

ANTIMICROBIAL ACTIVITY OF COPPER(II) COMPLEX OF 1-(1H-BENZIMIDAZOL-2-YL)-N-(TETRAHYDROFURAN-2-YLMETHYL)METHANAMINE

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ABSTRACT

A mononuclear copper(II) complex $[\text{Cu}(\text{L})(\text{Cl})]\text{Cl}$ where L is 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine) was synthesized. The synthesized complex was characterized using various physicochemical techniques like cyclic voltammetry and elemental analysis, ESI-MS, UV-Visible, Infra red and EPR. The antimicrobial activities of the ligand and their metal complexes were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

Keywords: antimicrobial, copper, benzimidazol.

1. INTRODUCTION

Copper is a biologically relevant element and many enzymes that depend on copper for their activity have been identified (Barton, 1986; Dervan, 1986; Dhar and Chakravarty, 2003; Garcia-Raso, *et al.*, 2003), Copper(II) is a substitutionally labile metal ion. So multidentate ligands are believed to be better than bidentate ligands in keeping the copper(II) ion chelated in solution. Typically, upon association with dioxygen or hydrogen peroxide these copper complexes are thought to perform reactive intermediates. Sigman *et al.* have shown that the bis(phen) copper complex acts as an efficient nuclease by oxidative cleavage mechanism in the presence of molecular oxygen and a reducing agent (Hemmer, *et al.*, 2001; Navarro, 2003; Sreedhara *et al.*, 2000). Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause disease. So more interests have been shown in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells. The biological studies of metal complexes highlighted the potential of antioxidant activity of copper(II) complex with bioactive ligand. The present work stems from our interest to design copper(II) complex with tetrahydro furyl amine based ligand. We have synthesized a copper complex $[\text{Cu}(\text{L1})(\text{Cl})]\text{Cl}$ where L1 is tetrahydro furyl amine based unsymmetrical tridentate ligand.

2. MATERIALS AND METHODS

1-(tetrahydrofuran-2-yl) methanamine, was procured from Sigma Aldrich, USA and used as received. Other materials like sodium borohydride and solvents like methanol, acetonitrile and dichloromethane were of reagent grade. Benzimidazole carbaldehyde was prepared using published procedure.¹ UV-visible spectrum of the complex was recorded on a Perkin-Elmer Lambda 35 double beam spectrophotometer at 25°C. Electron paramagnetic resonance spectrum of the copper(II) complexes were obtained on a Varian E 112 EPR spectrometer. IR spectrum was recorded as KBr pellets in the 400-4000 cm^{-1} region using a Shimadzu FT-IR 8000 spectrophotometer. Positive ion electrospray ionization mass spectrum of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer.

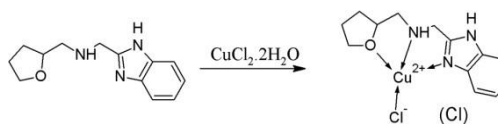


Figure 1. Structure of Copper Complex

2.1 Synthesis of 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine (L)

Benzimidazole-2-aldehyde (0.767 g, 5 mmol) and tetrahydrofurfuryl amine (0.505 g, 5 mmol) were mixed in methanol (20 mL) and stirred well for one day. Sodium borohydride (0.28 g, 7.5 mmol) was added to the above solution at 0°C and the reaction mixture was stirred overnight at room temperature. The reaction mixture was

rotaevaporated to dryness and the residue was dissolved in water (15 mL) and extracted with dichloromethane. The organic layer was dried and the solvent was evaporated to give the ligand as brown oil, which was used as such for the preparation of complex. Yield: 1.016 g (88 %).

2.2 Synthesis of [Cu (L) (Cl)] Cl (1)

The complex was prepared in good yield from the reaction of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in methanol with L. The ligand, L (0.68 g, 3 mmol) and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.5 g, 3 mmol) were dissolved in methanol individually and the solutions were warmed. To the hot solution of L5, copper chloride was added slowly and stirred for 3 hours. The resulting solution was cooled to room temperature and the green colored copper-L complex separated out was filtered and dried. Yield: 0.921 g (84 %). Anal. Calc. for $\text{C}_{13}\text{H}_{17}\text{Cl}_2\text{CuN}_3\text{O}$: C, 42.69; H, 4.68; N, 11.49; Cu, 17.37; Found: C, 42.67; H, 4.62; N, 11.43; Cu, 17.31 %. FT-IR (KBr pellet) cm^{-1} : 3302, 3067, 1624, 1589, 1093, 748, 621. UV (nm): 277, 365, 682. ESI-MS: $m/z = 365.27$ [M - Cl]⁺.

2.3 Antimicrobial Assay

2.3.1. Micro-organisms used

Five species of bacteria, two gram positive (*Streptococcus faecalis* & *Bacillus subtilis*) and three gram negative (*Escherichia coli*, *Klebsiella pneumonia* & *Salmonella paratyphi*) were obtained from KMCH, Coimbatore.

