Electronic Supporting Information

Recognition of fluoride anion at low ppm level inside living cell and from fluorosis affected tooth and saliva samples†

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1. Experimental

Materials

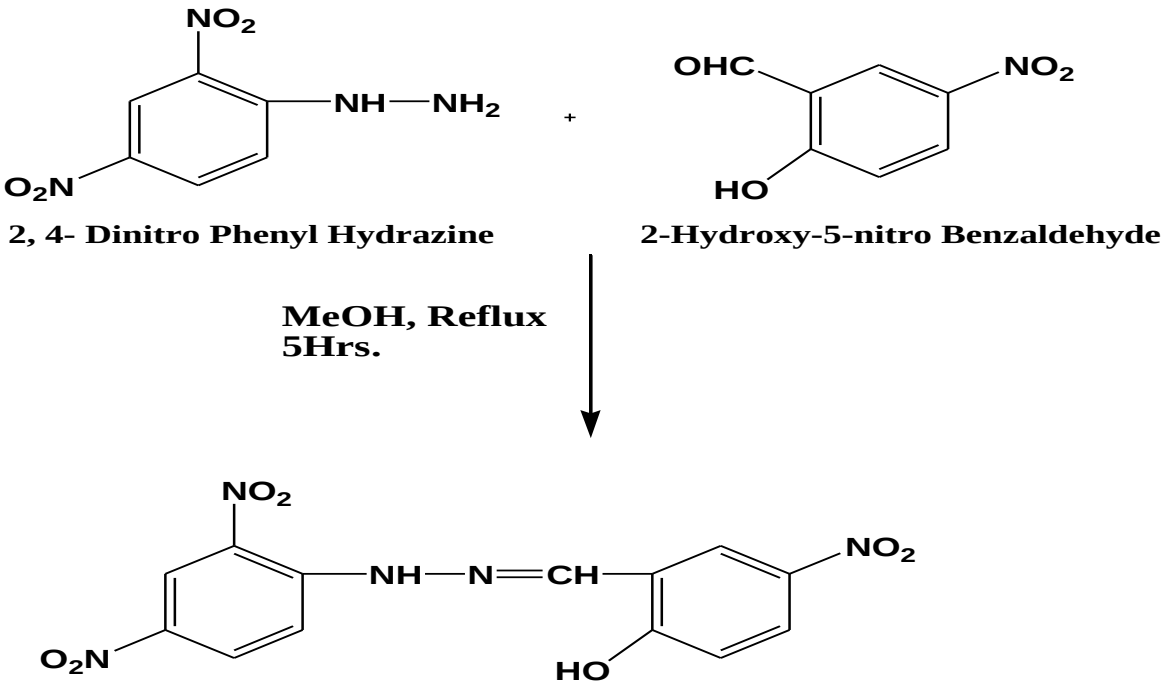
All the starting materials (reagents, solvents) were commercially available and all reagents and solvents purchased were of analytical grade and used without further purification. 5-nitro-2-hydroxy benzaldehyde was purchased from Sigma-Aldrich and 2, 4- dinitro phenyl hydrazine was from HIMEDIA chemical company. All tetrabutyl ammonium anionic salts are purchased from Sigma Aldrich company. Solvents like Methanol, DMSO, Acetonitrile, Water were of spectroscopic grade and purchased from Merck India Pvt. Ltd. and used without any further purification. To prepare test kit Whatman Cellulose paper was used.

Instrumentation

A Perkin Elmer 2400C elemental analyser was used to collect the microanalytical (C, H. N) data. UV-Vis spectroscopy was done on a ALS-SEC2000 and CARY 60 spectrophotometer. IR spectra were carried out in a Perkin Elmer FT-IR spectrometer (spectrum 65) (Using KBr pellets). ESI-MS mass spectra were recorded on an Advion made compact mass spectrometer (serial no. 3013-0140). Fluorescence measurement were done with a Perkin Elmer LS45 spectrofluorimeter. ^{1}H NMR experiments were carried out on a Bruker made 400 and 300MHz NMR spectrometer.

2. Preparation of L

2-hydroxy-5-nitrobenzaldehyde (167mg, 1 mmol) and 2, 4-dinitro phenyl hydrazine (198mg, 1 mmol) in 1:1 ratio were refluxed in MeOH for 5 hours (Scheme S1). After completion of the reaction, excess solvent was removed under reduced pressure and a solid yellow colored product was obtained. The crude product was repeatedly recrystallized from tetra hydrofuran (THF) and after 7 days of the layering of THF solution of **L** with hexane in 1:1 ratio finally produces single crystals which are suitable for X-ray study. Yield ~90%. I.R analysis (KBr pellet): 3293cm^{-1}(s), 3096cm^{-1}(s), 1610cm^{-1}(s), 1513 cm^{-1}(s), 1343cm^{-1}(s), 1147cm^{-1}(s), 1114cm^{-1}(s), 947cm^{-1}(s). Elemental analysis calculated for $C_{13}H_9N_5O_7$: C: 45.08%; H: 2.60%; N: 20.23%. Found: C: 45.10%;H: 2.63%;N: 20.26%. ESI-MS: m/z =347.25, found 346.53($L-H^+$) (Fig. S1).



Scheme S1 Synthesis of sensor **L**.

