Mycobiology 37(2) : 141-146 (2009)
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Antifungal Activities of Copper(II) with Biosensitive Macrocyclic Schiff Base Ligands Derived from 4-Aminoantipyrine Derivatives

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(Received April 16, 2009. Accepted June 4, 2009)

Novel copper(II) complexes have been synthesized from the macrocyclic Schiff bases derived from Knoevenagel condensed β-ketoanilides (obtained by the condensation of acetoacetanilide and substituted benzaldehydes), 4-aminoantipyrine and *o*-phenylene diamine. The structural features have been determined from their analytical and spectral data. All the Cu(II) complexes exhibit square planar geometry. Their high molar conductance values support their 1 : 2 electrolytic nature. The magnetic moment data provide evidence for the monomeric nature of the complexes. The X-band ESR spectra of the $[CuL^1](OAc)_2$ in DMSO solution at 300 and 77 K were recorded and their salient features are reported. The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by well diffusion method. A comparative study of inhibition values of the Schiff bases and their complexes indicate that complexes exhibit higher antimicrobial activity than the Schiff bases. Copper ions proved to be essential for the growth-inhibitor effect. The extent of inhibition appeared to be strongly dependent on the initial cell density and on the growth medium.

KEYWORDS : Biological screening, Inhibition, Schiff bases, Well diffusion

Many metal complexes have powerful antimicrobial activities and are already in common day-to-day use in medicinal field such as silver bandages for treatment of burns, zinc antiseptic creams, bismuth drugs for the treatment of ulcers and metal clusters as anti-HIV drugs. The potential for further development of metal-based drugs and treatments as an antimicrobial agent is enormous and also of great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities. The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their wide applications in various fields like biological, analytical and therapeutical (Agarwal *et al*., 2006; Rosu *et al*., 2006). Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic and anti-inflammatory agents (Kamalakannan *et al*., 2002; Argüelles *et al*., 2007; Singh *et al*., 2007). Redox active complexes can provide an alternative tool for redox regulation as a therapeutic basis, interfering in oxidative trigger mechanisms in cells. Specific ligands can be useful in the modulation of metal ion reactivity, by modifying their redox potential, hydrophilic or lipophylic characteristics or saturating its coordination sphere and therefore avoiding undesirable interactions with cell components. Particularly, copper based compounds have been investigated that endogenous metals may be less toxic (Sorenson, 1984). Cu^{2+} is a host of low-molecular-weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers and cancers (Sorenson, 1976; Brown *et al*., 1980). With the increasing incidence of deep mycosis in recent years, there has been an increasing emphasis on screening new and more effective antimicrobial drugs with low toxicity. The Schiff bases and their complexes were recently reported to be significant antifungal agents (Chandra *et al*., 2009). So we have evaluated the in vitro antifungal activity of our complexes against various fungi. In the present work, we report here the synthesis and structural characterization of copper(II) complexes with Schiff bases In addition, the results of their antifungal and antibacterial activities are also reported.

Materials and Methods

Apparatus and reagents. The chemicals used were of AnalaR grade. Copper(II) acetates were obtained from Merck. Acetonitrile was dried over phosphorous pentaoxide and distilled repeatedly to obtain a highly pure prod-

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uct. Micro analytical data and FAB Mass spectra of the compounds were recorded at the Regional Sophisticated Instrumentation Center, Central Drug Research Institute (RSIC, CDRI), Lucknow. The amount of copper present in the complexes was estimated using ammonium oxalate method. The FAB mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/ data system 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. Molar conductance of the complexes was measured in DMSO solution using a coronation digital conductivity meter. The IR spectra of the samples were recorded on a Perkin-Elmer 783 spectrophotometer in 4000~400 cm^{-1} range using KBr disc. Electronic spectra were recorded with a Systronics 2201 Double beam UV-Vis., spectrophotometer in the 200-1100 nm region. The magnetic susceptibility values were calculated using the relation $\mu_{eff} = 2.83\ (\chi_m.T)$. The diamagnetic corrections were made by Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. The ESR spectra of the $[CuL^1](OAc)_2$ complex were recorded at 300 and 77 K on a Varian E112 X-band spectrometer. Cyclic voltammetric measurements were performed using a glassy carbon working electrode, Pt wire auxiliary electrode and an Ag/AgCl reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. All solutions were purged with N_2 for 30 min prior to each set of experiments.

