CHAPTER-VI

IONIC LIQUID TAGGED AZO-AZOMETHINE BASED Zn(II) COMPLEX: SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITY

6.1. Introduction

Azo compounds containing conjugated chromophoric azo (-N=N-) group are an important class of organic colorants which are mostly studied during recent years [1-2]. These colorant compounds are widely used as a class of dyes due to their versatile application in various fields such as the dyeing of textile fiber, coloring of different materials, plastics, cosmetics industries, biological-medical studies and advanced application in organic synthesis [3-4]. Other applications of azo dyes includes in emerging technologies like liquid crystals, Organic photoconductors and nonlinear optics [5]. Furthermore, these compounds were found to possess a variety of biological activities such as antibacterial, antifungal, antiviral to anti-inflammatory [6-9]. Interestingly, Azo compounds are involve in a variety of biological reactions, such as inhibition of DNA, RNA and protein synthesis, nitrogen fixation and carcinogenesis [10]. In addition, the "privilege ligand" Schiff bases, which are formed by the condensation of carbonyl compound and primary amine, are important intermediates for the synthesis of some bioactive compounds [11]. In Schiff bases, it has been suggested that the azomehine linkage (-C=N) are responsible for many biological activities like antibacterial, antitumor, antiviral, anti HIV-1, herbicidal etc [12-16]. Also among the various organic chelating ligands, the Schiff bases bearing azomethine linkage is an excellent class of chelating ligands which are able to coordinate various metal ions and stabilize them in various oxidation states and enabling the application of Schiff base metal complexes in variety of catalytic organic transformations [17]. The Schiff base metal complexes are also used in many analytical fields such as colorometric sensing of ions, corrosion inhibitors and metal extractions etc [18]. During past decade, considerable attention has been paid to the synthesis and study of azo-azomethine compounds containing hydroxyl groups for intermolecular proton transfer reaction [19]. The

thermochromic and/ or potochromic behavior of these classes of compounds can be exploited from the electronic structure of such dyes [20]. In our ongoing interest in the stynthesis and DNA binding activity of metal complexes, we report here the synthesis and physico-chemical properties of ionic liquid tagged azo-azomethine ligand by condensation of azo-coupled salicyldehyde with amino functionalized ionic liquid followed by complexation.

6.2. Experimental Section:

6.2.1. Synthesis of Azo derivative of Salicylaldehyde (1a):

The azo-derivative of salicylaldehyde was prepared by diazotization of salicyldehyde with 4-chloro aniline. In a typical experiment, salicyldehyde (1.22gm, 10 mmol) was dissolved in water (15 ml) containing 0.4 gm (10 mmol) of NaOH and 4.24 gm (40 mmol) of Na_2CO_3 during the period of 30 minutes at 0°C in a 50 ml beaker. In another beaker, 4-chloro aniline (10mmol) was dissolved in water by adding concentrated HCl in a requisite amount. The solution was then cooled on an ice bath at 0°C and solid $NaNO_2$ (10 mmol) was added to the solution slowly with constant stirring. The salicylaldehyde solution was added drop wise to the resulting diazonium chloride solution with constant stirring and the temperature was maintained at 0°C during the coupling time. The reaction mixture was stirred for one hour at 0°C and then allowed to warm slowly to room temperature. The product was collected by filtration and washed with 100ml of 10% NaCl solution.

2-hydroxy 5-(4-chlorophenylazo)benzaldehyde: yellow solid. Yield 82%, M.P-168-171°C, (IR, KBr cm^{-1}); 3300 (O-H), 1668 (CHO), 1590 (N=N), 1399, 940 (N=N, bending); Anal. calcd. for $C_{13}H_9ClN_2O_2$: C, 59.88; H, 3.45; Cl, 13.60; N, 10.74; O, 12.28. Found: C, 59.60; H, 3.24; Cl, 13.10; N, 10.59; O, 12.03%.

6.2.2. Synthesis of amino functionalized ionic liquid (1b):

The amino functionalized ionic liquid has been prepared by following the literature procedure given elsewhere [21]. 1-methylimidazole (4.1 gm, 0.05mol) was taken in 20 ml of acetonitrile and 2-bromoethylamine hydrobromide (10.25 gm, 0.05mol) was added to it. The resulting reaction mixture was then heated with stirring at 80°C for 4 h. After completion of the reaction, the solvent was removed by distillation and the oily product was washed with ethanol. The oily residue was dissolved in 40 ml of CH_3CN/H_2O (1:1, v/v) oily and KPF_6 (9.20 gm, 0.05mol) was added to the solution. The mixture was left for 24 h at room temperature and NaOH (2 gm, 0.05mol) was added to neutralize the solution. Solvents were then removed by vacuum distillation and the product was washed successively with $CHCl_3$ (20 ml) followed by methanol (2 ml). The salts thus separated were filtered and solvents were evaporated. The yellow oil thus obtained was washed with chloroform and diethyl ether. After drying for several hours under vacuum, the desired product 1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate ([2-aemim] $[PF_6]$) has been obtained as yellow oil.

