Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 149 (2015) 869–874

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Amido-Schiff base derivatives as colorimetric fluoride sensor: Effect of nitro substitution on the sensitivity and color change

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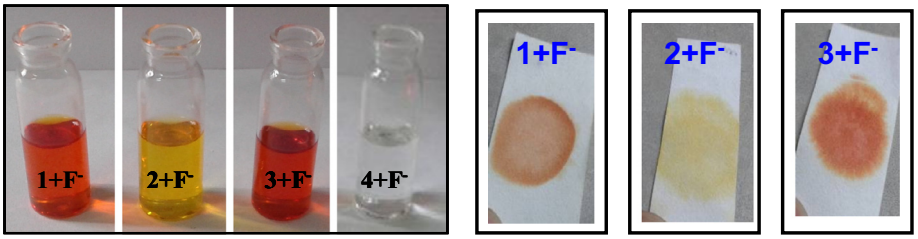
HIGHLIGHTS

- Synthesized colorimetric sensors for detection of biologically important anions.
- Color change of the sensor in presence of F^- was detected by naked-eye.
- Used of sensors as test kit for the detection of fluoride ion.
- Nitro group positional dependency on sensitivity and color change of the sensors.

GRAPHICAL ABSTRACT

Amido-Schiff base derivatives (**1**, **2** and **3**) can detect fluoride ion selectively through visual color change in both solution phase and solid phase.



ARTICLE INFO

Article history:
Received 27 September 2014
Received in revised form 1 April 2015
Accepted 16 April 2015
Available online 2 May 2015

Keywords:
Colorimetric sensing
Fluoride detection
Amido-Schiff base
Nitro-substitution
Test kits

ABSTRACT

A series of Schiff bases synthesized by the condensation of benzohydrazide and $-NO_2$ substituted benzaldehyde have been used as selective fluoride ion sensor. Test paper coated with these synthetic Schiff bases (test kits) can detect fluoride ion selectively with a drastic color change and detection can be achieved by just using the naked-eye without the help of any optical instrument. Interestingly, the position of $-NO_2$ group in the amido Schiff bases has an effect on the sensitivity as well as on the change of color of species.

Introduction

In the past two decades there have been a great deal of works focused on the design and synthesis of artificial receptors for selective detection of anions because of their crucial role in the biological, medical and environmental sciences. In particular, development of chemosensors for fluoride ion are quite intriguing due to its beneficial effects in human physiology [1–6]. Moreover, fluoride ion acts as an active constituent in most of the drug molecules and is used for the treatment of hypnotics, anesthetics, psychiatric drugs and cockroach poisons [7–8]. Because of significant importances, detection of fluoride with a easily synthesized receptor and minimal instrumental assistance is highly desirable towards practical applications. For this purpose colorimetric chemosensor is of great interest in recent years [9–11]. In general, a colorimetric chemosensor is composed of two main fragments, (1) a binding sites that interact with anions and (2) a signaling part connected to the binding site which can show the color changes in the anion recognition phenomenon [12–14].

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http://dx.doi.org/10.1016/j.saa.2015.04.025
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With regard to the above facts, the receptors with a combination of different types of anion-binding groups (e.g. amide, urea/thiourea, pyrrole and imidazolium groups) and nitro-phenyl as the signaling unit have been explored [15–19]. However, most of them suffer from interference of other anions. Specifically, discrimination of fluoride from $H_2PO_4^-$ and AcO^- with similar basicity is rather problematic [20–22]. Furthermore, these receptors are commonly used in solution which significantly restrict their uses. It is still a challenge to design and synthesize artificial receptor with high selectivity and sensitivity for fluoride ion both in solid and solution phase. Inspite of these limitations, detection of fluoride in solid state using test kit have attracted increasing attention because of their practical application. Till now, the reports on test kit for the detection of fluoride are limited [23–25].

To overcome this problem we have synthesized and characterized a series of simple benzohydrazide derived Schiff bases as molecular receptor. These newly designed molecules selectively detect fluoride ion by naked-eye as well as by change in the optical signal monitored by UV–Vis spectroscopy. Interestingly, test paper coated with the above amido-Schiff base (test kits) can recognize F^- ion visually. Here we have shown that the color change and sensitivity of the sensors depend on the position of nitro-group in the signaling unit.