Synthesis of Knoevenagel condensate *β*-ketoanilide. Condensation of acetoacetanilide with *p*-hydroxybenzaldehyde (L^1)/benzaldehyde (L^2)/*p*-nitrobenzaldehyde (L^3) was performed by heating equimolar amounts (10 mmol) under reflux in 50 *ml* ethanol, in the presence of 5 drops of piperidine as the catalyst for 5 h. The solution was then cooled and the condensed product was separated by adding 5 *ml* of toluene and 30 *ml* of petroleum ether (40~60°C). The yellow colour solid Knoevenagel condensate *β*-ketoanilide was isolated by filtration, washed and recrystallised from ethanol.

Synthesis of Schiff bases. Knoevenagel condensate *β*-ketoanilide (10 mmol) was dissolved in ethanol (30 *ml*) and refluxed with 4-aminoantipyrine (20 mmol) in ethanol (20 *ml*) with the addition 1 g of anhydrous K_2CO_3 for about 8 h. The solvent was reduced to one-third and the pasty mass so obtained was treated with hot water and set aside in refrigerator for 24 h. The solid material formed was removed by filtration and recrystallised from ethanol.

Synthesis of macrocyclic ligands. An ethanolic solution of Schiff base (10 mmol) was added to the ethanolic solution of *o*-phenylene diamine (10 mmol) and refluxed

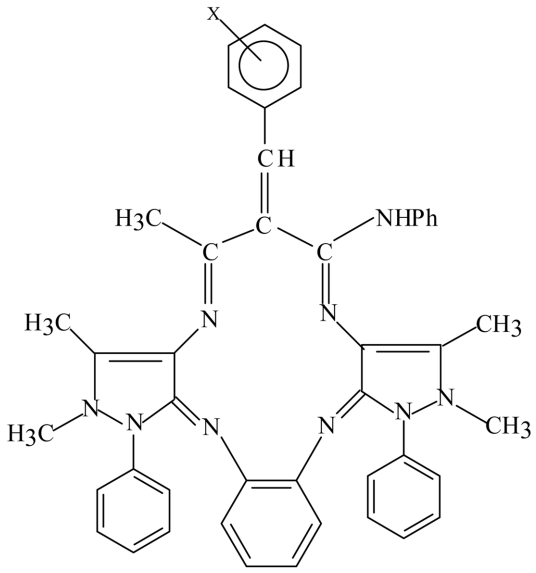

Fig. 1. Structure of macrocyclic ligands. L^1 = -OH; L^2 = -H; L^3 = $-NO_2$.

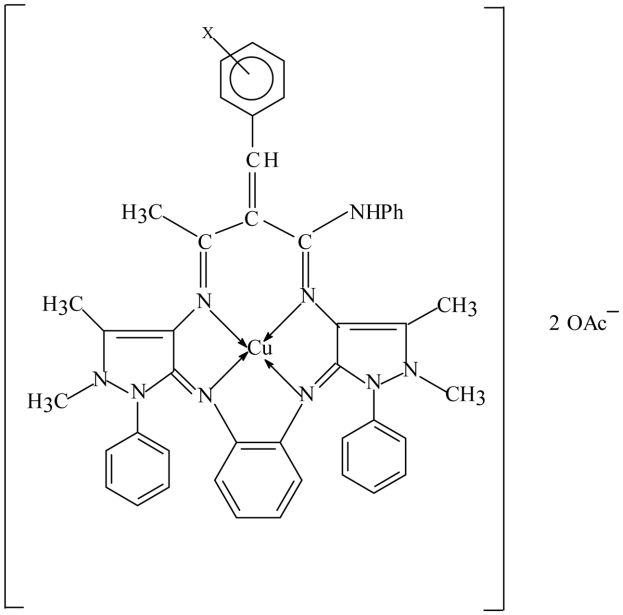

Fig. 2. Structure of macrocyclic copper complexes.

for 6 h. Then the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered and washed thoroughly with ethanol and dried *in vacuo*.