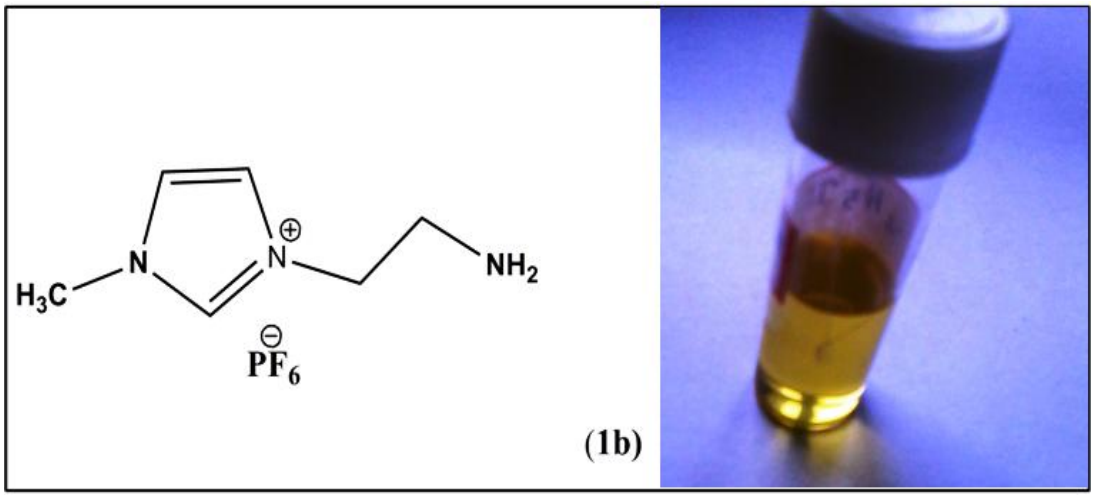


Fig.6.1. Structure of [2-aemim]PF_6.

1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate, ([2aemim] $[PF_6]$) (1b): Yellow oil; 9.35 g (yield, 69 %). Anal. Calcd. for $C_6H_{12}F_6N_3P$ (271): C, 26.58; H, 4.46; N, 15.50. Found: C, 26.32; H, 4.42; N, 15.36 %. FT-

IR (KBr, cm^{-1}): 3429 (N-H), 3106, 2365, 1617 (C=N), 1175, 846 (P-F). ^{1}H NMR (400 MHz, D_2O) δ: 3.26 (t, 2H, NH_2-CH_2); 3.83 (s, 3H, CH_3), 4.44 (t, 1H, N-CH_2), 7.39 (s, 1H, NC*H*), 7.48 (s, 1H, NC*H*), 8.52 (s, 2H, NH_2), 8.79 (s, 1H, N(*H*)CN); ^{13}C-NMR (400 MHz, D_2O) δ: 136.87, 124.53, 122.97, 53.35, 36.01, 27.88. ESI-MS (CH_3OH, *m/z*): 126.20 [$(M\text{-}PF_6)^+$]. The FT-IR and ESI-MS spectra are given below.

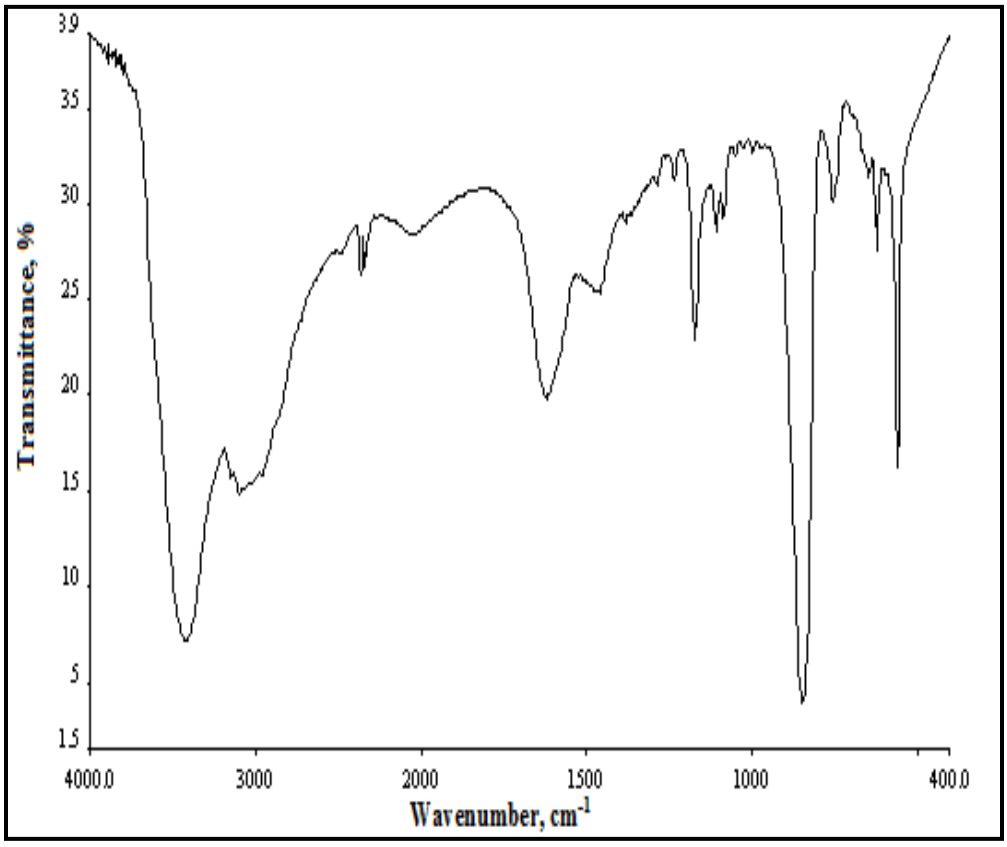


Fig.6.2. FT-IR spectrum of [2-aemim]PF_6.