Experimental

Instrumentation

Electronic absorption spectra were recorded by a Hitachi UV–Vis (Model U-3501) spectrophotometer. IR spectra (KBr pellet, 4000–400 cm^{-1}) were recorded on a Parkin Elmer modal 782 infrared spectrophotometer (resolution 4 cm^{-1}). ^1H NMR spectra were recorded on a Bruker, Avance 300 spectrometer, where chemical shifts (δ in ppm) were determined with respect to tetramethylsilane (TMS) as internal standards.

Reagents

All reagents and solvents were used as received from commercial sources without further purification. Benzoic acid, hydrazine and *o*/*m*/*p* substituted benzaldehyde were purchased from Sigma Aldrich Chemicals. Spectroscopic grade solvents were purchased from Spectrochem and were used after proper distillation. The anions, tetrabutylammonium fluoride ($Bu_4\ N^+F^-$) hydrate (98%), tetrabutylammonium acetate ($Bu_4N^+AcO^-$) (97%), tetrabutylammonium dihydrogenphosphate ($Bu_4N^+H_2PO_4^-$) (97%), tetrabutylammonium chloride ($Bu_4N^+Cl^-$) hydrate (98%), tetrabutylammonium bromide ($Bu_4N^+Br^-$) (98%), tetrabutylammonium bromide ($Bu_4N^+I^-$) (98%) tetrabutylammonium sulfite ($Bu_4N^+HSO_3^-$) (97%), and tetrabutylammonium Nitrate ($Bu_4N^+NO_3^-$) (97%), were received from Sigma–Aldrich Chemical Company Pvt. Ltd.

Synthesis

Benzoic acid hydrazide

The benzoic acid hydrazides were prepared according to the literature procedure [26,27] by refluxing ethyl benzoate (0.1 mol) with hydrazine hydrate (0.15 mol) in presence of ethanol for 12 h then the reaction mixture was allow to cool. The solid product was collected by filtration. The crude product was crystallized from ethanol.

*Receptor **1**. Synthesis of N-(2-nitrobenzylidene)benzohydrazide*

Benzoic acid hydrazide (0.200 g, 1.47 mmol) was reacted with *o*-nitrobenzaldehyde (0.300 g, 2.0 mmol) in methanol at room temperature. After 30 min very light yellow solid was obtained. The product thus obtained was filtered and then dried under vacuum (yield: 0.320 g, 76%). ^1H NMR in d_6-DMSO, 300 MHz, δ (ppm): 12.21 (s, 1H, –CONH–), 8.86 (s, 1H, —CH═N—), 8.12 (d, J = 7.5 Hz,1H), 8.06 (d, J = 8.2 Hz,1H), 7.92 (d, J = 7.2 Hz,2H), 7.81 (t, J = 7.5 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.52–7.42 (m, 3H), ^{13}C NMR (75.5 MHz, d_6-DMSO, 20 °C) δ (ppm): 124.78, 127.21, 128.15, 128.76, 130.91, 131.29, 133.43, 133.87, 143.61, 148.39, 162.88. IR (KBr): 3158.5, 3016.6, 2848.6, 1652.4, 1646.1, 1566.9, 1526.0, 1344.8, 1306.1, 1293.8, 1152.4, 1074.4 cm^{-1}.

*Receptor **2**. Sythesis of N-(3-nitrobenzylidene)benzohydrazide*

Receptor **2** was prepared by similar procedure as was done in case of compound **1**. Here *m*-nitrobenzaldehyde is used instead of *o*-nitrobenzaldehyde (yield: 70%). ^1H NMR in d_6-DMSO, 300 MHz, δ (ppm): 12.12 (s, 1H, –CONH–), 8.54 (s, 2H, —CH═N—, *o*-ArH-NO_2), 8.25 (d, J = 7.5 Hz, 1H), 8.14 (d, J = 7.5 Hz,1H), 7.91 (d, J = 7.2 Hz, 2H), 7.74 (t, J = 7.5 Hz, 1H), 7.62–7.50 (m, 3H), ^{13}C NMR (75.5 MHz, d_6-DMSO, 20 °C) δ (ppm): 124.28, 127.21, 128.05, 128.66, 131.11, 131.89, 134.13, 134.87, 143.41, 148.31, 161.78. IR (KBr): 3207.6, 3071.1, 1654.4, 1650, 1560.2, 1517.2, 1353.4, 1280.0, 1137.3, 1075.1 cm^{-1}.

