

Synthesis and Characterization of 1,2,3-Triazole Containing Fe(II) Sensor

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A new *bis*(1,2,3-triazolyl) imine based probe was designed and synthesized. Chemical structure of the probe was confirmed by IR, ^1H and ^{13}C NMR spectroscopy. The probe was investigated for its recognition abilities in aqueous-organic mixture against various cations and anions. It shows a highly selective colorimetric response to Fe(II) ion by changing the colour from colourless to brownish pink. Chemosensitivity of the probe was investigated by absorption spectrometric titration with the Fe(II) ions. ^1H NMR titration studies indicated imine nitrogen and one of the nitrogen in triazole ring was involved in complex formation with Fe^{2+} ion. Energy optimization studies by DFT method exhibits a marginal energy gap between ligand and Fe(II) complex (0.1166 eV) confirms the formation of metal ligand complex.

Keywords: Colorimetric chemo sensor, Chromogenic Fe^{2+} sensor, 1,2,3-Triazole, Absorption spectroscopy, Energy minimization.

INTRODUCTION

Iron is the most important, essential and abundantly available metal ion in vertebrates and plants, involved many biological processes like the construction of haemoglobin [1], electron transport processes [2,3], oxygen uptake and transport in the human cellular metabolic system [4-6]. It is also toxic when in excess in many ways for example Parkinson's [7], Alzheimer diseases [8], anaemia [9], kidney disorder [10,11] and liver damage [12], *etc.* Though Fe^{2+} plays an important role in natural processes and a dietary requirement of most organism, it creates hurdles to achieve good bleaching level and uniform dyeing in textile processing [13]. It is also considered a potent contaminant in ground water [14]. Comparatively, reports for Fe^{2+} selective sensors are very limited; hence it is necessary to develop an efficient sensor system to detect Fe^{2+} ions. Among the various available methods to detect Fe^{2+} , chromogenic analysis gains attraction due to its simplicity and portability. Majority of the chromogenic sensor systems so far reported for Fe^{2+} are based on complex formation using electron donor moieties like pyridine [11], bipyridine [12,13] and terpyridine [14-18] based ligands. But in those heterocyclic donors, most of them are sensitive to more than one ion [19]. Apart from that, few Schiff base sensors were also reported [19-21]

that also contains pyridine based binding sites. A few research papers published with other binding sites like fluoran dye [22], imidazole [23], pyrimidine [24], fluorogenic and functionalized gold nanoparticle sensing probes have also been reported for Fe^{2+} ions [25-28]. 1,2,3-Triazole is an important chelating system with strong chemical stability and excellent biocompatibility as it mimics the peptide linkage of the protein [29]. It has been very much used as a linker between chemical and biological entities [30]. 1,2,3-Triazole moiety is established as an effective chemosensor for various ions like Cu^{2+} [31], Al^{3+} [32], Zn^{2+} [33], Hg^{2+} [34], Fe^{3+} [35], Ca^{2+} [36], Pb^{2+} [37]. In this paper, we report a colorimetric sensor based on 1,2,3-triazole as an integral part of the sensing molecule. This sensor is highly selective towards Fe^{2+} and produces a distinct pink colour when in contact with the analyte.

EXPERIMENTAL

All the chemicals were purchased from Loba Chemicals or SRL, India and used as received. FTIR spectra were recorded on Agilent FT-IR Spectrometer with KBr pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 NMR Spectrometer using TMS as internal standard and CDCl_3 as solvent. UV/Vis measurements were recorded on Elico double beam

spectrophotometer at room temperature. Column chromatography was performed on silica gel (60–200 mesh) from Merck Company. All the reactions were followed by TLC using Merck 0.2 mm silica gel 60 GF254 analytical aluminum plates. Cation solutions were freshly prepared as 1×10^{-3} M stocks from the corresponding perchlorate salts and anion solutions were prepared at the same concentration from its quaternary ammonium or sodium (N_3^- , acetate) salts. Appropriate amount of ligand was dissolved in 8:2 water:acetonitrile mixture and sonicated to ensure complete dissolution. This solution was diluted in 0.1 M HEPES buffer at pH 7.2 at room temperature.

