an ion pair with Cu^{2+} in solution. Intensity of the d–d band increased slightly, possibly due to interaction between **2a** and Cu^{2+} ions.

In another experiment, **2a** was titrated with a mixture of F^- and Cu^{2+} (using 15, 30, 45 and 60 equiv.). When the mixture of F^- with Cu^{2+} was introduced to the DMSO solution of **2a**, the intensity of the band at 260 nm increased and enhancement of the d–d band occurred, due to formation of a Cu^{2+} complex with **2a**. The formation of the band at 565 nm was not observed in this case because F^- in solution engaged the metal ions in ion-pair formation rather than in complexation with these receptors. Similar changes were observed for **2b** and **2c** (see Supplementary Material, figures S17–S22). We repeated similar experiments with F^- and Co^{2+} showing the receptors **2a**–**2c** can be utilized for the detection of Co^{2+} in the presence of competing F^- (mixture of F^- and Co^{2+}) in DMSO by change in absorption spectra.

4. Conclusion

Receptors **2a**–**2c** are prepared via diazo coupling and Schiff base condensation, and possess the ability to sense both anions and cations colorimetrically. These Schiff base receptors form metal complexes and sense anions through hydrogen bonding interaction through the phenolic OH. In **2a**, the presence of the electron-withdrawing NO_2 enhances hydrogen bonding and exhibits significant difference in anion binding by providing colorimetric spectral responses. These receptors sense Cu^{2+} selectively in the presence of F^-, confirmed by the absorbance spectra of the competing studies.

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