*Receptor **3**. Synthesis of N-(4-nitrobenzylidene)benzohydrazide*

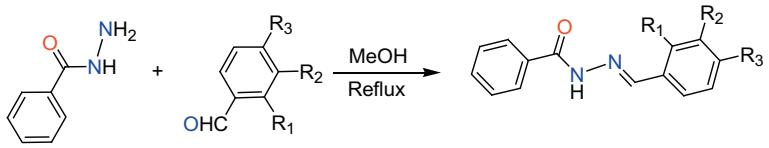
Using similar procedure receptor **3** was prepared by using *p*-nitrobenzaldehyde (yield: 80%). ^1H NMR in d_6-DMSO, 300 MHz, δ (ppm): 12.16 (s, 1H, –CONH–), 8.52 (s, 1H, —CH═N—), 8.28 (d, J = 8.1 Hz, 2H), 7.98 (d, J = 8.1 Hz, 2H), 7.91 (d, J = 7.5 Hz, 2H), 7.61–7.51 (m, 3H). ^{13}C NMR (75.5 MHz, d_6-DMSO, 20 °C) δ (ppm): 124.58, 127.41, 128.54, 129.16, 130.81, 131.29, 132.83, 133.67, 142.91, 148.09, 161.88. IR (KBr): 3176.7, 3017.9, 2836.6, 1654.1, 1647.5, 1569.8, 1522.7, 1343.3, 1305.9, 1150.7, 1104.1 cm^{-1}.

*Receptor **4**. Sythesis of N-benzylidenebenzohydrazide*

Using similar procedure benzaldehyde is used for the preparation of receptor **4**. (yield: 74%). ^1H NMR in d_6-DMSO, 300 MHz, δ (ppm): 11.86 (s, 1H, –CONH–), 8.44 (s, 1H, —CH═N—), 7.90 (d, J = 7.2 Hz, 2H), 7.72 (d, J = 5.1 Hz, 2H), 7.58–7.44 (m, 6H); ^{13}C NMR (75.5 MHz, d_6-DMSO, 20 °C) δ (ppm): 127.32, 127.82, 128.71, 129.07, 130.32, 131.99, 133.63, 134.52, 148.08, 164.05. IR (KBr): 3180.98, 3059.9, 3028.50, 2836.68, 1640.51, 1600.60, 1577.28, 1551.95, 1486.50, 1446.28, 1363.80, 1140.92, 1057.82 cm^{-1}.

Results and discussion

As shown in Scheme 1, Schiff bases (**1, 2, 3** and **4**) have been synthesized following two steps. Firstly, benzohydrazide has been synthesized from the ester of benzoic acid, then condensation with four different aldehydes (*o*-nitro benzaldehyde, *m*-nitro benzaldehyde, *p*-nitro benzaldehyde and benzaldehyde) produced the corresponding Schiff bases. All these Schiff bases (**1, 2, 3** and **4**) have been characterized by ^1H, ^{13}C NMR and IR spectroscopic methods. The ^1H NMR spectra of receptors **1**, **2**, **3** and **4** show two characteristic peaks in the range of 11.85–12.21 ppm and 8.44–8.86 ppm which are attributed to the protons of amido and imino groups, respectively (Figs. S1–S4). In case of compound **2** the peak at 8.54 ppm of —CH═N— is merged with the ortho hydrogen of the m-nitro substituted aromatic ring. The presence of amide (–CO–) and imino groups (—CH═N—) in the molecules is confirmed by the vibrations at 1654 and 1647 cm^{-1}, respectively, in the FT-IR spectrum [23].



Receptors	R_1	R_2	R_3
1	$-NO_2$	–H	–H
2	–H	$-NO_2$	–H
3	–H	–H	$-NO_2$
4	–H	–H	–H

Scheme 1. Syntheses of receptor **1**, **2**, **3** and **4**.

Naked-eye detection of anions

The anion binding study of the receptors (10^{-5} M) have been investigated by naked eye in acetonitrile solvent. In this experiment, visual inspection of receptors **1** and **3** show a vivid color change from colorless to red upon addition of F^- ion (10^{-5} M) to their acetonitrile solution (Figs. 1 and S5). But in the same experimental condition receptor **2** shows a color change from colorless to yellow (Fig. S6). The color of the solutions are intensified with increasing F^- ion concentration. It is to mention that the color intensity between compound **1** and compound **3** cannot be distinguished visually by addition of F^- ion. On the contrary, other anions such as CH_3COO^-, $H_2PO_4^-$, Cl^-, Br^-, I^-, HSO_3^- and NO_3^- do not respond under the similar experimental condition. That means the interaction of these anions with the receptors is too small to show any observable change. As expected the compound **4** does not show any color change in presence of any of the anions mentioned above (Fig. S7). Therefore, among the four receptors only **1**, **2** and **3** can detect F^- selectively (Fig. S8).