Synthesis of metal complexes. A solution of macrocyclic ligand (5 mmol) in ethanol (20 *ml*) was added to a solution of copper acetate (5 mmol) in ethanol (10 *ml*) and the mixture was refluxed for 6 h and concentrated to one-third volume and kept at 0°C for 4 h. The solid product formed was filtered, washed with ethanol and dried *in vacuo*.

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

S. No	Compound	Found (calc)%				Λ_m mhocm^2 mol^{-1}	Magnetic moment μ_{eff} (BM)
		M	C	H	N		
1	L^1	–	75.2 (74.8)	6.4 (6.0)	17.3 (17.1)	–	–
2	$[CuL^1](OAc)_2$	7.2 (6.9)	65.5 (65.2)	5.9 (5.5)	14.0 (13.7)	115	1.96
3	L^2	–	76.5 (76.3)	6.3 (6.0)	18.1 (17.8)	–	–
4	$[CuL^2](OAc)_2$	7.3 (7.1)	66.5 (66.1)	5.9 (5.4)	14.5 (14.2)	84	1.85
5	L^3	–	71.9 (71.7)	6.0 (5.5)	18.9 (18.6)	–	–
6	$[CuL^3](OAc)_2$	7.1 (6.8)	63.2 (62.9)	5.4 (5.1)	15.5 (15.0)	120	1.82

Antimicrobial activity. The *in vitro* evaluation of antimicrobial activity was carried out. The purpose of the screening program is to provide antimicrobial efficiencies of the investigated compounds. The prepared compounds were tested against some fungi and bacteria to provide the minimum inhibitory concentration (MIC) for each compound. The *in vitro* antimicrobial activities of the investigated compounds were tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by the well diffusion method. For preparation of plates and inoculation, 1.0 *ml* of inocula were added to 50 *ml* of agar media (50°C) and mixed. The agar was poured into 120 mm petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, fill wells were filled up to the surface of agar with 0.1 *ml* of the test compounds dissolved in DMSO (200 μmol/*ml*). The plates were left, on a leveled surface, incubated for 24 h at 30°C for bacteria and 48 h for fungi and the diameter of the inhibition zones were read. DMSO (0.1 *ml*) alone was used as control under the same conditions for each organism. By subtracting the diameter of inhibition zone resulting with DMSO from that obtained in each case. The results were compared with a similar run of standards of antibacterial and antifungal drugs. The MIC (as the lowest concentration of drug in the medium that showed no microbial growth by visual observation) of the complexes was determined by serial dilution technique (Reiner, 1982).

Results and Discussion

The analytical, molar conductance and magnetic moment values are summarized in Table 1. Efforts to grow good crystals of the ligands and their metal chelates for X-ray diffraction studies were unsuccessful due to their poor solubility in common organic solvents. All the complexes are stable at room temperature, insoluble in water but soluble in DMSO and MeCN. Elemental analysis values are in close agreement with the values calculated for molecular formula assigned to these complexes. The molar conductance data for the metal complexes measured in DMSO solution for the 0.001 M solutions are given in Table 1. The values fall in the range of 84~120 $\Omega^{-1}cm^2$ mol^{-1}, which is the expected range of 70~135 $\Omega^{-1}cm^2mol^{-1}$ for the complexes to behave as 1 : 2 electrolytic nature (Geary, 1979). Thus, the present complexes have non-electrolytic nature as evidenced by the involvement of acetate group in coordination. This result was confirmed from the chemical analysis of CH_3COO^- ion is not precipitated by addition of $FeCl_3$. All the complexes did not show electrolytic properties. The magnetic moments (Table 1) of all the Cu(II) complexes under the present study were found to be in the range of 1.82~1.96 B.M. at room temperature, suggesting a square-planar geometry around the copper ion (Harikumaran Nair *et al*., 2005).

