Synthesis, structural characterization and antibacterial studies of some biosensitive mixed ligand copper(II) complexes

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A series of copper(II) complexes of mixed ligands with Schiff bases derived from 2-aminophenol/2-aminobenzoic acid with substituted benzaldehydes and 1,10-phenanthroline have been synthesized. Microanalytical, magnetic susceptibility, IR, UV-vis, CV, ESR and mass spectral techniques have been used to confirm the structure of these complexes. Their low electrical conductance values indicate that all the complexes are non-electrolytes. The monomeric nature of the complexes is evidenced from their magnetic susceptibility values. Electronic and EPR spectra of the complexes suggest that they have distorted octahedral geometry. The electrochemical behavior, the anodic and cathodic potential and the number of electron transfer have been calculated using cyclic voltammogram. The synthesized compounds have been tested against microorganisms such as Escherichia coli, Bacillus subtilis, Micrococcus luteus and Proteus vulgaris. A comparative study of the MIC values of the ligands and the complexes indicate that the complexes exhibit higher antibacterial activity than the free ligand and control.

Experimental

All reagents were Merck products and used as supplied. For the voltammetric experiments, tetrabutylammonium perchlorate (TBAP) used as supporting electrolyte, was purchased from Sigma. Anhydrous grade methanol and DMSO were obtained from Fisher Scientific Company. Microanalytical data and FAB mass spectra of the compounds were recorded at the Regional Sophisticated Instrumentation Center, Central Drug Research Institute (RSIC, CDRI), Lucknow. The FAB mass spectrum of the complex was recorded on a JEOL SX 102/D-A-6000 mass spectrometer in 4000-200 cm⁻¹ range using KBr pellet. The UV-vis spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer in 4000-200 cm⁻¹ range using argon/xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using m-nitrobenzylalcohol (NBA) as the matrix. The IR spectra of the samples were recorded on a Shimadzu FTIR-8400S spectrophotometer in 4000-200 cm⁻¹ range using KBr pellet. The UV-vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer using DMSO as solvent. The X-band ESR spectra of the complexes were recorded at 300 K and 77 K at IIT, Mumbai, using TCNE (tetracyanoethylene) as the g-marker. Magnetic susceptibility measurements of the complexes were carried out by Guoy balance using copper sulphate as the calibrant. Electrochemical studies were carried out using EG&G Princeton Applied Research Potentiostat/Galvanostat (model 273A), controlled by M270 software. CV measurements were performed using a glassy carbon working electrode, platinum wire auxiliary electrode and an Ag/AgCl reference electrode. All solutions were purged with N₂ for 30 min prior to each set of experiments. The molar conductance of the complexes was measured using a Systronic conductivity bridge.

Synthesis of Schiff bases and Cu(II) complexes

The Schiff bases were synthesized by stirring a mixture of benzaldehyde (1.06 g, 0.01 M)/3-nitrobenzaldehyde (1.51 g, 0.01 M)/4-methoxybenzaldehyde (1.36 g, 0.01 M) with 2-aminophenol (1.09 g, 0.01 M)/2-aminobenzoic acid (1.37 g, 0.01 M) in 50 mL of ethanol medium. The solid product formed was removed by filtration and recrystallized from ethanol.
A solution of Schiff base(s) (0.001 M) and 1,10-phenanthroline (0.198 g, 0.001 M) in 40 mL of ethanol was added to an ethanolic solution of CuCl$_2$.2H$_2$O (1:1:1 molar ratio) and the mixture was stirred for 1 h. The solid product so formed was separated by filtration and washed thoroughly with ethanol and dried in vacuo.

**Antibacterial activity**

The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria *E. coli*, *B. subtilis*, *M. luteus* and *P. vulgaris* by the well diffusion method using agar nutrient as the medium. The stock solutions were prepared by dissolving the compounds (0.001-0.007 M) in 1 mL of DMSO. In a typical procedure, a well (50 µL) was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated at 35°C for 24 h. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. Antibacterial activity was indicated by the presence of clear inhibition of zone around the well.

