Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

1. Materials

2-Hydroxy naphthaldehyde (Alfa Aesar, Germany), 4, 5-diamino pyrimidine (SRL, India) and $Al(NO_3)_3 \cdot 9H_2O$ (Merck, India) have been used as received. All other chemicals and solvents are of analytical grade and used without further purification. Solvents used are of spectroscopic grade. Mili-Q Milipore® 18.2 MΩ cm^{-1} water has been used throughout all the experiments.

2. Apparatus

^{1}HNMR spectra have been recorded in DMSO-d_6 with a Bruker Advance 600 MHz using TMS as the internal standard. Absorption and fluorescence spectra are recorded on Shimadzu Multi Spec 1501 absorption spectrophotometer and Hitachi F-4500 fluorescence spectrophotometer, respectively. Mass spectra are recorded in QTOF Micro YA 263 mass spectrometer in ESI positive mode. IR spectra are recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Thermogravimetric analysis has been performed with a Perkin Elmer TG/DTA lab system l (Technology by SII). Structures of **L** and its Al^{3+} complex have been optimized by DFT and TDDFT using Gaussian '03 software package [29]. The fluorescence 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 is equipped with a mercury 50 W lamp.

*3. Synthesis of **(E)-1-((4-aminopyrimidin-5yl)imino)methyl)naphthalene-2ol (L)** (Scheme 1)*

Methanol solutions of 2-hydroxy naphthaldehyde (172.18 mg, 1 mmol) and 4,5-diamino pyrimidine (110.12 mg, 1 mmol) have been mixed slowly followed by reflux for 6 h. The reaction mixture is kept overnight to get pure crystalline **L.** It's probable molecular structure and purity are determined by ^{1}HNMR (ESI, Fig. S11 and S12), FTIR (ESI, Fig. S13) and QTOF –MS

ES^+ (ESI, Fig. S14). Yield 84 %; M. P. 217^{o}C (± 4^{o}C); ^{1}HNMR (600MHz, DMSO-d_6)**:** δ(ppm); 14.322 (s, 1H, OH), 9.670 (s, 1H, -CH=N), 8.519 – 8.562 (d, 1H, ArH), 8.316 (s, 1H, heterocyclic), 8.215 (s,1H, heterocyclic), 8.060 – 8.030 (d, 1H, ArH), 7.915 – 7.888 (d, 1H, ArH), 7.615 – 7.564 (t, 1H, ArH), 7.445 – 7.396 (t, 1H, ArH), 7.239 – 7.209 (d, 1H, ArH), 6.991 (s, 2H, - NH_2); QTOF –MS ES^+: $[M + H]^+$ = 265.12 (100%) ; elemental analysis as calculated for $C_{15}H_{12}N_4O$(%): C, 68.17; H, 4.58; N, 21.20. Found (%): C,68.15; H,4.59; N, 21.17. FTIR (cm^{-1}): υ(NH, stretching) 3310.30, υ(NH, bending) 1655.60, υ(OH, hydrogen bonded) 3152.21 , υ(C=N) 1581.63.

4. *Synthesis of* ***[Al (L) (NO_3) (H_2O) (DMF)]*** *complex*

A solution of Al $(NO3)_3$,$9H_2O$ (187.3 mg, 0.5 mmol) in DMF is added slowly to a solution of **L** in DMF (132 mg, 0.5 mmol) under stirring condition and continued for 15 minutes. Then the mixture is heated at 80^0 C for 2 h to get a clear wine color solution which yielded a brown colored compound after vacuum distillation. Its molecular structure and purity have been confirmed by FTIR (ESI, Fig. S15) and QTOF – MS ES^+ (ESI, Fig. S16). Yield: 80 %. QTOF – MS ES^+: 444.06 was found for $C_{18}H_{21}AlN_6O_6$: FTIR (cm^{-1}): υ(NH) 3440.30, υ(OH) 3340.15 , υ(C=N) 1618.85 & 1602.97; υ(-NO_3^-) 1384.78.

4. Cell studies

To detect intracellular Al^{3+}, *Candida albicans* cells (IMTECH No. 3018) from exponentially growing culture in yeast extract glucose broth medium (pH 6.0, incubation temperature, 37^{0}C) have been centrifuged at 3000 rpm for 10 minutes, washed twice with 0.1 M HEPES buffer at pH 7.4. Then, it was treated with 20 μM Al^{3+} salt for 45 minutes in 0.1 M HEPES buffer (pH 7.4) containing 0.01 % Triton X100 as permeability enhancing agent. After incubation the cells

are washed again with HEPES buffer at pH 7.4 and incubated with **L (**10 μM in DMSO) for 20 minutes and mounted on a grease free glass slide to observe under fluorescence microscope equipped with UV filter. Cells without Al^{3+} treatment but incubated with **L** are used as control.

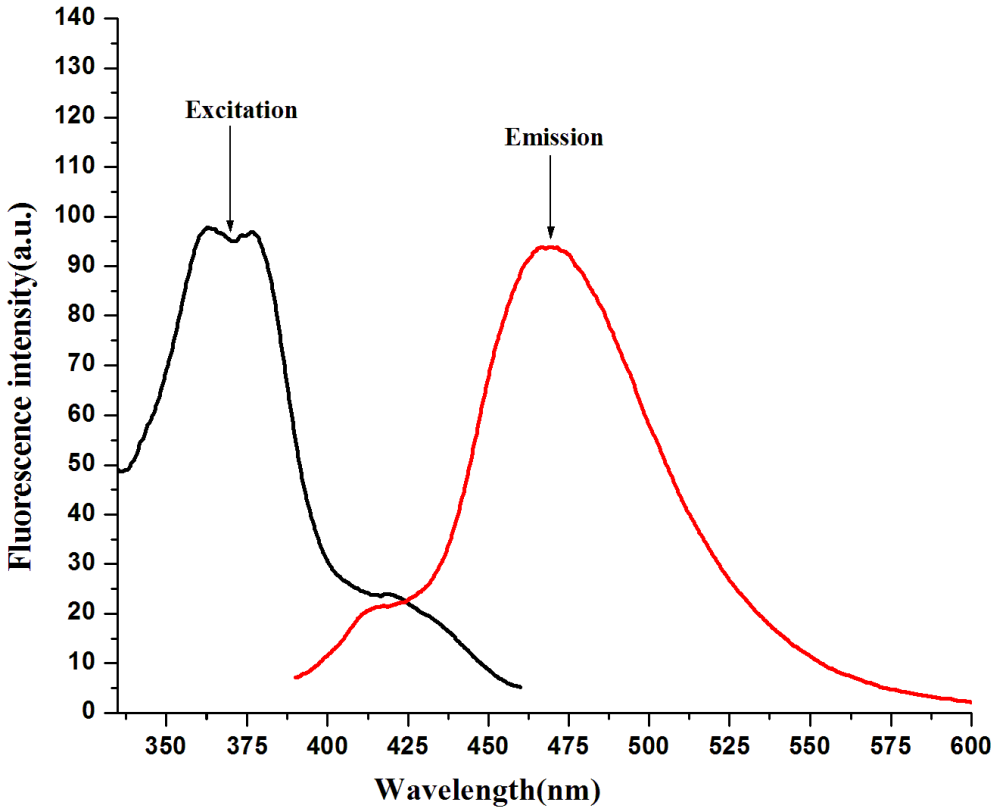


Fig. S1 Excitation (λ = 365 nm) and emission (λ = 470 nm) spectra of **L** (10 μM)

