SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NOVEL SCHIFF BASE COPPER(II) COMPLEXES

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ABSTRACT

Five novel copper(II) complexes have been synthesized using Schiff base ligands, synthesized by the condensation reaction of anthranilic acid and Knoevenagel β-ketoanilide condensates (obtained by the condensation of acetacetonilide and substituted benzaldehydes). The ligands and copper(II) complexes have been characterized on the basis of Microanalytical, Mass, UV-Vis., IR, 1H NMR, ESR, XRD and CV spectral studies, as well as magnetic susceptibility and conductivity data. On the basis of spectral studies, a square-planar geometry has been proposed for the copper(II) complexes. From the XRD data, the CuL2 complex has the crystallite size of 50 nm respectively. The in vitro antimicrobial activity of the compounds is tested against the bacteria Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa and fungi Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataiola and Candida albicans by well diffusion method. The complexes show stronger antimicrobial activity than the free ligands. They represent a novel class of metal-based antimicrobial agents which provide opportunities for a large number of synthetic variations for modulation of the activities.

Keywords: Schiff base ligands, square-planar, Knoevenagel.

INTRODUCTION

Drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria, fungi, parasite and virus. Infectious diseases like diarrhoea, dysentery, tuberculosis, acute respiratory tract infections, AIDS and recently SARS are global threat and their incidences are increasing significantly day by day. Although a number of chemotherapeutic agents are available in market places, the pathogenic organisms are developing resistance to these agents. So, it is important to find out safer, more effective and inexpensive chemotherapeutic agents.

Metals have an esteemed place within medical biochemistry, although until recently this has been restricted predominantly to organic drugs. Recently however, more research has been done in the area of inorganic chemistry, which has led to developments in cancer care, infection control, diabetes, ulcers and neurological, cardiovascular and anti-inflammatory drugs. Metal coordination complexes have been widely studied for their antimicrobial and anticancer properties. Many drugs possess modified pharmacological and toxicological properties when administered in the form of metallic complexes. Platinum anticancer drugs are now the widely used anticancer drugs in the world e.g. cisplatin, carboplatin, oxaliplatin, tetraplatin etc. This inspires synthetic chemists to search for new metal complexes for bioactive compounds and copper in particular has attracted the researchers. Probably the most widely studied cation in this respect is Cu2+, since a host of low-molecular-weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers and cancers. Metal complexes of copper containing nitrogen and oxygen donor ligands are found to be effective catalysts for oxidation of olefins, etc. The coordination environment around copper plays the key role in stabilizing its different oxidation states and hence dictates the redox properties of the central atoms. The treatment with copper complexes produces remarkable pharmacological effects, which are not observed when the parent ligands or inorganic forms of copper are used.

Various copper complexes have been reported to inhibit bacterial, fungal, yeast, algal, mycoplasma, and viral growth, as well as to cause the death of these organisms. This prompted us to investigate some novel copper(II) complexes using Schiff base ligands. For, the coordination chemistry of Schiff base ligands is of considerable interest due to their biological importance. A literature search revealed that no work has been done on the Schiff base ligands having anthranilic acid and Knoevenagel condensed β-ketoanilides. Hence, in this paper we describe the synthesis of these ligands. Further insight into the bonding and possible geometrical structure has been made by Microanalytical, Mass, IR, UV-Vis., 1H NMR, ESR,XRD and CV spectral studies, as well as magnetic and conductivity data. The in vitro antimicrobial activity of the compounds is also tested against few bacteria and fungi.