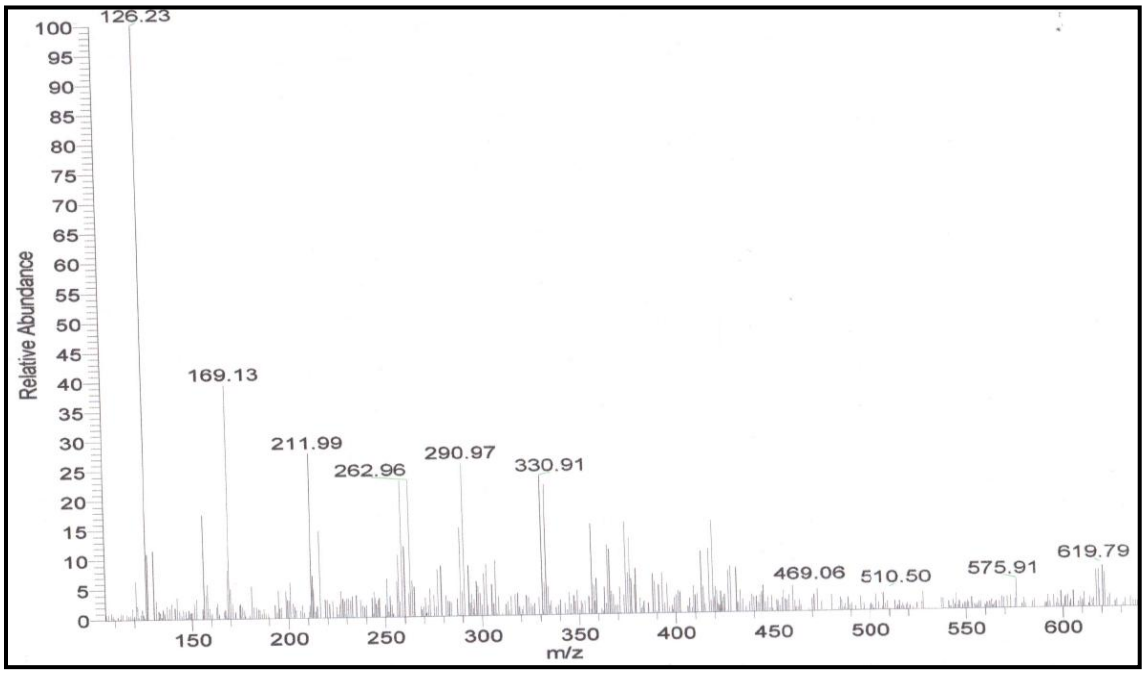
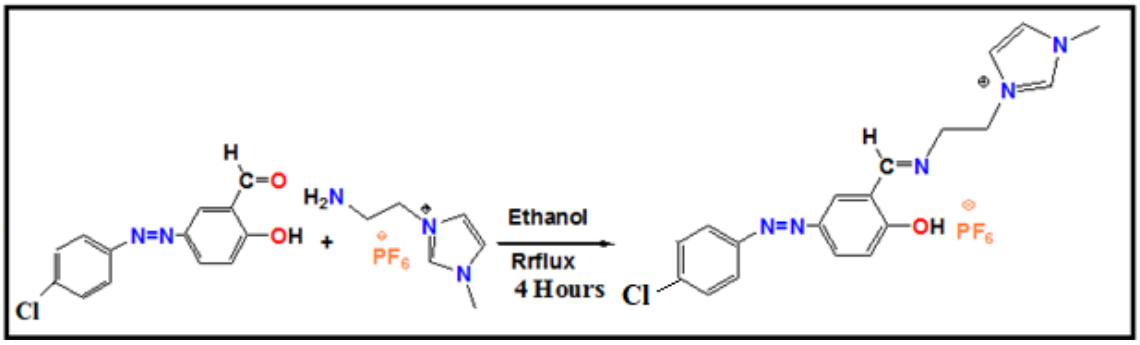


Fig. 6.3. ESI-MS spectrum of [2-aemim]PF_6.

6.2.3. Synthesis of ionic liquid tagged azo-azomethine ligand (L1):

In a typical reaction procedure 1-(2-Aminoethyl)-3-methylimidazolium hexafluoro-phosphate, ([2aemim] [PF_6]) (5 mmol) in absolute ethanol was added to stirring solution of azo-coupled salicyldehyde precursor, (5mmol) in 20 ml of absolute ethanol during a period of 10 min [22]. The reaction mixture was then refluxed in an oil bath for 6 h at 90°C with constant stirring with the help of magnetic stirrer. The solution was cooled to room temperature and the solid product thus obtained was filtered. The solid product was washed with little ethanol and diethyl ether respectively. Finally the resulted ionic liquid tagged azo-azomethine derivatives were recyrstallized from hot ethanol solution and dried over silica under vacuum.