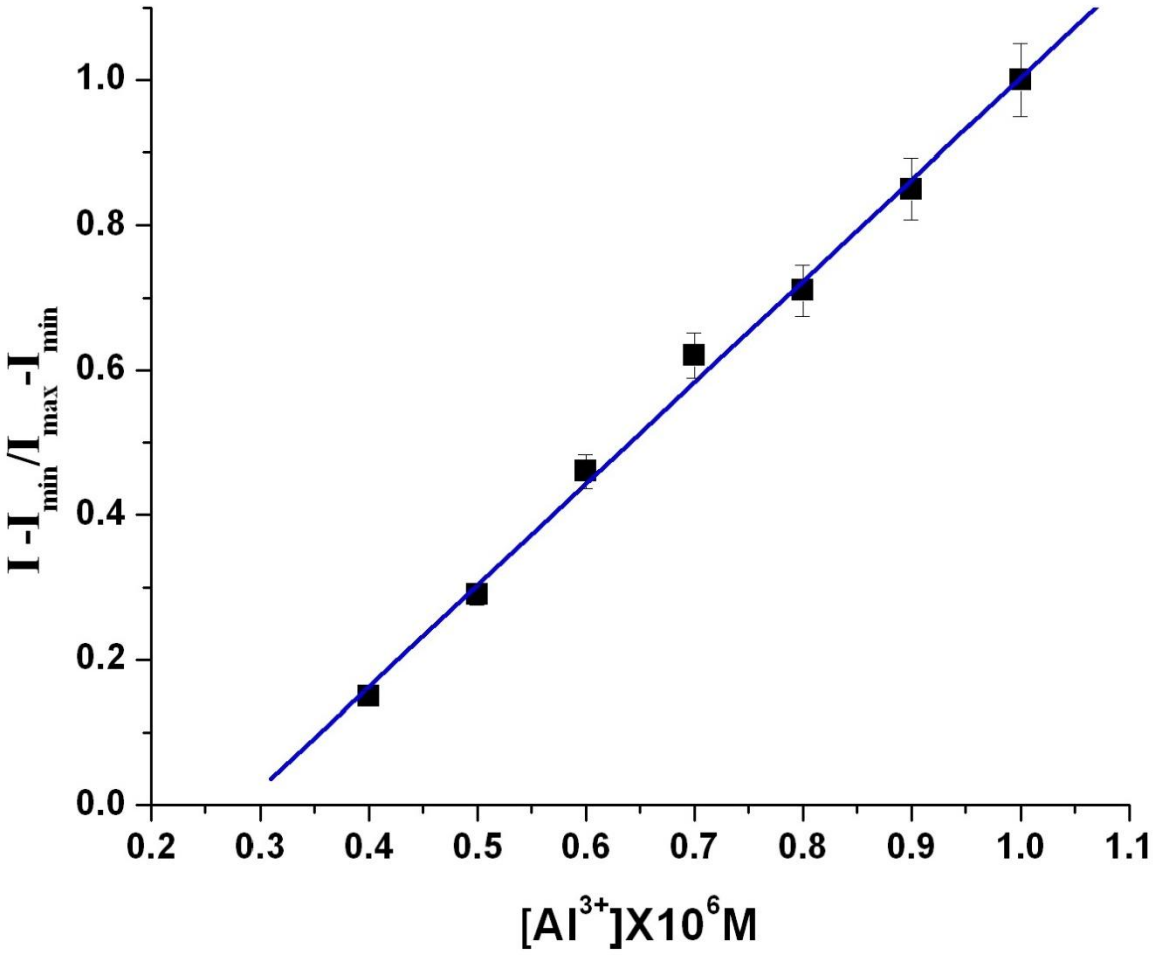


Fig. S2 Emission (at 505 nm) of **L** (1 μM) at different concentrations of Al^{3+} (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 μM)added, normalized between the minimum emission (0.0 μM Al^{3+}) and the maximum emission intensity in DMSO. The detection limit was determined to be 2.9×10^{-7} M.

