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Synthesis, Spectroscopic Characterization, *In Silico* DNA Studies and Antibacterial Activites of Copper(Ii) and Zinc(Ii) Complexes derived from Thiazole based Pyrazolone Derivatives

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ABSTRACT

Copper(II) and zinc(II) complexes of bidentate Schiff base ligand, synthesized via condensation of 4-aminoantipyrine and 2-aminothiazole. The ligand is characterized on the basis of elemental analysis, mass, IR, ^{1}H NMR, UV-Visible and ESR spectra. The molar conductance data reveals that metal complexes are non-electrolytes. From the elemental analyses, 1:1 [M]:[L] complexes are prepared with the general formulae [MLCl] (M = Cu(II) and Zn(II)). Schiff base ligand act as tridentate. From the magnetic susceptibility data suggests that the geometrical structures of these complexes are square planar. The interaction of the metal complexes with DNA was performed by molecular docking studies. The synthesized ligand, in comparison to its metal complexes is screened for its antibacterial activity against bacterial species, *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus.*

Keywords: Schiff Base, ESR, Molecular docking, antibacterial activity

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INTRODUCTION

The Schiff bases with sulphur and nitrogen donor atoms in their structures act as superior chelating agents for the transition and non-transition metal ions [1-4]. Coordination of such compounds with metal ions, such as copper, nickel and iron, often enhance their activities [5], as has been reported for pathogenic fungi [6]. Azomethine linkage is an important for biological activity; several azomethines were reported to possess important antibacterial [7-9], antifungal [10-11], , anticancer[12], and diuretic activities [13]. With the rising occurrence of deep mycosis, there has been increasing emphasis on the screening of new and more effective antimicrobial drugs with low toxicity. Schiff bases and their complexes were recently established to have significant antitumor and biological activity [14-15]. Antipyrine derivatives are reported to exhibit analgesic and anti-inflammatory effects [16], antiviral [17], antibacterial [18], anticonvulsant [19] and herbicidal [20] activities. Transition metal complexes with ligands derived from 4-aminoantipyrine have important biological activity [21-24]. This prompted us to synthesize a novel series of heterocyclic Schiff bases containing the antipyrinyl moiety. The present study reports the synthesis, characterization, *in silico* DNA studies and antimicrobial activity of copper and zinc complexes containing bidentate Schiff base derived from 4-aminoantipyrine and 2-aminothiazole.

EXPERIMENTAL

Chemicals and Instrumentation

All chemicals used were of the analytical reagent (AR) grade and of highest purity available. All reagents such as 4-aminoantipyrine, 2-aminothiazole and metal(II) chlorides were procured from Merck products. IR spectra were recorded on a Perkin Elmer 783 instrument in anhydrous KBr pellets. UV-Visible spectra of the complexes were recorded on Perkin Elmer Lambda EZ201 spectrophotometer in DMSO solutions. ^{1}H-NMR spectra was recorded on a Bruker 300 MHz instrument using $CDCl_3$ as a solvent and TMS as an internal standard. Molar conductivity was determined using Systronic Conductivity Bridge with a dip type cell using 10^{-3} M solution of complexes in DMSO. Magnetic susceptibility was measured with a Guoy balance.

Synthesis of Schiff base (L)

Hot ethanolic solution of 4-aminoantipyrine (2.03 g, 10 mM) was mixed with hot solution of 2-aminothiazole (1.01 g, 10 mM) in 50mL ethanol. The resulting mixture was left under reflux for 4 h and the formed solid the solid product separated was filtered and recrystallized from ethanol. The outline synthesis of ligand is shown in **Scheme 1**.

Schiff base (L): Yield: 82%; M.P: 178±2°C; Elemental analysis: Analytical Calculated for $C_{14}H_{15}N_5S$: C, 58.92; H, 5.3; N, 24.54. Found: C, 58.76; H, 5.14; N, 24.42%. UV-Visible: 302 nm.