Synthesis of benzazide (A): Aniline (0.01 mol) is dissolved in 30 mL of conc. HCl and mixture was cooled (0–5 °C). A solution of NaNO_2 (0.012 mol) in water (5 mL) was added in a drop-wise manner to the reaction mixture in such a way that the temperature of the mixture is controlled between 5–7 °C. After being stirred well for 10 min, a cold solution of NaN_3 (0.02 mol) in water (20 mL) was added to the reaction mixture and stirred for 0.5 h. An oily layered was formed was then extracted with ether several times and the combined extracts are washed with NaHCO_3 , brine solution and dried over Na_2SO_4 . The solvent was removed under vacuum, a yellow oil (A) obtained was pure enough for further steps.

^1H NMR (CDCl_3 , δ ppm): 6.9 to 7.24 (d, 3H, aromatic H). ^{13}C NMR (CDCl_3 , δ ppm): 134, 130, 118 (aromatic carbon), 137 (aromatic *ipso* carbon). FT-IR (cm^{-1}): 2105 ($-\text{N}_3$), 3030 (aromatic CH– stretching).

Synthesis of (1-phenyl-1H-1,2,3-triazol-4-yl)methanol (B): The synthesized compound A (0.001 mol) was dissolved in dry THF and added with propargyl alcohol (0.001 mol) along with CuI (0.001 mol) and triethylamine (0.5 mL). The reaction mixture was stirred at room temperature for 12 h and followed by TLC and the solution was filtered after completion of the reaction. THF was removed under vacuum yielded pale brown coloured crude product. It was purified by column chromatography over silica gel using a chloroform-methanol mixture (9:1).

^1H NMR (CDCl_3 , δ ppm): 7.47 to 7.88 (d, 3H, aromatic H) and 8.66 (s, 1H, triazole) and 4.66 (s, 2H, CH_2) and 5.3 (s, 1H, -OH). ^{13}C NMR (CDCl_3 , δ ppm): 120, 128, 129 (aromatic carbon) and 136 (aromatic *ipso* carbon) and 149 (triazole *ipso* carbon) and 54 (methylene carbon). FT-IR (cm^{-1}): 3109 (triazole CH stretching) and 3030 (aromatic CH– stretching) and 2920 (CH_2 -stretching) and 3350 (OH stretching).

Synthesis of 1-phenyl-1H-1,2,3-triazole-4-carbaldehyde (C): Compound B (0.0017 mol) was dissolved in dry dichloromethane (30 mL) and pyridinium chloro chromate (0.002 mol) was added and stirred at room temperature. The progress of the reaction was checked by TLC. The reaction mixture was filtered after the reaction was completed. The solvent in the filtrate was removed under vacuum. The residue was purified by column chromatography over silica gel using chloroform as eluent. The product was collected as a pale white solid.

^1H NMR (CDCl_3 , δ ppm): 7.52 to 7.65 (d, 3H, aromatic H) and 7.98 (s, 1H, triazole) and 9.55 (s, 1H, CHO). ^{13}C NMR (CDCl_3 , δ ppm): 120.67, 126.09, 129.45 (aromatic carbon) and 135.94 (aromatic *ipso* carbon) and 147.52 (triazole *ipso* carbon) and 184.92 (aldehydic carbon). FT-IR (cm^{-1}): 3110

(triazole CH stretching) and 3032 (aromatic CH– stretching) and 1730 (C=O stretching).

Synthesis of (1E,1'E)-N,N'-(ethane-1,2-diyl)bis(1-(1-phenyl-1H-1,2,3-triazol-4-yl)methanimine) (D): Compound C (0.002 mol) was dissolved in ethanol (50 mL) and ethylene diamine (0.001 mol) and 3 drops of glacial acetic acid were added with stirring. The mixture was refluxed in an oil bath for another 6 h and concentrated to half of its volume. The mixture was kept at the refrigerator for overnight. A white solid obtained was filtered and recrystallized in hot ethanol yielded the target compound D.

