Materials and methods

High-purity HEPES, indole 3-carboxyaldehyde and N-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Sigma Aldrich (India). NaN_3 was purchased from Merck (India). Solvents used were of spectroscopic grade. Other chemicals were of analytical reagent grade and had been used without further purification except when specified. Mili-Q Milipore® 18.2 MΩ cm^{-1} water was used throughout all the experiments. A JASCO (model V-570) UV–vis. spectrophotometer was used for recording absorption spectra. FTIR spectra were recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Mass spectra were performed on a QTOF Micro YA 263 mass spectrometer in ES positive mode. ^{1}H NMR spectra were recorded using Bruker Advance 600 (600MHz) in DMSO-d_6. Elemental analysis was performed using Perkin Elmer CHN-Analyzer with first 2000-Analysis kit. The steady-state fluorescence spectra were recorded with a Hitachi F-4500 spectrofluorimeter. pH measurements were performed with Systronics digital pH meter (model 335). All spectra were recorded at room temperature except fluorescence microscope images. All the fluorescence and absorption spectra were recorded after 5 minutes of mixing of azide ion with **L** (Fig. S21).

***Synthesis of* N-(2-(1H-indol-3-yl-)methyleneamino)ethyl)naphthalene-1-amine (L)**

An ethanol solution of indole 3-carboxyaldehyde (560 mg, 3.858 mmol) was added drop wise under stirring condition to a solution (20 mL in ethanol) of N-(1-naphthyl)ethylenediamine dihydrochloride (1g, 3.858 mmol) in presence of $NaHCO_3$ (648 mg, 7.716 mmol) (Scheme 1). Stirring was continued for further 30 minutes followed by reflux for 8 h. Upon removal of $NaHCO_3$, a brown color solution was obtained which on slow evaporation of the solvent, yielded a brown residue. The residue was recrystallized from ethanol. Yield 89 % , M. P. 153^{o}C (± 4^{o}C);

^{1}HNMR (600MHz, DMSO-d_6) (Fig. S14, ESI): 3.535 (2H, q, J = 6.6 Hz); 3.861 (2H, t, J = 6.0 Hz); 6.2 (1H, t, J = 5.4 Hz); 6.65 (1H, d, J = 7.2 Hz); 7.11 (2H, m, J = 7.2 Hz); 7.18 (1H, t, J = 1.2 Hz); 7.307(2H, m, J = 7.8 Hz); 7.756 (2H, m, J = 7.2 Hz); 8.128 (2H, d, J = 10.2 Hz); 8.269 (1H, d, J = 7.8) ;8.285 (1H, t, J = 9.6); 8.522 (1H, s); 11.510 (1H, s); ^{13}CNMR (300MHz, DMSO-d_6) (Fig. S20, ESI): 40.85, 59.20, 103.39, 111.90, 114.57, 115.60, 120.44, 121.41, 121.76, 122.26, 123.13, 124.11, 125.14, 125.68, 126.99, 128.13, 131.17, 134.17, 137.23, 144.12, 157.06. QTOF – MS ES^{+} (Fig. S10, ESI): $[M + Na]^{+}$ = 336.20 ; elemental analysis data as calculated for $C_{21}H_{19}N_3$ (%): C, 80.48; H, 6.11; N, 13.41. Found (%): C, 80.67; H, 6.18; N, 13.34. FTIR (cm^{-1}) (Fig. S11, ESI): υ(NH) 3399.92, υ(C=N) 1635.34.

Synthesis of [L- N_3 -H_2O] assembly

10 mL ethanolic solution of NaN_3 (0.0415 g, 0.638 mmol) was added slowly to a magnetically stirred solution (10 mL) of **L** (0.2 g, 0.638 mmol) in ethanol. Stirring was continued for 7 h. On slow evaporation of the solvent, a brown color compound was obtained. The compound was collected from ethanol. QTOF –MS ES^{+} (Fig.S12, ESI): $[M + Na]^{+}$ = 397.30. FTIR (cm^{-1}) (Fig.S13, ESI): υ(NH) 3437.49, υ(OH), 3437.49; υ(C=N), 1638.23; υ(-N_3^{-}), 2046.1, 2141.

Cell imaging studies

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.

Preparation and imaging of cells

A fungal strain (ASF-11) was isolated from Arctic glacier sample and grown in potato dextrose broth medium supplemented with 0.1 % yeast extract at 10 ^{0}C for three days. After three days

incubation, 1 mL culture broth containing the mycelium of the test strain was centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded and the mycellial pellet was washed twice in normal saline and then incubated in a solution of NaN_3 (200 μM) for 1h at 10 ^{0}C. After incubation, they were again washed in normal saline and then photographed using UV filter under the fluorescence microscope in presence and absence of the probe **L**.

Pollen grains were obtained from freshly collected mature buds of *Allamanda puberula* (Aapocynaceae), a common ornamental plant with bell shaped bright yellow flower by crashing stamens on a sterile petriplate and suspending them in normal saline. After crashing the stamina debrishes were removed by filtering through a thin layer of non absorbant cotton and then the suspended pollens were collected by centrifugation at 5000 rpm for 5 minutes. The pollen pellet was then washed twice in normal saline and then incubated in a solution of NaN_3 (200 μM) for 1 h at ambient temperature. After incubation they were again washed in normal saline as mentioned above and then photographed under the fluorescence microscope using UV filter in presence and absence of **L**.

Candida albicans cells (IMTECH No. 3018) from exponentially growing culture in yeast extract glucose broth medium (pH 6.0, incubation temperature, 37^{0}C) were centrifuged at 3000 rpm for 10 minutes, washed twice with 0.1 M HEPES buffer (pH 7.4). The cells were then treated with **L** (10 μM) for 30 minutes in 0.1 M HEPES buffer (pH 7.4) containing 0.01 % Triton X100 as permeability enhancing agent. After incubation, the cells were washed with HEPES buffer and incubated with NaN_3 (200 μM) for 30 minutes. Cells thus obtained were mounted on grease free glass slide and observed under the fluorescence microscope having UV filter. Cells incubated with **L** but without N_3^- were used as control.

A Gram positive bacterial strain was isolated from a tea garden soil and later identified as *Bacillus sp*. on the basis of 16S rDNA sequence homology and physio-biochemical characteristics. The bacteria isolate was grown in nutrient broth (pH 7.00 at 37 ^{0}C for 24 h. After 24 h incubation 1 mL culture broth containing the bacterial cells was centrifuged at 3000 rpm for 5 minutes, and the supernatant was discarded. The cell pellet was washed twice in normal saline and then incubated in a solution of NaN_3 (200 μM) for 1 h at 37 ^{0}C. After incubation they were again washed in normal saline as mentioned above and then photographed under a fluorescence microscope using UV filter in presence and absence of **L**. Both N_3^- treated and untreated cells were stained with **L** and observed under fluorescence microscope.

Calculation of Quantum Yield

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation,

$$\phi_{\text{sample}} = \frac{\text{OD}_{\text{standard}} \times A_{\text{sample}}}{\text{OD}_{\text{sample}} \times A_{\text{standard}}} \times \phi_{\text{standard}}$$

where A was the area under the fluorescence spectral curve and OD was optical density of the compound at the excitation wavelength. Anthracene was used as quantum yield standard (quantum yield is 0.27 in ethanol)[1] for measuring the quantum yields of ligand(**L**) and its **L**-N_3-H_2O complex.