UV–Vis spectroscopic titration

In order to understand the interaction between the anions and the receptors properly, UV–Vis experiment has been monitored in acetonitrile solvent. Receptor **1** (*N-(2-nitrobenzylidene)benzohydrazide*) exhibits a strong absorption band at 274 nm and a shoulder at 343 nm (Fig. 2). Upon adding increasing amount of F^- ion to **1** (1.0×10^{-6} M) in CH_3CN solution, the peak at 274 nm gradually decreases, shoulder band slightly is shifted to red with increasing intensity and a new broad absorption peak at 440 nm appears accompanied by a visual color change from colorless to red color solution. Spectral change clearly indicates interaction of receptor with fluoride ion and the obvious choice is the formation of complex between the receptor and the fluoride ion indicated by the presence of a distinct isobestic point at 317 nm. In case of receptor **3** (*N-(4-nitrobenzylidene)benzohydrazide*) the UV–Vis spectral pattern is slightly different as was observed in case of receptor **1.** No shoulder band was observed in case of receptor **3**. Fig. 3 shows the change in UV–Vis spectra of receptor **3** (1.0×10^{-6} M) during titration with F^- ion. Upon addition of F^- ion the peak of the receptors at 330 nm decreases its intensity and a new peak at 466 nm appears with an isosbestic point at 370 nm exhibiting a color change from colorless to red. Interestingly, the band position of the fluoride complex of **3** is in the red side than the fluoride complex of **1** which indicates more stabilization of the system. During complexation steric crowding in the ortho substituted nitro compound (receptor **1**) may insist the nitro group to be twisted form thereby reducing the resonance stabilization of the complex leading to blue sided absorption band. It is important to mention that the UV–Vis response of receptor **1** and **3** is different on addition of F^- ion in contrast with the "naked eye" experiment which cannot distinguish between receptor **1** and **3** in presence of F^- ion.

In absence of anions, receptor **2** (*N-(3-nitrobenzylidene)benzohydrazide*, 1.0×10^{-5} M) exhibits a strong band at 290 nm. On addition of F^- ion to receptor **2** the absorbance at 290 nm decreases and a new absorbance band appears at 365 nm with a distinct isosbestic point at 370 nm (Fig. 4) showing a visual color change from colorless to yellow. The band position of fluoride complex of receptor **2** compared to the band position of fluoride complex of compound **1** and **3** indicates that complex of receptor **2** is least stable**.** Weaker acidity of the amide proton may be the reason.

In UV–Vis spectra receptor **4** **(***N-benzylidenebenzohydrazide*) shows a strong peak at 290 nm (Fig. S12). With the addition of increasing amount of F^- ion no spectral modification has been observed which indicates there is no interaction between the receptor **4** and fluoride ion. Under similar experimental condition, on addition of other anions such as AcO^-, $H_2PO_4^-$, Cl^-, Br^-, I^-, HSO_3^- and NO_3^- no noticeable spectral changes were observed with **1**, **2**,**3** and **4** indicating no interaction or complexation of these anions with the above the compounds (Figs. S9–S12). The appreciable spectral changes of **1**, **2** and **3** in presence of fluoride also indicate that it can detect fluoride ion selectively (Figs. 5 and S13, S14).

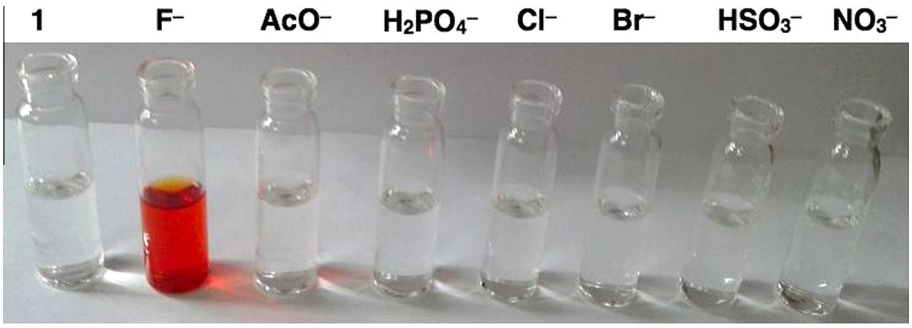


Fig. 1. Naked-eye color change of receptor **1** (1.0×10^{-5} M) after addition of 2 equivalent of various anions in CH_3CN.