Mass spectra. Mass spectra provide a vital clue for elucidating the structure of compounds. A fast atom bombardment mass spectrum was obtained for the macrocyclic Schiff base L^3. This spectrum showed a peak at m/z 709 [M^+], as expected for a monomeric formulation of the respective ring. Also the fast atom bombardment mass spectrum of its $[CuL^3](OAc)_2$ complex exhibited a peak at m/z 906 [M^+], which confirms the stoichiometric composition of the complex formation. The intensity of these peaks reflects the stability and abundance of the ions (Hamming and Foster, 1972).

IR spectra. In order to study the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes. The IR spectra of the free ligands show the characteristic >C=N bands in the 1658~1634 cm^{-1} region which are shifted to lower frequencies in the spectra of the metal complexes (1636~1620 cm^{-1}) (Silverstein and Webster, 2007). The complexes also display bands at 1620~1615, 1326~1318 cm^{-1} due to the asymmetric and symmetric stretching vibration of the acetate group with $\Delta\upsilon$ = 294 cm^{-1} indicating the coordination of acetate group (Silverstein and Webster, 2007). The IR spectra of the metal complexes also show some new bands in the 480~450 cm^{-1} and 440~400 cm^{-1} regions, which may probably due to the formation of M-O and M-N bands (Chandra *et*

Table 2. Electronic absorption spectral data of the complexes at 300 K

S. No	Compound	Solvent	Absorption (cm^{-1})	Band assignment	Geometry
1	L^1	$CHCl_3$	29480	INCT	--
2	L^2	$CHCl_3$	28642	INCT	--
3	L^3	$CHCl_3$	31210	INCT	--
4	$[CuL^1](OAc)_2$	DMSO	28654	INCT	
			21890	$^2B_{1g} \rightarrow {}^2E_g$	Square-planar
			18250	$^2B_{1g} \rightarrow {}^2A_{1g}$	
5	$[CuL^2]$ $(OAc)_2$	DMSO	32345	INCT	Square-planar
			20940	$^2B_{1g} \rightarrow {}^2E_g$	
			17946	$^2B_{1g} \rightarrow {}^2A_{1g}$	
6	$[CuL^3](OAc)_2$	DMSO	31170	INCT	Square-planar
			20850	$^2B_{1g} \rightarrow {}^2E_g$	
			17840	$^2B_{1g} \rightarrow {}^2A_{1g}$	

al., 2007; Nakamoto, 1978), respectively. The band observed at 1540 cm^{-1} is due to the $\nu_{C=C}$ stretching of the aromatic ring system. The band observed at 871 cm^{-1} is due to ν_{C-N} stretching vibration of the aromatic nitro group. In all the metal-Schiff base complexes most of the band shifts observed in the wave number region 1150~994 cm^{-1} are in agreement with the structural changes observed in the molecular carbon skeleton after complexation, which cause some changes in (C-C) bond lengths.

Electronic spectra. The electronic absorption spectra of $L^1/L^2/L^3$ and their copper complexes were recorded at 300 K using suitable solvent. The solvent, absorption region, assignment of the absorption bands and the proposed geometry of the complexes are given in Table 2. From the table, we concluded that all the complexes are having square-planar geometry around the copper atom (Lever, 1968).

