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**SYNTHESIS, STRUCTURAL ELUCIDATION, ANTIBACTERIAL AND ANTIOXIDANT
ACTIVITY OF CU(II) AND ZN(II) COMPLEXES DERIVED FROM THIOPHENE – 2
CARBOXALDEHYDE**

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ABSTRACT

The hexa-coordinated mixed ligand Cu(II) and Zn(II) complexes of the type [MLX]Cl₂ where X=2,2'-bipyridine and ligand (L) have been synthesized from the condensation of thiophene-2-carboxaldehyde with *o*-phenylenediamine. They were characterized by molar conductance, magnetic susceptibility, infrared, electronic absorption, proton magnetic resonance and mass spectral studies. An octahedral geometry has been proposed for all these complexes. The high molar conductance studies of the complexes show their electrolytic nature. The infrared spectra of the complexes show that the imine group of the Schiff base coordinates with the metal ion. The electronic absorption spectra of the complexes show the charge transfer, d-d transitions consistent with their proposed geometry. The proton magnetic resonance spectrum of the ligand shows the presence of CH=N group in the ligand. The mass spectral data also confirm the proposed structure of ligand and its complexes. The mixed ligand complexes possess good antibacterial activities. The above complexes also exhibited DPPH radical scavenging activity.

Keywords: Antibacterial activity, DPPH radical scavenging activity,

Mixed ligand complexes.

1. INTRODUCTION

Schiff bases derived from aromatic amines and aromatic aldehydes or ketones have a wide variety of applications in many fields like biological, inorganic and analytical chemistry (Krishnamoorthy P, et al., 2011). They play important roles in coordination chemistry as they easily form stable complexes with most transition metal ions (Spinu C, Pleniceanu M and Tigae C, 2008). Insufficient levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells. Oxidative stress seems to play a significant role in many human diseases including cancers. Antioxidants are frequently added to industrial products such as stabilizers in fuels and lubricants to prevent oxidation and in gasolines to prevent polymerization. Copper(II) and Zinc(II) complexes with organic molecules are used in clinical medicine. Our focus is to investigate the interaction between copper and zinc with mixed ligand. We are also interested in studies related to the antibacterial and antioxidant activity of Cu(II) and Zn(II) complexes. Bearing these facts in mind, the hexa-coordinated mixed ligand Cu(II) and Zn(II) complexes were synthesised from the diimino tetradentate Schiff base

which was derived from the condensation of thiophene-2-carboxaldehyde with *o*-phenylenediamine and bipyridine. The complexes possess potent antibacterial and antioxidant activities.

2. EXPERIMENTAL

2.1. Materials and methods

All chemicals used in the present work, viz., thiophene-2-carboxaldehyde, *o*-phenylenediamine, 2,2'-bipyridine, copper and zinc chlorides were of analytical reagent grade (Merck, Germany). Common solvents like ethanol, methanol, and DMSO used at various stages of this work were purified according to the standard procedures described in Weissenburg series (Weissenberger A, Proskauer ES, Riddick JA and Toops EE, 1955) and in quantitative analysis by Vogel (Vogel AI, 1989). Elemental analysis (C, H, N and S) were performed using a Carlo Erba 1108 analyzer. The molar conductivity of the complex in DMSO (10^{-3} mol/dm³) was measured using a Systronic Model-304 digital direct reading conductivity meter. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature on powder sample of the

complex. Copper sulphate was used as calibrant. Infra-red spectra of the Schiff base and its metal complexes were recorded as KBr discs in the range 400–4000 cm^{-1} on a Shimadzu spectrophotometer. The electronic absorption spectra of the Schiff base and its metal complexes were recorded on a Shimadzu UV-1601 spectrophotometer. The ^1H NMR spectra of the Schiff base and its zinc complex in DMSO-d_6 were recorded using tetramethylsilane as internal standard. Electrospray ionisation mass spectrometry (ESI-MS) analysis was performed in the positive ion mode on a liquid chromatography-ion trap mass spectrometer.

2.2. Estimation of metal

The metals were estimated gravimetrically as their oxides by fusion with AnalaR ammonium oxalate. In a typical experiment, about 0.3g of the dried complex was accurately weighed in a previously weighed silica crucible. AnalaR ammonium oxalate, roughly three parts by weight of the complex, was added and the mixture was incinerated slowly at first and then strongly using a Bunsen burner for 3h. It was then cooled in a desiccator and weighed. The procedure was repeated till the final oxide weight was constant. From the weight, the percentage of metal in the complex was calculated.

2.3. Determination of Chloride Content

The chloride present in the complexes was determined gravimetrically as silver nitrate test (Vogel AI, 1989).