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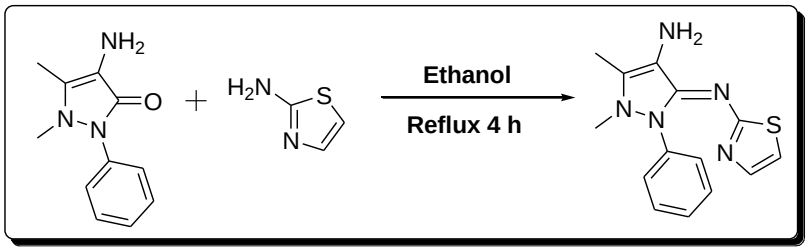

Scheme 1. Outline synthesis of Schiff base ligand (L).

Synthesis of metal complexes

The metal complexes of the Schiff base (L) were prepared by the addition of hot solution of the appropriate metal chloride (1 mM) in an ethanol to the hot solution of the Schiff base (1 mM) in the same solvent (25 mL). The resulting mixture was stirred under reflux for 1 h whereupon the complexes precipitated. They were collected by filtration and purified by washing with an ethanol and diethyl ether.

[$CuLCl_2$]: Yield: 70%; Elemental analysis: Analytical Calculated for $C_{14}H_{15}ClCuN_5S$: Cu, 16.53; C, 43.75; H, 3.93; N, 18.22. Found: Cu, 16.08; C, 43.16; H, 3.72; N, 18.42 %. μ_{eff}(BM) = 1.82; Λ_m(mho cm^2 mol^{-1})= 14; m/z =384; UV–Vis: 312 and 438 nm.

[$ZnLCl_2$]: Yield: 62%; Elemental analysis: Analytical Calculated for $C_{14}H_{15}ClZnN_5S$: Zn, 16.93; C, 43.54; H, 3.91; N, 18.13. Found: Zn, 16.36; C, 43.06; H, 3.62; N, 18.24%. diamagnetic; Λ_m(mho cm^2 mol^{-1})= 22; m/z =386.

In silico DNA–metal complex interaction

The DNA sequence was selected based on the literatures [25-27]. The selected DNA sequence was subjected into DNA sequence to structure web server [28] for generating the DNA structure based on experimental fiber-diffraction studies [29]. The structure of the metal complexes was drawn using ChemDraw Ultra10.0 and three-dimensional structure of metal complexes was prepared by using Molinspiration Galaxy 3D Structure Generator v2011.02 beta [30]. The DNA-metal complex interaction was studied using Patch dock web server [31]. The PyMol visualize [32] was used to visualize the DNA-metal complex interaction.

Antibacterial activity

Fresh bacterial cultures of 2 gram negative bacteria namely *Escherichia coli* (MTCC 733), *Pseudomonas aeruginosa (MTCC*1688) and 2 gram positive bacteria *Bacillus subtilis* (MTCC 41) and *Staphylococcus aureus* (MTCC 96) were used for the antibacterial test. Fresh bacterial cultures were used for the antimicrobial activity. The colonies of the strains were inoculated to Brain Heart Infusion broth and incubated at 37ºC for 24 hin orbit shaker at 200 and rpm. Turbidity was adjusted with sterile broth to corresponds to the 0.5 McFarland standards before swabbing; standard inoculum of the microorganism was of 1.5×10^{6} colony forming units (CFU

mL^{-1}) diluted to 1:100 and given suspension of turbidity equal to a McFarland standard 0.5. The turbidity was adjusted to match a McFarland 0.5 mL of 1.175% w/v (0.048 M) $BaCl_2.H_2O$ to 99.5 mL of 1% w/v (0.36) sulphuric acid. The *in vitro* antimicrobial activities of test compounds were determined by the well diffusion method as described by Perez *et al* [33]. Standard antibiotic Tetracycline was used as reference. Organisms (24 h old culture) were swabbed on the Mueller Hinton Agar (MHA) plates with sterilized cotton swab sticks. Wells (9 mm diameter) were cut using a sterile cork borer. Stock solutions of all compounds (25) were diluted in dimethyl sulfoxide (DMSO). The stock solutions were prepared to 3 mg of Compound/ 2 mL of DMSO concentration. From the stock solution, different diluted measurements such as 20 µL, 40 µL 60 µL (20 µL diluted sample contain 30 µg of the test compound) were immediately dispensed into agar wells of culture inoculated plates (MHA) using sterilized microtips. The plates were incubated at 37°C for overnight. The antibacterial activity was measured as the diameter of the inhibition zone including the diameter of the well.