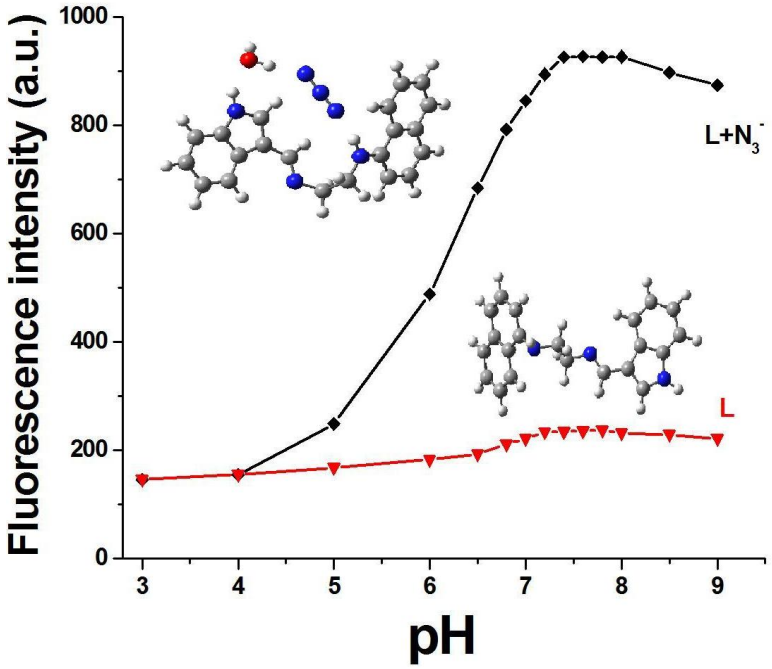


Fig.S1. Effect of pH on the fluorescence intensity of **L** (10 μM) and [**L** - N_3^-] system in ethanol – H_2O (4/1, v/v) solution. λ_{ex} = 330 nm and λ_{em} = 428 nm.

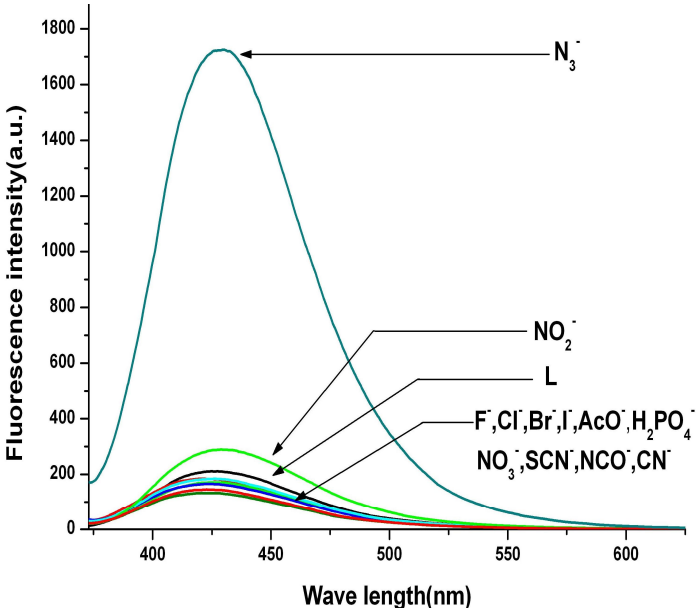


Fig. S2. Fluorescence spectra of **L** (10 μM) in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4) in presence of 7000 μM N_3^-, 700 equivalent NO_3^-, NO_2^-, SCN^-, NCO^-, CN^-, $H_2PO_4^{2-}$,CH_3COO^-, F^-, Cl^-, Br^-, I^-. λ_{ex} = 330 nm and λ_{em} = 428 nm.

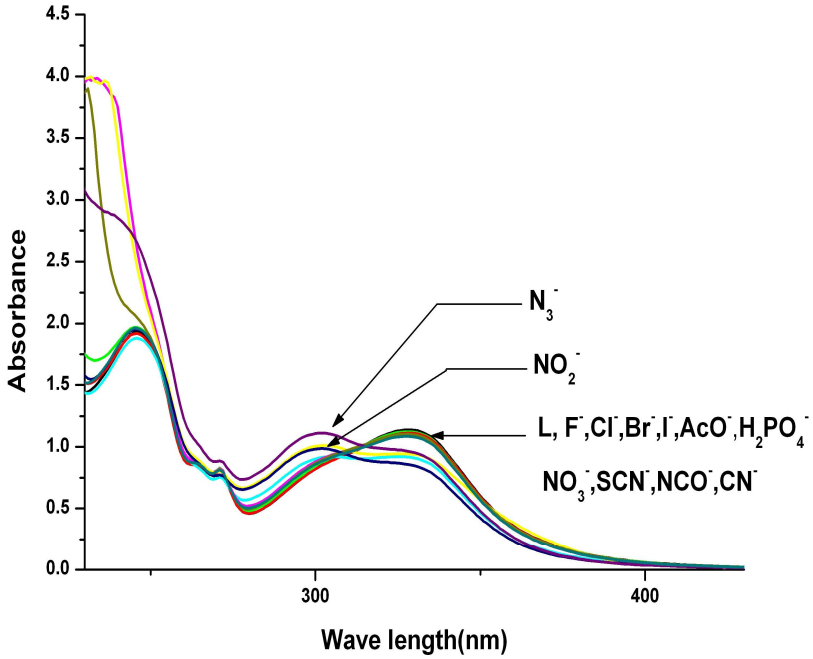


Fig. S3. UV-vis. spectra of **L** (10 μM) in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4) in presence of 7000 μM N_3^-, 700 equivalent NO_3^-, NO_2^-, SCN^-, NCO^-, CN^-, $H_2PO_4^{2-}$, CH_3COO^-, F^-, Cl^-, Br^-, I^-.

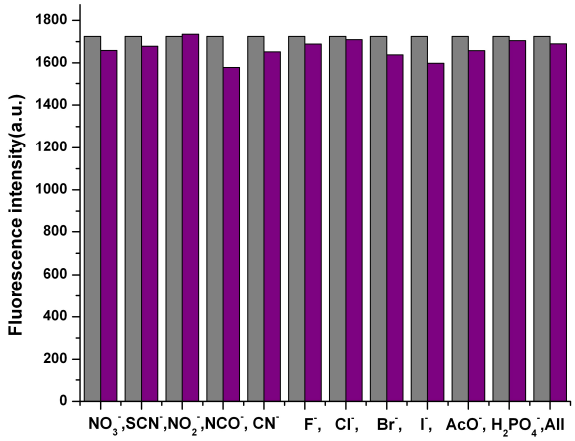


Fig. S4. Relative fluorescence intensity of [**L** - N_3^-] system in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4) in presence of various anions. Purple bar: **L** (10.0 μM) with 700 equivalent of N_3^-. Gray bar: 10.0 μM of **L** with 700 equivalent of N_3^- and 700 equivalent of foreign anion. **All** is mixture of SCN^-, NCO^-, $H_2PO_4^{2-}$, NO_2^-, CN^-, CH_3COO^- with mixture of **L** (10.0 μM) and N_3^- (700 equivalent).

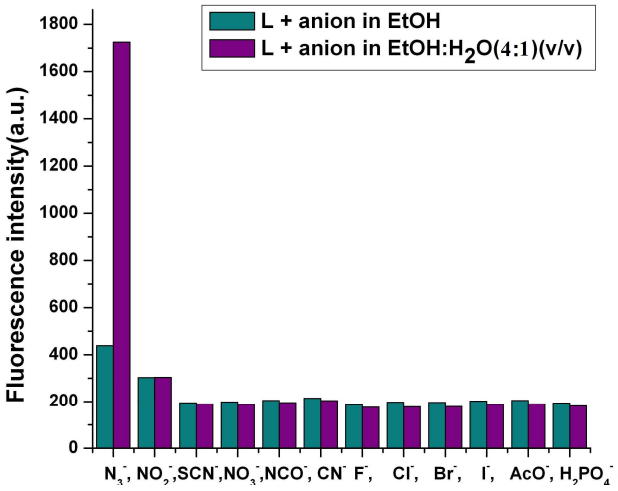


Fig. S5. Relative emission intensity of **L** in presence of different anions (in HEPES buffer, 0.1 M). [**L**] = 10.0 μM), [Anion] = 7000 μM.