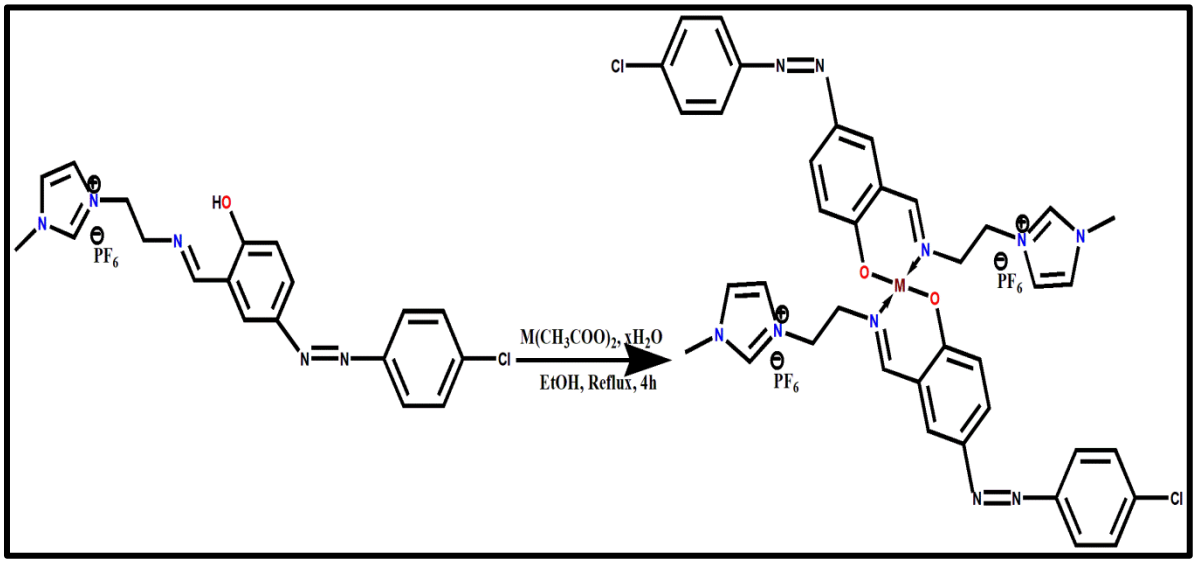


Scheme.6.1. Synthesis of ionic liquid tagged azo-azomethine ligand

1-[2-(2-hydroxy-3-methoxy-5-(4-chlorophenylazo) benzaldeneamino)ethyl]-3-methyl-3H-imidazole-3-ium hexafluorophosphate: brown solid. Yield: 62%, (IR, KBr cm^{-1}); 3200 (O-H), 1645 (C=N), 1614 (C=C), 1453 (N=N), 1168 (N=N, bending); 838 (PF_6). ^{1}H NMR (300 MHz, d_6-DMSO, ppm): δ 3.37 (s, 3H, NCH_3), 3.69 (t, 2H, N-CH_2 J_1=6.00 Hz, J_2=6.00 Hz), 4.61 (t, 2H, N-CH_2, J_1=6.00 Hz, J_2=5.10 Hz), 7.62 (s, 1H, NCH), 7.73 (s, 1H, NCH), 9.17 (s, 1H, N(H) CN), 8.56 (s, 1H, HC=N), 13.50 (s, 1H, broad, OH), 7.41-7.89 (m, 6H, Ar-H)) [62-65]. Anal. calcd. for $C_{20}H_{21}ClF_6N_5O_2P$: C, 44.17; H, 3.89; Cl, 6.52; F, 20.96; N, 12.88; O, 5.88; P, 5.70. Found: C, 44.02; H, 3.67; Cl, 6.32; F, 20.77; N, 12.56; O, 5.78; P, 5.61%.

6.2.1.4. Synthesis of Zn(II) metal complex:

Hot ethanolic solution of Zn(II) acetate was added dropwise to the equimolar ethanolic solution of synthesized ligand L1. The resulting mixture was then stirred and refluxed (at 40-50 °C) for 2 h whereupon the Zn(II) metal complex was precipitated out. The dark brown colored solid complex was separated by filtration, washed with ethanol and dried in vacuum desiccator.



Scheme.6.2. Synthesis of azo-azomethine ligand based Zn(II) complex.

6.3. Results and discussion:

The formation of the investigated azo-azomethine ligand based Zn(II) complex is represented in Scheme.6.2.

6.3.1. Characterization of the ligand and its Zn(II) complex:

The newly synthesized ligand and its corresponding Zn(II) complex have been characterized by different analytical and spectroscopic techniques (IR, NMR, UV-Visible *etc*.).

6.3.1.1. IR spectral studies:

In order to ascertain the binding mode in the synthesized azo imidazole ligand and its metal complex, the IR spectra of the compounds were closely analyzed [23-27]. In the solid state IR spectrum of the ligand (L1) a strong band at 3200cm^{-1} can be assigned to stretching frequency of OH group. However this OH band gets disaapered in the spectra of complex suggesting the involvement of OH group in complexation. The appearance of absorption band at 1645cm^{-1} in the spectra of L1can be assigned to the stretching vibration of azomethine linkage ν(-C=N). This band gets shifted to 1629 cm^{-1} in the synthesized complex indicating the participation of the azomethine nitrogen in

complexation The presence of band at 1616 cm^{-1} can be assigned to ν(C=C) stretching frequency. Also, the N=N stretching frequency of the synthesized compound is found at 1453cm^{-1} and the N=N bending vibration is found at 1168 cm^{-1}. Band at 838 cm^{-1} can be assigned for stretching vibration of PF_6 group. The participation of phenolic O and azomethine N in complexation are confirmed by the development of two non-ligand bands in the spectra of complex at 515 cm^{-1} and 412 cm^{-1} due to ν(Zn-O) and ν(Zn-N), respectively.