^1H NMR (CDCl_3 , δ ppm): 7.48 to 7.82 (d, 3H, aromatic H) and 8.58 (s, 1H, triazole) and 8.87 (s, 1H, imine) and 3.75 (CH_2). ^{13}C NMR (CDCl_3 , δ ppm): 120.75, 121.06, 126.60 (aromatic carbon) and 136.6 (aromatic *ipso* carbon) and 144.4 (triazole *ipso* carbon) and 29.71 (methylene carbon) and 147.52 (imine). FT-IR (cm^{-1}): 3060 (triazole CH stretching) and 3123 (aromatic CH–stretching) and 1640 (C=N stretching).

Binding titration: The stock solutions of ligand (D) were prepared by dissolving compound D in $\text{CH}_3\text{CN}/\text{water}$ (2:8, v/v) containing HEPES buffer (pH = 7.2). The cationic stocks were prepared in distilled water and taken in five times to the concentration of ligand for UV-visible absorption spectral analysis. For titration experiments, each time 3 mL solution of compound D was filled in a quartz cell and added the metal ion solutions by stepwise addition of different equivalents using a micro-syringe. After each addition of analyte ion, the solution was well stirred to ensure complete reaction.

RESULTS AND DISCUSSION

The target imine ligand (D) was synthesized from starting compound aniline through four steps. The scheme of the synthesis of the target ligand is presented in Fig. 1. All the compounds were sufficiently purified before proceeding to the next step except in the first step because the product benzazide (A) obtained was pure enough and starts decomposing on storage. Aniline on diazotization followed by nucleophilic substitution with azide produces azido benzene, which on further reaction with propargyl alcohol under click reaction conditions [38] yielded methanolic derivative of 1,2,3-triazole. It was further oxidized to get 1,2,3-triazole containing aldehyde functionality. The target ligand was prepared by the reaction of triazole containing aldehyde with aliphatic diamine. Spectroscopic investigation of all the precursors and final compound confirms the chemical structure of the synthesized compounds. The disappearance of azide stretching frequency at 2105 cm^{-1} and appearance a new weak stretching absorption at 3109 cm^{-1} in IR spectrum of compound B indicated the conversion of azide group to 1,2,3-triazole ring and further it was confirmed by observing a resonating singlet for one proton around 8.5 δ ppm in compounds B, C and D. Formation imine linkage was confirmed by observing a singlet at 8.9 ppm.

In order to investigate the recognition abilities of the ligand D, it has been carried out a series of experiments in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (2:8/v:v) aqueous/organic HEPES buffered solution at pH 7.0. The experiments were carried out by dissolving the ligand in the aqueous/organic mixture at 1.23×10^{-3} M concentration buffered at pH 7.0 with HEPS and sonicated for 2 min