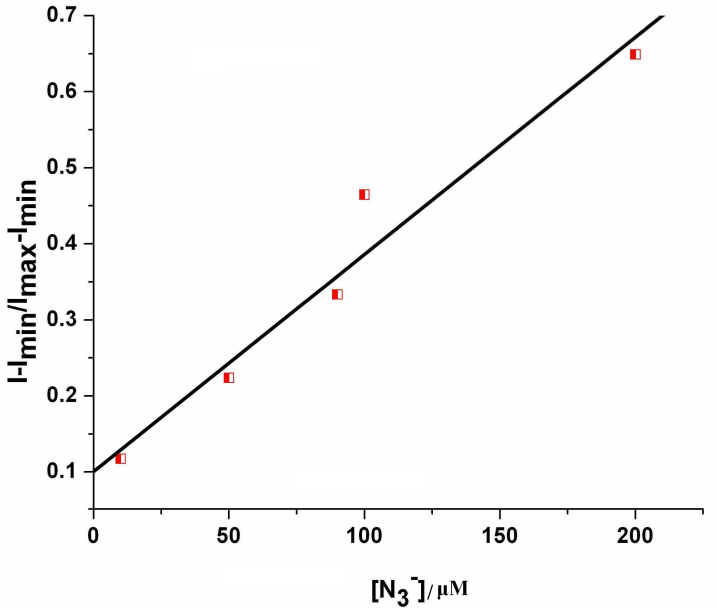


Fig. S6. Emission intensities of **L** (10.0 μM) as a function of externally added [N_3^-] in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4).

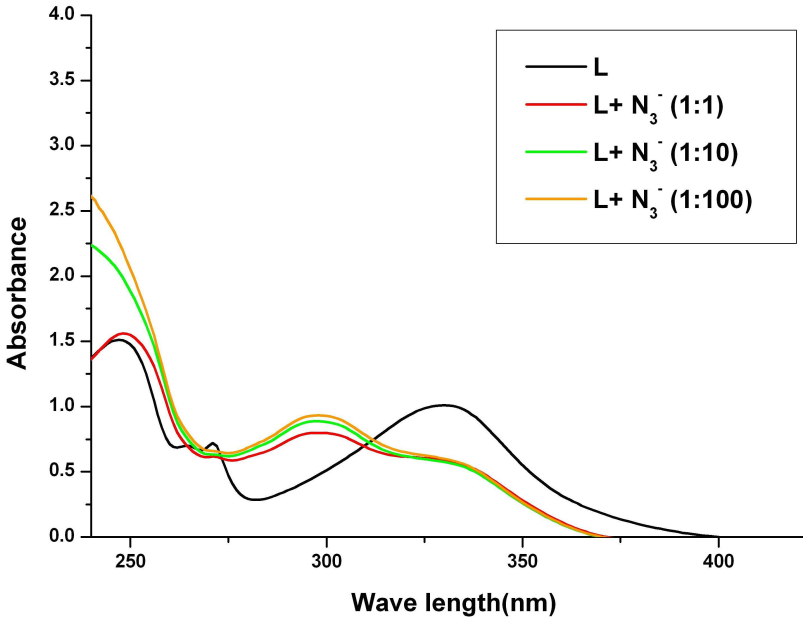


Fig. S7. Absorption spectra of **L** (10 μM) as a function of [N_3^-] in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4)

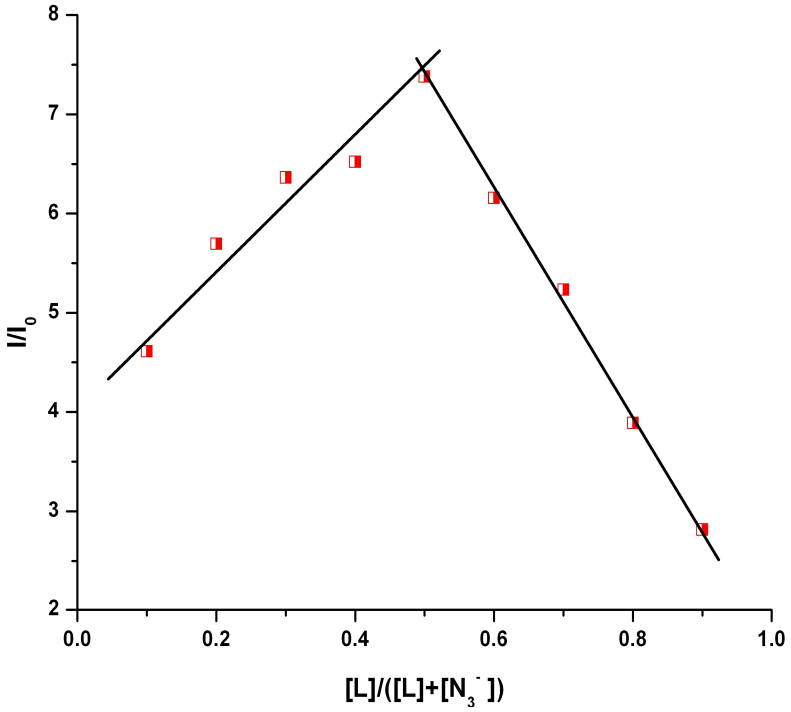


Fig. S8. Job's plot for determination of stoichiometry of [**L-** N_3^-] system in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4)

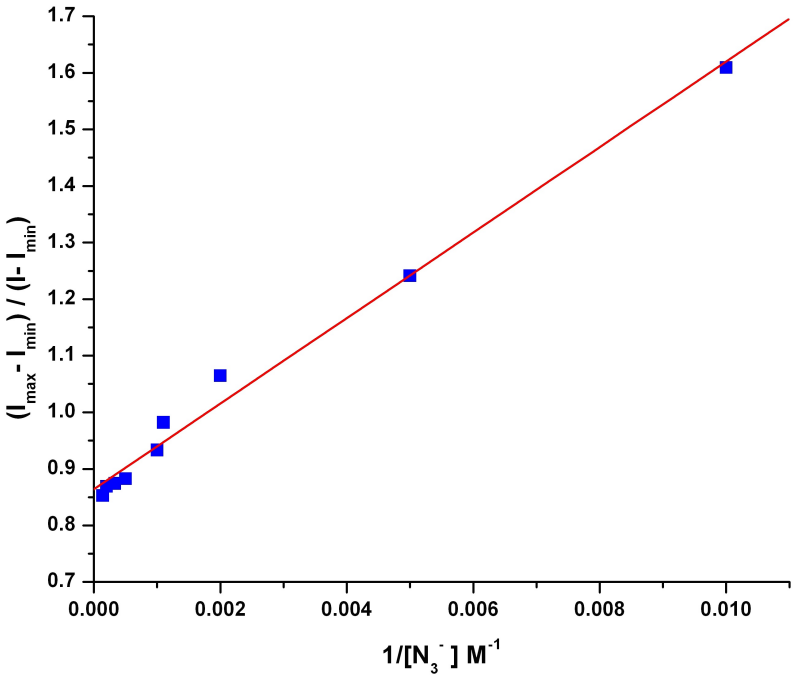


Fig. S9. Determination of association constant of **L** for N_3^- in ethanol/water (4/1, v/v, HEPES buffer, 0.1 M, pH 7.4)