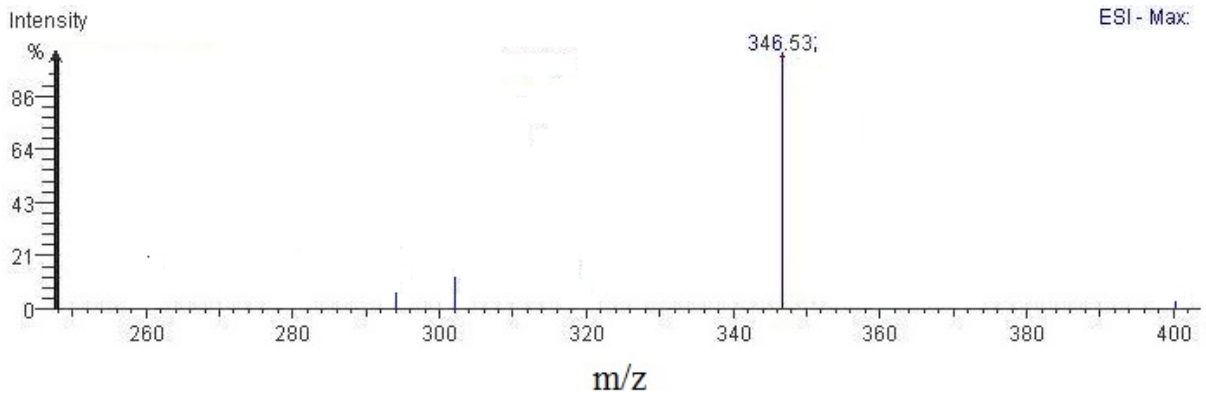


Fig. S1 ESI-MS spectrum of **L** in acetonitrile.

3. Crystallographic data

The imine bond length in hydrazone moiety agrees well with the previously reported -C=N- bond distances[ref 1] (Table S2).

Table S1. Crystallographic data and details of the Structure Determination for sensor **L**

Crystal Data

Formula	$C_{13}H_9N_5O_7$
Formula Weight	347.25
Crystal System	Monoclinic
Space group	P21/n (No. 14)
a, b, c [Angstrom]	12.745(5) 8.223(5) 13.910(5)
alpha, beta, gamma [deg]	90 112.259(5) 90
V [Ang**3]	1349.2(11)
Z	4
D(calc) [g/cm**3]	1.709
Mu(CuKa) [/mm]	1.236
F(000)	712
Crystal Size [mm]	0.12 x 0.16 x 0.23

Data Collection

Temperature (K)	150
Radiation [Angstrom]	CuKa 1.54180
Theta Min-Max [Deg]	1.9, 26.0
Dataset	-15: 15 ; -7: 9 ; -17: 14
Tot., Uniq. Data, R(int)	8759, 2611, 0.018
Observed data [I > 2.0 sigma(I)]	2409

Refinement

N_{ref}, N_{par}	2611, 230
R, wR2, S	0.0350, 0.1011, 1.05
w = 1/[\s^2^(Fo^2^)+(0.0607P)^2^+0.3980P] where P=(Fo^2^+2Fc^2^)/3	
Max. and Av. Shift/Error	0.00, 0.00
Min. and Max. Resd. Dens. [e/Ang^3]	-0.22, 0.25

Table S2. Bond distances (Å) of sensor **L**

O1	-C1	1.3442(18)	C3	-C4	1.394(2)
O2	-N1	1.2289(19)	C4	-C5	1.384(2)
O3	-N1	1.2238(17)	C5	-C6	1.393(2)
O4	-N4	1.2243(19)	C6	-C7	1.455(2)
O5	-N4	1.2359(18)	C8	-C13	1.422(2)
O6	-N5	1.2222(18)	C8	-C9	1.4180(19)
O7	-N5	1.221(2)	C9	-C10	1.369(2)
O1	-H101	0.90(2)	C10	-C11	1.401(2)
N1	-C4	1.460(2)	C11	-C12	1.375(2)
N2	-C7	1.2895(18)	C12	-C13	1.388(2)
N2	-N3	1.3704(18)	C2	-H2	0.9500
N3	-C8	1.3542(19)	C3	-H3A	0.9500
N4	-C13	1.455(2)	C5	-H5	0.9500
N5	-C11	1.458(2)	C7	-H7	0.9500
N3	-H3	0.8800	C9	-H9	0.9500
C1	-C6	1.4129(19)	C10	-H10	0.9500
C1	-C2	1.402(2)	C12	-H12	0.9500
C2	-C3	1.376(2)			