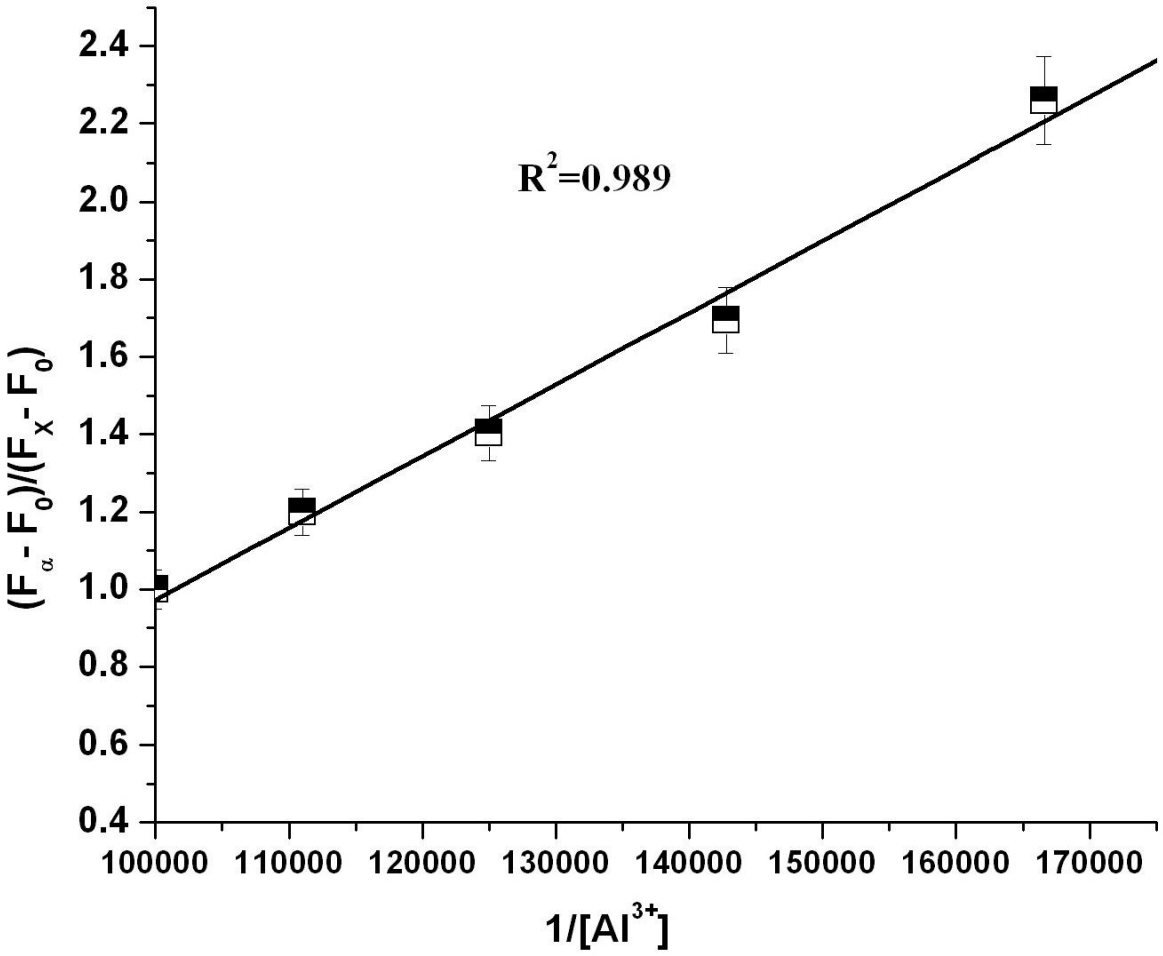


Fig. S3 Benesi-Hildebrand plot for determination of binding constant (λ_{em} = 505 nm).

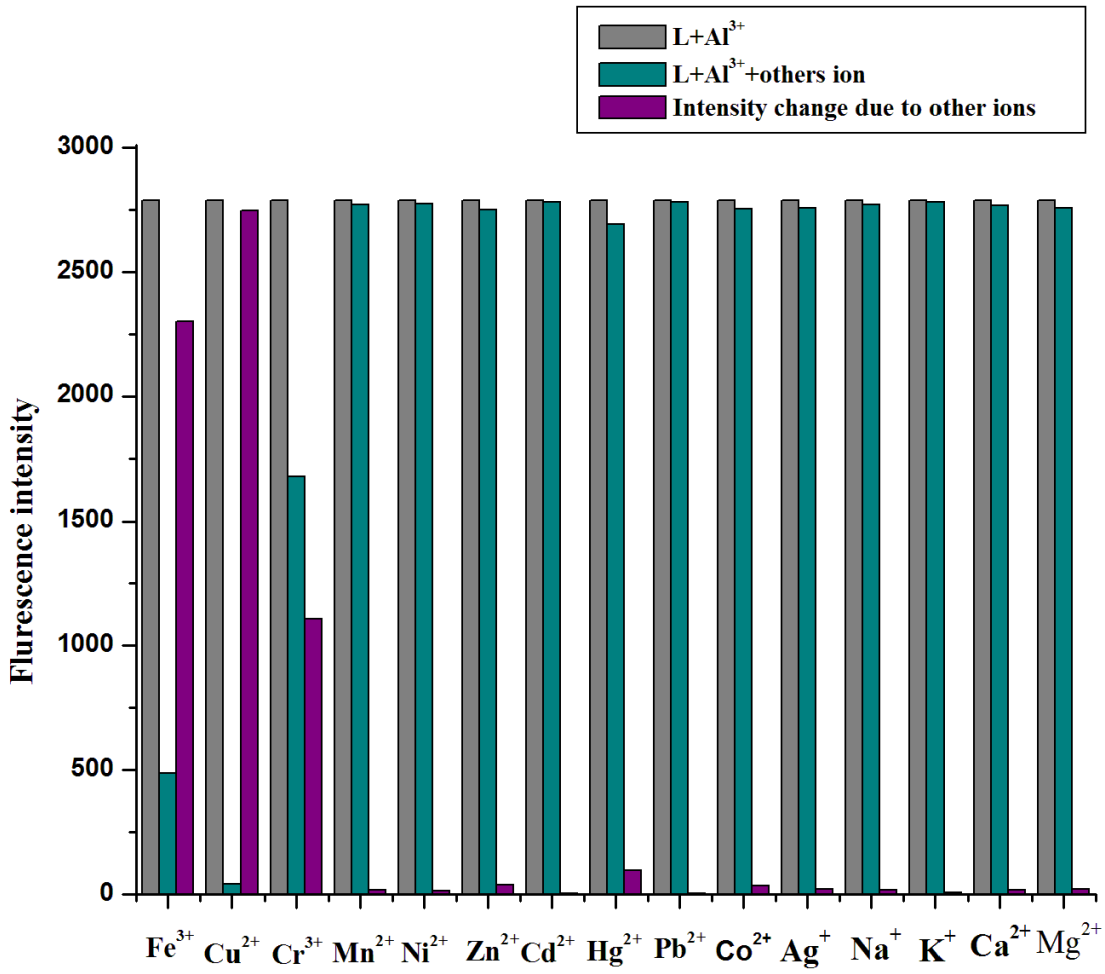


Fig. S4 Effect of foreign cations (50 μM) on the emission intensity of [**L**- Al^{3+}] system (10 μM) in DMSO solution.