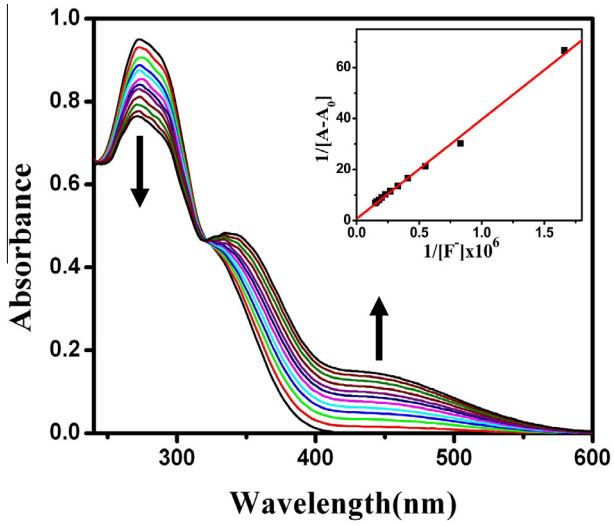


Fig. 2. UV–Vis spectral changes of receptor **1** (1.0×10^{-6} M) upon addition of F^- ion (0–2 equiv.) in CH_3CN. Inset: B–H plot for the titration of **1** with F^- ion.

Determination of stoichiometries and association constants

With the help of UV–Vis titration the stoichiometry and the association constant between the compounds and fluoride has been calculated. The binding constants (K) of all the three compounds (**1**, **2** and **3**) and F^- complexes have been determined by using Benesi–Hildebrand (B–H) relation [28,29]. The complexation between the receptors and F^- ion takes place following the given complexation equilibrium

$$L + mX^{n-} \leftrightarrow (X_mL)^{mn-}$$

$$K = \frac{[(X_mL)^{mn-}]}{[L][X^{n-}]^m}$$

Benesi–Hildebrand relation with $m = 1$ for 1:1 complex can be expressed in terms of optical density (A) as follow

$$A = \frac{A_0 + A_1K[X^{n-}]}{1 + K[X^{n-}]}$$

$$\text{Or, } \frac{1}{(A - A_0)} = \frac{1}{(A_1 - A_0)} + \frac{1}{(A_1 - A_0)K[X^{n-}]}$$

where $[X^{n-}]$, $[L]$, and $[(X_mL)^{mn-}]$ are the concentration of fluoride added, compounds and the complex between fluoride and the compounds, respectively. A_0, A and A_1 indicate optical density or absorbance at a particular wavelength of the compounds without fluoride, absorbance after adding fluoride at every successive step and excess amount of anion added, respectively. The terms K, m and n are binding constant or association constant of the complex, moles of fluoride and charge of fluoride, respectively. The binding constant or association constant K (M^{-1} or M^{-2}) is determined from the ratio of intercept and slope of Benesi–Hildebrand plot of the optical density. As shown in Fig. 2 (inset), the Benesi–Hildebrand (B–H) plot of $1/[A - A_0]$ vs $1/[F^-]$ for the titration of **1** and F^- ion provides a straight line, indicating 1:1 complex formation with association constant (K) 1.8×10^4 M^{-1}. Receptor **2** and **3** can also form 1:1 adduct with fluoride having association constants 1.62×10^3 and 1.24×10^4 respectively (Figs. 3 and 4 (inset)). The association constant values of all the three fluoride complexes clearly indicate that the stability of the complex of **1** and **3** is comparable and higher than the complex of compound **2**.

^{1}H NMR titration spectra

To further look into the binding nature of the receptors and anions, ^{1}H NMR titration experiments have been performed in d_6-DMSO. ^{1}H NMR titration experiment of **1** shows (Fig. 6) that the proton H_a at δ 12.11 ppm corresponding to –NH peak of receptor **1** disappears on adding TBAF (1 equiv.) as a result of fast proton exchange. The signal at δ 8.05 for H_d corresponding to nitro phenyl group is slightly shifted to upfield. On further addition of 2 equiv. of F ions the H_d proton is found to merge with the H_e and H_f proton of the phenyl ring. At 5 equivalent of F^- ions the H_d proton is further shifted to upfield direction and then is separated from the H_e and H_f proton. As the concentration of F^- ion has been increased from 1 equiv. to 5 equiv., the signal at δ 8.78 corresponding to imine proton H_b is slowly upfielded owing to the formation of exocyclic double bond during detection process. The upfield shift of aromatic protons may be explained in terms of deprotonation of the amide proton which leads to increase in electron density over the receptor and as a consequence upfield shift is observed.

