

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

Elayaperumal, R¹, M. Kiruthika² and P. Dharmalingam*

¹Department of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli.

²Department of Chemistry, Kongunadu Arts and Science College, Coimbatore.

*Department of Chemistry, Urumu Dhanalakshmi College, Tiruchirappalli.

*E-mail: relaya82@gmail.com

ABSTRACT

A mononuclear copper(II) complex of 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine) was synthesized and characterized by various physicochemical techniques like cyclic voltammetry and elemental analysis, ESI-MS, UV-Visible, Infra red and EPR spectroscopy. The antibacterial activities of the ligand and its complex were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

Keywords: Copper(II), tetrahydrofuran, disc diffusion, antimicrobial.

1. INTRODUCTION

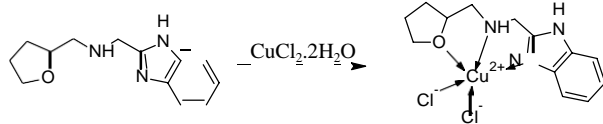
Copper is an essential trace element in plants and animals, but not some microorganisms. Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase. Copper proteins have diverse roles in biological electron transport and oxygen transportation, processes that exploit the easy interconversion of Cu(I) and Cu(II) (Lippard and Berg, 1994). It is well known that the derivatives of thiosemicarbazones possess the antibacterial activity. In addition, there are many studies that show the antimicrobial activity of copper(II) complexes with these ligands (Rauf *et al.*, 2009). Common anti-bacterial agents have been also used as ligands to complex with copper ions. It was noticed that the antimicrobial activity against *M. Smegmatis* of a metal ion complex in comparison to free ciprofloxacin, a bacterial gyrase inhibitor, increased three times. It may result from facilitated diffusion of the drug through the cell membrane, presumably by an increase in the lipophilicity of the drug. The activity against *Streptococcus* can also be influenced by the slow release of the ligands inside the bacterial cell. This was noticed with the copper complex of isoniazide and ethambutol. It seems that intercellular reduction of Cu(II) into Cu(I) can activate the oxygen which is toxic for bacteria. Moreover, a copper(II) complex of sulfacetamide, (N-[4-(amino-fenil)sulfonyl]acetamide), has been intensively used in treatment of ophthalmic and dermatologic infections (Mitra and Pandey, 1992). Further studies of the copper(II) complexes of sulfacetamide and sulfanilamide and sulfisoxazole

have shown promising results. The biological studies of metal complexes highlighted the potential of antioxidant activity of copper(II) complex with bioactive ligand (Rao *et al.*, 2010). In the present work, we synthesized and characterized a copper(II) complex of the ligand 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine and also the antimicrobial activity was explored. The synthetic route for the present complex is shown in scheme 1.

2. MATERIALS AND METHODS

1-(tetrahydrofuran-2-yl)methanamine and copper(II) chloride 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 (Sathyaraj *et al.*, 2010). 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) complex was 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. Cyclic voltammetry study of the complex was carried out by using three electrode system in a single compartment comprising of glassy-carbon working electrode and potentials were referenced to standard calomel electrode. Minimum quantity of the complex was dissolved in DMSO and decimolar solution of tetra butyl ammonium perchlorate was added. Positive ion electrospray

ionization mass spectrum of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer.



Scheme 1. Synthesis of Copper(II) Complex.

2.1. Synthesis of 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl) methanamine (L1)

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 a brown oil, which was used as such for the preparation of complex. Yield: 0.1.016 g (88 %).

2.2. Synthesis of [Cu(L1)(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 L1. The ligand, L1 (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 L1, copper chloride was added slowly and stirred for 3 hours. The resulting solution was cooled to room temperature and the green colored copper-L1 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} : 3248, 2954, 1620, 1452, 752, 631. ESI-MS: $m/z = 367.27$ $[\text{M} - \text{L} \cdot \text{Cl}]^+$.

2.3. Antibacterial 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 L1 was prepared by condensing tetrahydro furfuryl amine with benzimidazol-2-aldehyde to form Schiff base followed by reduction with Sodium borohydride. The copper(II) complex(1) was synthesized by the reaction between copper(II) chloride and L1 in equimolar quantities using methanol as solvent. The present complex was obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. The analytical data obtained for the new complex agree well with the proposed molecular formula. The synthetic scheme for the present complex is shown in scheme 1. The ESI mass spectrum of $[\text{Cu}(\text{L1})(\text{Cl})](\text{Cl})$ displayed the molecular ion peak at m/z 367.27 which is reliable with the proposed molecular formula of the copper (II) complex.

3.2. Electronic Spectral Analysis

The electronic spectrum of the present complex shows two bands at 270.8 and 277.4 nm, which can be attributed to intra ligand transitions of the ligand. Broad metal to ligand charge transfer (MLCT) transition has been observed at 364.6 nm. Complex 1 also exhibits its ligand field transition as broad band at 682 nm. Three d-d transitions are possible for copper (II) complex. They are $d_{xz}, d_{yz} - d_{x^2-y^2}$, $d_z^2 - d_{x^2-y^2}$ and $d_{xy} - d_{x^2-y^2}$. 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.