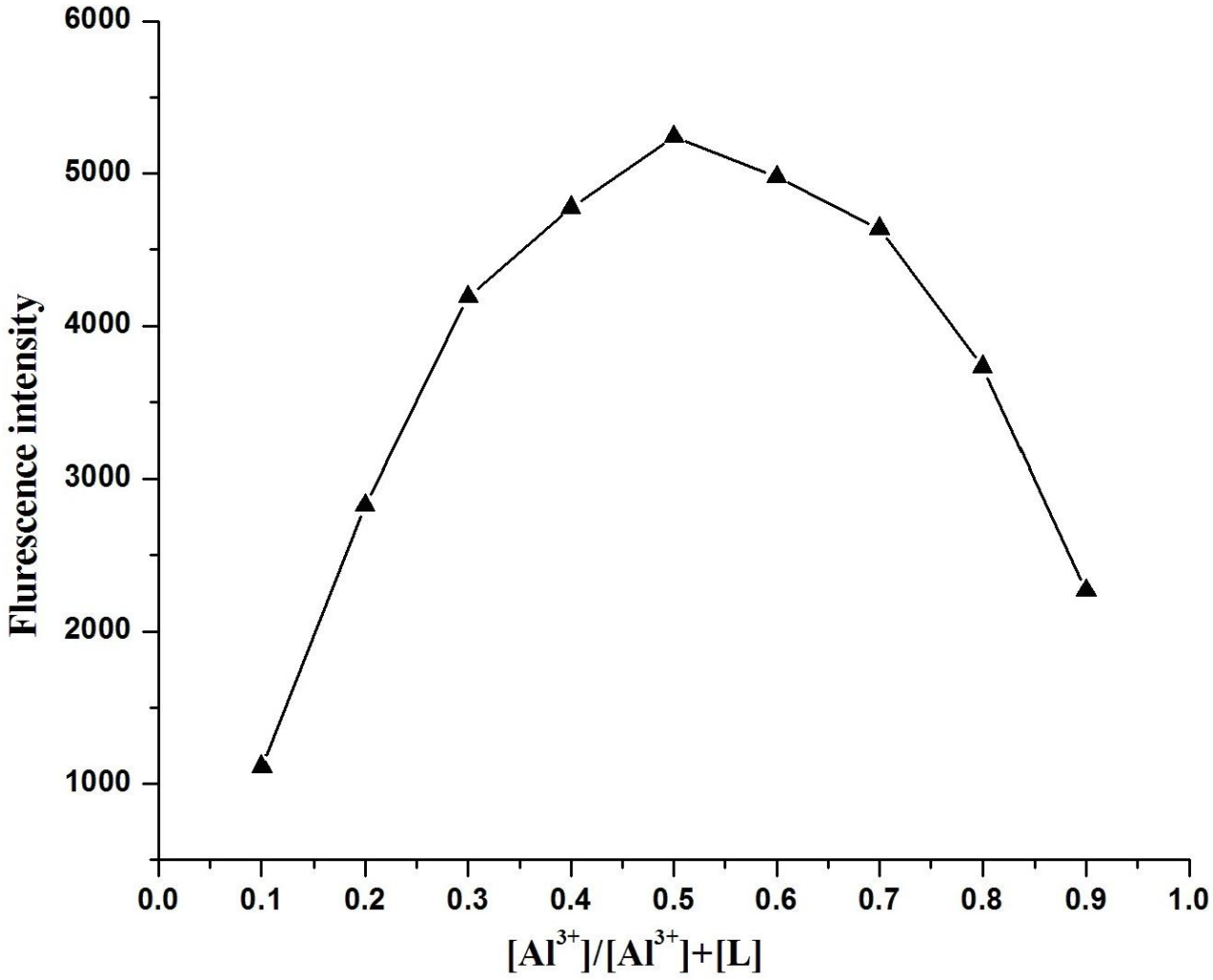


Fig. S5 Job's plot in DMSO solution.

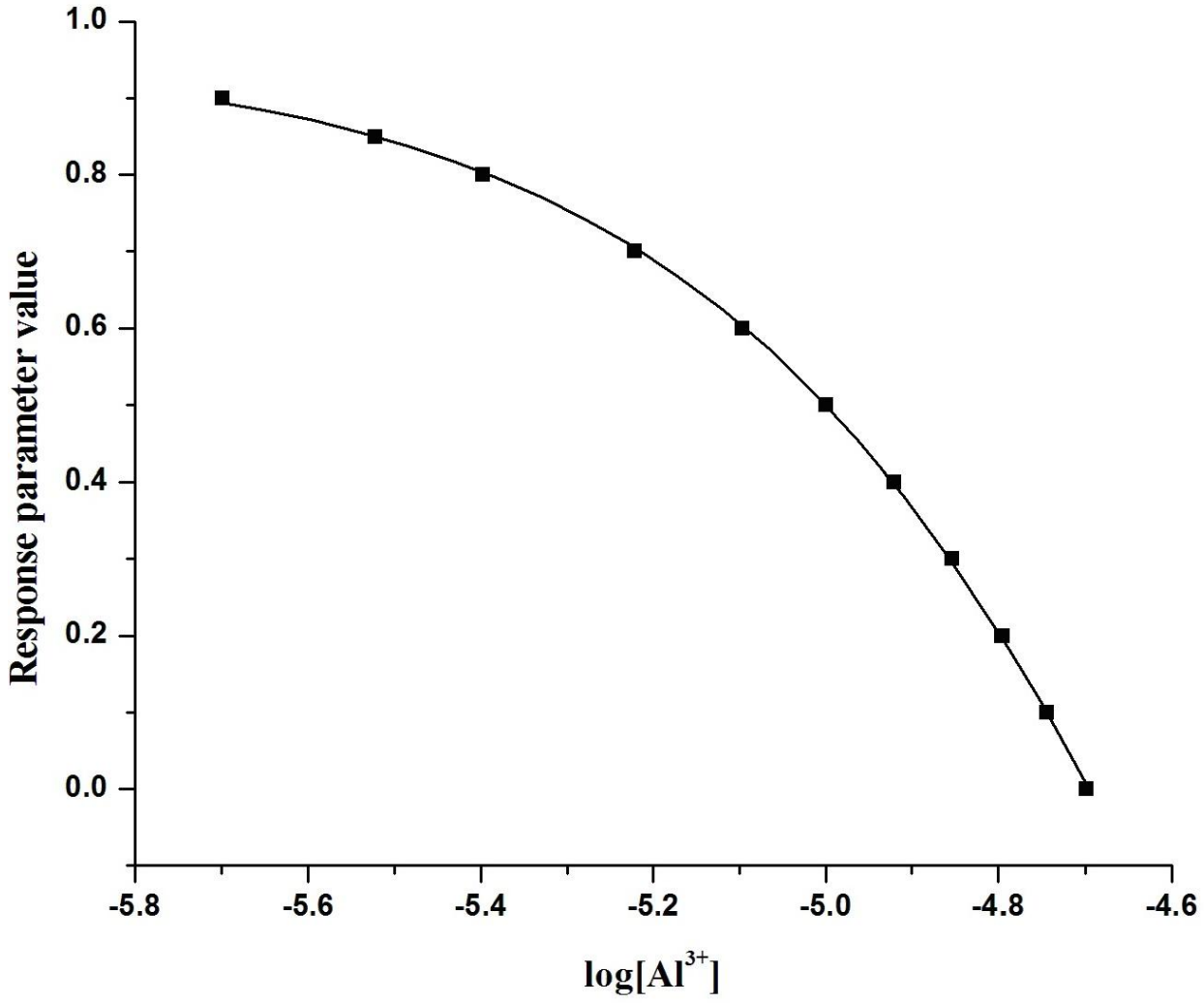


Fig. S6 Response parameter values (α) as a function of log[Al^{3+}]. α is defined as the ratio between the free [**L**] and the initial concentration of [**L**].

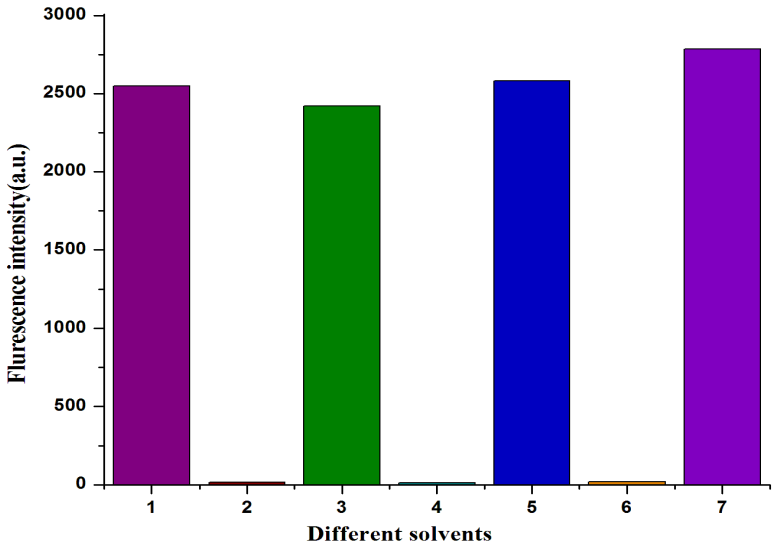


Fig. S7 Fluorescence intensity of [**L** -Al^{3+}] system (20 μM) in different solvents. CH_3OH (1), H_2O (2), CH_3CN (3), $CHCl_3$ (4), DMF (5), Toluene (6), DMSO (7).