In case of receptor **3** the –NH peak at δ 12.17 vanishes with the addition of TBAF (1 equiv.) during ^{1}H NMR titration (Fig. 7). In the same time a slight upfield shift of H_d proton (δ 7.99) of nitro phenyl group is observed. As a result the peak of H_d and H_e proton merge together at δ 7.91 ppm. On further addition of TBAF (2 equiv.), the H_d proton is shifted again in the upfield direction. Finally, on

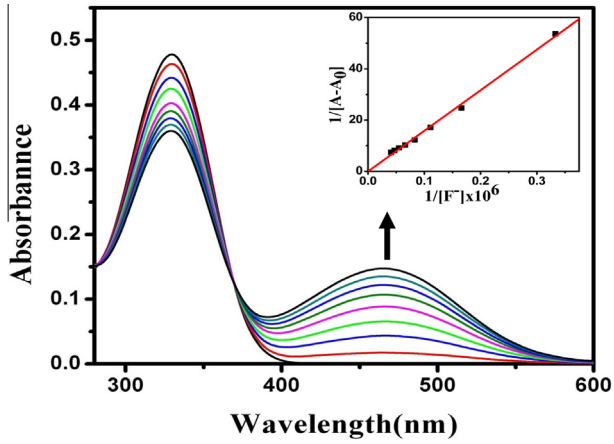


Fig. 3. UV–Vis spectral changes of receptor **3** (1.0×10^{-6} M) upon addition of F^- ion (0–2 equiv.) in CH_3CN. Inset: B–H plot for the titration of **3** with F^- ion.

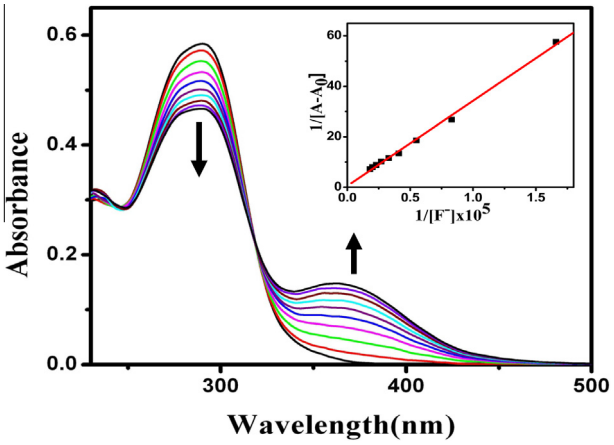


Fig. 4. UV–Vis spectral changes of receptor **2** (1.0×10^{-5} M) upon addition of F^- ion (0–2 equiv.) in CH_3CN. (Inset) B–H plot for the titration of **2** with F^- ion.

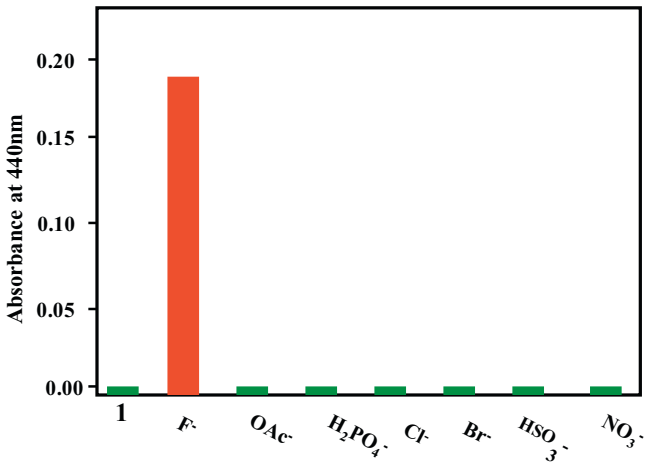


Fig. 5. Change in absorbance (λ at 440 nm) of **1** (10^{-6} M) upon addition of different anions in acetonitrile solvent.