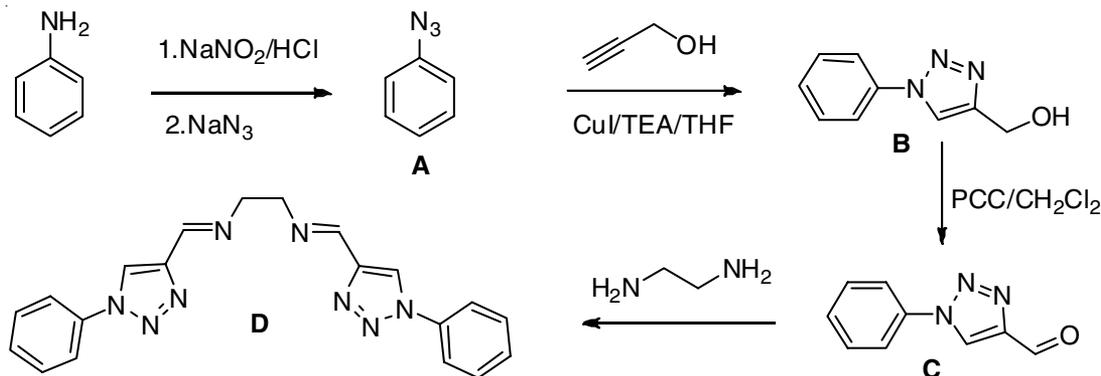
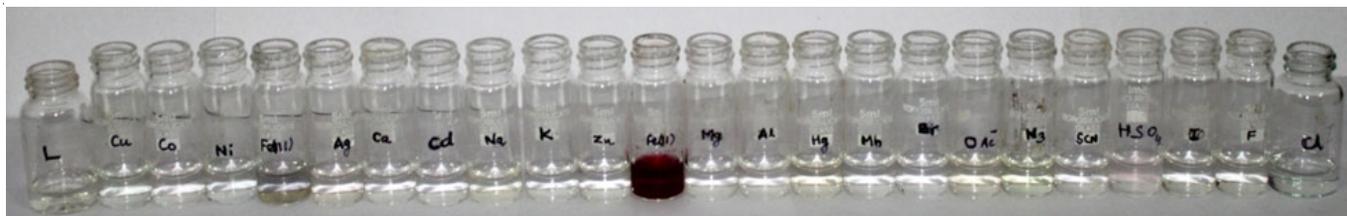
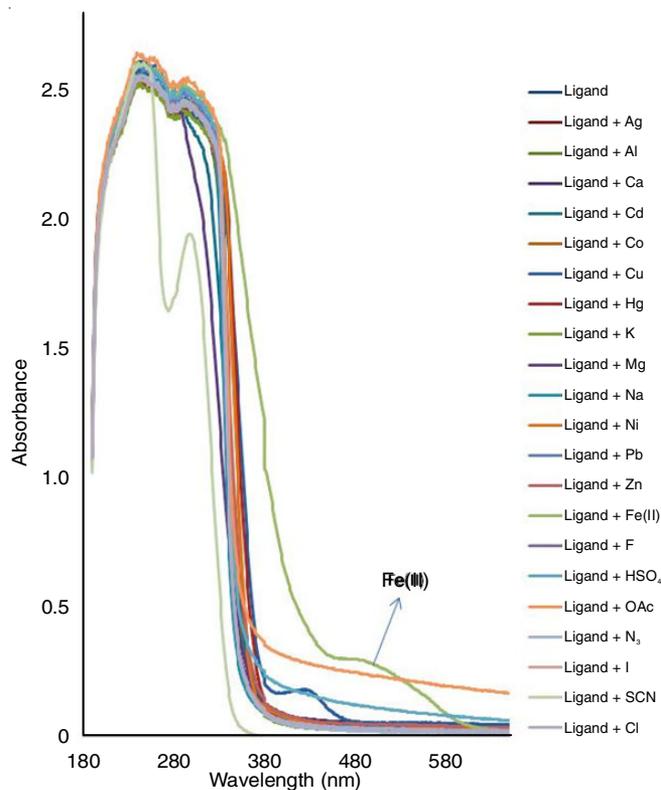
Fig. 1. Scheme of the synthesis of ligand **D**

Fig. 2. Colour change while adding various metals ions to ligand solution

for ensuring complete dissolution. Then it was added with various aqueous perchlorate cation solutions (Al^{3+} , Ca^{2+} , Mg^{2+} , Mn^{2+} , Cd^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Hg^{2+} , Zn^{2+} , Pb^{2+}) and quaternary ammonium salts of halogens, SCN^- and the sodium salt of N_3^- and OAc^- , approximately five times the concentration of ligand. The mixtures were gently shaken and leave it for another 1 min. The ligand responded only with Fe^{2+} ion by changing its colour from colourless to pink (Fig. 2). All other cation and anion solutions did not bring any changes to naked eye observation and ligand was sensitive to only Fe^{2+} .

Further, the recognition was studied using electronic spectra. UV-visible spectra of ligand and its recognition are presented in Fig. 3. UV-visible spectrum of compound **D** exhibits λ_{max} at 300 nm due to its π - π^* transition of aromatic ring π electrons. Upon adding different cations it produces the change in absorption pattern according to its binding nature. No significant change in spectra was observed except in the case of Cu^{2+} and Fe^{2+} . Cu^{2+} produces a slight red shift λ_{max} from 300 nm to 320 nm. But it does not produce any significant colour change while in the case of Fe^{2+} it exhibited a remarkable colour change from colourless to dark pink and in electronic absorption spectra; a new λ_{max} appears in low intensity at 520 nm. The red shift, while the ligand interacts with $\text{Fe}(\text{II})$ ion, was significant around 220 nm due to d - d transition in L-Fe complex. It has been noted that there is no change in the higher energy region of the spectra suggested that the π electrons of the ligand have not participated in the complex formation and only non-bonding electrons on nitrogen atom might be involved.