3.3. IR and EPR Spectral Analysis

IR spectrum of complex 1 supports the coordination of N-H group of ligand (L1) as there is lowering of $\nu_{\text{N-H}}$ from 3360-3248 cm^{-1} . The absorption found at 1091-1042 cm^{-1} is attributed to the presence of the coordinated furan ring through oxygen atom. The absorption in low frequency region at 752-630 cm^{-1} is attributed to the positive shifting of in-plane ring deformation vibration of the imidazole ring indicating the imidazole nitrogen coordination. For the complex 1, the calculated g_{\parallel} and g_{\perp} values are 2.274 and 2.0871 respectively. The g_{\parallel} value is g_{\perp} predicting the presence of the unpaired electron in the $d_{x^2-y^2}$ orbital. The g_{\parallel} value is slightly less than 2.3 indicating that the metal-ligand bonding is predominantly covalent in nature. The G value is found to be 3.225 which is much less than 4 indicating that there is a strong interaction between two copper centres in the solid state. The g_{\parallel} value is higher than 2.2 predicting the complex to have a pseudo-tetrahedral geometry.

3.4. Electrochemical Behavior of Copper Complex

The redox behavior of copper complex is studied with the help of cyclic voltammetry. Cyclic voltammogram of the copper complex was recorded in DMSO (Dimethyl sulphoxide) solution at 300 K using tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. Complex 1, records E_{pc} and E_{pa} values at -0.523 and -0.2824 V respectively. These peak potential values signify the existence of $\text{Cu}^{\text{II}}/\text{Cu}^{\text{I}}$ redox couple. The reduction of Cu^{II} to Cu^{I} occurs at the cathodic peak potential of -0.523 V and reoxidation of Cu^{I} occurs at the anodic peak potential of -0.2824 V on scan reversal. The peak separation is found at 240 mV. This indicates a quasi-reversible one electron redox process. The $E_{1/2}$ value is measured at 0.12 V. This positive value of $E_{1/2}$ shows that the reduction of Cu^{II} in the complex is difficult as it is stabilized by the sigma bonding ligands. The peak current ratio for this complex is measured at 1.09. This value is slightly higher than unity and it indicates that the electron transfer is not followed by chemical reaction.

3.5. Antibacterial 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. Antibacterial activity shown in Table 1 clearly indicates that the inhibitions are much larger by

copper complexes as compare to the metal free ligand. The observed zone of inhibition order of complex 1 was *S. faecalis* > *B. subtilis* > *K. pneumonia* > *S. paratyphi* > *E. coli*. The increased activity of the metal complex 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 (Arjmand *et al.*, 2005).

Table 1. Antibacterial Activity of Complex 1.

S. No.	Bacteria	Zone of inhibition (mm)		
		Ampicillin	L1	Complex 1
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±0.04	8.64±0.31	9.0±0.38
4	<i>Salmonella paratyphi</i>	15.0±0.57	6.71±0.23	8.0±0.13
5	<i>Escherichia coli</i>	16.3±0.15	5.44±0.05	6.0±0.26

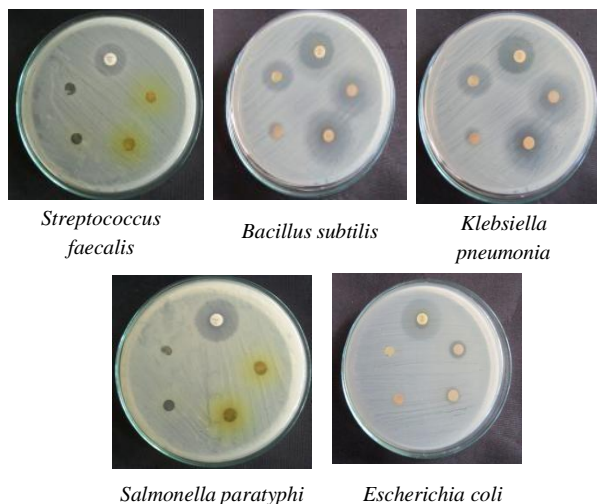


Fig. 1. Antibacterial Activity of Complex 1.

4. CONCLUSION

In the present work mononuclear copper(II) complex of tridentate reduced Schiff base ligand 1-(1*H*-benzimidazol-2-yl)-*N*-(tetrahydrofuran-2-ylmethyl)methanamine has been isolated and

characterized by various physico-chemical techniques. The antibacterial activity of the complex has also been evaluated and found that the Cu(II) complex showed better biological activity when compared to that of the ligand.

ACKNOWLEDGEMENT

The authors thank the Head, Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore for antibacterial studies.

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