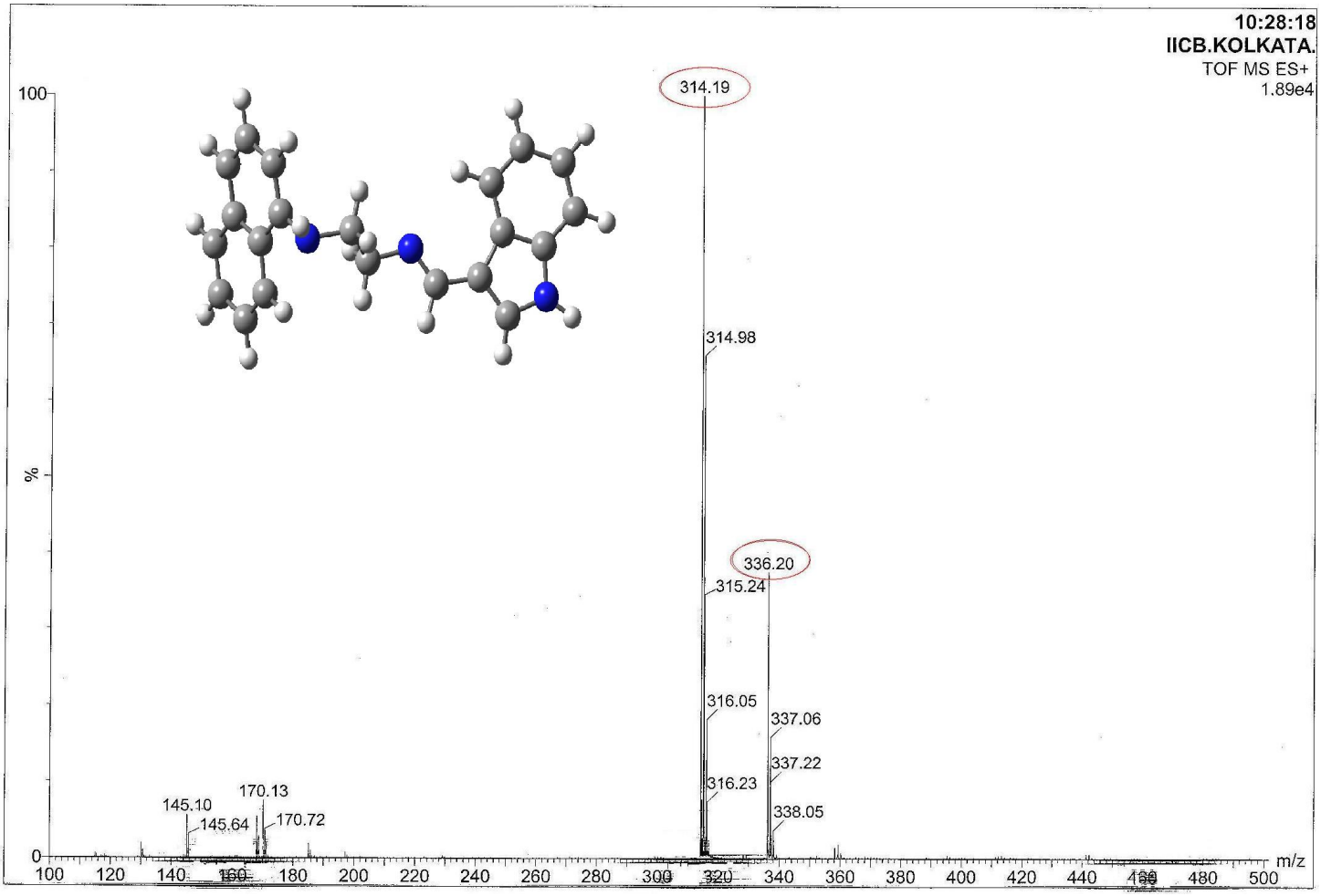
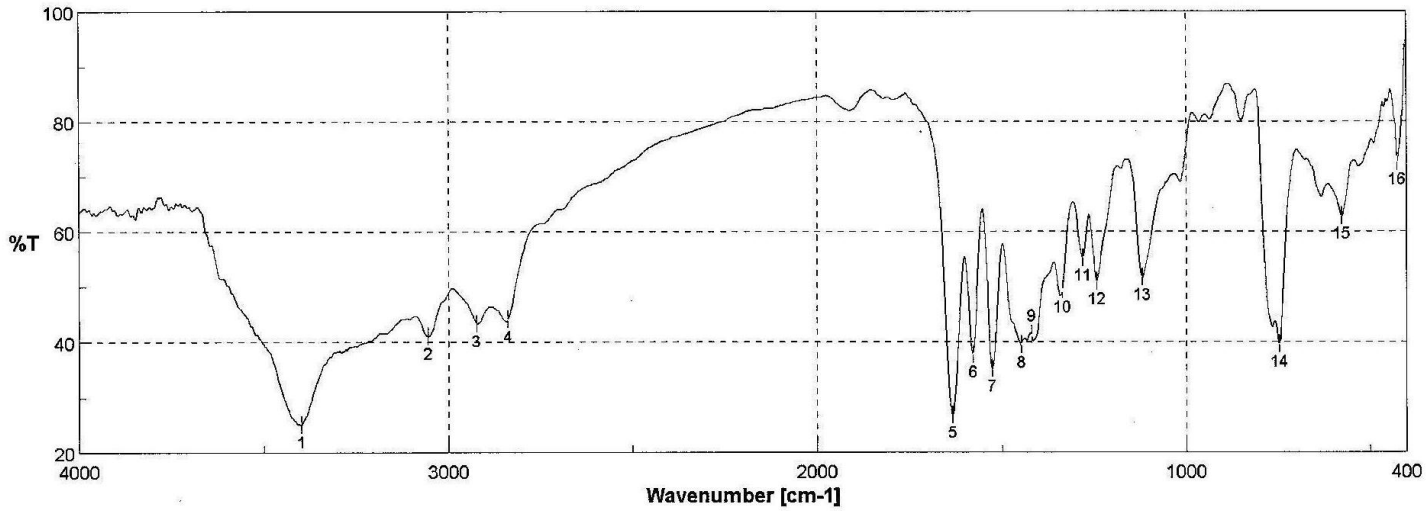


Fig. S10. QTOF-MS spectrum of **L**



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Zero Filling	ON	Apodization	Cosine
Gain	Auto (16)	Scanning Speed	Auto (2 mm/sec)
Date/Time	12/7/2010 6:01PM	Update	12/7/2010 6:03PM
Operator	ARNAB		
File Name	Memory#5		
Sample Name	NEDAIA		
Comment	Nil		

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3398.92	25.1206	2	3056.62	41.1482	3	2924.52	43.4371	4	2838.7	44.2035	5	1635.34	26.9138
6	1579.41	37.8401	7	1526.38	35.2176	8	1448.28	39.5615	9	1420.32	41.3555	10	1335.46	49.4516
11	1280.5	55.1914	12	1241.93	50.9793	13	1118.51	51.7745	14	746.317	39.6521	15	576.612	62.7841