The binding property of ligand **D** with Fe^{2+} was further studied by UV-visible titration experiments (Fig. 4). Upon incremental addition of Fe^{2+} , the absorption peak at 512 nm gradually increases which indicated the formation of a **D**- Fe^{2+} complex. The addition of EDTA to a mixture of ligand **D** and Fe^{2+} bring back to colourless solution instantly and diminution of the absorption intensity at 512 nm decreases to initial level

Fig. 3. Electronic spectral studies of ligand **D** and various metal ions

was observed, which indicated the regeneration of the ligand **D**. The absorption band was again reappearing by the addition of Fe^{2+} again. Such reversibility and regeneration are important for the fabrication of devices to sense the ions.

Fig. 5 shows the change in absorbance intensities at 512 nm while adding Fe^{2+} ion with ligand **D**. With the incremental addition of Fe^{2+} , the absorbance intensity of ligand **D** at around 512 nm was gradually increased and it was evidenced by observing

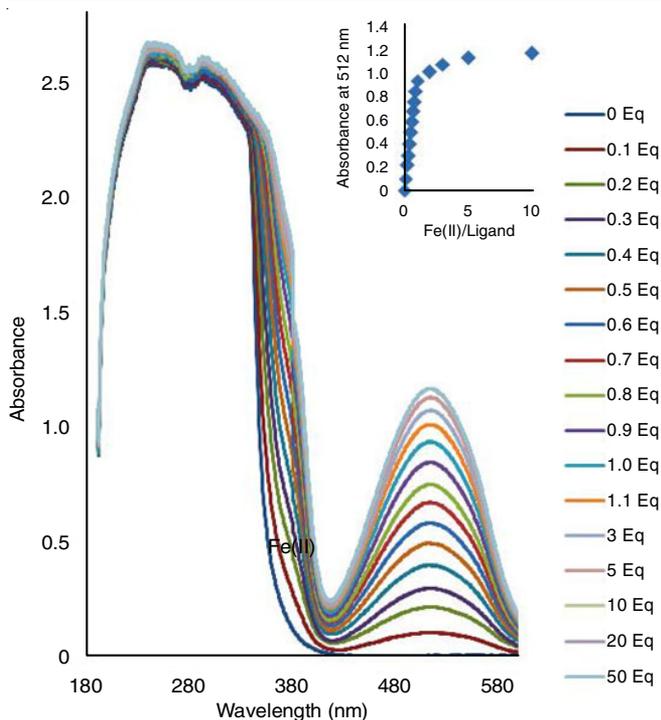


Fig. 4. Binding titration study of the ligand with Fe^{2+} ion

the colour of the solution gradually becomes colourless to dark pink. The increase in absorbance intensity attained saturation after the addition of slightly more than one equivalent of Fe^{2+} ions. Upon further addition, the intensity did not increase, which clearly indicated that the formation of 1:1 complex.

The infrared spectra of the ligand and its complex with Fe(II) ion are shown in Fig. 6. Absorbance at 1595, 1498 and 1636 cm^{-1} confirmed the presence of 1,2,3-triazole and imine C=N groups respectively. A new strong absorption in the case of metal complex at 1145, 1085 cm^{-1} shows metal coordination through nitrogen of triazole ring occurs by the formation of C-N- Fe(II) bond.

To get further information for the binding mode of ligand **D** with Fe^{2+} , $^1\text{H NMR}$ titration study was carried out (Fig. 6). Upon the addition of 0.4, 0.8 and 1.1 equivalent of Fe^{2+} to ligand **D**, a peak resonating for methylene at 3.85 ppm was shifted to up field (3.25 ppm) and the resonating peak of a proton 'Hc' in the 1,2,3-triazole ring appeared at 8.6 ppm in

ligand **D**, which on addition of Fe^{2+} ion shifted to downfield at 8.7 ppm. Meanwhile, the imine proton initially appeared at 8.9 ppm for pure ligand was shifted to slight up field. The shift of methylene and imine proton attached to electronegative nitrogen upon addition of metal ion might be due to the loss of electron density of non-bonding lone pair of nitrogen for the complex formation with metal ion. Meanwhile the triazole ring proton was shifted to downfield by deshielding effect. This indicated the ligand- Fe^{2+} complex could be formed through the participation of imine nitrogen and triazole nitrogen of the Ligand. Further Job's plot showed a 1:1 stoichiometric ratio of **D**- Fe^{2+} complex (Fig. 4) indicated the formation of the mono-nuclear complex.