Table S3. Bond angles (º) of sensor **L**

C1	-O1	-H101	107.5(14)	N3	-C8	-C13	122.75(11)
O2	-N1	-O3	122.88(12)	N3	-C8	-C9	120.87(12)
O2	-N1	-C4	118.50(11)	C9	-C8	-C13	116.35(12)
O3	-N1	-C4	118.62(12)	C8	-C9	-C10	121.86(12)
N3	-N2	-C7	115.43(10)	C9	-C10	-C11	119.48(12)
N2	-N3	-C8	120.67(10)	C10	-C11	-C12	121.33(12)
O4	-N4	-C13	118.56(11)	N5	-C11	-C10	119.59(12)
O5	-N4	-C13	118.81(11)	N5	-C11	-C12	119.08(12)
O4	-N4	-O5	122.59(12)	C11	-C12	-C13	118.90(13)
O6	-N5	-C11	118.21(13)	N4	-C13	-C8	121.71(12)
O7	-N5	-C11	118.37(12)	N4	-C13	-C12	116.25(12)
O6	-N5	-O7	123.42(13)	C8	-C13	-C12	122.04(12)
N2	-N3	-H3	120.00	C1	-C2	-H2	120.00
C8	-N3	-H3	120.00	C3	-C2	-H2	120.00
O1	-C1	-C2	117.81(11)	C2	-C3	-H3A	121.00
O1	-C1	-C6	122.18(12)	C4	-C3	-H3A	121.00
C2	-C1	-C6	120.01(12)	C4	-C5	-H5	120.00
C1	-C2	-C3	120.77(12)	C6	-C5	-H5	120.00
C2	-C3	-C4	118.72(12)	N2	-C7	-H7	119.00
N1	-C4	-C5	118.43(11)	C6	-C7	-H7	119.00
N1	-C4	-C3	119.81(12)	C8	-C9	-H9	119.00
C3	-C4	-C5	121.75(12)	C10	-C9	-H9	119.00
C4	-C5	-C6	119.95(11)	C9	-C10	-H10	120.00
C1	-C6	-C5	118.77(12)	C11	-C10	-H10	120.00
C5	-C6	-C7	118.18(11)	C11	-C12	-H12	121.00
C1	-C6	-C7	123.00(11)	C13	-C12	-H12	121.00
N2	-C7	-C6	121.11(11)				

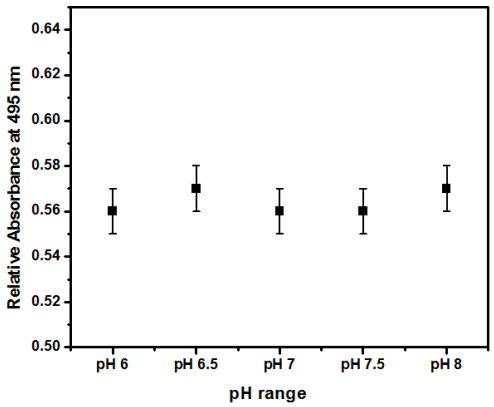
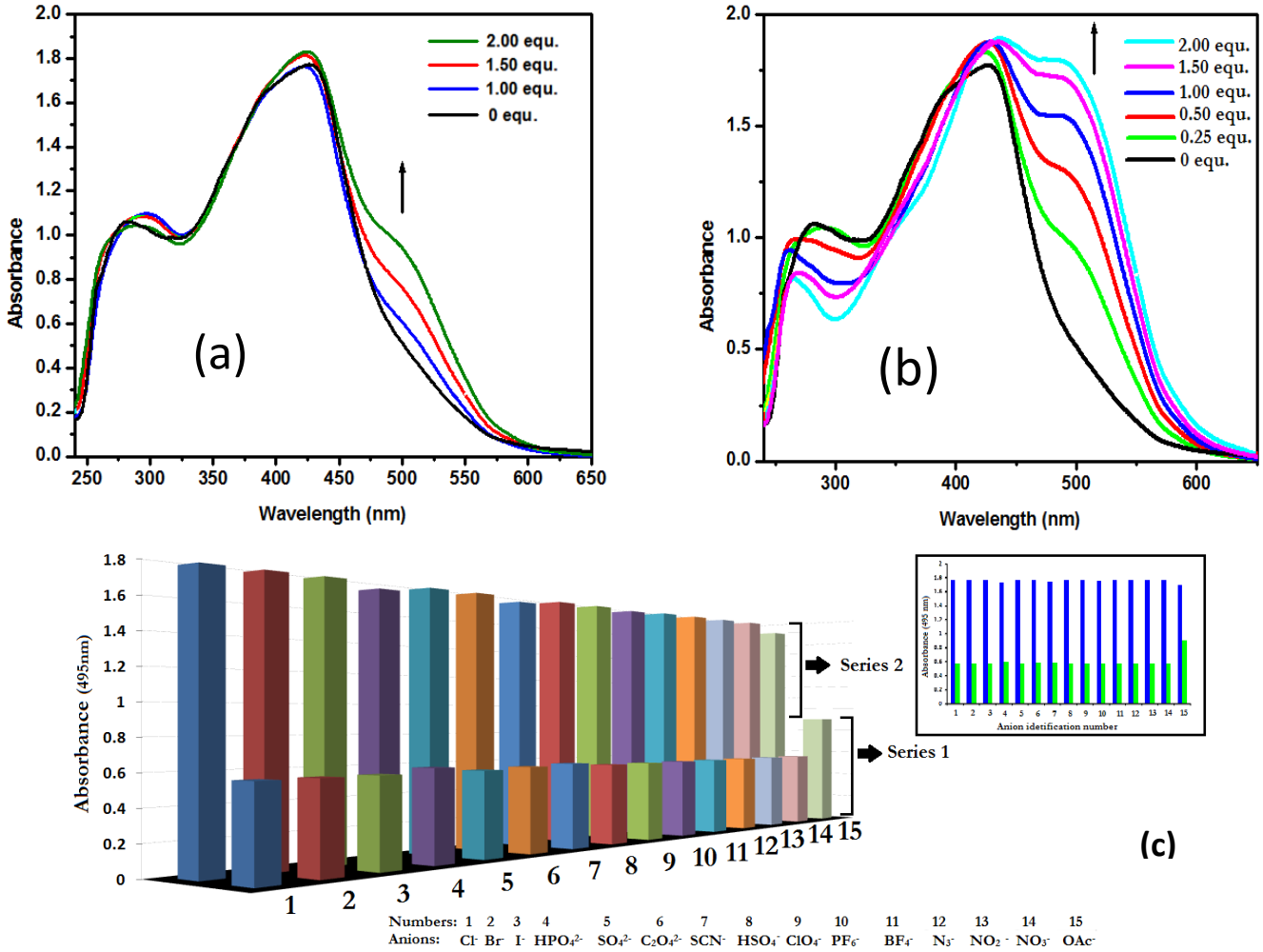


Fig. S2 pH influence on sensor **L** with error bars.