EXPERIMENTAL

All reagents were of Merck products and used as supplied. For the voltammetric experiments, tetrabutylammoniumperchlorate (TBAP) used as supporting electrolyte, was purchased from Sigma. Anhydrous grade methanol and DMSO were obtained from Fisher Scientific Company. Micro analytical data and FAB Mass spectra of the compounds were recorded at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute (SAIF, CDRI), Lucknow. The FAB mass spectrum of the complex was recorded on a JOEL SX 102/DA-6000 mass spectrometer/data system using 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 4000-400 cm−1 range in a KBr pellet. The UV-Vis. spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using copper sulphate as the calibrant. The values were corrected for diamagnetism by applying Pascal’s constants. Electrochemical studies were carried out using BAS CV-50 cyclic voltammogram. 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 Systronic conductivity bridge. The 400 MHz 1H NMR spectra of the Schiff bases and their zinc complexes were recorded at Indian Institute of Technology, Chennai. ESR spectra of the paramagnetic complexes were recorded at liquid nitrogen temperature as a corresponding class in the X-band region on a Varian E-112 spectrometer equipped with 100 kHz field modulation at IIT, Chennai. The computer controlled X-ray diffractometer system JEOL JDX 8030 was used to record the data for powder copper complex, recorded at Central Electrochemical Research Institute (CECRI), Karaikudi.

In vitro antimicrobial activity

In vitro antimicrobial assay was performed by well diffusion method. The complexes and ligands were tested against bacteria such as Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus by the well diffusion method using nutrient agar as the medium and fungi such as Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataiola and Candida albicans, cultured on potato dextrose agar as medium. In a typical procedure, a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated at 30°C for 72 h. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone developed on the plate was measured. The inhibition zone developed on the plate was measured. The MIC of the complexes was determined by serial dilution technique.
The absence of this band accompanied by the appearance of two characteristic frequencies of the azomethine group confirms the involvement of the azomethine nitrogen in the formation of the Schiff base products. This band is shifted towards lower frequencies compared with the free ligand, indicating the formation of a new covalent bond between the metal and the nitrogen atom. The observed shifts suggest a change in the electronic structure of the Schiff base, possibly due to the coordination of the nitrogen atom to the metal center.

### RESULTS AND DISCUSSION

All the complexes are stable at room temperature, insoluble in water but soluble in DMSO and chloroform. The physical properties and analytical data of the complexes are given in Table 1. The elemental analysis data of the complexes are in good agreement with theoretical values. These complexes showed lower conductance values (5-20 ohm cm) indicating their non-electrolytic nature. The magnetic moments (Table 1) of all the Cu(II) complexes under the present study were found to be in the range of 1.79-1.86 B.M. at room temperature, suggesting monomeric nature of the complexes.

### Mass spectra

The fast atom bombardment mass spectrum of the Schiff base (L) showed a peak at m/z 549 [M]+, as expected for a monomeric formulation of the respective ring. Its CuL 

### Electrochemical behaviour

The cyclic voltammogram of the CuL complex in acetonitrile solution at 300 K in the potential range +0.4 to -0.8 V at scan rate 0.1 V/s was recorded and data of all the complexes were presented (Figure 5). It shows a well-defined redox process corresponding to the formation of the quasi-reversible Cu(II)/Cu(I) couple. The anodic peak at Ep = -0.15 V versus Ag/AgCl and the associated cathodic peak at Ep = -0.32 V correspond to the Cu(II)/Cu(I) couple. The CuL complex exhibits a quasi-reversible behaviour as indicated by the non-equivalent current intensity of cathodic and anodic peaks. The quasi-reversible behaviour of this complex is also supported by the presence of the characteristic peaks at 8.2 and 10.8 ppm, which are attributed to the methyl protons due to the methyl group in the acetoacetanilide moiety and –COOH of the anthranilic acid moiety. This peak is also supported by the 1H NMR spectra of the other ligands.
large peak separation of Ep and Ep.

**Antimicrobial activity**

The *in vitro* antimicrobial activity of the compounds was tested against the bacteria *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae* and *Seudomonas aeruginosa* and fungi *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataiola* and *Candida albicans* by well diffusion method. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Tables 5 and 6. A comparative study of the ligand and its complexes (MIC values) indicates that complexes exhibit higher antifungal activity than the free ligand. From the MIC values (Tables 5 and 6), it was found that the compound 6, CuL, was more potent among the other investigated complexes and the standard. Further studies are required to explore these complexes as drugs.