Fig. S11. FTIR spectrum of **L**

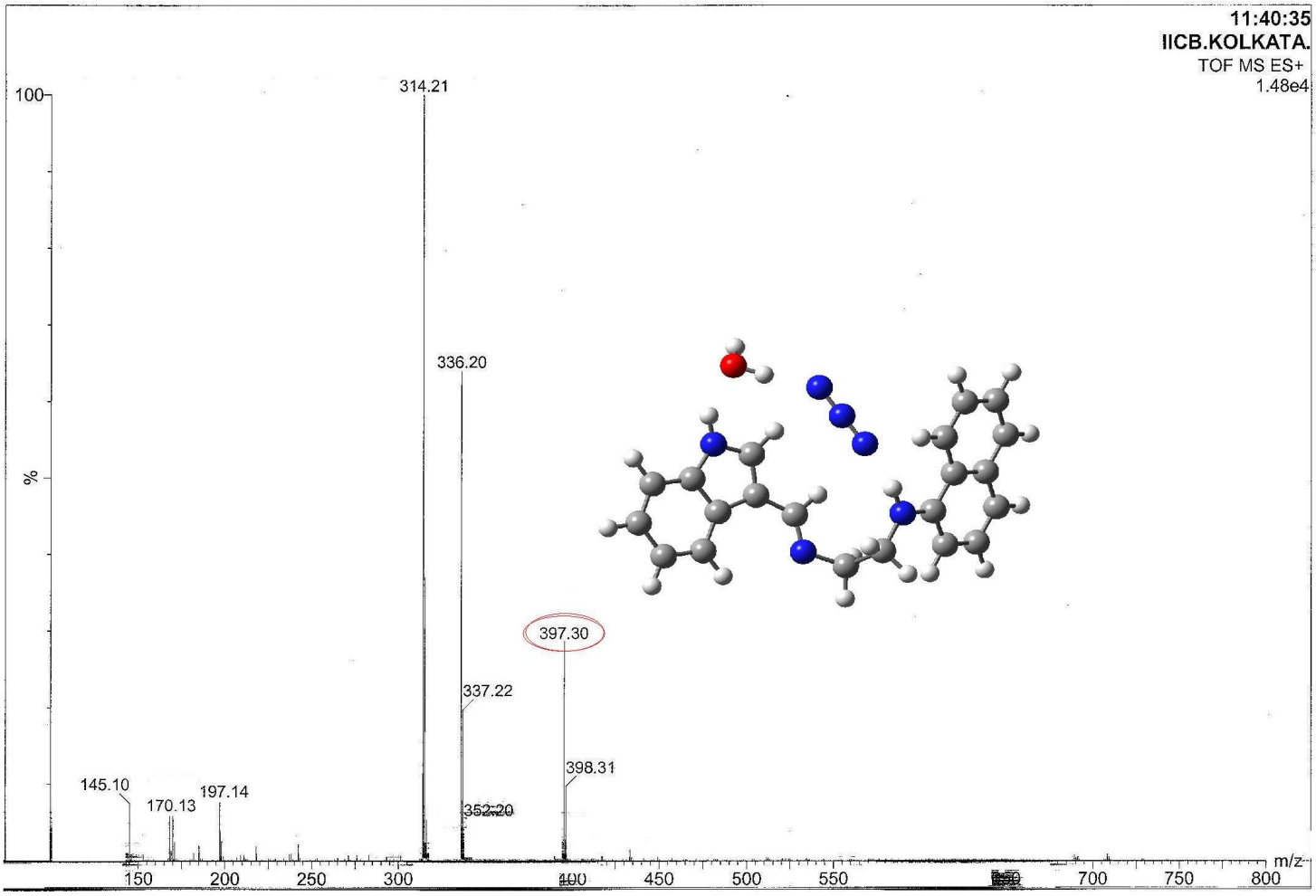
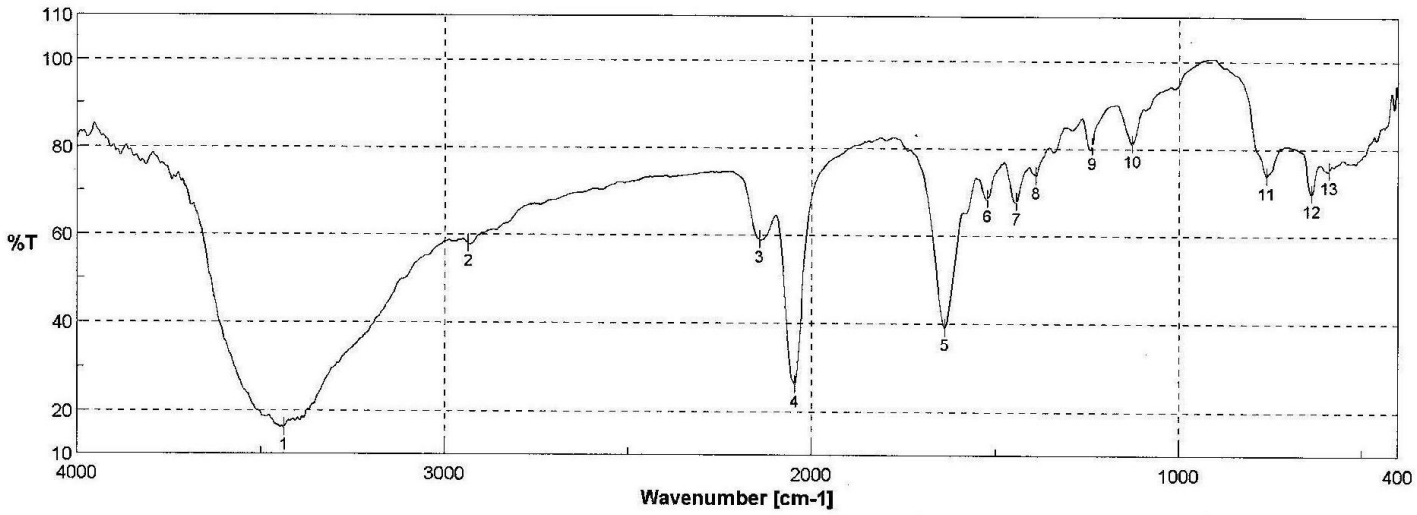


Fig.S12. QTOF-MS spectrum of **L**-N_3^--H_2O-Na^+-H^+ system



Accumulation	30	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	Auto (16)	Scanning Speed	Auto (2 mm/sec)
Date/Time	12/7/2010 6:12PM	Update	12/7/2010 6:15PM
Operator	ARNAB		
File Name	Memory#5		
Sample Name	NEDAIA		
Comment	Nil		

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3437.49	16.2155	2	2936.09	57.9366	3	2141.56	58.9436	4	2046.1	26.294	5	1638.23	39.2313
6	1522.52	68.4286	7	1442.49	67.7862	8	1390.42	73.8694	9	1237.11	80.5407	10	1127.19	81.1636
11	760.78	73.809	12	637.358	69.7352	13	589.147	75.1033						