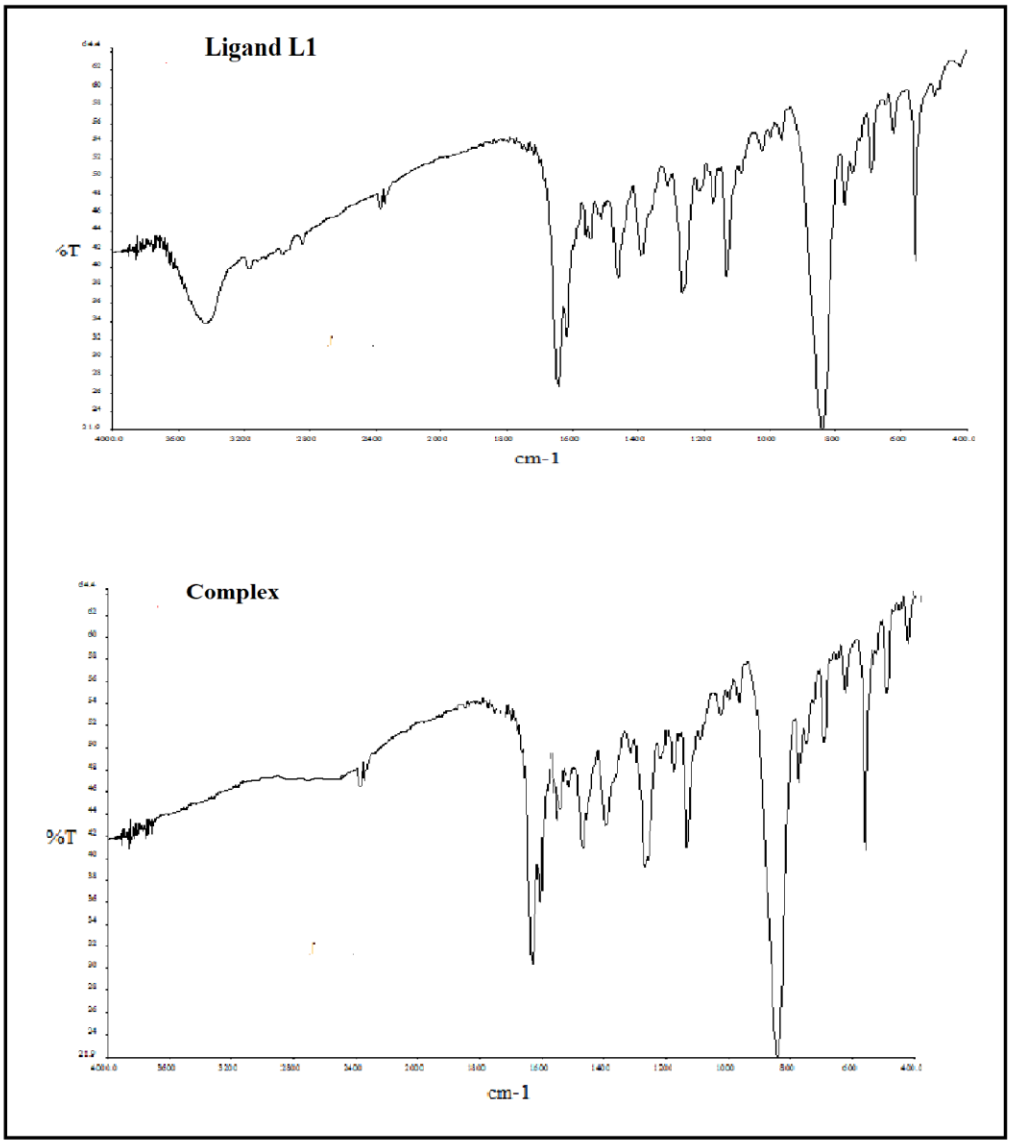


Fig.6.4. FTIR spectra of ligand and its Zn(II) complex.

6.3.1.2. ^{1}H NMR spectral studies:

The ^{1}H-NMR spectra of the synthesized ligand recorded in DMSO-d_6 at ambient temperature, displays a group of signals corresponding to the hydrogen. In the ^{1}H-NMR spectrum of L1, the signal corresponds to OH proton exhibited a slightly broad singlet peak at δ 13.50 ppm and the signal for CH=N proton appeared as a singlet at δ 8.56 ppm. The appearance of the peaks like singlet at δ 7.62 ppm for (NCH) proton, singlet at δ 9.17 ppm for N (H) CN proton is in good agreement with the proposed structure for L1. Signal for NCH_3 protons appeared at δ 3.37. The aromatic protons appeared as multiplet in the range δ 7.41-7.89 ppm. The signal at 13.50 ppm (-OH gr.) get disappeared in the complex while the signal at 8.56 ppm (-CH=N) shifted to 8.18ppm [28-29]. Both these observation confirms complexation.