Compounds containing >C=N group have enhanced antimicrobial activity than >C=C group. The growth of certain microorganisms take place even in the absence of O₂. Hence, compounds containing >C=C group capable of absorbing O₂ are not related with the growth of microorganisms.

Such increased activity of the complexes can be explained on the basis of Overtone’s concept and Tweedy’s Chelation theory. According to Overtone’s concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials which makes liposolubility an important factor, which controls the antifungal activity. On chelation, the polarity of the copper ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the copper ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity 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 the proteins that restrict further growth of the organism.

**Effect of substituents**

Generally, the nature (electron withdrawing and electron releasing substituents) and position of substituents present in the phenyl ring decide the antimicrobial activities. It is well known that the substituents at o-position have lower antimicrobial activity whereas the substituents at m- and p-positions have higher antimicrobial activity. It is obvious that inhibitory action gets enhanced with the introduction of electron-withdrawing nitro and chloro groups in the phenyl ring. However, electron-releasing substituents such as methoxy and hydroxyl groups are less active compared to unsubstituted phenyl ring. The lower activity of complexes could also be accounted on the basis of low lipid solubility. Hence, the metal ion cannot reach the desirable site of action of the cell wall to interfere with the normal cell activity. The nature of metal ion also plays a decisive role in determining antifungal properties.

In the present study, the order of the antimicrobial activity of the synthesized compounds (based on the substituent present in the phenyl ring) is as follows:

**-NO₂ > -Cl > -H > -OCH₃ > -OH**

**Effect of hetero atoms**

From the observation, the higher inhibition of microbial growth is due to uncoordinated hetero atoms and carboxylic moieties. In the complexes, the ligands have uncoordinated donor atoms which enhance the activity of the complexes by bonding with trace elements present in microorganisms that combine with the uncoordinated site and may inhibit the growth of fungi.

The mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine group (>C=N) with the active centers of cell constituents, resulting in interferences with the normal cell process. Although the exact mechanism is not understood biochemically, mode of action of antimicrobials may involve various targets in microorganisms.

(i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered or they may disorganize the lipoprotein leading to the cell death.

(ii) Deactivation of various cellular enzymes, which plays a vital role in different metabolic pathways of these microorganisms.

(iii) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired.

(iv) Formation of a hydrogen bond through the azomethine group with the active centre of cell constituents, resulting in interference with the normal cell process.

From the observed cyclic voltammetric behaviour, the redox properties of copper ion, may also contribute to their inherent toxicity. For example, redox cycling between Cu(II) and Cu(I) can catalyze the production of highly reactive hydroxyl radicals, which can subsequently damage lipids, proteins, DNA and other biomolecules.

Several mechanisms may be responsible for the cytotoxic effect of the metal chelates. After penetration of the complex into the organism, the cellular oxygen oxidizes the cellular mercaptide compounds (RSH), and the electron transfer process is mediated through the central Cu(II) ion of the complex. Probably, the intracellular Cu(II) complex undergoes reduction to Cu(I) complex by the different sulphhydryl group-containing compounds (RSSR). The reduced Cu(I) complexes may catalyze the reduction of O₂ to O₂⁻, while Cu(II) complexes may catalyze the dismutation of O₂⁻ to H₂O₂. In this situation, the Cu(I)/Cu(II) couple is involved as a redox center. It is known that by changing the ligand environment around the central copper ion, the redox potential of the couple can be significantly changed. The O₂⁻ and H₂O₂ produced by such redox reactions cause cell toxicity by their potential oxidizing effect on vital cell components such as lipoid acid, etc. In addition, the reduced Cu(I) complex may inhibit DNA synthesis, energy, or ATP production by inhibition of mitochondrial respiration and destruction of cell viability.