4. Sampling process:

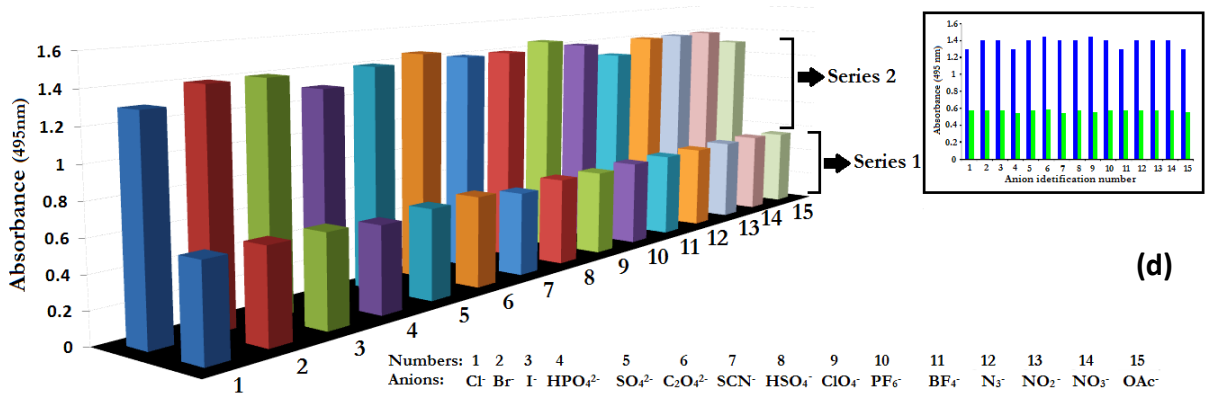
The saliva specimens were collected from one of the reputed dental clinics of district Burdwan, West Bengal, India. All the samples were collected in presence of experienced dentist and with permission of the patients. Collection was performed in the morning between 8.00 -9.00 AM before eating any sorts of food or drinking any beverages (Tea, coffee *etc.*) Samples were collected following a mouth wash treatment of the patient with 10mL pure drinking water twice to washout any remaining food fragments, exfoliated cells. After 5 minutes break, 0.25-0.30 mL saliva sample of patient is sufficient enough to carry out the further clinical diagnosis of fluorosis.

5. UV-Vis titration of L with $TBA^{+}OAc^{-}/OH^{-}$ in DMSO



Series 1: Addition of other anions results in no change on initial absorbance at 495 nm for **L** (except OAc^{-} where a feeble change is noticed) in DMSO.

Series 2: When F^{-} is added on other anions containing solution of **L** in DMSO, it results in an appreciable enhancement of OD value at 495 nm for **L**.



Series 1: Addition of other anions results in no change on initial absorbance at 495 nm for **L** in DMSO:Water (1:1)

Series 2: When F^- is added on other anions containing solution of **L** in DMSO:Water (1:1) it results in an appreciable enhancement of OD value at 495 nm for **L**.

Fig. S3 (a) UV-Vis titration of **L** ($1x10^{-4}$ M) with TBA^+OAc^- ($1x10^{-4}$ M) in DMSO (0-2 equivalent); (b) UV-Vis titration of **L** ($1x10^{-4}$ M) with TBA^+OH^- ($1x10^{-4}$ M) in DMSO (0-2 equivalent); (c) 3D patterns of interference study for TBA^+F^- with other anions in DMSO (inset shows 2D plots); (d) 3D patterns of interference study for TBA^+F^- with other anions in DMSO:Water (1:1) medium (inset shows 2D plots).

6. (i) Benesi-Hildebrand Equation and Plot

The association equilibrium constant of a complex formed in between the receptors and the incoming anions has been determined from the following complex equilibrium.

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

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

For 1:1 type complex formation with m=1 following the Benesi-Hildebrand relation, can be expressed in terms of optical density (A) as follows:

$$A = \frac{A_o + A_1 K[X^{n-}]}{1 + K[X^{n-}]}$$

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 the added anion, chemo sensor and the complexation between anion and concerned chemo sensor, respectively. A_o, A and A_1 indicates the optical density or absorbance at a particular wavelength of **L** without adding F^- anion, absorbance after adding anion at every successive step and excess amount of added anion, respectively. The binding or association equilibrium constant or association constant K (M^{-1} or M^{-2}) is determined from the ratio of intercept and slope of Benesi-Hildebrand plot of optical density.