2.3.2. Preparation of Inoculum

A loopful of strain was inoculated in 30 mL of nutrient broth in a conical flask and incubated on a rotary shaker at 37°C for 24 hours to activate the strain.

2.3.3. Bioassay

The bioassay used was the standard Agar Disc Diffusion assay. Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Subsequently, the sterilized filter paper discs were completely saturated with the test compound. The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37°C. Each compound was tested against each organism in triplicate. Methanol was used as negative control. Standard discs of Ampicillin served as positive antibacterial control. The test materials having antimicrobial activity inhibited the growth of the micro organisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was

determined by measuring the diameter of zone of inhibition in mm.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization

Ligand L was synthesized by condensing tetrahydro furfuryl amine with benzimidazole aldehyde to form Schiff base followed by reduction with sodium borohydride. It was characterized by ESI-MS and ¹H NMR spectra. The copper(II) complex of the ligand was prepared by the reaction between

copper(II) chloride and the corresponding ligand in equimolar quantities using methanol as solvent. The complex was obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. The structure for the present complex is shown in figure 1.

The ESI mass spectra of [Cu(L)(Cl)]Cl displayed the molecular ion peak at m/z 367.27 which is reliable with the proposed molecular formula of the corresponding copper (II) complex. The electronic spectrum of the complex shows a low energy ligand field (LF) band (682 nm) and a high energy ligand based band (277 nm). Broad ligand field transition has been observed for all the four complexes in the region of 682 nm. Three d-d transitions are possible for copper (II) complexes. They are $d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$, $d_z^2 \rightarrow d_{x^2-y^2}$ and $d_{xy} \rightarrow d_{x^2-y^2}$. However, only a single broad band is observed for the copper (II) complex. This indicates the total sum of all the above transitions. The broadness associated with the d-d bands is generally taken as an indication of the geometrical distortion of the complex from perfect planar symmetry.

IR spectra provide the valuable information about the nature of the binding mode and functional group attached to the metal ion. The peak observed at 1620 cm^{-1} have been assigned to the C=N stretching frequencies of benzimidazole group. IR peak observed in the region of 3248 cm^{-1} indicates the stretching vibration of NH group of ligand L.

The epr spectrum of complex shows axial signal at 300 K from a static copper (II) centre with dx^2-y^2 as the ground state. The g value is 2.07. The broad epr spectrum and its g value confirm the formation of the copper (II) complex. Also it confirms that the complex is paramagnetic.

The redox behavior of copper complex is studied with the help of cyclic voltammetry. Copper complex shows an irreversible peak at 0.51 V at a scan rate of 100 mVs^{-1} . The redox process is assigned to $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ couple (Mistra and Pandey, 1992).

3.2. Antimicrobial Activity

The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria: *Salmonella paratyphi*, *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus subtilis* by the disc diffusion method. In the modified disc diffusion assay the majority of the complexes showed some activity in the screen. Results displayed in Table 1, clearly indicate that the inhibitions are much larger by metal complexes as compare to the metal free ligand.

The observed zone of inhibition order of present complex was *S. faecalis* > *B. subtilis* > *K. pneumonia* > *S. paratyphi* > *E. coli*. Interestingly the copper complex showed an efficient inhibitory activity against the bacterial pathogen *Streptococcus faecalis*.

The increased activity of the metal chelates can be explained on the basis of chelation theory. Also activity increases with concentration of the metal complexes. The chelation tends to make the ligands act as more powerful and potent bacterial agents, thus killing of more bacteria than the ligand. It is observed that in complexes the positive charge of the metal partially shared with the donor atoms present in the ligand and there may be π -electron delocalization over the whole chelate ring. Such an electron delocalization enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Table 1. Antimicrobial activity of copper complex

S. No	Bacteria	Control Ampicillin (mm)	Zone of inhibition (mm)	
			L5	12
1	<i>Streptococcus faecalis</i>	13.2±0.51	10.17±0.57	17.3±0.9
2	<i>Bacillus subtilis</i>	14.5±0.4	7.49±0.34	13.6±0.29
3	<i>Klebsiella pneumonia</i>	15±0.04	8.64±0.31	9±0.38
4	<i>Salmonella paratyphi</i>	15±0.57	6.71±0.23	8±0.13
5	<i>Escherichia coli</i>	16.3±0.15	5.44±0.05	6±0.26

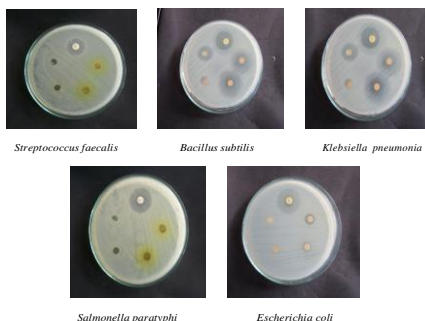


Figure 1. Antimicrobial activity of copper complex

4. CONCLUSION

In summary, we have synthesized and characterized a new mononuclear copper complex having tridentate reduced Schiff base. The antimicrobial activities of the ligand and their metal complexes were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

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