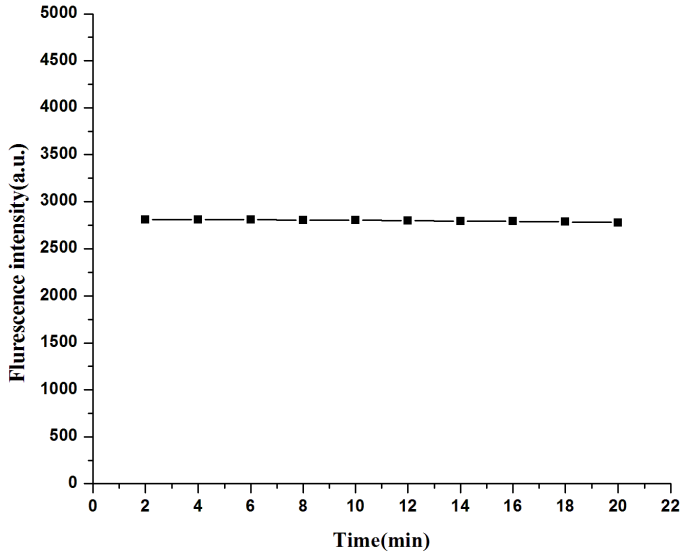


Fig. S8 Plot of fluorescence intensity as a function of time.

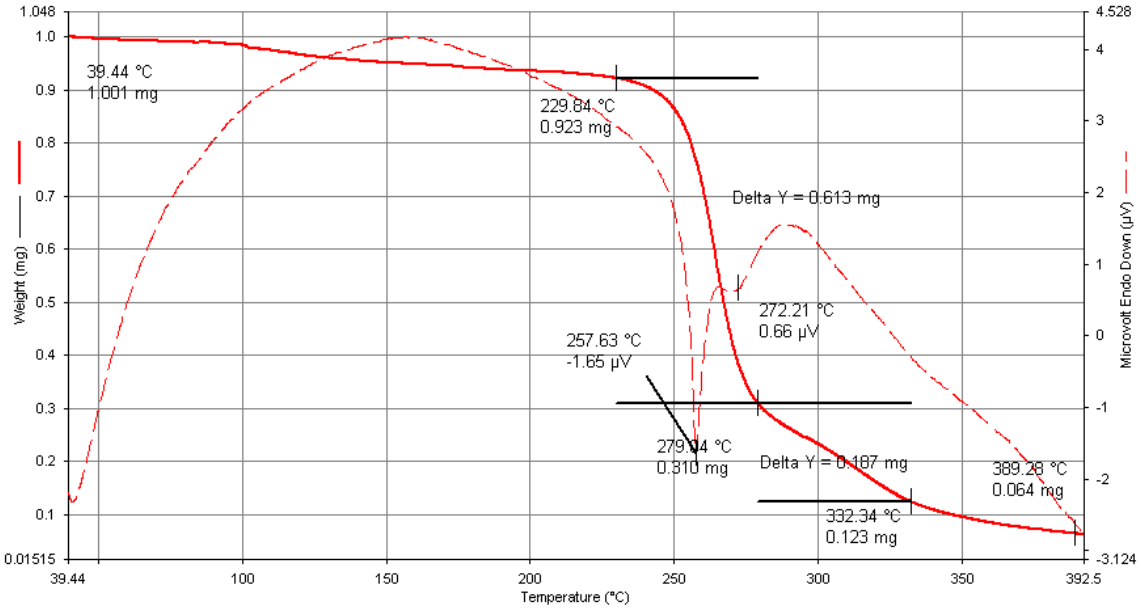


Fig. S9 Thermogravimetric analysis of **L**

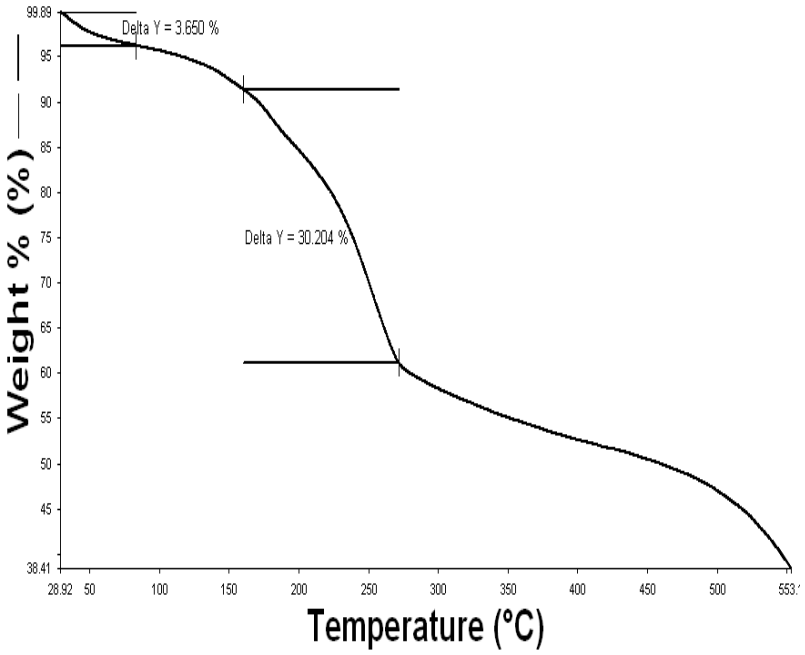


Fig. S10 Thermo-gravimetric analysis of [Al(**L**)(NO_3) (DMF)(H_2O)] complex.