Fig.S13. FTIR spectrum of **L**-N_3^--H_2O system

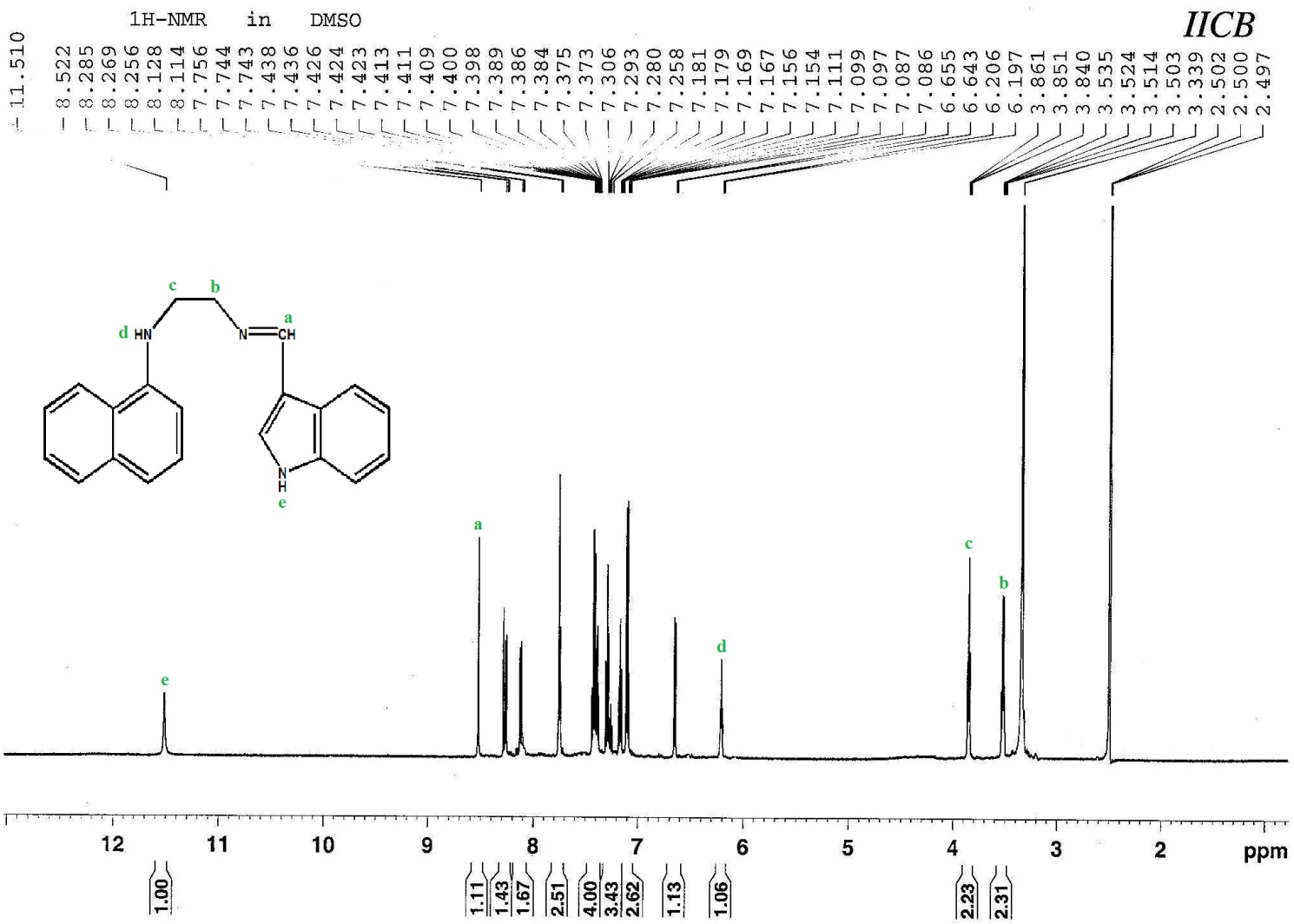


Fig. S14. ^{1}H NMR spectra of **L** in DMSO-d_6

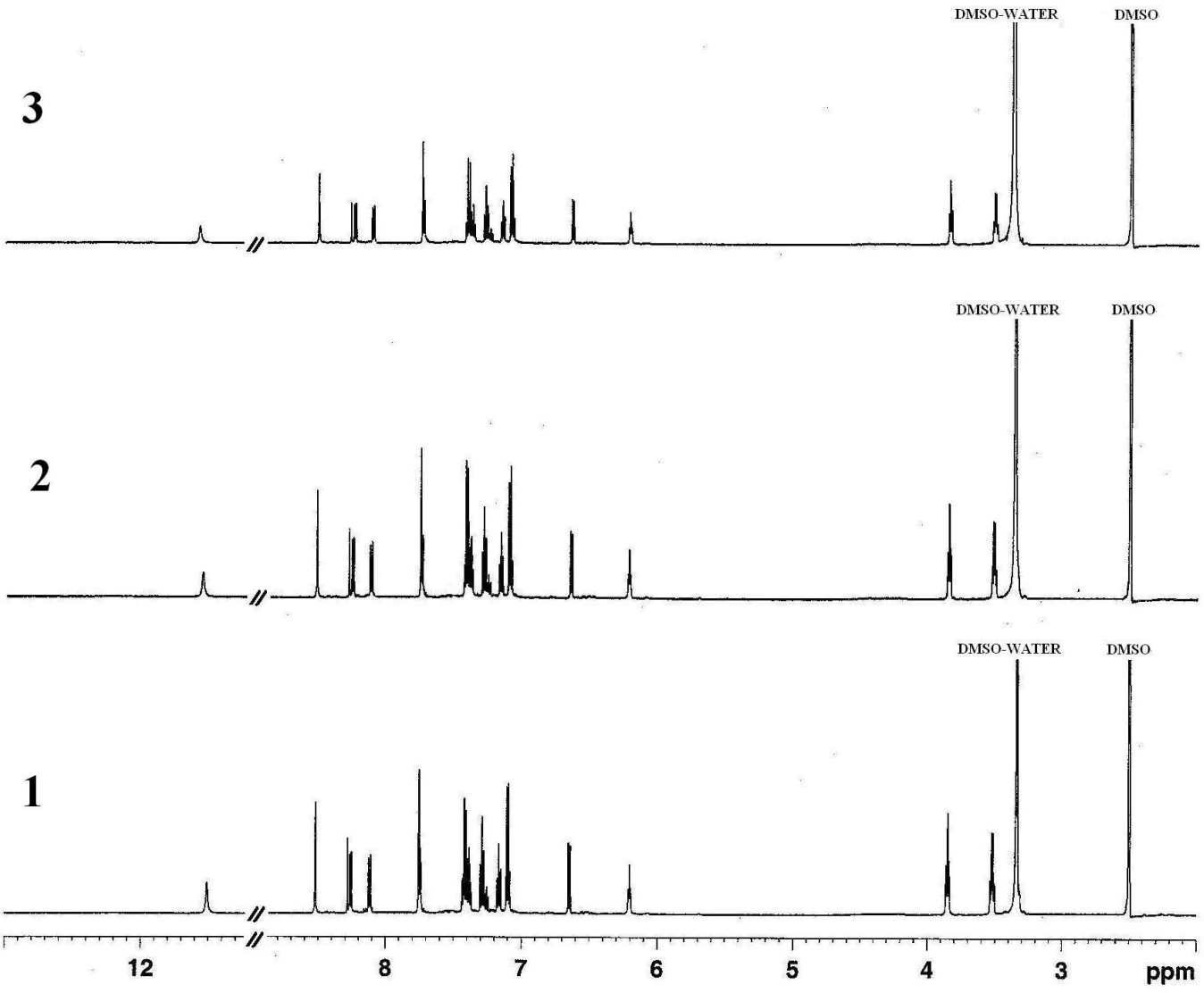


Fig. S15. ^{1}H NMR spectra of **L** (1); **L** with 2.5 equivalent of N_3^- (2); **L** with 5 equivalent of N_3^- (3) Solvent: DMSO-*d*6

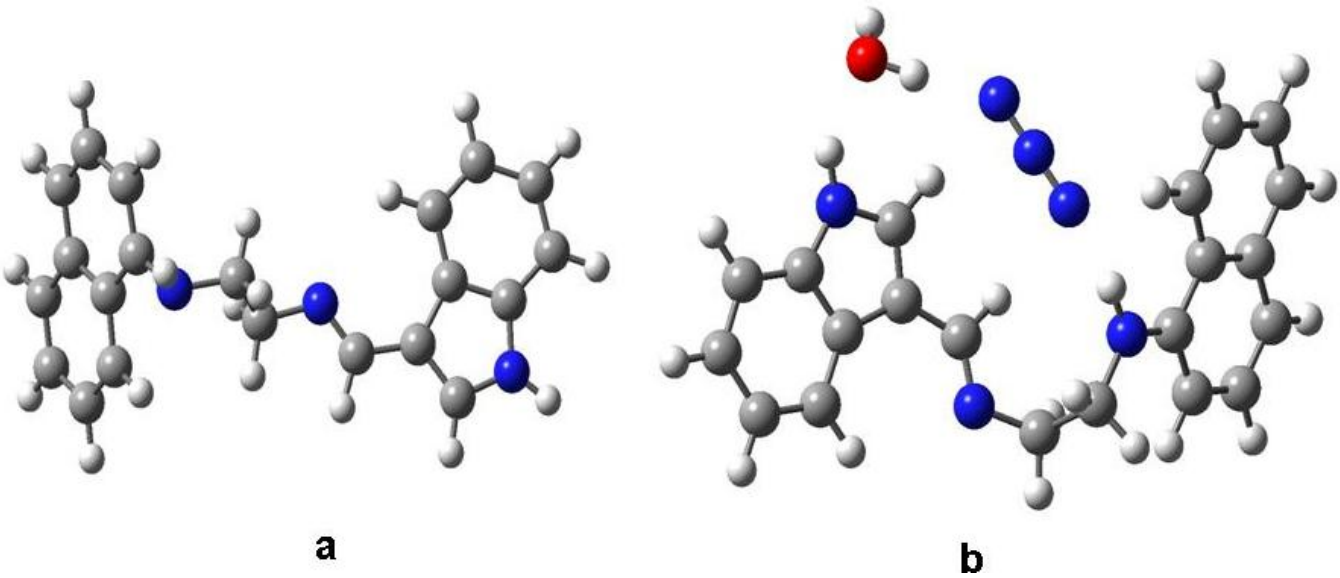


Fig.S16. Energy optimized geometry of **L** (a) and [**L**-N_3^--H_2O] system (b)