2.4. Synthesis of ligand (L)

An ethanolic solution of thiophene-2-carboxaldehyde (0.04mol, 3.67mL) was added dropwise to an ethanolic solution of *o*-phenylenediamine (0.02mol, 2.163g) with continuous stirring and reaction mixture was refluxed for ca. 5h. The yellow product obtained was filtered and recrystallized from ethanol and dried in desiccator over anhydrous calcium chloride. Yield = 83%. The schematic representation of the Schiff base is given in figure 1.

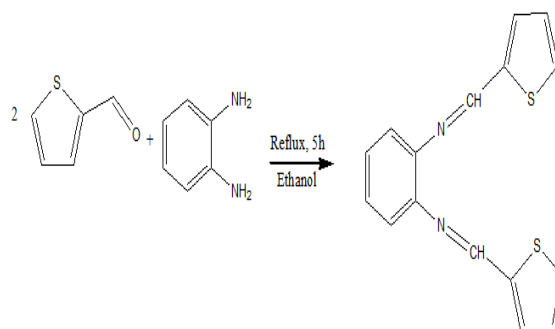


Figure 1. Synthesis of the Schiff base

2.5. Synthesis of metal complexes

An ethanolic solution of the Schiff base (0.002 mol) was added to the ethanolic solution of metal (II) chlorides (0.002 mol) and refluxed for ca. 3 h. To the above mixture an ethanolic solution of 2,2'-bipyridine (0.002 mol) was added in a

1:1:1 molar ratio and refluxed for about ca.2 h. The solid product formed was filtered and washed with ethanol.

2.6. Biological Studies

2.6.1. Antibacterial Studies

The *in vitro* antibacterial screening of the Schiff base and their complexes were evaluated by the disc diffusion method against the human pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* (Raman N, et al., 2002). A loop full of bacterial cells from the nutrient agar plates was incubated into a nutrient broth (50 mL) in a 250 mL Erlenmeyer flask and incubated at 37 °C for 18 h with vigorous shaking. Using a sterile L-rod 18 h bacterial cultures (100 µL) were spread on nutrient agar and allowed to dry for 5 minutes. The 5 mm diameter and 1 mm thickness filter-paper discs of uniform size are impregnated with different concentrations of Schiff base and its complexes and then placed on the surface of an agar plate that has been seeded with the organism to be tested. Then the plates were incubated at 37 °C for 24 h. During this period, the test solution diffused and inhibits the growth of the inoculated bacteria. The zone of inhibition, developed on the plate was measured in mm. *Streptomycin* was used as standard.

2.6.2. Antioxidant Studies (DPPH assay):

DPPH scavenging activity was carried out by using the method of Kirby Schmidt (1997) with slight modification. Different concentrations (1000, 500, 250, 125µg/mL) of Schiff base and its complexes were weighed respectively and dissolved in DMSO. Then 5mL of 0.1mM ethanolic solution of DPPH (1, 1, diphenyl - 2 - picrylhydrazyl) was added to each of the test tube containing the sample and the tubes were shaken vigorously. They were then allowed to stand at 35°C for 30 minutes. The control was prepared without any compound and ethanol was used for base line corrections in absorbance (OD) of samples measured at 517 nm. Radical scavenging activities were expressed as % scavenging activity and were calculated by the following formula.

$$\% \text{ Radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3. RESULTS AND DISCUSSION

The complexes of the type [MLX]Cl₂ where, M=Cu(II) and Zn(II) L=tetradentate Schiff base, X= 2,2'-bipyridine were synthesised by the reaction of tetradentate ligand, metal(II)chlorides and 2,2'-bipyridine in a

1:1:1 molar ratio in ethanol. In DMSO the complexes showed high molar conductance value indicates the electrolytic nature.

3.1. Infrared Spectral Studies

The IR spectrum of the free ligand shows band at 1660 cm^{-1} is characteristics of the azomethine moiety. This band is shifted to lower frequency in the complexes which indicates the coordination of metal to the azomethine nitrogen. The absorption band at 848 cm^{-1} is due to thiophene ring $\nu_{\text{C-S-C}}$. This band shifts to the lower wave number in the complexes. This shows the sulfur in the thiophene is participated in the complexation (Spinu C, Kriza A, 2000). The appearance of two new bands in the regions $510\text{-}495\text{ cm}^{-1}$ and $421\text{-}418\text{ cm}^{-1}$ in the spectra of complexes is due to $\nu_{\text{M-N}}$ and $\nu_{\text{M-S}}$ stretching vibrations respectively also confirmed the formation of metal complexes (Chandra S and Kumar R, 2004). The FT IR Spectral data are summarized in Table 1.