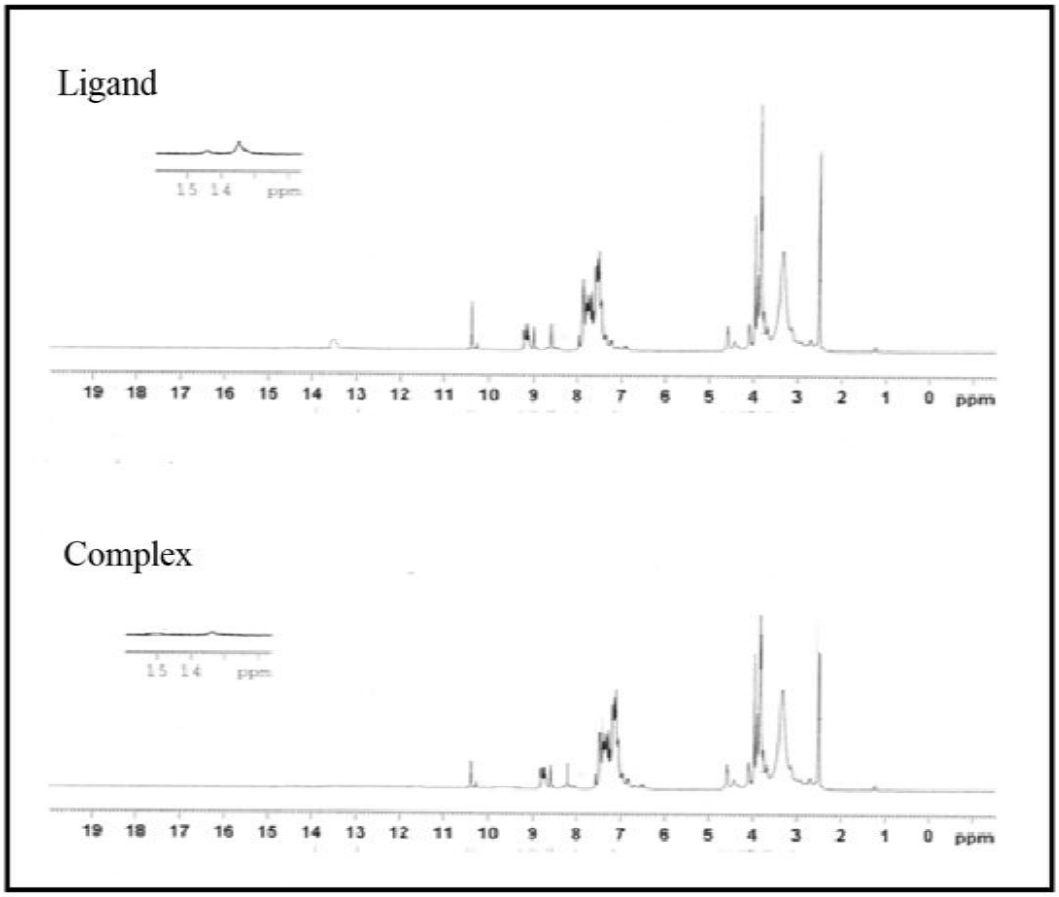


Fig.6.5. NMR spectra of Ligand and its Zn(II) complex.

6.3.1.3. Electronic absorption spectra:

UV-Visible spectrum of the synthesized azo-azomethine derivative was recorded in Methanol at room temperature. The electronic absorption spectra of the synthesized ligand displayed three peaks appear at 275 nm, 335 nm and 438 nm; these peaks were attributed to $\pi \rightarrow \pi^*$ transition of the aromatic rings, $n \rightarrow \pi^*$ transition of azomethine moiety and $n \rightarrow \pi^*$ transition of azo nitrogen (–N=N–), respectively [30-34]. Upon complexation the peaks for azomethine moiety and azo nitrogen (–N=N–) shifted to 355 nm and 460 nm respectively.

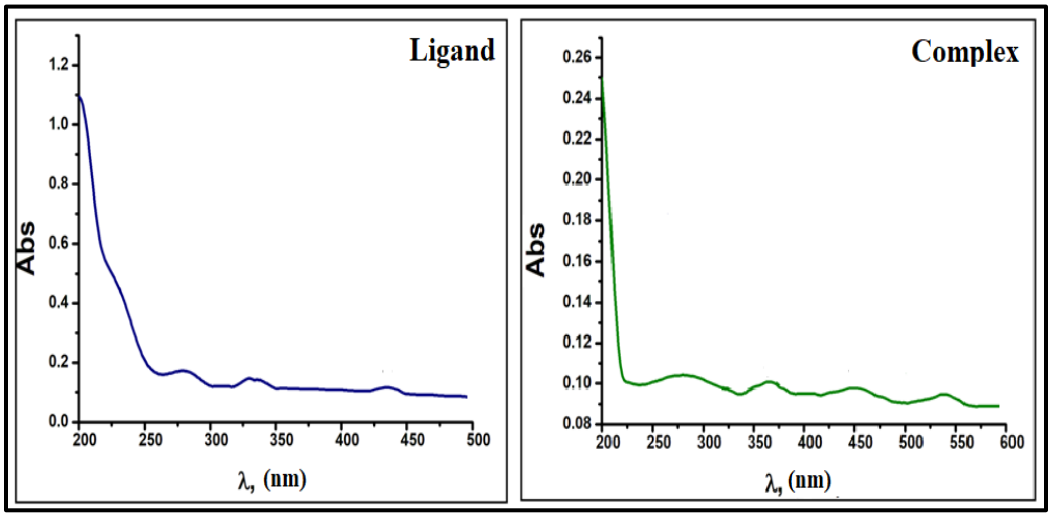


Fig.6.6. Absorption spectra of azo-azomethine based ligand and its Zn(II) complex.

6.3.2. DNA binding study:

6.3.2.1. Electronic absorption titration:

To study the binding mode of the metal complexes with DNA, electronic absorption spectroscopy serves as a most common tool. When a metal complex binds to DNA through intercalative mode (because of strong stacking interaction between an aromatic chromophore and DNA base pairs) results into shift in both absorbance and wavelength. Absorption spectrum of the complex in presence of DNA is shown in Fig.6.7.

