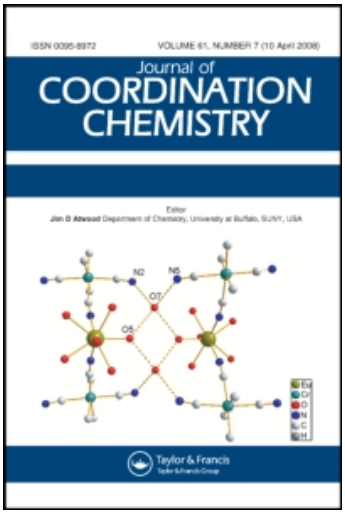

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Synthesis, spectral characterization and antimicrobial activity of macrocyclic Schiff-base copper(II) complexes containing polycrystalline nanosized grains

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Synthesis, spectral characterization and antimicrobial activity of macrocyclic Schiff-base copper(II) complexes containing polycrystalline nanosized grains

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Schiff-base copper(II) complexes were prepared using macrocyclic ligands, synthesized by condensation of diethylmalonate with Schiff bases derived from *o*-phenylenediamine and Knoevenagel condensed β-ketoanilides (obtained by the condensation of acetoacetanilide and substituted benzaldehydes). The ligands and their copper complexes were characterized by microanalytical, mass, UV–Vis, IR, ^{1}H-NMR, ESR and CV studies, as well as conductivity data. Microanalytical, mass and magnetic moment analyses are consistent with formation of monomeric $[CuL]Cl_2$. Spectral studies indicate square-planar geometry around copper. The smaller grain sizes found from XRD data suggest that these complexes are polycrystalline with nanosized grains. The SEM images of $[CuL^1]Cl_2$ have leaf-like morphology. The *in vitro* biological screening of the investigated compounds against the bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and fungi *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* were tested by the well diffusion method to assess growth inhibition. A comparative study of MIC values of the Schiff-base ligands and their complexes indicate that the complexes exhibit higher antimicrobial activity than the free ligands.

Keywords: Schiff base; Square planar; Biological screening; Antimicrobial activity

1. Introduction

Schiff-base complexes have applications in oxidation catalysis and electrochemistry [1–4]. Organic chelating ligands containing amide have the ability to form metal complexes and exhibit a range of biological activities [2–4] with much interest in synthesis and structural elucidation of transition metal complexes containing amide [5–12]. Macrocyclic ligands are of interest because of their unique coordination chemistry [13–16], behaving as model ligands for natural enzymes, as metal ion selective ligands and as metal chelating agents for medical purposes. A literature search revealed that no work had been done on the condensation product of diethylmalonate with Schiff bases formed using *o*-phenylenediamine and Knoevenagel condensed β-ketoanilides.

As part of our continuing efforts to investigate transition metal complexes of Schiff bases, synthesis of tetradentate ligands (N_4 type) derived from condensation of

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diethylmalonate with Schiff bases formed using *o*-phenylene diamine and Knoevenagel condensed β-ketoanilides (obtained by the condensation of acetoacetanilide and substituted benzaldehydes) and their complexes with copper(II) have been carried out. The structural features of these complexes were examined by analytical and spectral techniques and the redox behavior of the copper complexes using cyclic voltammetry. The *in vitro* antimicrobial behavior of the investigated compounds was studied by the well diffusion method.

2. Experimental

All reagents used for preparation ligands and complexes were Sigma and Merck products. For the voltammetric experiments, tetrabutylammonium perchlorate (TBAP) used as supporting electrolyte was purchased from Sigma. Anhydrous grade ethanol, CH_3CN and DMSO were obtained from Fisher Scientific Company. The purities of ligands and their complexes were evaluated by thin layer chromatography. Microanalytical data, ^{1}H-NMR 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 spectra of the ligands and their complexes were 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. IR spectra of the samples were recorded on a Shimadzu FTIR-8400S spectrophotometer in the 4000–200 cm^{-1} range using KBr pellets. UV–Vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Magnetic susceptibility measurements were carried out by Gouy balance using copper sulfate as the calibrant. The values were corrected for diamagnetism by applying Pascal's constants. Electrochemical studies were carried out using a BAS CV-50W electrochemical analyzer. 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 N_2 for 30 min prior to each set of experiments. The molar conductances of the complexes were measured using a Systronic conductivity bridge. The computer controlled X-ray diffractometer system JEOL JDX 8030 was used to record powder data for the copper complex, at the Central Electrochemical Research Institute (CECRI), Karaikudi. SEM images were recorded using a Hitachi SEM analyzer.