Compounds that showed positive antibacterial with the disc diffusion assay were subjected to the broth dilution method for the quantitative measurement of microstatic (inhibitory) activity. The lowest concentration which completely inhibited visible microbial growth was recorded as the minimum inhibitory concentration (MIC, µg/mL).

**Results and discussion**

Bidentate Schiff base ligands derived from substituted benzaldehyde and 2-aminobenzoic acid/2-aminophenol and its Cu(II) complexes were synthesized as described above. The complexes were found to be air stable and insoluble in water, but soluble in DMSO and DMF. The analytical data are in good agreement with theoretical values for the proposed Cu(II) complexes (Table 1).

In order to study the bonding mode of the Schiff base to the metal complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes. The IR spectrum of the ligand shows a broad band in the region 3250-3450 cm$^{-1}$ assignable to –OH groups. The absence of this band, noted in the spectra of the complexes, indicates the deprotonation of the –OH groups on complexation. The shifting of υ(CO) to lower frequencies (1355-1345 cm$^{-1}$) and appearance of new bands around 1580 cm$^{-1}$ in all the complexes due to υ(C-O-M) reveals the participation of phenoxy oxygen in coordination. The ligand shows its characteristics –CH=N- bands in the region 1620-1590 cm$^{-1}$, which are also shifted to lower frequencies in the spectra of all complexes (1580-1550 cm$^{-1}$). The IR spectra of the metal chelates also show some new bands in the region 305-450 cm$^{-1}$ which are due to the formation of M-O, M-N and M-Cl bands respectively. The sharp peaks around 900-800 cm$^{-1}$ in all the complexes are due to rocking mode of coordinating water molecules.

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**Table 1 — Physical characterization, analytical, molar conductance and magnetic susceptibility data of the complexes**

<table>
<thead>
<tr>
<th>Comp. (Colour)</th>
<th>Melt/decomp. temp. (°C)</th>
<th>Yield (%)</th>
<th>Found (Calc.) (%)</th>
<th>$\Lambda_m$ (ohm$^{-1}$ cm$^2$ mol$^{-1}$)</th>
<th>$\mu_{\text{eff}}$ (BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>[CuL$^1$(phen)Cl(H$_2$O)] (Brown)</td>
<td>265</td>
<td>58</td>
<td>12.7 (12.9)</td>
<td>60.3 (60.9)</td>
<td>3.8 (4.1)</td>
</tr>
<tr>
<td>[CuL$^2$(phen)Cl(H$_2$O)] (Brown)</td>
<td>above 280</td>
<td>61</td>
<td>11.3 (11.8)</td>
<td>55.1 (55.8)</td>
<td>3.2 (3.5)</td>
</tr>
<tr>
<td>[CuL$^3$(phen)Cl(H$_2$O)] (Brown)</td>
<td>above 280</td>
<td>65</td>
<td>11.8 (12.2)</td>
<td>59.2 (59.7)</td>
<td>3.8 (4.2)</td>
</tr>
<tr>
<td>[CuL$^4$(phen)Cl(H$_2$O)] (Green)</td>
<td>254</td>
<td>59</td>
<td>11.7 (12.2)</td>
<td>59.3 (59.9)</td>
<td>3.4 (3.8)</td>
</tr>
<tr>
<td>[CuL$^5$(phen)Cl(H$_2$O)] (Green)</td>
<td>above 280</td>
<td>54</td>
<td>10.6 (11.2)</td>
<td>49.7 (55.7)</td>
<td>3.1 (3.4)</td>
</tr>
<tr>
<td>[CuL$^6$(phen)Cl(H$_2$O)] (Green)</td>
<td>above 280</td>
<td>63</td>
<td>11.1 (11.5)</td>
<td>58.2 (58.8)</td>
<td>3.3 (4.0)</td>
</tr>
</tbody>
</table>
The $^1$H-NMR spectrum of the ligand ($L^2$) in CDCl$_3$ shows signals for the phenyl multiplet at 7.1 – 7.8 δ range and azomethine proton at 8.4 δ. The peak at 7.8 δ is attributed to the phenolic –OH group present in the ligand. Other ligands also show similar patterns.