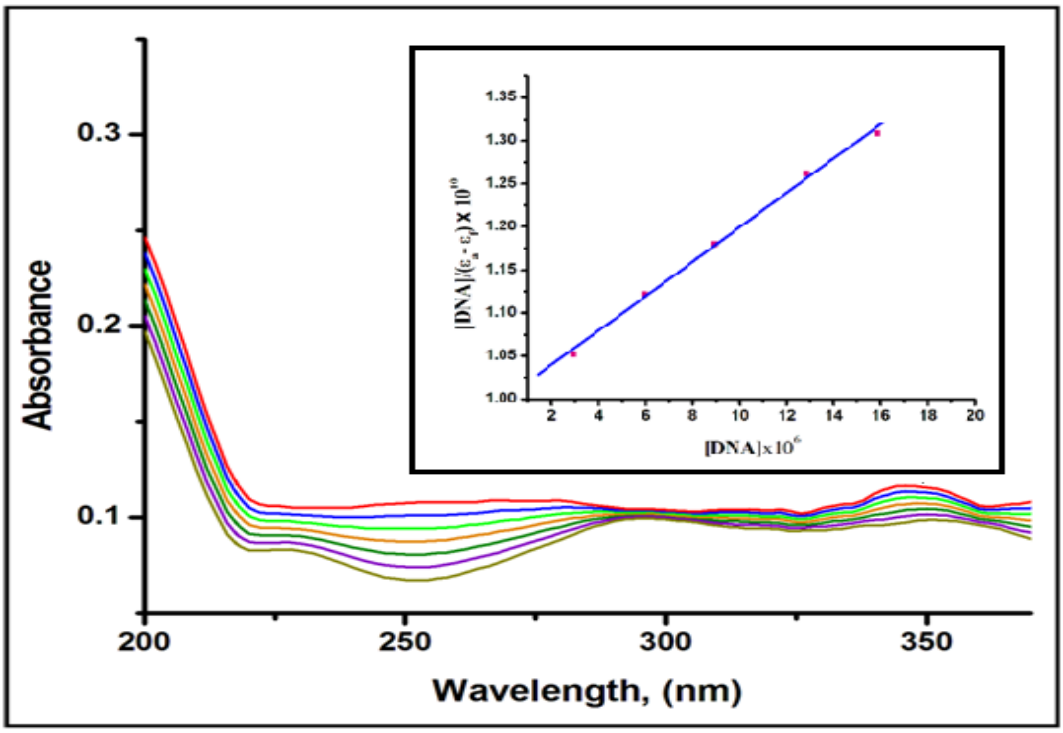


Fig.6.7. Absorption spectra of Zn(II) complex (in absence and presence of increasing amount of CT DNA; Inset: plot for binding constant (K_b).

On successive addition of DNA in the complex solution chamber, change in both absorbance and wavelength (2.5 nm for azomethine band) were observed suggesting an intecalative mode of binding. In order to calculate the binding ability of metal complex to CT-DNA, the intrinsic binding constant (K_b) was determined using Wolfe-Shimer equation [35]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

and the value of K_b is $(1.26\pm0.02) \times 10^4\ M^{-1}$.

6.3.2.2. Fluorescence emission spectroscopy:

To affirm the mode of binding of synthesized complex to CT-DNA, a competitive binding experiment was performed using Ethidium Bromide displacement strategy. EB-DNA couple shows an intense emission band at 592 nm because of intercalation of the planar phenanthridine ring of EB between adjacent DNA base pairs. A noticeable

quenching of the EB-DNA emission band may be seen if a foreign molecule having the ability to intercalate to DNA equally or stronger than EB, is added into EB-DNA solution [35].

The addition of complex solution in EB-DNA solution resulted in significant quenching in the emission intensity (shown in Fig.6.8). This lowering in emission intensity of EB-DNA couple upon addition of complex suggests displacement of EB which can be delineated as intercalative mode of binding. The quenching constant or quenching strength (K_{sv}) of synthesized complex toward EB-DNA conjugate was further calculated using the classical Stern‑Volmer equation and calculated value of K_{sv} for the synthesized complex is $(1.8\pm0.3)\times10^4$ M^{-1}.