2.1. In vitro *antimicrobial activity*

Bacterial and fungal cultures were obtained from the Department of Microbiology, VHNSN College, Virudhunagar and were identified using standard laboratory techniques. *In vitro* antibacterial and antifungal assay was performed by the well diffusion method. Both positive (streptomycin for bacteria and nystatin for fungi) and negative (CH_3CN) controls were used in the technique. The complexes and ligands were tested against the bacteria, cultured on nutrient agar as medium and fungi cultured on potato dextrose agar as medium.

In a typical procedure, a well was made on the agar medium inoculated with the fungi. 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 fungi was affected. The inhibition zone developed on the plate was measured. The MIC of the complexes was determined by serial dilution technique [17].

2.2. *Synthesis of Knoevenagel condensate β-ketoanilide*

Condensation of acetoacetanilide (0.177 g) with 4-methoxybenzaldehyde (L^1) (0.136 g)/2-chlorobenzaldehyde (L^2) (0.140 g)/benzaldehyde (L^3) (0.106 g)/3-nitrobenzaldehyde (L^4) (0.151 g) was performed by heating equimolar amounts (10 mM) for 5 h under reflux in 50 mL ethanol, in the presence of 5 drops of piperidine as the catalyst. 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 Knoevenagel condensate β-ketoanilide was isolated by filtration, washed and recrystallized from ethanol.

2.3. *Synthesis of Schiff bases*

Knoevenagel condensate β-ketoanilide(s) (0.30 g, 10 mM) was dissolved in ethanol (30 mL) and refluxed with *o*-phenylenediamine (0.216 g, 20 mM) in ethanol (20 mL) with addition (1.0 g) of anhydrous K_2CO_3 for about 6 h. The solvent was reduced to one-third and the pasty mass so obtained was treated with hot water and set aside in a refrigerator for 10 h. The solid material was removed by filtration and recrystallized from ethanol.

2.4. *Synthesis of macrocyclic ligands*

An ethanolic solution of Schiff base(s) (0.380 g, 10 mM) was added to ethanolic solution of diethylmalonate (0.160 g, 10 mM) and refluxed for 3 h. Then the solution was reduced to one-third on a water bath. The precipitated complex was filtered and washed thoroughly with ethanol and dried *in vacuo*.

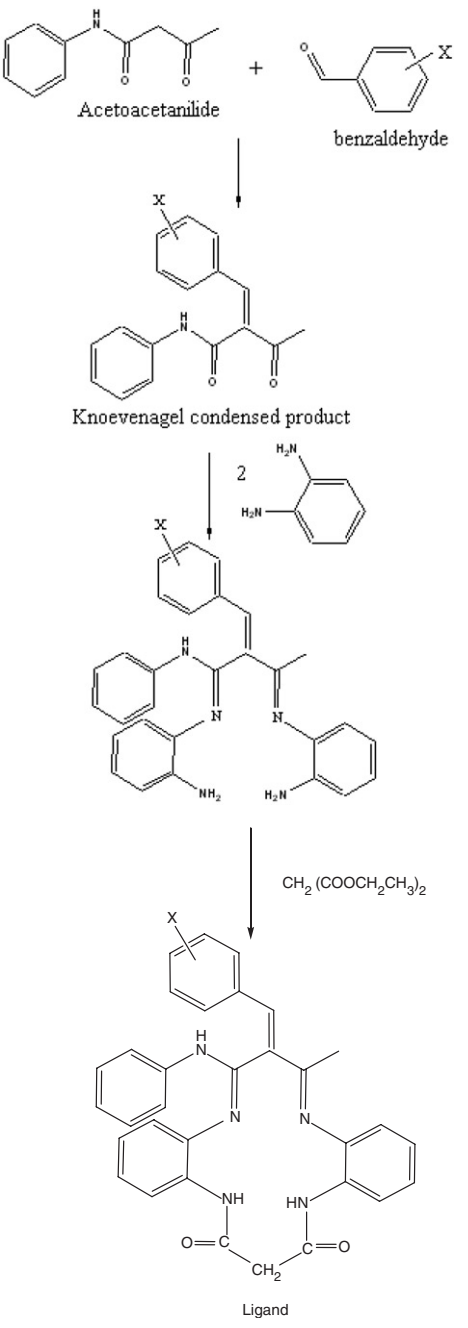
The outline of the synthesis is given in scheme 1.