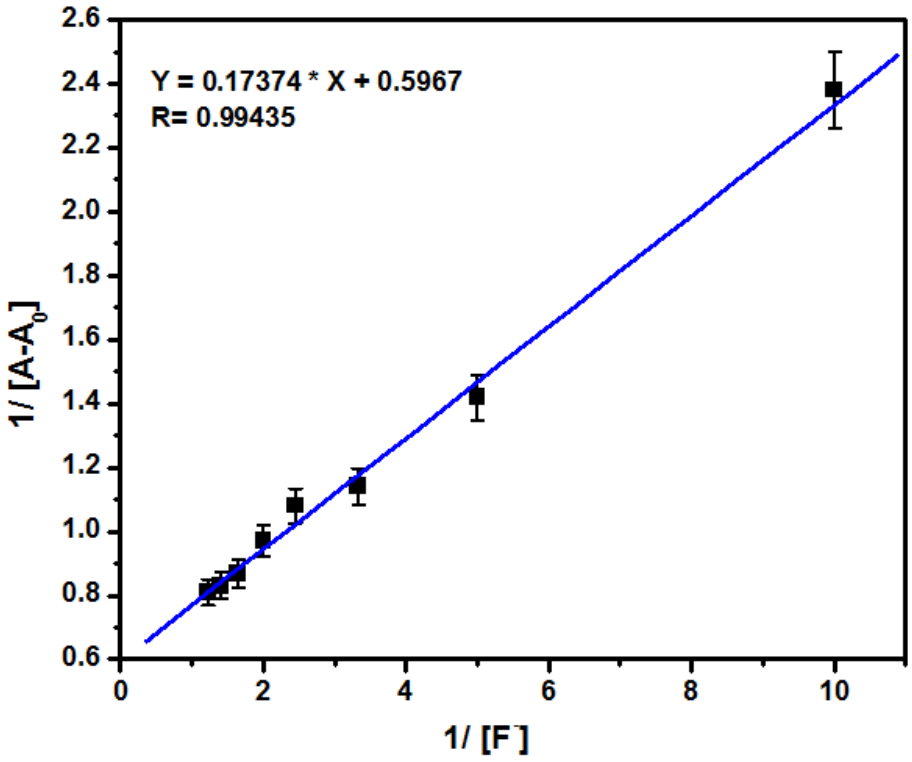


Fig. S4a B-H plot of sensor **L** *vs.*(TBA^+F^-).

As shown in the Fig. S4a, the Benesi-Hildebrand (B-H) plot of $1/[A-A_0]$ *vs* $1/[F^-]$ for the titration of sensor **L** and TBA^+F^- provides a straight line (best fitted), indicating a 1:1 type complex formation with association equilibrium constant K = 3.35 x 10^4 M^{-1}**.**

(ii) Binding equilibrium constant determination using UV-Vis absorption studies[ref2,3]

Association equilibrium constants can also be calculated using the equation 1 for optical titrations originally taken from Connors [ref3], where [L] = [anion] and data are fitted using Origin version 6.0.

$$\frac{b}{\Delta A} = \frac{1}{S_t K_a \Delta\varepsilon} \times \frac{1}{[L]} + \frac{1}{S_t \Delta\varepsilon} \qquad \textbf{(1)}$$

$\Delta A = A_{substrate+anion} - A_{substrate}$, b = path length (1cm here), S_t = total concentration of substrate, K_a = association equilibrium constant, $\Delta\varepsilon = \varepsilon_{substrate+anion} - \varepsilon_{substrate} - \varepsilon_{anion}$.

$$Slope = \frac{1}{S_t K_a \Delta\varepsilon} \quad \text{and} \quad Intercept = \frac{1}{S_t \Delta\varepsilon}$$

Thus,

$$K_a = \frac{Intercept}{Slope}$$

Plotting 1/Δ*A* against 1/[L] gives the straight line curve. Association equilibrium constant can be obtained from intercept and slope ratio. Correlation coefficient (R>0.99) establish the formation of 1:1 complex between **L** and F^-. The binding equilibrium constant is found to be 3.70 x 10^4 M^{-1}.

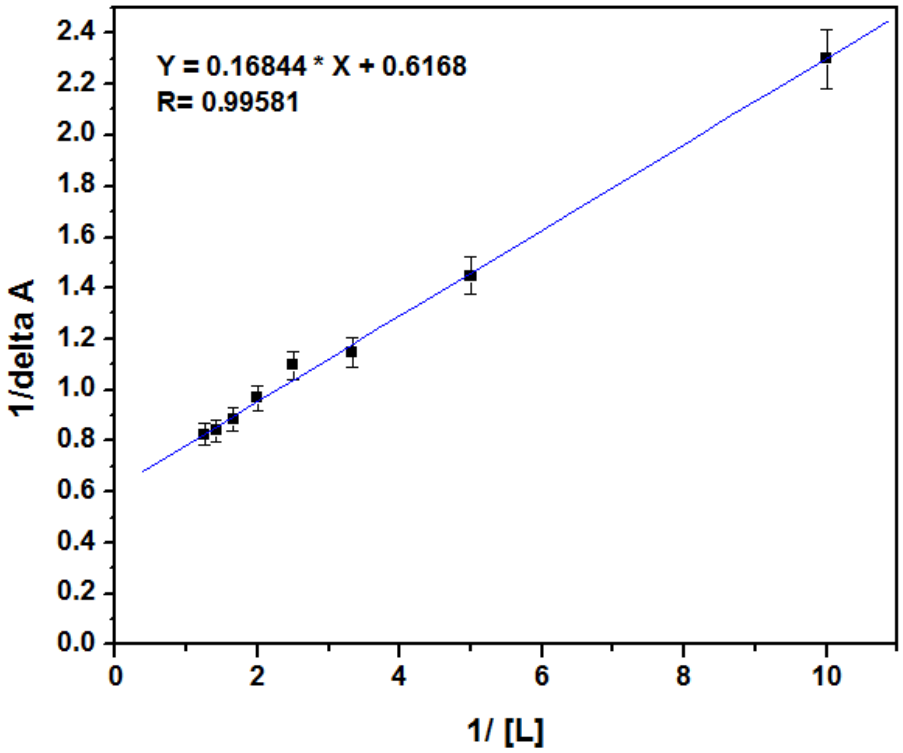


Fig. S4b Fitting line of UV-Vis absorption titration spectra of **L** with TBA^+F^-.