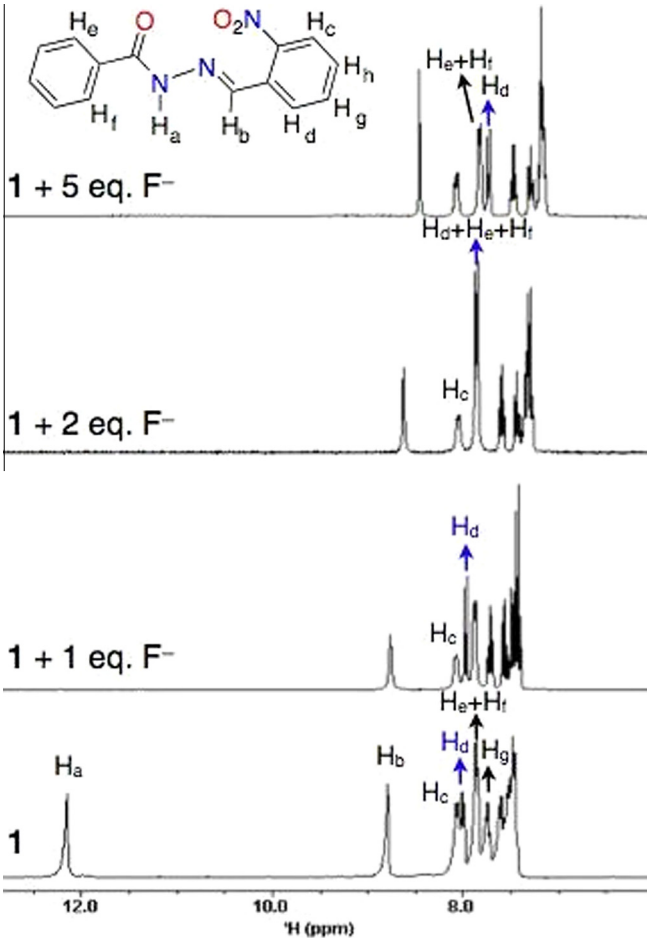


Fig. 6. Partial ^{1}H NMR spectra of compound **1** in DMSO-d_6 after addition of 0–5 equivalent of fluoride ion.

addition of 5 equiv of TBAF, the H_d proton appears prominently on the right side of H_e proton.

Now the ^{1}H NMR titration of compound **2** shows a slight different result. For compound **2** both the imine proton (H_b) and aromatic proton H_c appear as singlet at δ 8.55 ppm. When one equivalent of TBAF is added to the solution of compound **2** the –NH peak at δ 12.12 does not vinish completely and there is no significant shifts of the

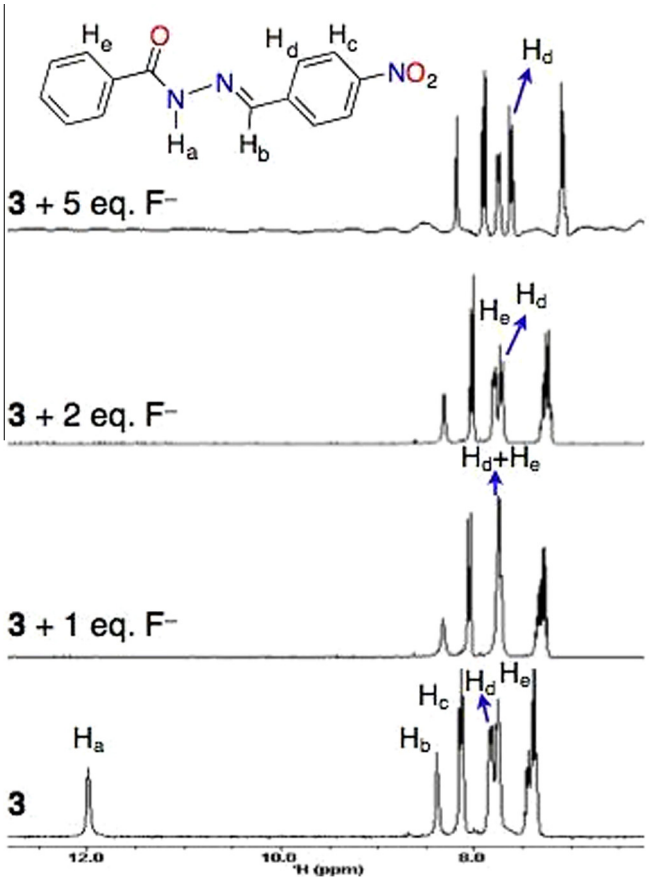


Fig. 7. Partial ^{1}H NMR spectra of compound **3** in DMSO-d_6 during addition of 0–5 equivalent of fluoride ion.

aromatic protons. However on addition of 5 equivalent of TBAF the H_c proton is shifted to upfield and separated from the H_b proton (Fig. S15).