The electronic spectra of the copper(II) complexes were recorded in DMSO solution. The Cu(II) ion with $d^9$ configuration in a complex can be either distorted octahedral or tetrahedral or rarely square-planar. The octahedrally coordinated Cu(II) ion has the ground state $^2E_g(t_{2g})^6(e_g)^3$. The only excited state should then be $^2T_g(t_{2g})^5(e_g)^4$, the energy difference being 10Dq. The Cu(II) complexes under the present investigation exhibit a broad band in the region 13,850-13,280 cm$^{-1}$. The broadness of the band may be due to Jahn-Teller distortion. These observations suggest that the complexes have distorted octahedral structures (Fig.1). The magnetic susceptibility values (Table 1) of the copper complexes further indicate the octahedral arrangement of the ligand around the central atom.

The FAB mass spectrum of copper(II) complex such as $[CuL^2(phen)Cl(H_2O)]$ showed molecular ion peak at $m/z = 531$, which corresponds to the molecular weight of compound supporting to the structure of the complexes. The fragment ion peaks, $m/z = 180$ and 242, correspond to the 1,10 phenanthroline and Schiff base ($L^2$) moiety present in the complex. This is also supported by the mass spectra of the other complexes.

The CV is the most versatile electroanalytical technique for the study of electroactive species. The redox properties of copper(II) complex have been studied by cyclic voltammetry experiments using glassy carbon working electrode in DMSO solvent at scan rate 100 mVs$^{-1}$. The cyclic voltammogram of the $[CuL^2(phen)Cl(H_2O)]$ in DMSO solution recorded over a potential range -0.4 V to –1.1 V shows two quasi-reversible peaks, one at cathodic direction and another at anodic direction. The quasi-reversible reduction peak at -0.92 V is due to the formation of Cu(II)/ Cu(I) while the other quasi-reversible oxidation peak at -0.56 V is due to the formation of Cu(I)/ Cu(II).

The ESR spectra of metal complexes provide information about hyperfine and superhyperfine structures which are of importance in studying the metal ion environment in the complex i.e., the
geometry, nature of the ligating sites of the Schiff base and the metal and the degree of covalency of the metal-ligand bonds. The X-band ESR spectrum of polycrystalline copper complex was recorded at 300 and 77 K (Fig. 2).

The ESR spectrum of copper complex, [CuL^2(phen)(H_2O)] at RT and LNT, exhibits three different g-values indicating magnetic anisotropy in the complex. The distorted structure of the complex is supported by its ESR spectrum recorded in solid state the complex. The distorted structure of the complex is explained on the basis of Overtone’s concept and increased activity of the metal chelates can be greater lipophilic nature of the complexes. Such complexes exhibit higher antibacterial activity than the free ligands. This is probably due to the metal complexes have higher activity than that of the free ligands. It has been observed from the results that the antibacterial activity of the copper complexes is higher than the free ligands and control.

**Antibacterial activity**

Antibacterial activity of the ligand and its complexes has been carried out against the bacteria, *E. coli*, *B. subtilis*, *M. luteus* and *P. vulgaris* using Muller-Hinton agar by well-diffusion method^23^ using DMSO as solvent.

A comparative study of MIC values of the Schiff base and its complexes indicate that the metal complexes exhibit higher antibacterial activity than the free ligands. It has been observed from the results that the metal complexes have higher activity than that of the free ligands. This is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone’s concept and chelation theory. According to Overtone’s concept of cell permeability the lipid membrane that surrounds the cell favours the passage of only lipid soluble materials due to which liposolubility is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilic property of the complex. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocks the metal binding sites on enzymes of microorganisms.

In the present study, six Cu(II) complexes have been synthesized using the Schiff bases (formed by the condensation of benzaldehyde/3-nitro-benzaldehydes/4-methoxy benzaldehydes with 2-aminophenol/2-aminobenzoic acid) and 1,10-phenanthroline. They are characterized by the usual spectral and analytical data. From the above data, it is concluded that all the complexes exhibit distorted octahedral geometry. The antibacterial activity of the copper complexes is higher than the free ligands and control.

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**References**