ESR spectra. The ESR spectrum of the $[CuL^1](OAc)_2$ complex was recorded in DMSO at 300 and 77 K. The spectrum at 300 K shows one intense absorption band at high field, which is isotropic due to the tumbling motion of the molecules. However, this complex in the frozen state shows four well resolved peaks with low intensities in the low field region and one intense peak in the high field region. The magnetic susceptibility value reveals that the copper complex has a magnetic moment of 1.96 B.M corresponding to one unpaired electron, indicating that the complex is mononuclear in nature. This fact was also evident from the absence of half field signal, observed in the spectrum at 1600 G due to the $m_s = \pm 2$ transitions, ruling out any Cu-Cu interaction (Hathaway and Billing, 1970). For the present Cu(II) complex, the observed g values are $g_{\parallel}(2.25) > g_{\perp}(2.04) > g_e(2.0023)$, which suggest that the unpaired electron lies in the $d_{x^2-y^2}$ orbital. The $A_{\parallel}$ and $A_{\perp}$ values in the order: $A_{\parallel}(144) > A_{\perp}(42)$ also indicate that the complex has square planar geometry and the system is axially symmetric (Kivelson and Neiman, 1961). In the axial spectra, the g-values are related with exchange interaction coupling constant (G) by the expression:

$$G = g_{\parallel} - 2/g_{\perp} - 2 \quad (1)$$

The observed G value of 6.25 suggests that the local tetragonal axes are aligned parallel or only slightly misaligned and the unpaired electron is present in $d_{x^2-y^2}$ orbital. This result also indicates that the exchange coupling effects are not operative in the present complex. Molecular orbital coefficients α^2 (in-plane s-bonding), β^2 (in-plane π-bonding) and γ^2 (out-plane p-bonding) were calculated using Eqs. (2)~(4).

$$\alpha^2 = -(A_{\parallel}/0.036) + (g_{\parallel} - 2.0036) + 3/7\,(g_{\perp} - 2.0036) + 0.04 \quad (2)$$

$$\beta^2 = (g_{\parallel} - 2.0036)E/-8\lambda\alpha^2 \quad (3)$$

$$\gamma^2 = (g(-2.0036)E/-2\lambda\alpha^2 \quad (4)$$

The α^2 value of 0.5 indicates complete covalent bonding, while that of 1.0 suggests complete ionic bonding. The observed value of 0.645 for the present complex indicates that the metal-polymer complex has some covalent character. The observed β^2 and γ^2 values of 1. 124 and 0.7427 indicate that there is interaction in the out-of-plane π-bonding, whereas the in-plane π-bonding is predominantly ionic. Significant information about the nature of bonding in the Cu(II) complex can be derived from the relative magnitudes of $K_{\parallel}$ and $K_{\perp}$.

$$K_{\parallel} = \alpha^2\beta^2 \quad (5)$$

$$K_{\perp} = \alpha^2\gamma^2 \quad (6)$$

For the present complex, the observed order: $K_{\parallel}(0.89) > K_{\perp}(0.57)$ implies a greater contribution from out-of plane π-bonding than from in in-plane π-bonding in metal-ligand π bonding.

Electrochemical behaviour. The expansion of bioinorganic chemistry in the last decades gave a strong impetus to the development of copper coordination chemistry, and

an enormous number of new complexes, with very interesting structures and properties, have been prepared. As a rule, their redox properties have been investigated by electrochemical techniques, especially the cyclic voltammetry of solution in appropriate solvents. The cyclic voltammogram of the $[CuL^1](OAc)_2$ complex in DMSO at 300 K in the potential range +0.9 to −0.2 V. It shows a well-defined redox process corresponding to the formation of the quasi-reversible couple copper(II)/ copper(III). The anodic peak at $Ep_a = 0.38$ V versus Ag/AgCl and the associated cathodic peak at $Ep_c = 0.19$ V correspond to the copper(II)/copper(III). The $[CuL^1](OAc)_2$ complex exhibits a quasi-reversible behaviour as indicated by the non-equivalent current intensity of cathodic and anodic peaks and also shows large peak separation indicates quasireversible behaviour.