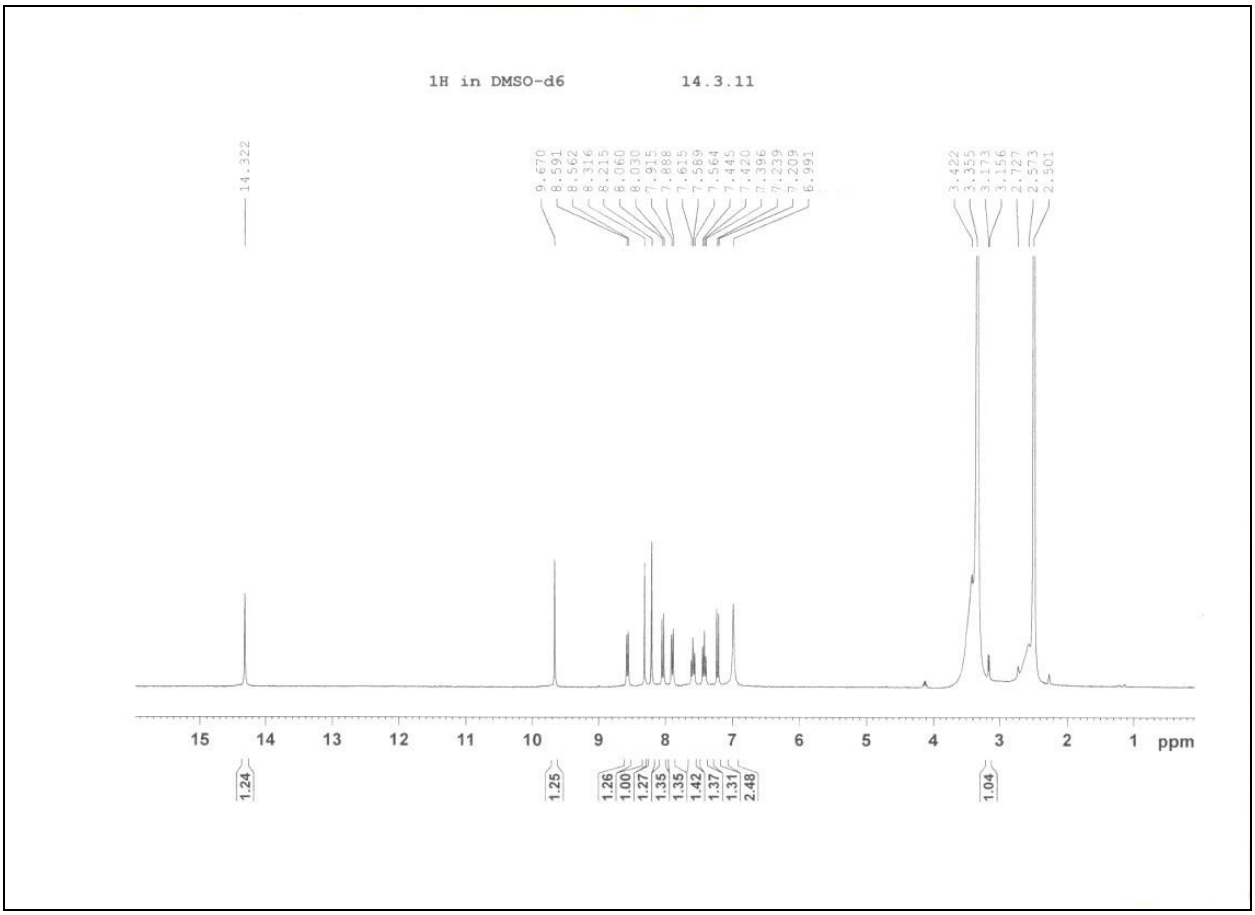


Fig. S11 ^{1}H NMR spectra of **L.**

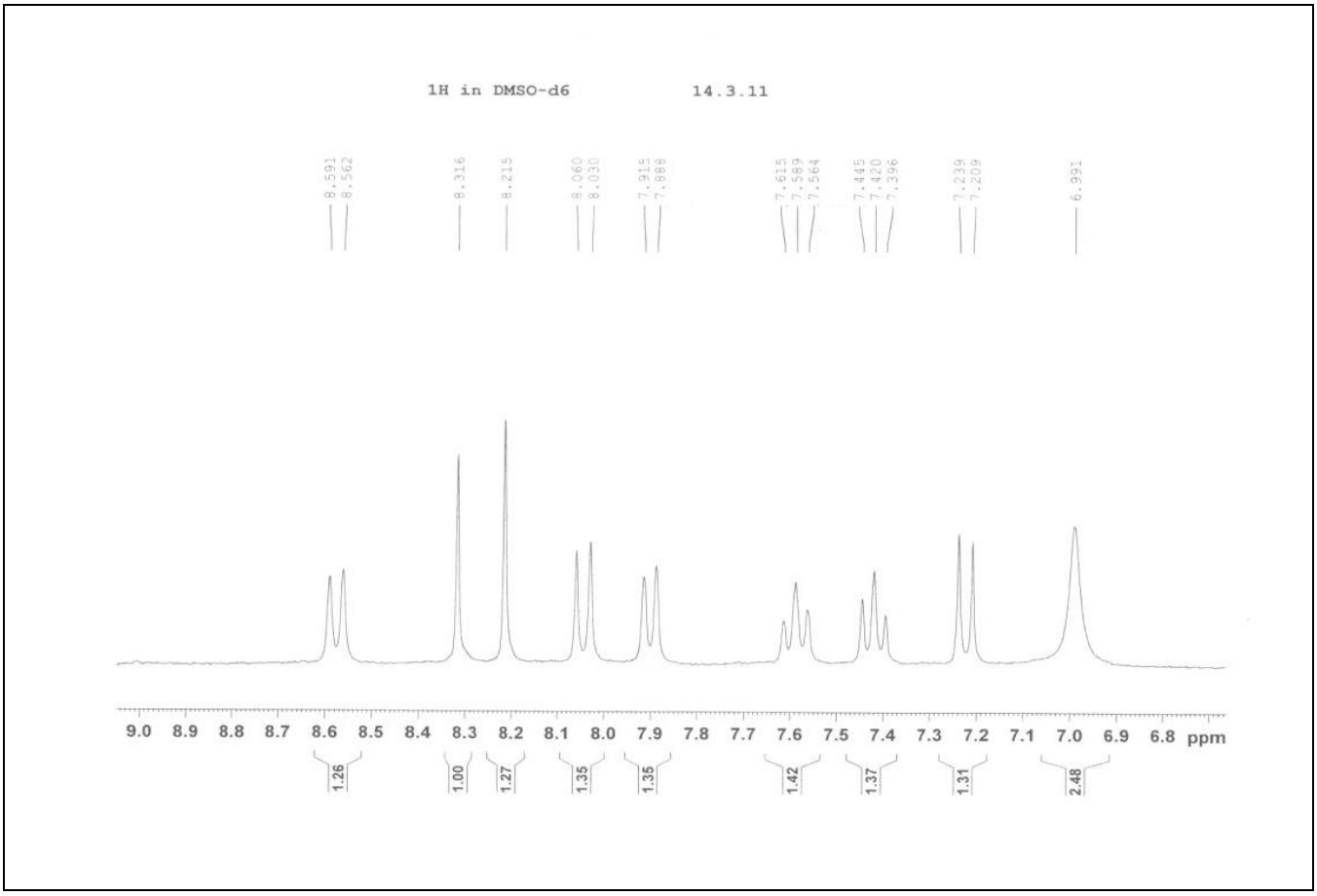


Fig. S12 Expanded aromatic region of ^{1}H NMR spectra of **L.**

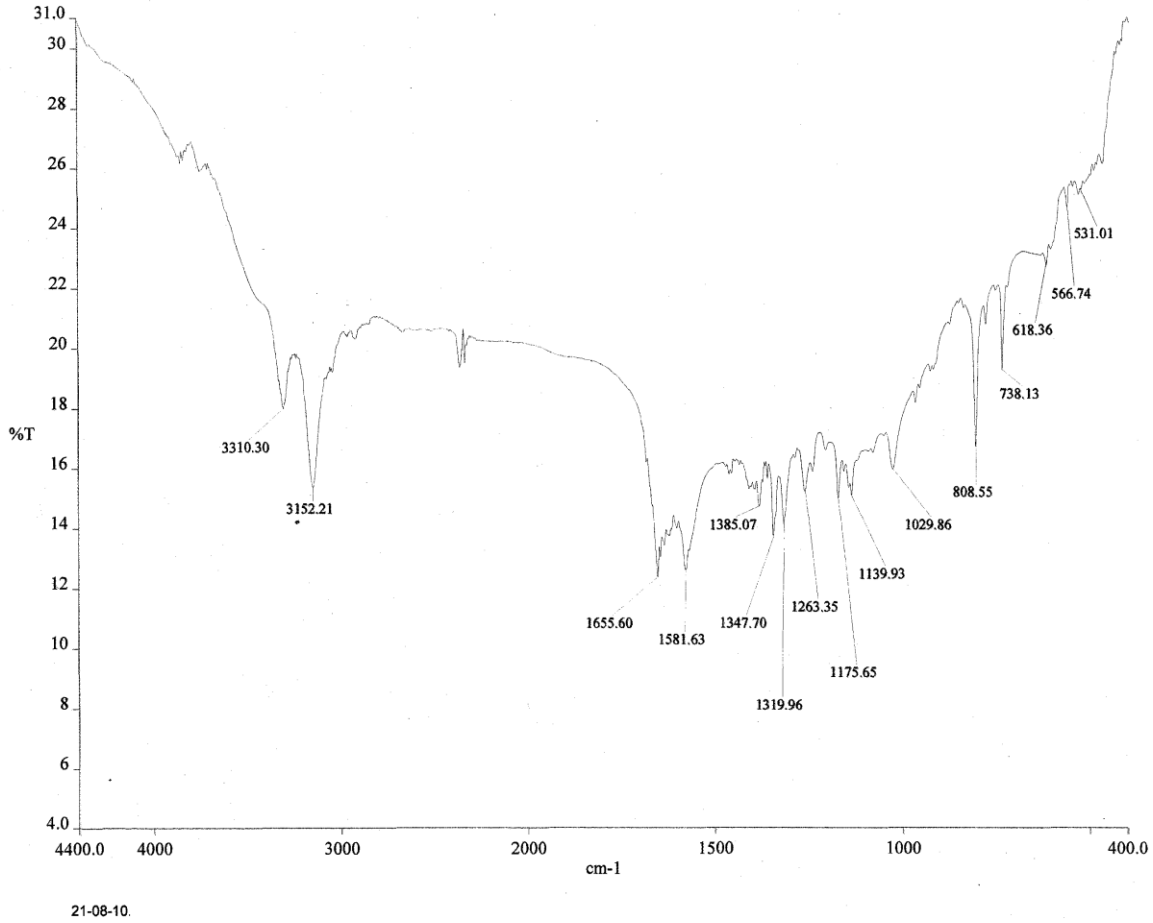


Fig. S13 FTIR spectra of **L.**