3.2. Electronic absorption Spectra and magnetic measurement

The UV-Vis. spectra of the ligand and its mixed ligand metal complexes were recorded in DMSO. The ligand shows the two bands at 42918 and 33113 cm^{-1} assigned for $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ electronic transitions respectively (Neelakantan MA

et.al,2008). The extension of the absorption spectra from UV region to visible region is due to ligand to metal charge transfer and d-d transition bands of the metal in the complexes. The spectrum of Cu(II) complex consists of broad, low intensity shoulder bands centred at $12,681\text{ cm}^{-1}$ and $23,616\text{ cm}^{-1}$. The ${}^2\text{E}_g$ and ${}^2\text{T}_{2g}$ states of the octahedral Cu(II) ion (d^9) split under the influence of the tetragonal distortion that causes three transitions ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$; ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$; and ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{2g}$. It could be concluded that all the three transitions lie within the two broad envelopes centred at the same range. The magnetic moment of 1.98 B.M. falls within the range normally observed for octahedral Cu(II) complex. Zn(II) complex shows two bands at $32250, 34489\text{ cm}^{-1}$ assigned for $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ electronic transition.

Table 1. IR Spectral data of the Ligand and its Metal Complexes:

Groups	$\nu(\text{C=N}) (\text{cm}^{-1})$	$\nu(\text{C-S-C}) (\text{cm}^{-1})$	$\nu(\text{M-N}) (\text{cm}^{-1})$	$\nu(\text{M-S}) (\text{cm}^{-1})$
Ligand	1660	848	-	-
[CuLX]Cl ₂	1629	825	49 8	419
[ZnLX]Cl ₂	1629	827	49 5	421

3.3. Proton Magnetic Resonance Spectra

The Proton Magnetic Resonance Spectra of ligand and its diamagnetic Zn (II) complex was recorded in DMSO-d₆. The thiophene proton appeared as a singlet at 6.9 ppm. The iminic proton appeared as a singlet at 7.9 ppm and the phenyl multiplet at 7.5- 7.7 ppm. In the ¹H-NMR spectrum of Zn (II) complex thiophene proton appeared as a singlet at 6.9-7.2 ppm. The iminic Proton appeared as a singlet at 8.5 ppm and phenyl multiplet at 7.5-7.7 ppm. The signal at 7.60 – 7.76 δ, 8,20–.39δ ppm was assigned to bipyridine proton.

3.4. Mass Spectra

The ESI mass spectra of the ligand and its Zn (II) complex were recorded and the obtained molecular ion peak confirmed the proposed formulae. The mass spectrum of the ligand exhibits peak at 296(M⁺) with 100% abundance which was also confirmed by ‘nitrogen rule’. The mass spectrum of the zinc complex shows peak at 588 (M⁺) with 100% abundance confirms the stoichiometry of metal complexes as [MLX] Cl₂ type. The mass spectra of ligand and its zinc complex are given in Figure 2.0 and 2.1

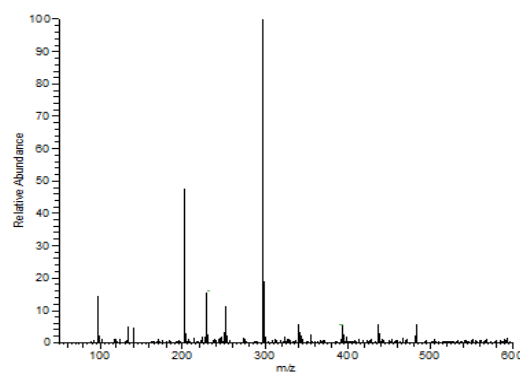


Figure 2.0. Mass spectrum of ligand

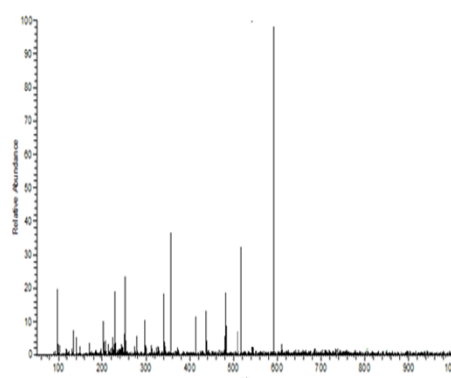
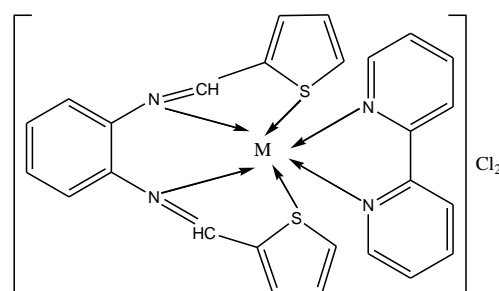