RESULTS AND DISCUSSION

The Schiff base was prepared according to **Scheme 1**. All the complexes are stable at room temperature, insoluble in water but soluble in DMSO and MeCN.

Molar conductance

The molar conductance data of the copper and zinc complexes were measured in DMSO solution for the 0.001M solutions. The values fall in the range of 14-22 Ω^{-1} cm^2 mol^{-1}, which is the expected range for non-electrolytes [34]. All the complexes did not show electrolytic properties.

Mass spectra

ESI-Mass spectra provide a vital clue for elucidating the structure of compounds. The mass spectra of the ligand and its copper and zinc complex were recorded and their stoichiometric compositions were compared. The Schiff base ligand showed molecular ion peak at 285 *m/z*, whereas its copper and zinc complex shows the molecular ion peak at 385 and 386 m/z, which confirms the stoichiometry of the complexes to be [MLCl] whereas M = Cu and Zn. Elemental analysis values are in good agreement with the values calculated from molecular formula assigned to these complexes, which is further supported by the ESI-mass studies.

IR Spectra

IR spectra give a lot of valuable information in coordination chemistry. In order to study the binding mode of the Schiff base to the copper in the complexes, the IR spectrum of the Schiff base ligand was compared with the spectra of the complexes. The IR spectra of the free ligands show the characteristic $\upsilon_{(C=N)}$ bands in the 1654 cm^{-1} region which is shifted to lower frequencies in the spectra of the metal complexes (1636–1620 cm^{-1}) representing the involvement of azomethine nitrogen in coordination to the metal ion [35-37]. The IR spectrum

of ligand shows a medium broad band at 3355 and 3310 cm^{-1} which attributed to NH_2 of the amino group. The contribution of the $\upsilon_{(NH2)}$ group is further confirmed by clarifying the effect of chelation on the in-plane bending, NH_2 vibration. The shift of this band, from1552 cm^{-1} in the free ligand to 1485–1537 cm^{-1} in the complexes indicates the participation of the NH_2 group in complex formation. Also the ligand shows a band at 1610 cm^{-1} which is attributed to $\upsilon_{(CH=N)}$ of the thiazole ring and $\upsilon_{(C=C)}$ at 1552 cm^{-1}. The stretching vibration appears at 772 cm^{-1} is due to $\upsilon_{(C-S)}$ of the thiazole ring. A shift in the band at 1608 cm^{-1} of the thiazole ring (1605–1575 cm^{-1}) in complexes suggesting the coordination via thiazole nitrogen (N → M)[5]. In all the complexes the $\upsilon_{(C-S)}$ remains unchanged indicating the sulphur does not involve in the coordination. Assignment of the proposed coordination sites is further supported by the appearance of medium bands at 510-500 and 570-550 cm^{-1}, which could be attributed to v (M–N) and v (M–O) respectively [38].

^{1}H-NMR spectra

The ^{1}H NMR spectrum of ligand in $CDCl_3$ gives the following signals: phenyl as multiplet at 7.25–7.75 ppm, =C–CH_3 at 2.43 ppm, –N–CH_3 at 3.05 ppm, N-CH=CH and S-CH=CH in thiazole as doublet at 8.91 and 8.32 ppm. The peak at 3.82 ppm is attributable to the amino group present in the 4-aminoantipyrine. The signals observed at 3.82 ppm for ligand, is assigned to NH_2 protons. This signal is found at 3.52 ppm in zinc complex. This indicates that the NH_2 group is coordinated to the Zn(II) ion.