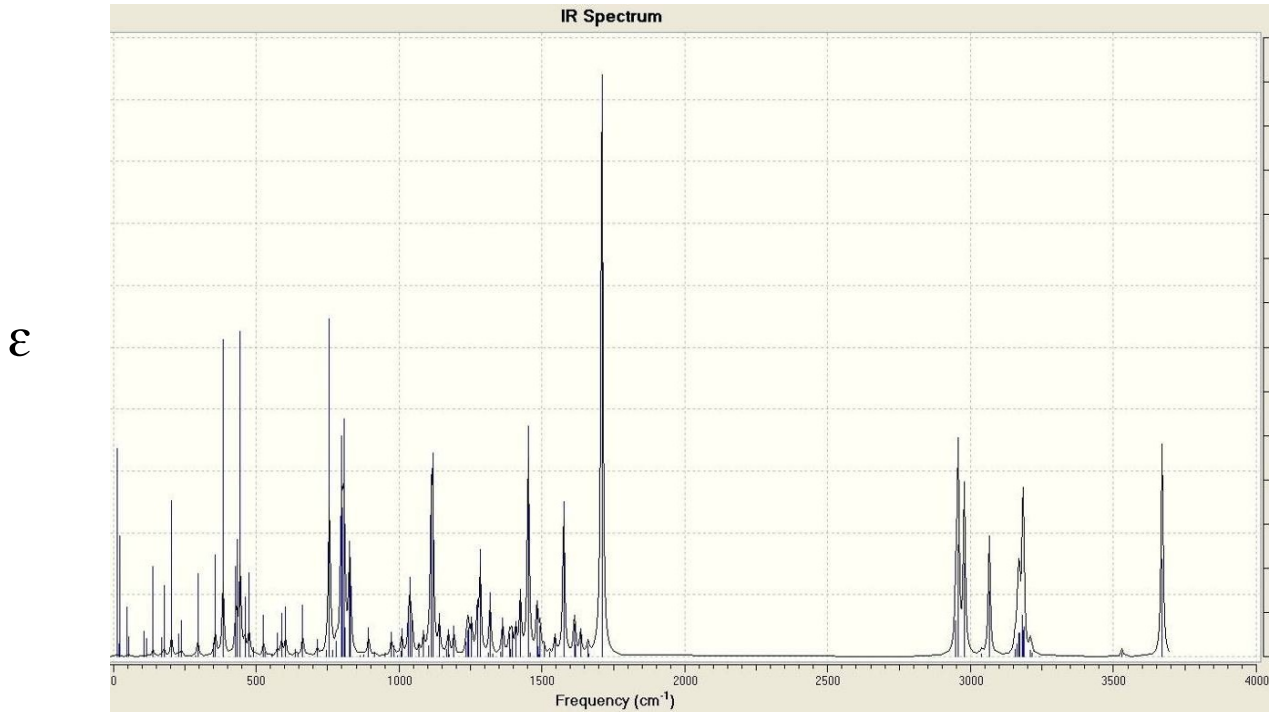


Fig.S17. Theoretical IR spectrum of **L** derived from DFT calculation

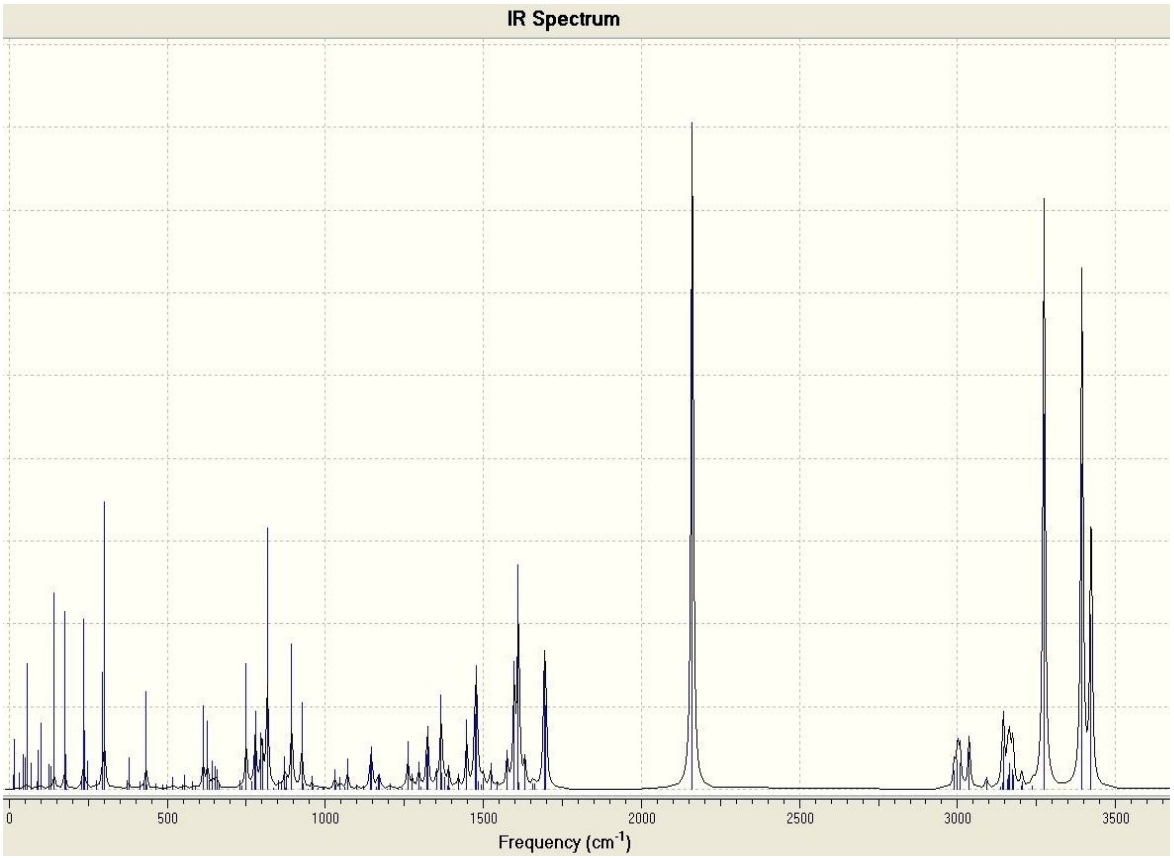


Fig.S18. Theoretical IR spectrum of [**L**-N_3^--H_2O] system derived from DFT calculation