2.5. *Synthesis of metal complexes*

A solution of macrocyclic ligand(s) (0.450 g, 5 mM) in ethanol (20 mL) was added to a solution of $CuCl_2$ (0.170 g, 5 mM) in ethanol (10 mL), the mixture refluxed for 6 h, concentrated to one-third volume and kept at 0°C for 2 h. The solid product was filtered, washed with ethanol and dried *in vacuo*.

3. Results and discussion

All the complexes are stable at room temperature, insoluble in water but soluble in CH_3CN, DMSO and chloroform. Formation and purification of the ligands and their



Scheme 1. Synthesis of the ligands.

copper complexes were performed using chromatographic techniques. The physical properties and analytical data of the complexes are listed in table 1. The elemental analytical data are in good agreement with theoretical values. These complexes showed high conductance values (95–136 $ohm^{-1}\,cm^2\,mol^{-1}$) indicating their 1 : 2 electrolytic

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

Compound	Found (Calcd %)				Λ_m ohm cm^2 mol^{-1}	Magnetic moment μ_{eff}(BM)
	M	C	H	N		
L^1	–	77.2 (77.4)	5.1 (5.5)	12.3 (12.4)	–	–
$[CuL^1]Cl_2$	12.7 (12.9)	69.5 (69.7)	4.6 (4.9)	11.0 (11.4)	115	1.81
L^2	–	72.9 (72.9)	4.6 (4.9)	11.4 (11.8)	–	–
$[CuL^2]Cl_2$	11.3 (11.8)	65.6 (65.8)	4.2 (4.5)	10.4 (10.5)	136	1.85
L^3	–	71.6 (71.9)	4.6 (4.6)	13.5 (13.8)	–	–
$[CuL^3]Cl_2$	11.8 (12.2)	64.9 (64.7)	4.1 (4.4)	12.3 (12.5)	106	1.79
L^4	–	75.8 (75.4)	5.1 (5.3)	11.6 (11.8)	–	–
$[CuL^4]Cl_2$	9.5 (9.7)	68.2 (68.5)	4.7 (5.0)	10.4 (10.6)	95	1.83

nature (i.e., anions are not coordinated to copper in these complexes). The magnetic moments (table 1) of the Cu(II) complexes were in the range 1.79–1.85 B.M. at room temperature, indicating the presence of one unpaired electron [18].

3.1. *Mass spectra*

The fast atom bombardment mass (FAB) spectra of L^1 and its copper complex, $[CuL^1]Cl_2$ recorded at room temperature as representative examples were used to compare the composition. The molecular ion peaks observed at m/z 606 [M^+] for L^1 and m/z 740 [M^+] for copper complex show the stoichiometry of the complex as $[CuL^1]Cl_2$. These peaks are in agreement with empirical formula from microanalytical data (table 1). The m/z of all the fragments of the L^1 and its copper complex with the relative intensities confirm the stoichiometry of the complexes is being of the type $[ML]Cl_2$. This is further supported by the mass spectra of all the complexes.

3.2. *IR spectra*

Infrared spectra gave important information regarding the skeleton of the ligands and their complexes. The IR spectra of the macrocyclic ligands show characteristic bands for ν(N–H) at 3324 cm^{-1}, ν(C=O) at 1625 cm^{-1} and ν(C–N) at 1595 cm^{-1}. In all the complexes, ν(N–H) bands shifted by 128–145 cm^{-1} to lower frequencies, due to coordination of NH [19]. The ν(C=N) bands were shifted by 18–46 cm^{-1} to lower frequencies, due to the participation of azomethine in coordination [20]. The ν(C=O) was not affected in all the complexes, indicating that the carbonyl groups are not involved in coordination to copper. Coordination of nitrogen to copper is supported by the appearance of a new band in the region 425 – 480 cm^{-1} assignable to ν(M–N) [20, 21]. These results, coupled with elemental analyses, indicate that the copper ions in

the complexes are bonded to the N_4 donor sites of the ligands *via* azomethine and amide nitrogens.