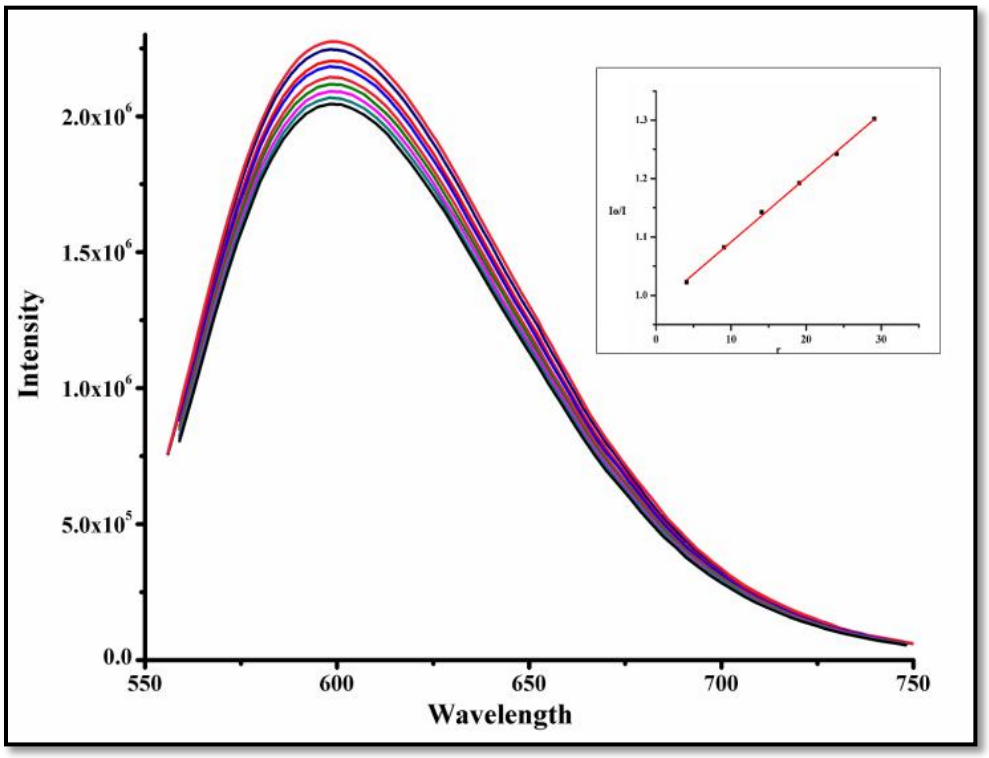


Fig.6.8. Emission spectra from EB bound to the DNA in the absence and presence of increasing amount of complex (0–30 μM), Inset: plot for quenching constant (Ksv).

6.3.3. Molecular docking study:

Prediction of protein-molecules interaction through molecular docking studies plays an important role to screen the potent molecules having an ability to alter the activity of biological receptor/enzymes. Thus Molecular docking plays an important role for drug design and it aims to achieve an optimize conformation for both protein and drug with relative orientation between them such that the free energy of the overall system is minimized [35]. In this context, we used molecular docking between the synthesized complex with receptor of breast cancer mutant 3hb5-oxidoreductase and diabetic molecular target 11-β-hydrosteroid dehydrogenase type 1 (shown in Fig. 6.9.). The docking study showed a favourable interaction between the metal complex and the receptors 11-β- hydrosteroid dehydrogenase 1 (2BEL) and Receptor breast cancer (3hb5). Based on the interaction (binding affinity) it can be proposed that the synthesized complex could alter the activity of 2BEL involved in glucose metabolism and breast cancer (3hb5) receptors and thus may have a potential to be used as antidiabetic and anticancer agents.

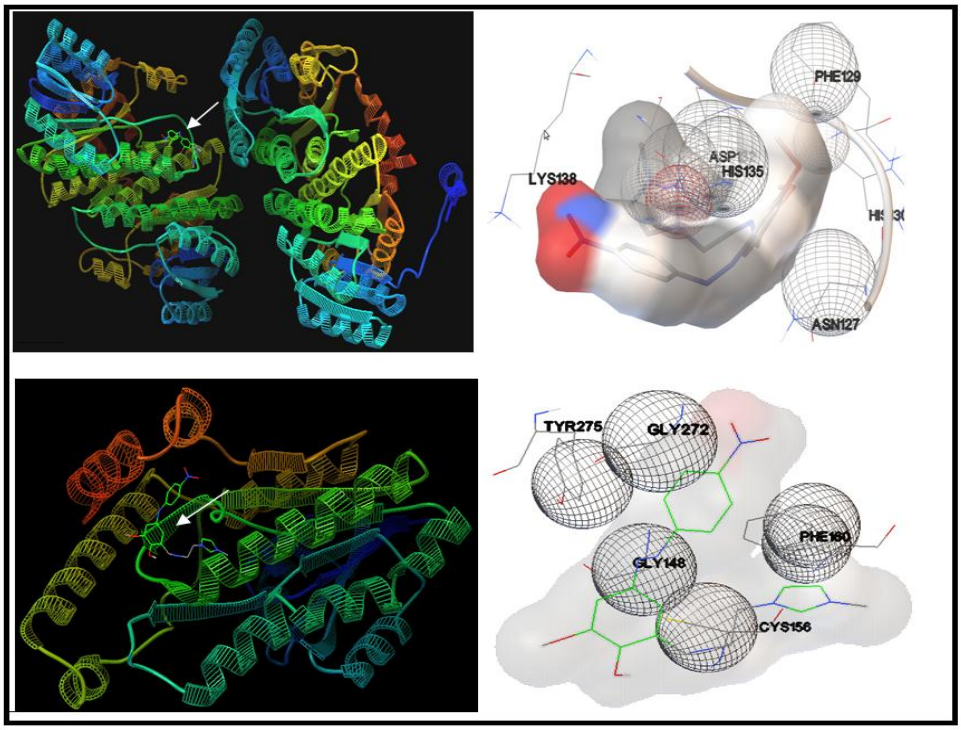


Fig.6.9. Molecular docking between the Zn(II) complex and (a) 3hb5, (b)2BEL.

7.5.6. Conclusion:

A novel ionic liquid tagged azo-azomethine Zn(II) complex have been prepared by the condensation reaction of azo-coupled salicylaldehyde precursor with amino functionalised ionic liquid and characterized using different analytical and spectroscopic tools (NMR, IR, UV-visible). The synthesized complex was tested for DNA interaction and the results showed that the complexes bind with CT-DNA through intercalative mode. The docking study showed a favourable interaction between the metal complex and the receptors 11-β- hydrosteroid dehydrogenase 1 (2BEL) and Receptor breast cancer (3hb5).

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