^{1}H NMR titration experiments of **4** shows that the proton H_a at δ 11.86 ppm corresponding to –NH of receptor **4** is present till the addition of 1 equivalent of TBAF. When excess amount of fluoride is added –NH peak disappears with no observable shift of the aromatic protons.

Analyzing the results of ^{1}H NMR titration and UV–Vis titration the binding mechanism of receptors can be proposed by considering the detection process as a two step process. Initially, hydrogen bonding occurs between the –NH– proton and F^- ion to form 1:1 adduct and then –NH– proton gets deprotonated resulting in increase of electron density over the receptor. As a result charge separation is introduced in the receptor which causes intramolecular charge transfer (ICT) between the electron deficient $-NO_2$ group and electron rich –N to show the UV–Vis and colorimetric changes [30–32].

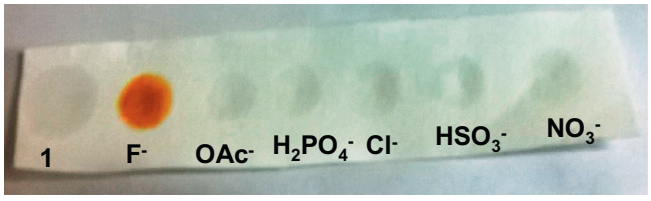


Fig. 8. Color change of test paper containing **1** (10^{-4} M) in presence of different anions.

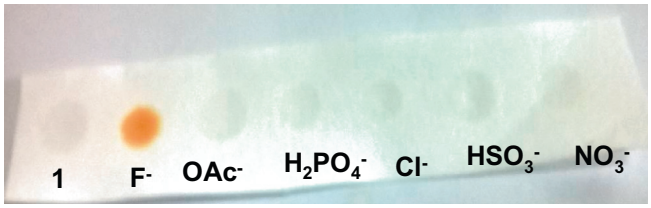


Fig. 9. Color change of test paper containing **2** (10^{-4} M) in presence of different anions.

In case of receptor **1** and **3** the electron deficient $-NO_2$ group is in ortho and para position of the phenyl ring, respectively. As a result better charge separation occurs which influences the ICT process resulting a color change from colorless to deep red (Schemes S1 and S3). In contrast, the receptor **2** possess $-NO_2$ group in meta position which has less influence on the ICT process and shows yellow color (Scheme S2). ICT process does not occur in receptor **4** due to the absence of $-NO_2$ group in the phenyl unit. As a result no color change has been observed.

Visual color changes on test papers

On the basis of the above observation, we have investigated the potential practical applications of **1** as an anion sensor. To explore this, we have prepared test kits using Whatman-40 test paper coated with CH_3CN solution of **1** (1×10^{-4} M) and then dried in air (test kits). For the detection of anion, the acetonitrile solution of anions (1×10^{-4} M) has been dropped onto the test paper and dried in hot air. Interestingly, only F^- ion exhibits a bright red color (Fig. 8). Other anions such as AcO^-, $H_2PO_4^-$, Cl^-, Br^-, I^-, HSO_3^-, and NO_3^- do not show any naked eye detectable color changes.

Under similar experimental condition test paper coated with compound **2** exhibites yellow color (Fig. 9), but compound **3** coated test paper (test kits) exhibites bright red color in presence of F^- ion (Fig. S16). In contrast, receptor **4** cannot be used as test kit for F^- ion. This experiment support that compounds **1–3** have the capability to detect F^- ion in solid state.

Conclusion

In summary, we have demonstrated that benzohydrazide base Schiff base **1–3** having electron deficient $-NO_2$ group in different position (ortho, meta and para) can selectively detect F^- ion by changing color, which can be visible by "naked-eye" without any spectroscopic instrumentation both in solution and solid supported materials (test kits). This easy to prepare, cheap test kit would be advantageous to detect F^- ion in economically poor and underdeveloped regions in the world.

Acknowledgment

S.G. & A.G. would like to acknowledge UGC and CSIR for Fellowship. M.A.A. thanks DST, Govt. of West Bengal, India (Project No. 376 (sanc.)/ST/P/S&T/9G-16/2013) for support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.04.025.

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