Electronic spectra

The electronic spectra can often give fast and reliable information about the ligand arrangements in the transition metal complexes. The UV-Vis spectrum of the copper complex in DMSO shows a broad band at 438 nm which can be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition (Lever, 1968) [39]. It reveals that the copper(II) complex exhibits in square planar geometry.

Electron spin resonance spectra

The EPR spectrum of copper complex provides valuable information in studying the metal ion environment. The EPR spectra were recorded in DMSO at RT (room temperature) and LNT (liquid nitrogen temperature). The spectrum of the copper complex at RT showed one intense absorption band in the high field and was isotropic due to the tumbling motion of the molecules. However, this complex at LNT showed well resolved peaks with low field region. The copper complex exhibited the $g_{||}$ value of 2.28 and $g_{\perp}$ value of 2.04. From the experimental values, it is clear that $g_{||}$ (2.28) > $g_{\perp}$ (2.04) > 2, which suggests that the complex is square planar. These values indicate that the Cu(II) lies predominantly in the $d_{x^2-y^2}$ orbital [40-43], as was evident from the value of the exchange interaction term G, estimated from the expression:

$$G = g_{||} - 2.0023 / g_{\perp} - 2.0023$$

It is reported that $g_{||}$ is 2.4 for copper–oxygen bonds, 2.3 for copper–nitrogen bonds. For mixed copper–nitrogen and copper–oxygen systems, there is a small difference in the point

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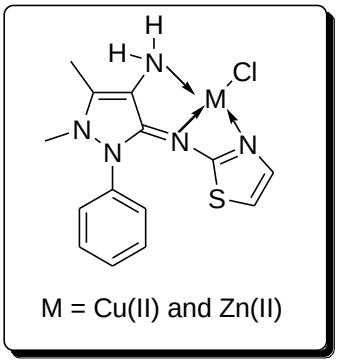
of symmetry from octahedral geometry. For the present copper complex, the $g_{||}$ value (2.36) is between 2.3–2.4. This shows that the complex contains mixed copper–nitrogen and copper–oxygen bonds. If G > 4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If G < 4.0, significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the copper complex (G = 7.3) suggests that the local tetragonal axes are aligned parallel or slightly misaligned, and the unpaired electron is present in the orbital. This result also indicates that the exchange coupling effects are not operative in the complex [44].

The proposed structure of the Schiff base complexes is shown in below:



Schiff base metal complexes

***In silico* DNA–metal complex interaction**

One hundred docking conformations were generated for each ligand with its DNA molecule. For each docking results, the best solution was inferred by highest value of shape complementarity score. The shape complementarity score of copper and zinc DNA-metal complexes was displayed in **Table 1**. Molecular docking (**Figure 1**) results suggested that the interaction between DNA and zinc complex is more stable (high shape complementarity score) rather than others. The order of the stability of all the four metal complexes is as follows: [ZnLCl] > [CuLCl].

Patch dock results suggested that the interaction between DNA and copper and zinc complexes are bound to “Minor groove” portion of DNA and hence they are called “Minor groove binders”.