Figure 2.1. Mass spectrum of zinc complex

Based on the elemental analysis, molar conductance, magnetic moments, IR, UV-Vis., proton NMR and Mass spectral data, the proposed structure of the complexes are given in figure 3.



where, M = Cu(II) and Zn(II) ions

Figure 3. Structure of synthesized complexes

3.5. Biological Studies

3.5.1. Antibacterial Studies

The ligand and its metal complexes have been screened *in-vitro* against Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli* and *Proteus mirabilis* species and the results are given in Table 2. As expected, overall ligand showed weak activity, whereas the metal complexes had the higher antimicrobial activities against both Gram-positive and Gram-negative bacteria. Since chelation of the central metal atom could enhance the lipophilic character which subsequently favors its permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganism. It is observed that copper (II) complex is effective against all the bacteria examined except *Bacillus subtilis* when compared to standard drug streptomycin. Zinc (II) complex exhibit moderate antibacterial activities against

Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*.

Table 2. Antibacterial activity of ligand and its metal (II) complexes (Zone of inhibition in mm)

Compound	Gram (+ve) Bacteria					
	<i>B. subtilis</i>			<i>S. aureus</i>		
	25 (µg)	50 (µg)	75 (µg)	25 (µg)	50 (µg)	75 (µg)
L	6	8	9	9	10	12
[CuLX]Cl ₂	14	16	22	17	23	26
[ZnLX] Cl ₂	8	10	12	10	11	12
Streptomycin	13	15	20	18	22	24

Compound	Gram (-ve) Bacteria					
	<i>E. coli</i>			<i>P. mirabilis</i>		
	25 (µg)	50 (µg)	75 (µg)	25 (µg)	50 (µg)	75 (µg)
L	10	11	12	8	9	11
[CuLX]Cl ₂	25	27	29	15	19	21
[ZnLX] Cl ₂	12	13	16	9	11	13
Streptomycin	22	23	26	15	18	20

3.5.2. Antioxidant Studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical in organic solvents; in alcoholic solution it appears purple and has

a single electron. This radical is able to react with any compound that releases a hydrogen atom or an electron, resulting in a color change from purple to yellow. The degree of discoloration indicates the scavenging potential of the samples in terms of hydrogen donating ability. Figure 4 shows the comparative effect of metal complexes on DPPH radical. From the IC_{50} value (Table 3) it is inferred that Copper (II) complex ($IC_{50} = 1.061 \mu M$) exhibits potent antioxidant activity when compared to Zinc complex. The increased activity of the complexes can be attributed to the presence of thiophene moiety which is coordinated with the metal ions which facilitate the release of hydrogen to reduce the DPPH radical.

Table 3. Antioxidant activity of [MLX]Cl₂

Concentration	[CuLX]Cl ₂	[ZnLX]Cl ₂
1000 $\mu g/mL$	60.8	52.4
500 $\mu g/mL$	48.3	43.7
250 $\mu g/mL$	41.6	36.9
125 $\mu g/mL$	35.0	29.3
IC_{50} (μM)	1.061	1.601

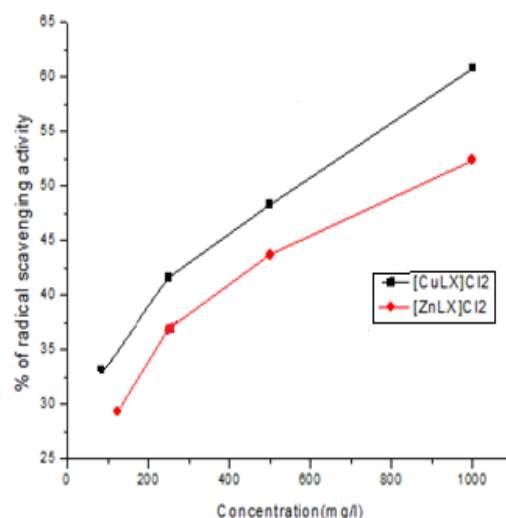


Figure 4. Antioxidant activity of mixed ligand complexes

4. CONCLUSION

We synthesized novel mixed ligand Cu(II) and Zn(II) complexes of [MLX]Cl₂ type derived from thiophene-2-carboxaldehyde, orthophenylenediamine and 2,2'-bipyridine. The UV-Vis., IR, Proton NMR, Mass spectral studies showed the complexes are in octahedral structure. The high conductance showed the complexes are in electrolytic nature. They exhibited antibacterial activities against the Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli* and *Proteus mirabilis*. The results showed that the Cu (II) complex exhibits higher antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*

when compared to the standard drug streptomycin. The other complex showed moderate activity. All the complexes demonstrated antioxidant properties and could be useful in combating the free radicals which exist in close relationship with cancerous cells. It was notable that the [CuLX]Cl₂ complex exhibited stronger antioxidant effects than other.

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