Density functional theory (DFT) calculations have been used to understand the behaviour of ligand **D** with the Fe^{2+} ions. Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) for ligand **D** and its metal complex **D**- Fe^{2+} have been generated from optimized structures (Fig. 7). The structural optimization and the computational calculations were carried out using Gaussian09 quantum chemistry package and results were viewed with GaussView 5. In this study, density functional theory supported B3LYP/6-31G(d,p) model chemistry was implemented for Schiff base ligands and LANL2DZ basis set to optimize the geometry of the metal complex. The metal atom present in the complex was treated under LANL2DZ whereas other atoms are manipulated with B3LYP/6-31G(d,p) level of theory. HOMO and LUMO pictures of ligand indicated that, the electron density of the ligand was evenly distributed all over but in the case of Fe^{2+} complex the electron density was accumulated on the metal centre shows the metal draws up the electron density that causes deshielding effect of protons attached to the nitrogen atom. In addition, donation of electron pair of one of the nitrogen in triazole causes upfield shift in NMR spectrum, which was evidenced in Fig. 6. Band gap energy of the ligand **D** was calculated as -0.2788 eV and for the metal complex as 0.16211 eV . This clearly indicated that theoretically, the formation of Ligand- Fe complex formation is feasible.

Conclusion

1,2,3-Triazole based new Schiff base ligand was successfully synthesized through four steps. In all stages of synthesis, intermediates and final compounds were thoroughly investi-