Table 1 Shape complementarity score of DNA-Metal Complexes

S. No	Metal Complexes	Shape-Complementarity Score
1.	DNA – CuLCl	3416
2.	DNA – ZnLCl	3604

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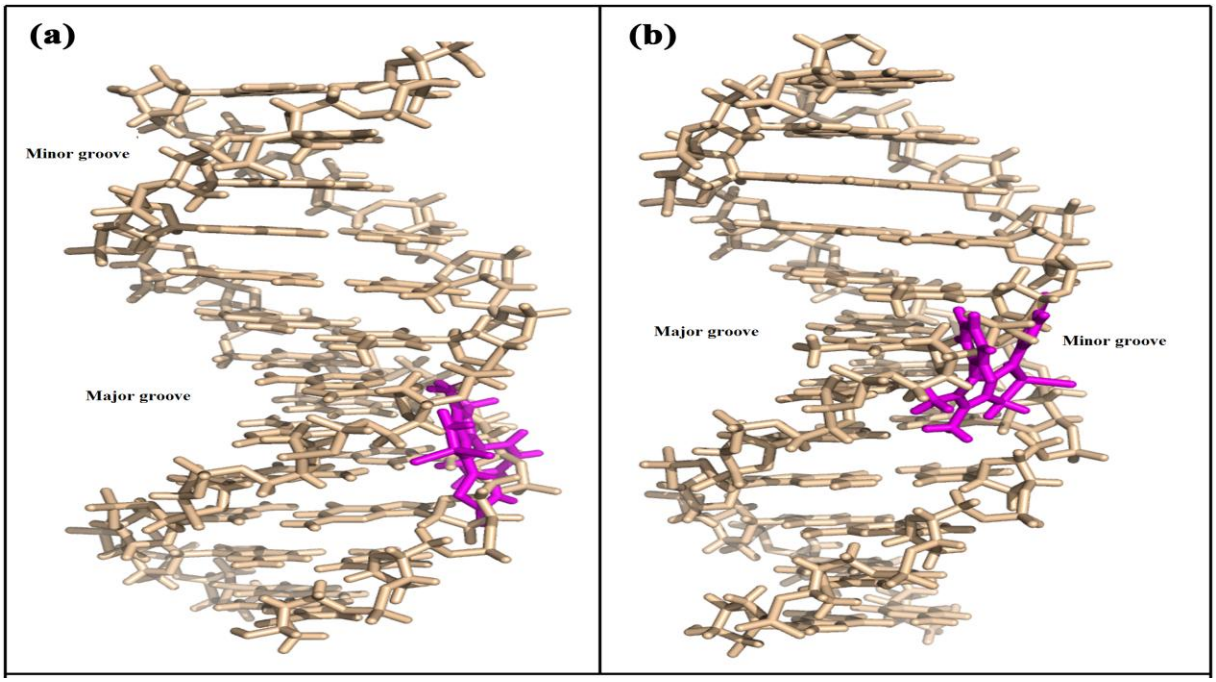

Figure 1. Molecular docking results DNA–metal complex interaction
(a) DNA–[CuLCl], (b) DNA–[ZnLCl] complex

Antibacterial activity

The Schiff base ligand and its metal complexes have been screened for their antibacterial activities against various pathogenic bacterias such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus. Tetracycline* was used as the standard for bacterial studies.

Zone of inhibition is illustrated in **Table 2.** It has been observed that the metal complexes have a higher activity than that of the free ligand. Probably this may be due to the greater lipophilic nature of the complexes. The increase in antibacterial activity is due to faster diffusion of metal complexes as a whole through the cell membrane or due to the combined activity effect of the metal and ligand. Such increased activity of the metal complexes can be explained on the basis of Overtone's concept [45] and Tweedy's chelation theory [46]

Table 2 Antibacterial activity data for the Schiff base and their metal complexes

Compounds	*E. coli*			*P. aeruginosa*			*B. subtilis*			*S. aureus*		
	30µg	60µg	90µL	30µg	60µg	90µg	30µg	60µg	90µg	30µg	60µg	90µg
Ligand	10	11	13	-	-	-	10	11	13	-	11	13
Cu(II) complex	14	16	20	-	11	14	11	13	18	13	15	19
Zn(II) complex	11	13	14	12	17	18	11	12	14	-	12	14

Zone of Inhibition (mm)

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CONCLUSION

Copper(II) and Zinc(II) complexes were synthesized from the Schiff base ligand derived from 4-aminoantipyrine and 2-aminothiazole. The structural features were derived from their elemental analyses, IR, UV-vis, NMR spectroscopy, ESR spectral analyses and conductivity measurements. The data of the complexes suggested square planar geometry for the metal complexes. The results of *in silico* DNA-metal complex interaction reveal that all the complexes can interact with DNA. The Schiff base behaves as a tridentate ligand. Evaluation of antibacterial activity of the complexes against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* exhibited that the complexes have potent biocidal activity than the ligand.

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