Antimicrobial activity. To contribute in the field of bioinorganic chemistry, consequently, the compounds synthesized have been evaluated for their antibacterial and antifungal studies. The antibacterial and antifungal tests were carried out using the well diffusion method. The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumaniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by the well diffusion method. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Tables 3 and 4. A comparative study of the ligands and their complexes (MIC values) indicates that complexes exhibit higher antimicrobial activity than the free ligands. In this study, the antimicrobial activity of the ligands may be due to the heteroaromatic residues. Compounds containing >C=N group have enhanced antimicrobial activity than >C=C< group. The growth of certain microorganisms takes place even in the absence of O_2. Hence, compounds containing >C=C< group though capable of absorbing O_2 are not related with the growth of microorganisms. The enhanced activity of the complexes can be explained on the basis of Overtone's concept (Anjaneyula and Rao, 1986) and Tweedy's Chelation theory (Dharamaraj *et al*., 2001). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials makes 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 the positive charge of the metal 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 permeation 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 restricts further growth of the organism. The increased activity of the complexes can also be explained on the basis of their high solubility, fit-

Table 3. Minimum inhibition of concentration of the synthesized compounds against growth of five fungi ($\mu g/ml$)

S. No	Compound	*A. niger*	*R. stolinifer*	*A. flavus*	*R. bataicola*	*C. albicans*
1	L^1	76	82	80	96	120
2	L^2	84	75	72	60	108
3	L^3	62	50	66	74	74
4	$[CuL^1](OAc)_2$	44	48	38	42	36
5	$[CuL^2](OAc)_2$	30	39	36	40	44
6	$[CuL^3](OAc)_2$	28	34	20	22	18
7	Nystatin	15	12	18	10	8
8	Ketoconazole	16	18	22	6	12
9	Clotrimazole	18	20	12	8	14

Table 4. Minimum inhibition of concentration of the synthesized compounds against growth of five bacteria ($\mu g/ml$)

S. No	Compound	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. aeruginosa*	*S. aureus*
1	L^1	65	50	85	42	56
2	L^2	72	65	56	60	49
3	L^3	69	56	59	72	54
4	$[CuL^1](OAc)_2$	34	42	50	44	56
5	$[CuL^2](OAc)_2$	30	38	46	36	44
6	$[CuL^3](OAc)_2$	26	32	24	18	20
7	Penicillin G	08	10	14	16	04
8	Ampicillin	06	18	04	12	20
9	Ofloxacin	14	09	14	06	10

ness of the particles, size of the metal ion and the presence of the bulkier organic moieties.

Effect of substituents. In the present study, the order of the antimicrobial activity of the synthesized compounds (based on the substituents present in the benzene ring) is as follows:

$NO_2 > H > OH$

Electron transfer as a possible mode of action. A wide variety of synthesized drug molecules have electron transfer capabilities which allow them to generate reactive oxygen species (ROS). In particular, many antibiotics that kill or inhibit bacteria, yeasts and cancer cells readily transfer electrons to oxygen making superoxide and hydrogen peroxide in the process. When suitable redox active forms of copper are available, to generating the highly damaging hydroxyl radical. This type of chemistry is very similar to that which evolved within phagocytic cells as part of their microbial killing. Redox processes could be involved in the observed biological activity, especially for the copper complexes. In the present study, the observed cyclic voltammetric behaviour, the redox properties of copper ion, may also contribute to their inherent toxicity. In our synthesized copper complexes generates Cu(I) oxidation state during electrolysis, it can catalyze the production of highly reactive hydroxyl radicals, which can subsequently damage lipids, proteins, DNA and other biomolecules.

In this study, the compounds are active against both types of the bacteria and as well as active against fungi, which may indicate a broad-spectrum affect. Among the studied compounds, especially $[CuL^3](OAc)_2$ (MIC 18 $\mu g/ml$), presented good activity against *Candida albicans*.

Ackowledgements

The authors express their sincere thanks to the UGC for financial assistance under UGC-Dr.D.S.Kothari Post Doctoral Fellowship Scheme (No. F.4-2/2007 (BSR)/13-32/2008 (BSR).

References

Agarwal, R. K., Singh, L. and Sharma, D. K. 2006. Synthesis, spectral, and biological properties of copper(II) complexes of thiosemicarbazones of schiff bases derived from 4-aminoantipyrine and aromatic aldehydes. *Bioinorg. Chem. Appl.* 2006:59509 1-10.