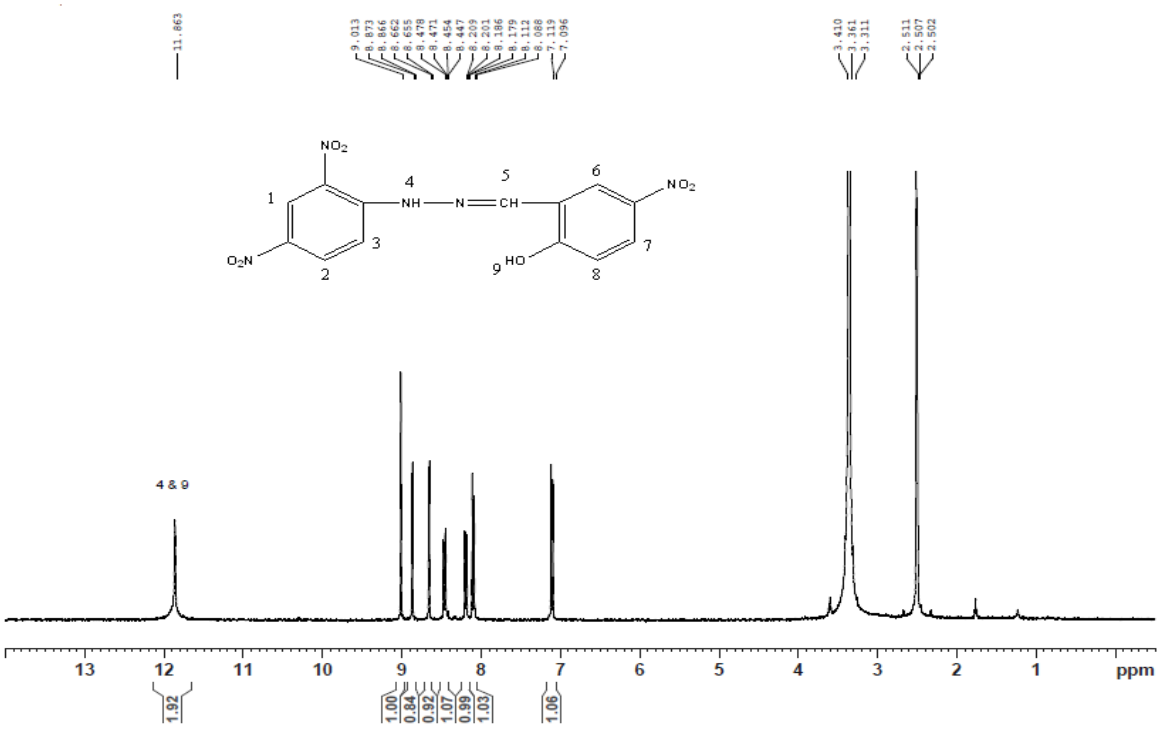


Fig. S5 ^{1}H-NMR of **L** in DMSO-d_6 (-OH and –NH protons are marked).