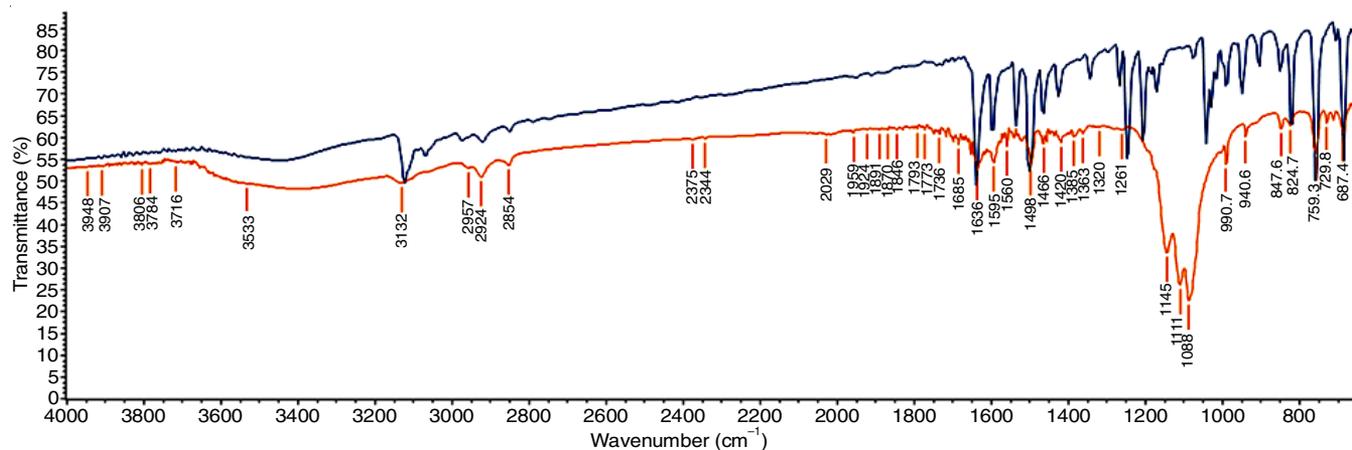
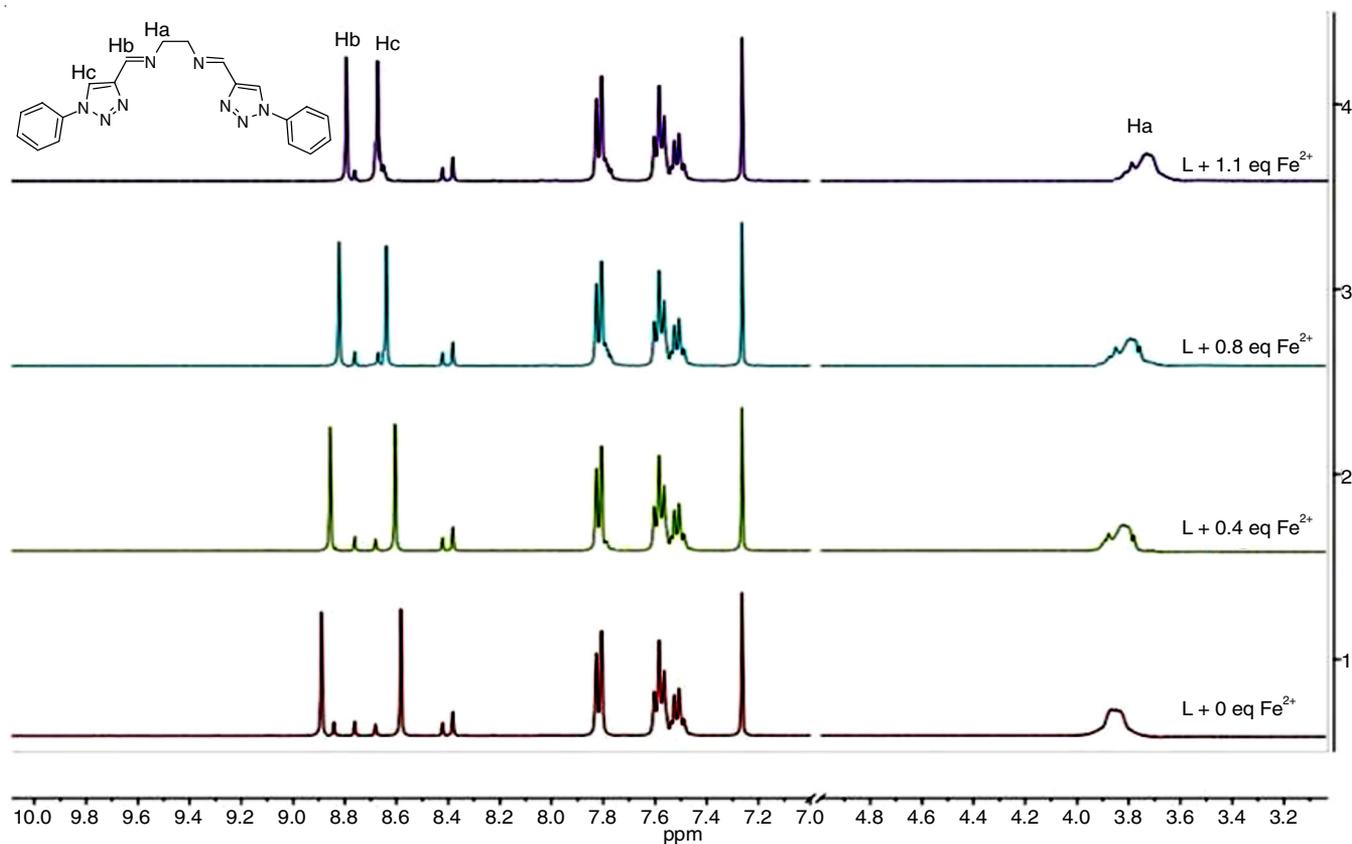
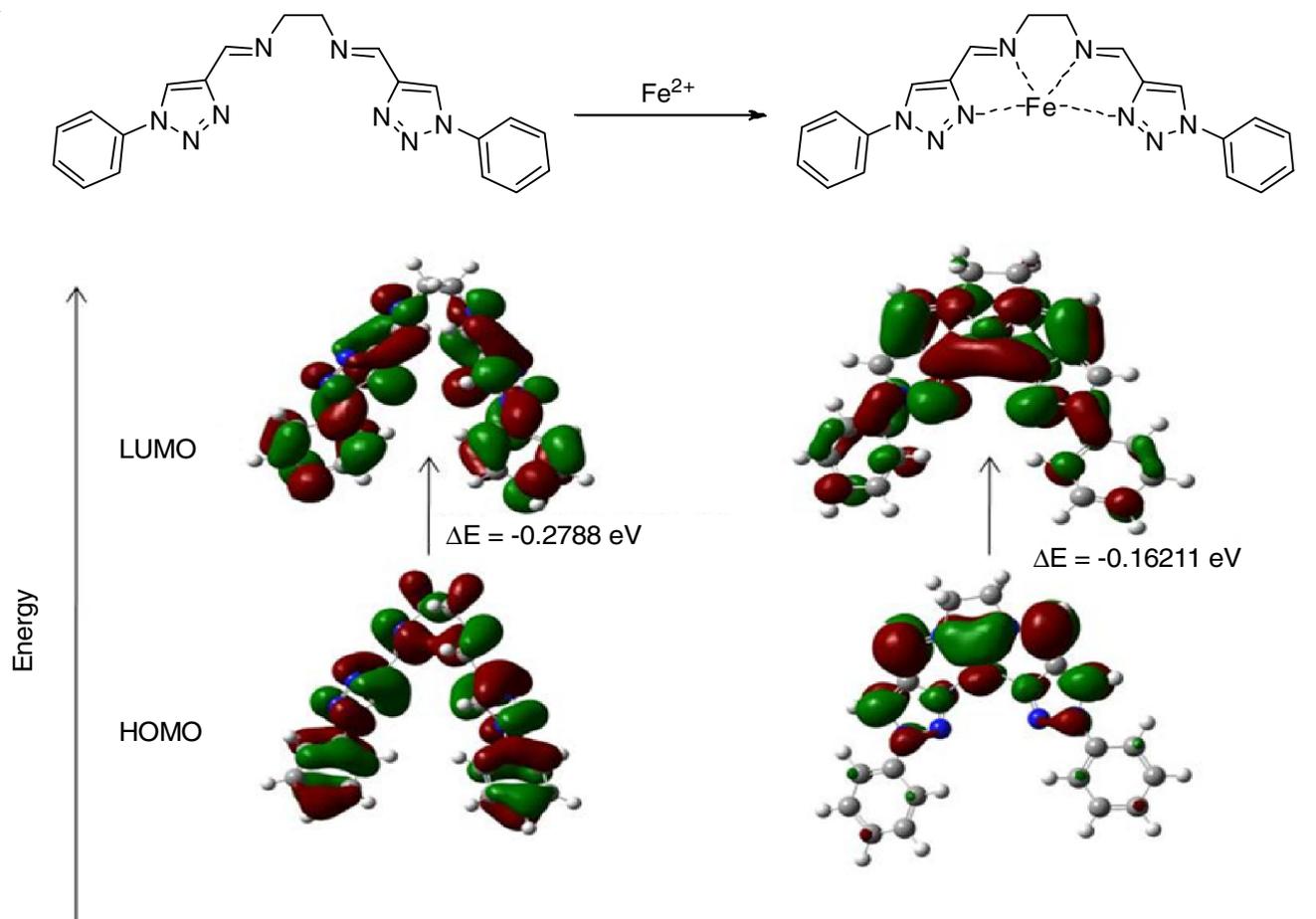


Fig. 5. IR spectra of ligand and its Fe^{2+} complex

Fig. 6. ^1H NMR spectral titration with Fe^{2+} ionFig. 7. Molecular modelling of ligand D and ligand- Fe^{2+} complex

gated using various spectral techniques and confirmed the chemical structure of the synthesized compounds. The synthesized ligand was investigated for its ion recognition abilities and found that it was very selective towards Fe²⁺ ion. Fe²⁺ exhibit distinct colour change detected by naked eye. Recognition ability was further confirmed by titration experiments and found to be 1:1 complex formation. ¹H NMR titration studies indicated imine nitrogen and nitrogen in triazole ring was involved in complex formation with Fe²⁺ ion. Molecular modeling studies also support the formation of ligand-Fe²⁺ metal complex formation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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