The probable cytotoxic reactions can be outlined as follows:

\[
\text{Cu(II) complex} \rightarrow \text{Cu(I) complex} \\
\text{(inside the organism)} \quad \text{(outside the organism)}
\]

**Step 2**

\[
\text{Cu(II) complex} + \text{RSH} \rightarrow \text{Cu(I) complex} + \text{RSSR} + \text{reduced oxygen containing radicals}
\]

**Step 3**

\[
\text{Cu(I) complex} \rightarrow \text{DNA} \rightarrow \text{inhibition of DNA synthesis}
\]

**Step 4**

\[
\text{Cu(I) complex} \rightarrow \text{Phosphorylation} \rightarrow \text{inhibition of oxidative phosphorylation}
\]

Oxidation of thiol compounds by cellular oxygen catalysed by a copper complex was demonstrated by the fact that during the reaction of bis(thiosemicarbazone)copper(II) complexes with Ehrlich cells, the cells showed a stimulated rate of oxygen consumption. Here, it is worth mentioning that such oxidation of cellular thiols through the catalytic role of copper is also assumed to be the key step of cytotoxic reactions in many anticancer drugs.

**Powder XRD study**

Single crystals of the complexes under study could not be obtained because the metal complexes were separated as powder rather than single crystals, in addition to their insolubility in most organic solvents, thus no definitive structure can be described. X-ray powder diffraction patterns in the 10° to 20° range of the compounds were carried in order to obtain an idea about the lattice dynamics of the compound. X-ray diffraction patterns of CuL complex was recorded between 10° and 90° (2θ) and is given in Figure 6. The value of (2θ), interplanar spacing d (Å), FWHM and the relative intensities of the compounds under study are compiled in Table 7. The X-ray powder diffraction pattern throws light only on the fact that each solid represents a definite compound of a definite structure which is not contaminated with starting materials.

Figure 6 shows the X-ray diffraction patterns of the CuL complex. Cu(II) complex shows sharp crystalline peaks. The crystallite size of the complex d<sub>cryst</sub> could be estimated from XRD patterns by applying full-width half maximum of the characteristic peak to Scherrer’s equation

\[
d_{\text{cryst}} = 0.9 \lambda / (\text{FWHM} \cos \theta) \quad \text{(1)}
\]

where λ is the wavelength used, FWHM is the full width at half maxima and θ is the diffraction angle. The CuL complex has the crystallite size of 50 nm respectively. It represents the mean size of every nanocrystallite complexes. All other complexes supports similar crystallite size pattern.

Comparing the X-ray diffraction pattern of the ligands and their copper complexes indicate that the interplanar spacing and the relative intensities are
different which could be attributed to the complex formation. Furthermore, correlation of analytical, spectroscopic and magnetic data enables us to predict the possible structure for the copper complexes.

CONCLUSION

Novel tetradentate Schiff base ligands derived from Knoevenagel condensates β-ketoanilides (obtained from acetoacetanilide and substituted benzaldehydes) and their copper(II) complexes have been characterized by analytical and spectral techniques. From the electronic absorption spectra and the stoichiometric analysis, square-planar geometry was assigned for the synthesized complexes. The very low conductivity values indicate that all the complexes are non-electrolytes. From their magnetic susceptibility and ESR spectral data the monomeric nature of the complexes was confirmed. Redox couple of the copper complexes was assigned as quasi-reversible from their cyclic voltammetric data. From the antimicrobial screening observation, cyclic voltammetric behaviour and the available literature knowledge, the above possible ways to inhibit the antimicrobial growth using synthesised copper(II) complexes were explained. The redox properties of copper ion, the presence of >C=N group, carboxylate moiety and also substituents are responsible for their increased antimicrobial activity. It is believed that the new derivatives can provide a wide choice and flexibility to change the structure in order to find a less toxic derivative with enhanced activity. From the XRD data, the CuL complex has the crystallite size of 50 nm respectively.

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