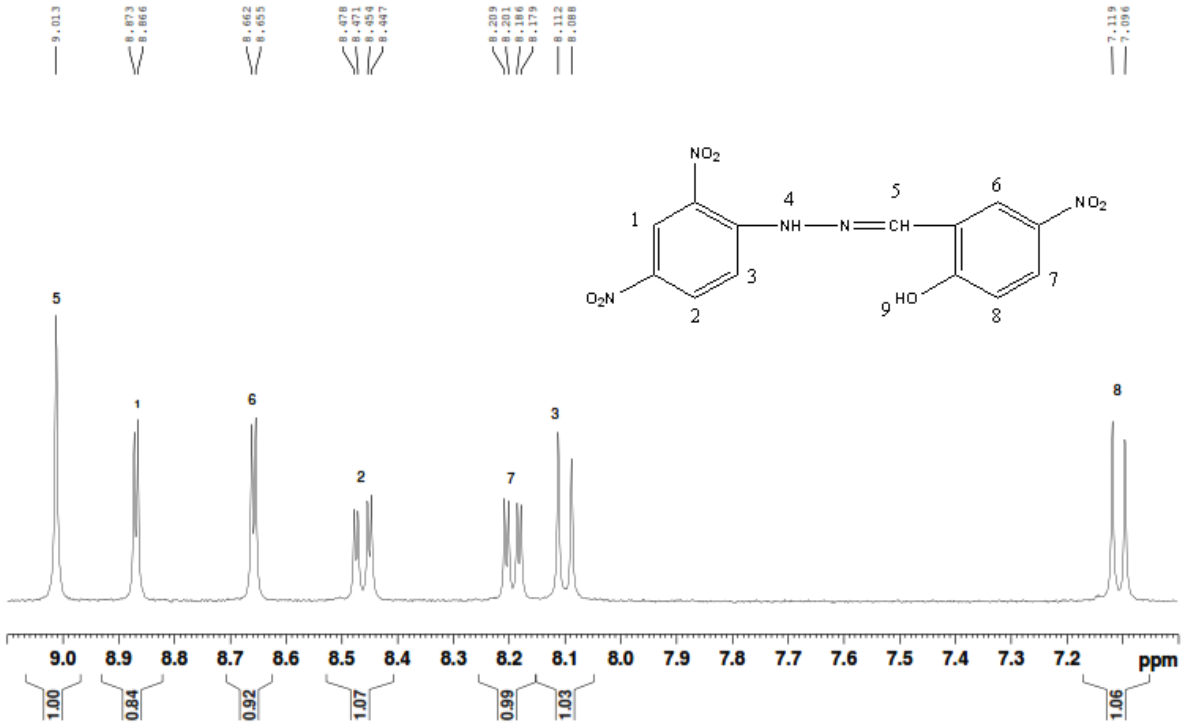


Fig. S6 ^{1}H- NMR of **L** in DMSO-d_6 (Aromatic region is expanded).

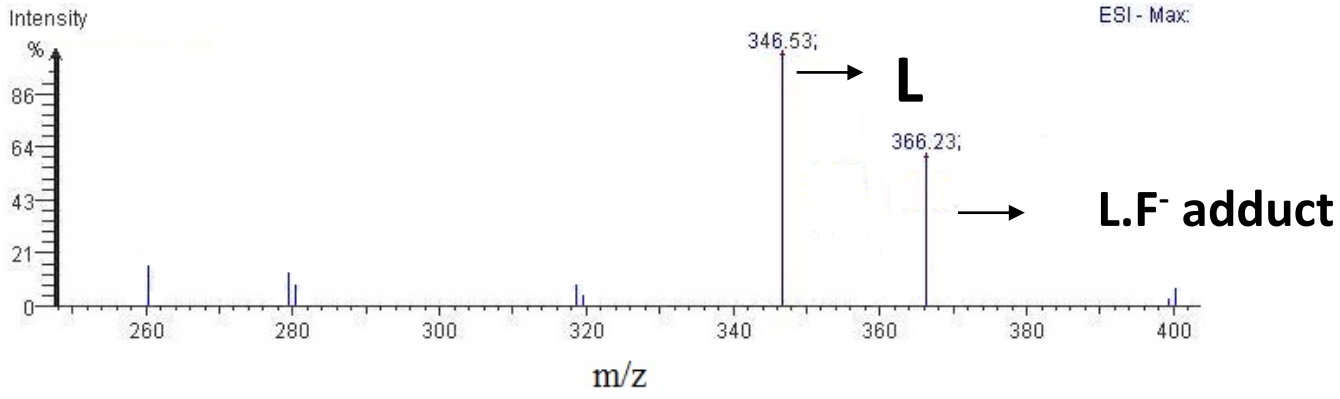


Fig. S7 ESI-MS of **L** and **L.**F^- adduct in acetonitrile.

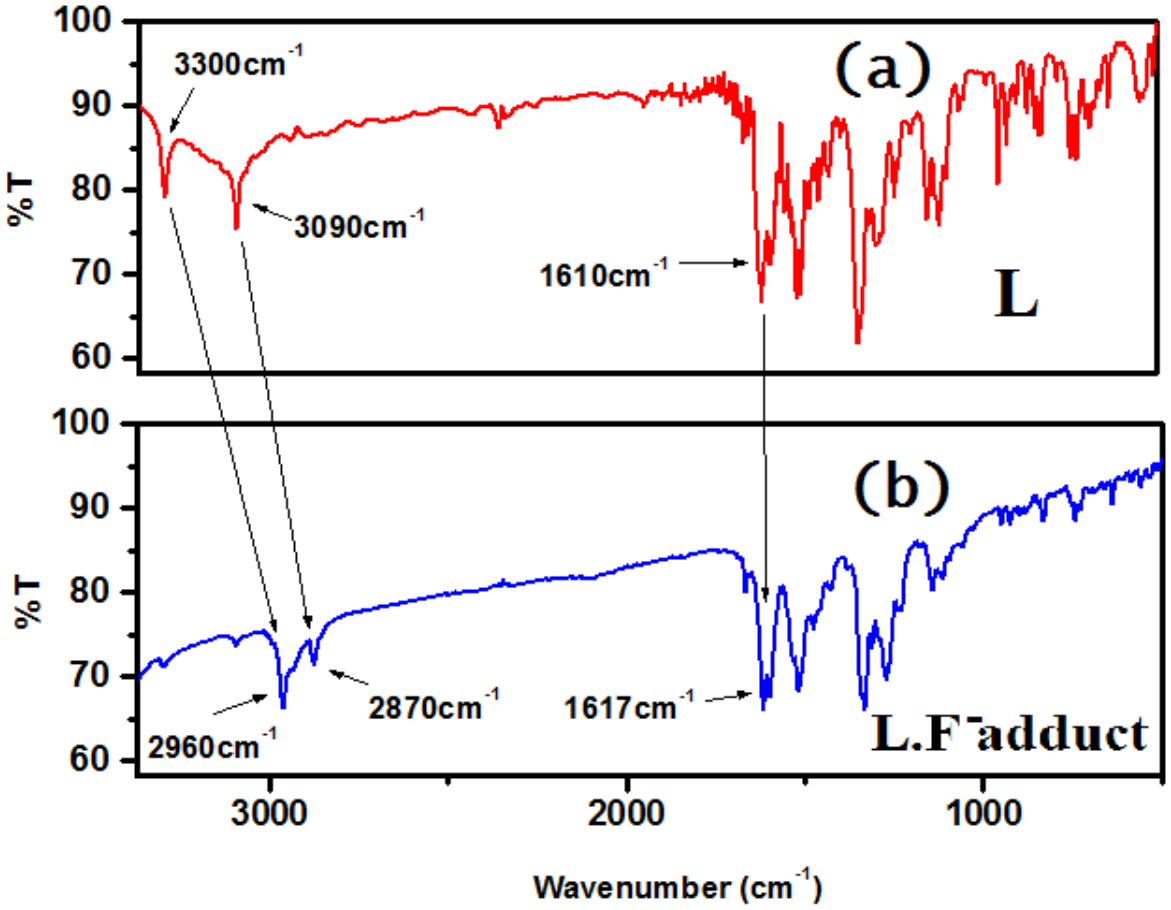
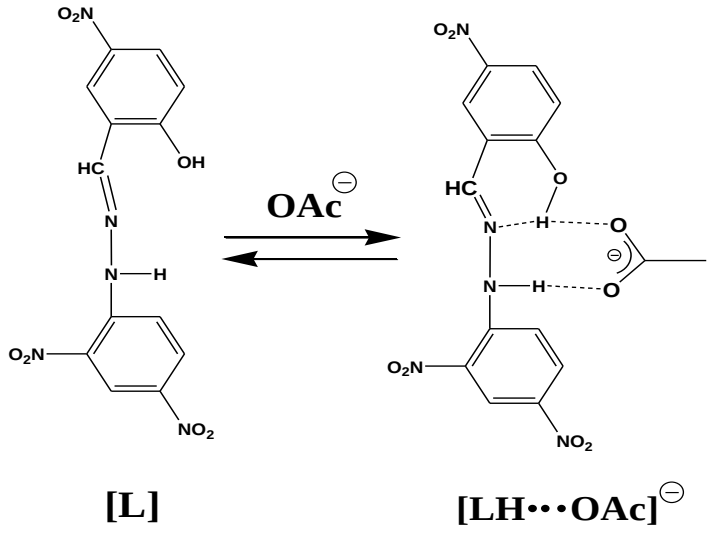