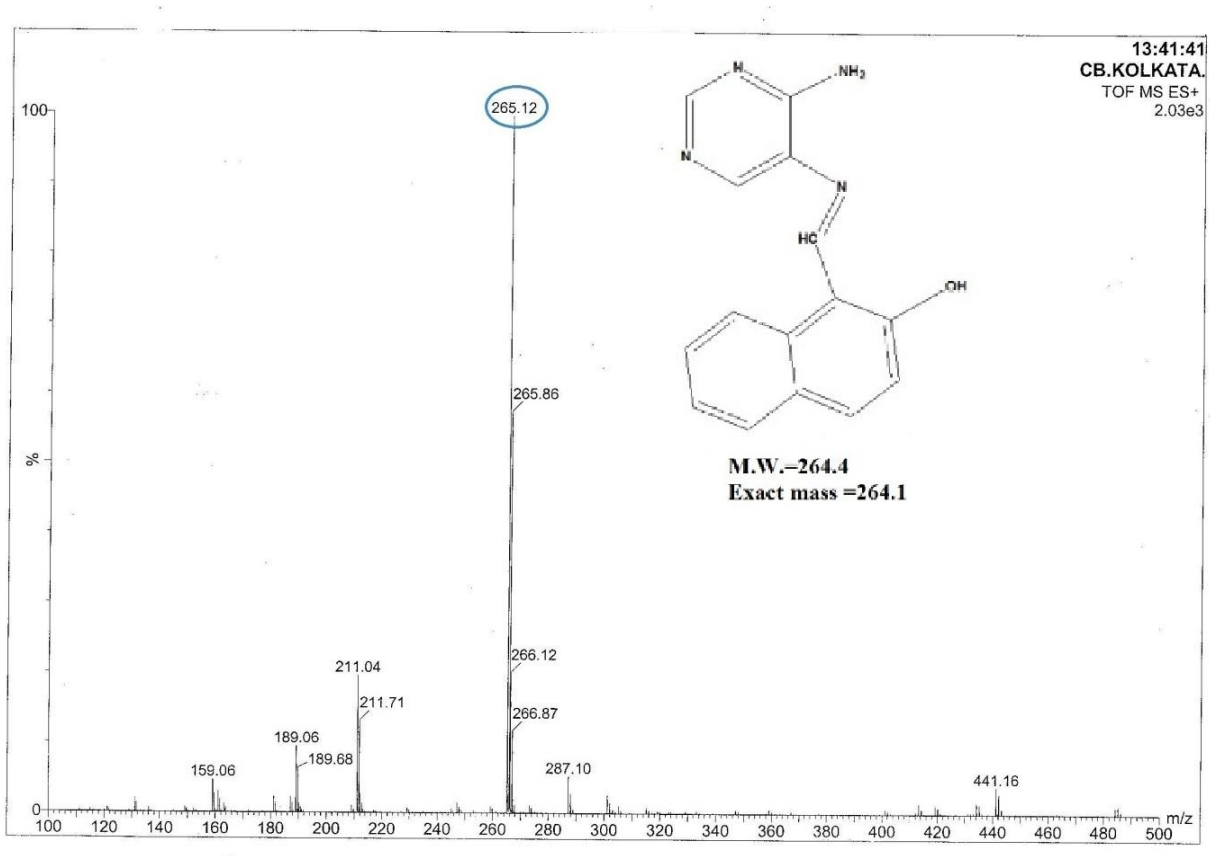


Fig. S14 QTOF-MS spectrum of **L.**

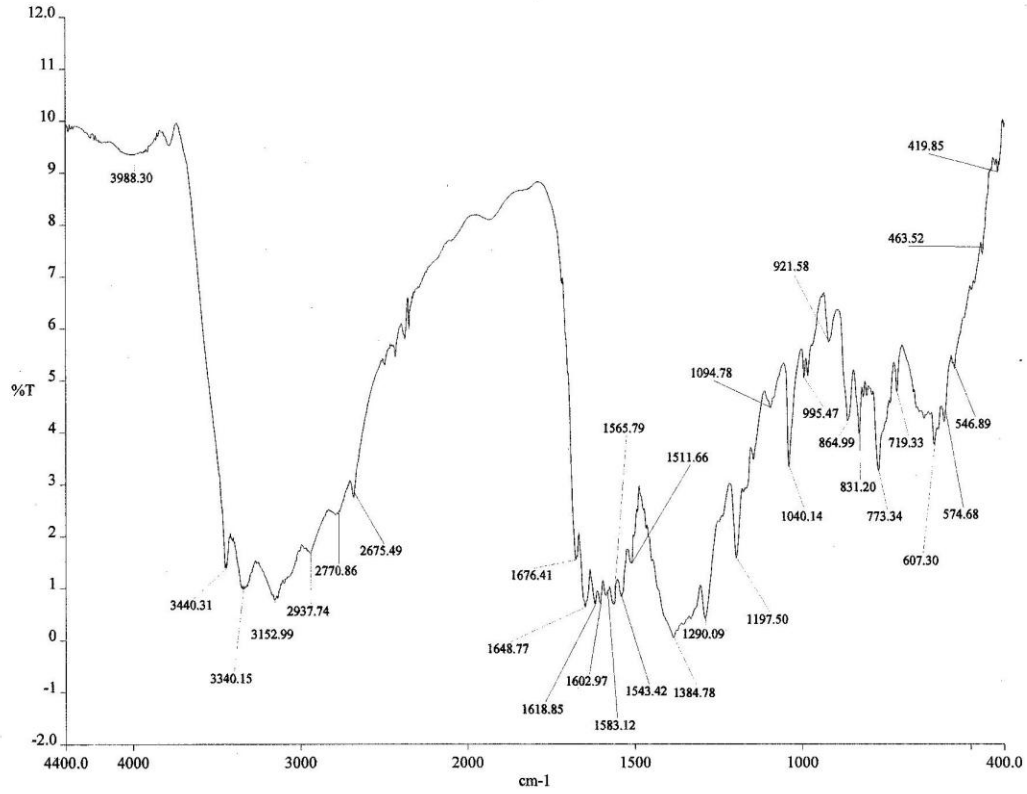


Fig. S15 FTIR spectra of [Al (**L**) (NO_3) (H_2O) (DMF)].

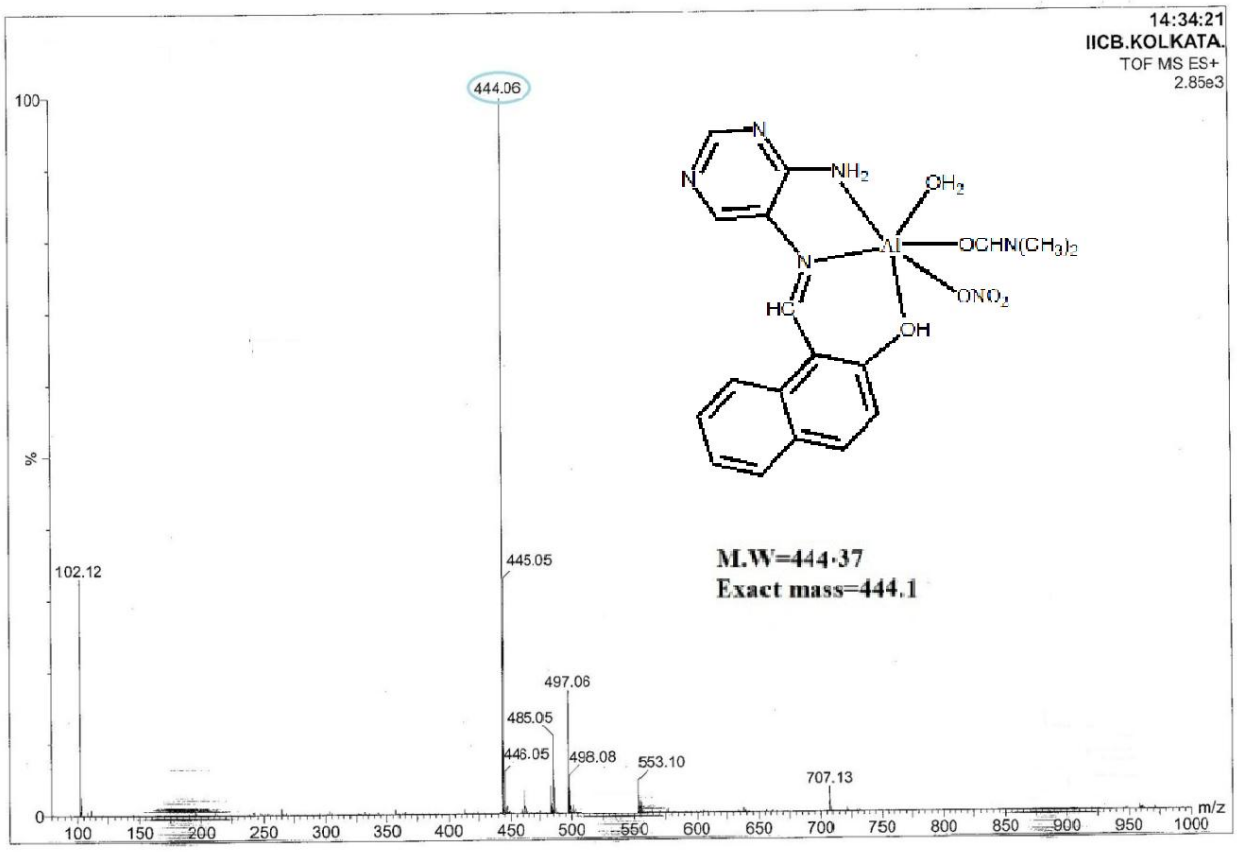
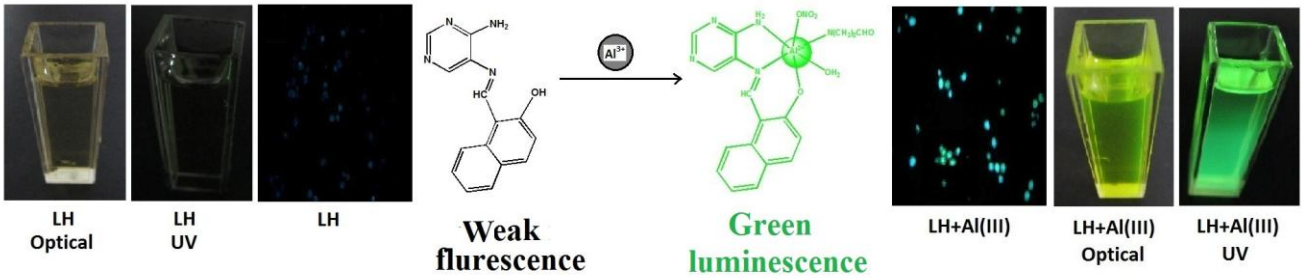


Fig. S16 QTOF-MS spectrum of complex [Al (**L**) (NO_3) (H_2O) (DMF)].



Graphical abstract

Naked eye detection of Al^{3+}using a turn-on fluorescent probe which produces green luminescence in presence of Al^{3+} and its application for living cell imaging.