Anjaneyula, Y. and Rao, R. P. 1986. Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetylacetone and various salicylic acids. *Synth. React. Inorg. Met.-Org. Chem.* 16:257-272.

Argüelles, M. C. R., Vázquez, S. M., Touceda, P. T., Matalobos, J. S., Deibe, A. M. G., Ferrari, M. B., Pelosi, G., Pelizzi, C. and Zani, F. 2007. Complexes of 2-thiophene carbonyl and isonicotinoyl hydrazones of 3-(N-methyl)isatin: A study of their antimicrobial activity. *J. Inorg. Biochem.* 101:138-147.

Brown, D. H., Lewis, A. J., Smith, W. E. and Teape, J. W. 1980. Anti-inflammatory effects of some copper complexes. *J. Med. Chem.* 23:729-734.

Chandra, S., Gupta, L. K. and Agrawal, S. 2007. Modern spectroscopic and biological approach in the characterization of a novel 14-membered $[N_4]$ macrocyclic ligand and its transition metal complexes. *Transition Met. Chem.* 32:240-245.

Chandra, S., Jain, D., Amit Kumar, S. and Sharma, P. 2009. Coordination modes of a schiff base pentadentate derivative of 4-aminoantipyrine with cobalt(II), nickel(II) and copper(II) metal ions: Synthesis, spectroscopic and antimicrobial studies. *Molecules* 14:174-190.

Dharamaraj, N., Viswanathamurthi, P. and Natarajan, K. 2001. Ruthenium (II) complexes containing bidendate Schiff bases and their antifungal activity. *Transition Met. Chem.* 26:105-109.

Geary, W. G. 1971. The use of conductivity measurements in organic solvents for the characterisation of coordination compounds. *Coord. Chem. Rev.* 7:81-122.

Hamming, M. and Foster, N. 1972. Interpretation of Mass Spectra of Organic Compounds; Academic Press: New York, USA.

Harikumaran Nair, M. L., Mathew, G. and Sudarasana Kumar, M. R. 2005. Synthesis and characterisation of some new Cu(II) complexes of azo dyes derived from 1,2-dihydro-1,5-dimethyl-2-phenyl-4-amino-3H-pyrazol-3-one. *Ind. J. Chem.* 44A:85-89.

Hathaway, B. J. and Billing, D. E. 1970. The electronic properties and stereochemistry of mono-nuclear complexes of the copper(II) ion. *Coord. Chem. Rev.* 5:143-207.

Kamalakannan, P. and Venkappayya, D. 2002. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. *J. Inorg. Biochem.* 21:22-37.

Kivelson, D. and Neiman, R. 1961. ESR studies on the bonding in copper complexes. *J. Chem. Phys.* 35:149-155.

Lever, A. B. P. 1968. Inorganic Electronic Spectroscopy; Elsevier, Amsterdam, Netherland. Nakamoto K. 1978. Infrared and Raman Spectra of Inorganic and Coordination Compounds, Wiley Interscience, New York, USA.

Reiner, R. 1982. In Antibiotics-an introduction. Roche Scientific Services, Switzerland 1:21-25.

Rodriguez-Arguelles, M. C., Lopez-Silva, E. C., Sanmartin, J., Bacchi, A., Pelizzi, C. and Zani, F. 2004. *Inorganic Chim. Acta* 357:2543-2552.

Rosu, T., Pasculescu, S., Lazar, V., Chifiriuc, C. and Cernat, R. 2006. Copper(II) complexes with ligands derived from 4-amino-2,3-dimethyl-1-phenyl-3-pyrazoline-5-one. *Molecules* 11:904-914.

Singh, K., Barwa, M. S. and Tyagi, P. 2007. Synthesis and characterization of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine. *Euro. J. Med. Chem.* 42:394-402.

Sorenson, J. R. J. 1976. Copper chelates as possible active forms of the anti-arthritic agents. *J. Med. Chem.* 19:135-148.

Sorenson, J. R. J. 1984. Copper complexes in biochemistry and pharmacology. *Chem. Britain* 1110-1113.