Fig. S8 FT-IR spectra of (a) **L** and (b) **L.**F^- adduct.



[L] **[LH···OAc]⊖**

Scheme S2 Probable **L**: OAc^- binding mode in DMSO.

7. Test Kit Preparation

For the preparation of Test kit, a dry cellulose test paper is soaked with a standard DMSO solution of **L** (1 x 10^{-4} M). The soaked test paper was air dried properly. Now, F^- anion solution in proper concentration was sprayed on

the test kit, notably, a sharp reddish brown colour was developed on the paper for F^- anion instantly but interesting enough, further addition of any other TBA salt solution of anions there were no noticeable colour change within the same time limit as of F^- anion testing. To reuse the test kit (after F^- detection) we may wash the test paper with distilled water and air dried properly and redo the same job again, *i.e.*, spraying the experimental test anion solution in same fashion on test kit. We can see the same reddish brown colour is regenerating and thus the test kit can be reused for several times.

8. Imaging System. The imaging system was comprised of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50 watt lamp.

9. Preparation of Cell image.

Candida albicans cells (IMTECH No. 3018) from exponentially growing culture in yeast extract glucose broth medium (pH 6.0 and incubation temperature 37^0C) were washed by suspending them in normal saline and centrifuged at 3000 rpm for 10 minutes. It was washed twice with 0.1 M HEPES buffer (pH 7.4). Then cells were treated with F^- solution (10 μM) for 1hr. After incubation, the cells were again washed with HEPES buffer and then incubated with **L** (10 μM) for another 1hr. Cells obtained this way are mounted on a grease free glass slide and observed under a Leica DM 1000 Fluorescence microscope with UV filter. Cells treated with F^- are used as control. [ref 4]

Preparation of pollen grains to detect intracellular F^-: Pollen grains of *Techoma stans* (Family: Bignoniaceae) are collected from fresh buds and washed twice with 0.1 M HEPES buffer at pH 7.4. These are treated with 10 μM F^- for 1hr in 0.1 M HEPES buffer (pH 7.4) containing 0.01% Triton X100 as a permeability enhancing agent. After incubation the pollens are washed again with HEPES buffer at pH 7.4 and incubated with **L** (10 μM) for 1hr. **L** treated pollens are washed by centrifugation (3000 rpm for 5 minutes) using HEPES buffer and are mounted on a grease free glass slide and observed under a Leica DM 1000 fluorescence microscope equipped with a UV filter. Cells treated with F^- are used as control.[ref 4]

References:

Ref 1. P.Banerjee, G. Mostafa, A. Castiñeiras ,S.Goswami, *Eur. J. Inorg. Chem.*, 2007, 412.

Ref 2. J. Xiong, L. Sun, Y. Liao, G-N. Li, J-L. Zuo and X-Z. You, Tetrahedron Lett., 2011, 52, 6157.

Ref 3. K. A. Conners, Binding Constants; John Wiley & Sons: New York, **1987**, pages 148 and 24.

Ref 4. A. Sahana, A. Banerjee, S. Lohar, S. Panja, S. K. Mukhopadhyay, J. S. Matalobos and D. Das, *Chem. Commun.*,2013, **49**, 7231.