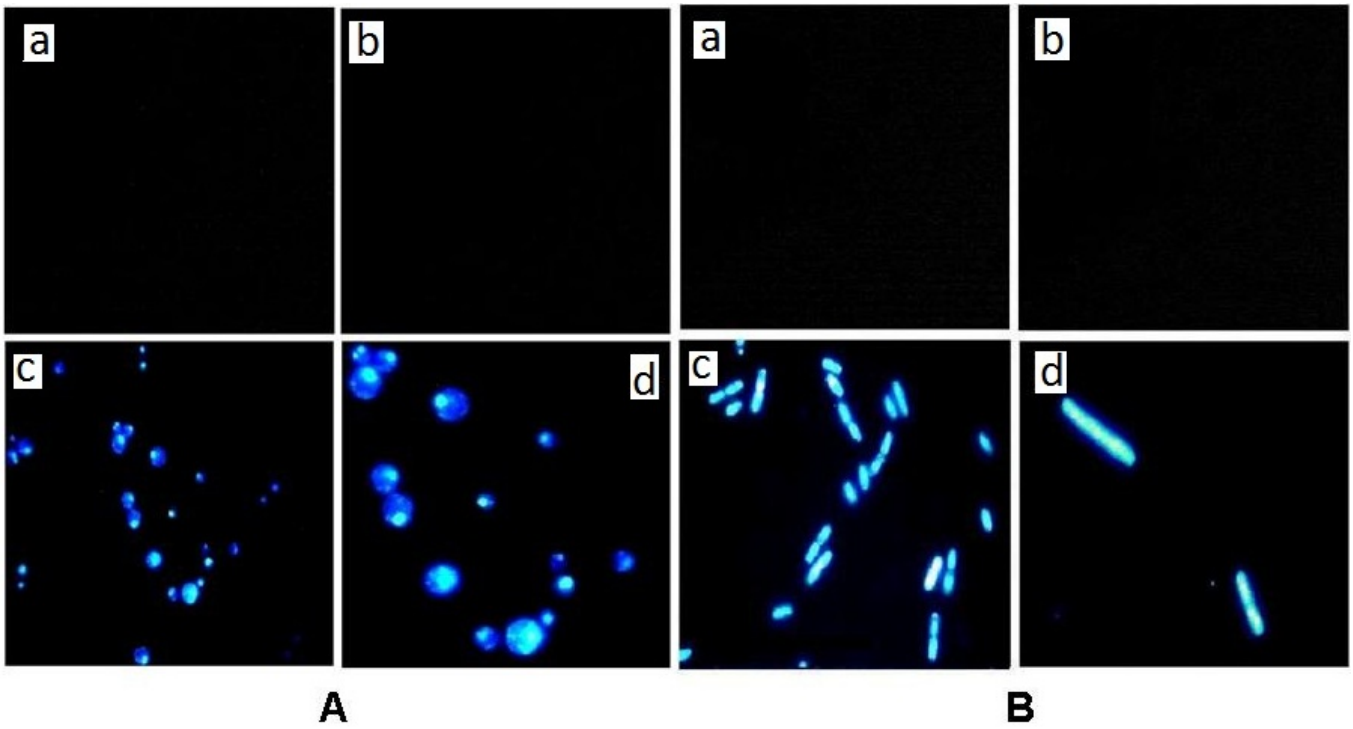


Fig. S19. (A) Fluorescence microscopy images of *Candida albicans* cells (IMTECH No. 3018) and (B) gram positive bacterial strain cells without **L** (a), cells loaded with probe **L** (10 μM) for 30 minutes under 100× objective lens (b), fluorescence image of **L**-stained cells pre-exposed to 200 μM N_3^- for 30 min under 100× objective lens (c), and expanded image of (c) (d)**.** Incubation was performed at 37 ◦C.

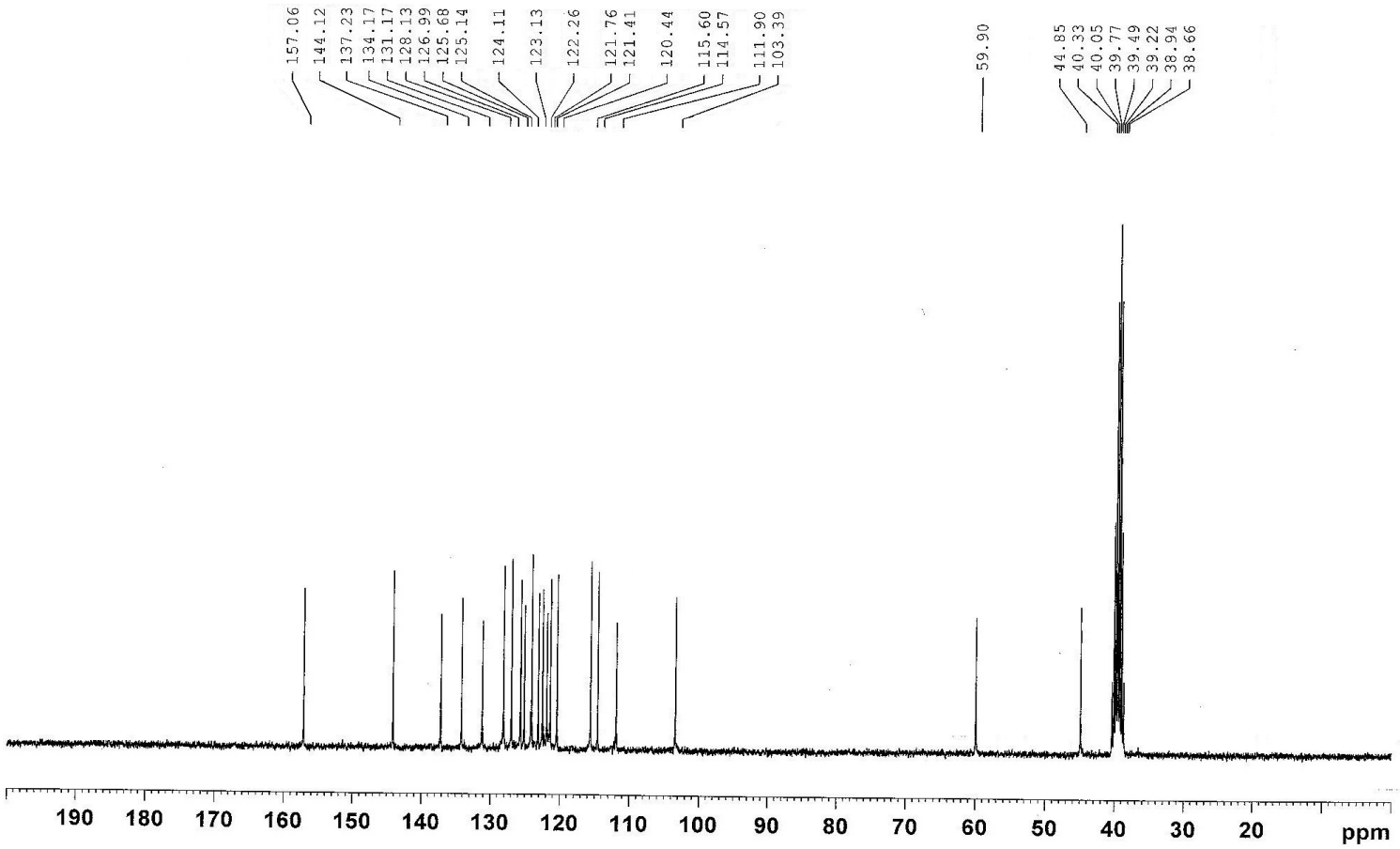


Fig. S20. ^{13}CNMR spectra of **L**

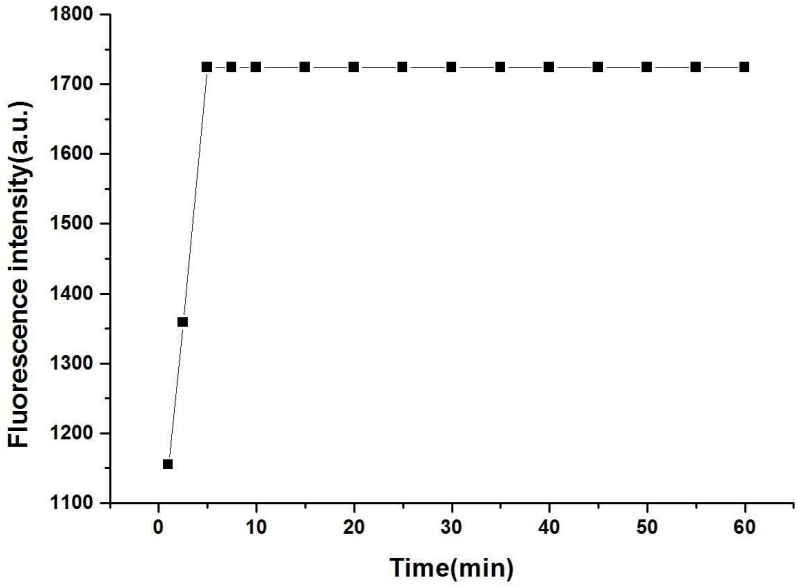


Fig. S21. Emission intensity of **L** (10μM) in presence of 7000 μM of azide ion as a function of time (minute)

1. W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229.