3.3. *Electronic absorption spectra*

Electronic absorption spectra of $L^1/L^2/L^3/L^4$ and their copper complexes were recorded at 300 K using suitable solvents. The solvent, absorption region, assignment of the absorption bands and the proposed geometry of the complexes are given in table 2. The absorption band observed at 28660 cm^{-1} for L^1 is assigned to the $\pi \rightarrow \pi^*$ transition of the azomethine chromophore. On complexation, this band shifted to lower wavelength, suggesting coordination of azomethine nitrogen with copper [22]. In general, due to Jahn–Teller distortion, square-planar Cu(II) complexes give a broad absorption band between 600 nm (16,667 cm^{-1}) and 700 nm (14,286 cm^{-1}) and the peak at 510 nm (19,608 cm^{-1}) merges with the broad band. In the electronic spectrum of $[CuL^1]Cl_2$ complex, the observed bands at 22,445 and 18,690 cm^{-1} are assigned to $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions, respectively [23, 24], consistent with square-planar geometry. All other complexes have similar absorptions confirming the square-planar geometry around copper.

3.4. *ESR spectra*

The X-band ESR spectrum of $[CuL^2]Cl_2$ in DMSO at 77 K shows well resolved hyperfine splitting. The magnetic susceptibility value (1.85 B.M.) corresponding to the one unpaired electron indicates that the complex is mononuclear. This was also evident from the absence of a half field signal, expected at 1600 G due to the $m_s = \pm 2$ transitions, ruling out any Cu–Cu interaction.

The observed g-tensor values are $g_{\parallel}(2.256) > g_{\perp}(2.052) > g_e(2.0036)$, suggesting square-planar complex. The ESR parameters of the complex coincide with related systems and suggest that the complex has square-planar geometry [25]. In the axial

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

Compound	Solvent	Absorption (cm^{-1})	Band assignment	Geometry
L^1	$CHCl_3$	28,660	INCT	–
L^2	$CHCl_3$	29,546	INCT	–
L^3	$CHCl_3$	30,250	INCT	–
L^4	$CHCl_3$	29,640	INCT	–
$[CuL^1]Cl_2$	CH_3CN	29,800	INCT	Square-planar
		22,445	$^2B_{1g} \rightarrow {}^2E_g$	
		18,690	$^2B_{1g} \rightarrow {}^2A_{1g}$	
$[CuL^2]Cl_2$	CH_3CN	30,350	INCT	Square-planar
		21,240	$^2B_{1g} \rightarrow {}^2E_g$	
		18,226	$^2B_{1g} \rightarrow {}^2A_{1g}$	
$[CuL^3]Cl_2$	CH_3CN	32,200	INCT	Square-planar
		21,850	$^2B_{1g} \rightarrow {}^2E_g$	
		17,450	$^2B_{1g} \rightarrow {}^2A_{1g}$	
$[CuL^4]Cl_2$	CH_3CN	30,255	INCT	Square-planar
		22,835	$^2B_{1g} \rightarrow {}^2E_g$	
		17,550	$^2B_{1g} \rightarrow {}^2A_{1g}$	

spectra, the g-values are related with exchange interaction coupling constant (G) by the expression,

$$G = \frac{g_{\parallel} - 2}{g_{\perp} - 2}$$

According to Hathaway [26], if the G value is larger than four, the exchange interaction is negligible because the local tetragonal axes are aligned parallel or slightly misaligned. If its value is less than four, the exchange interaction is considerable and the local tetragonal axes are misaligned. For the present copper complex, G is 4.9, suggesting that the local tetragonal axes are aligned parallel or slightly misaligned, consistent with a $d_{x^2-y^2}$ ground state.

Based on the above analytical and spectral studies, the proposed structures of the ligands and their complexes are shown in figures 1 and 2.

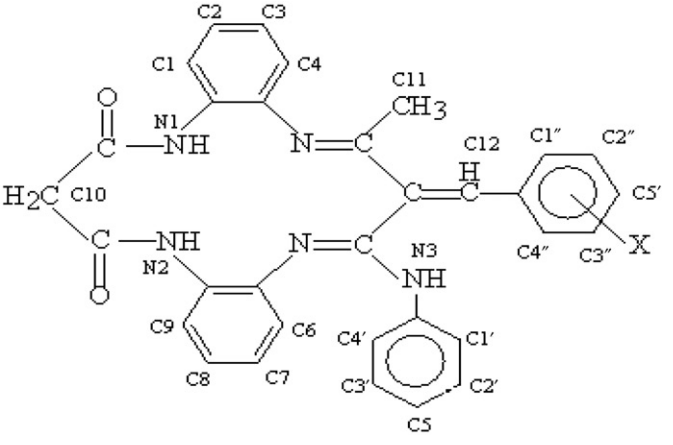


Figure 1. Structure of macrocyclic ligands.

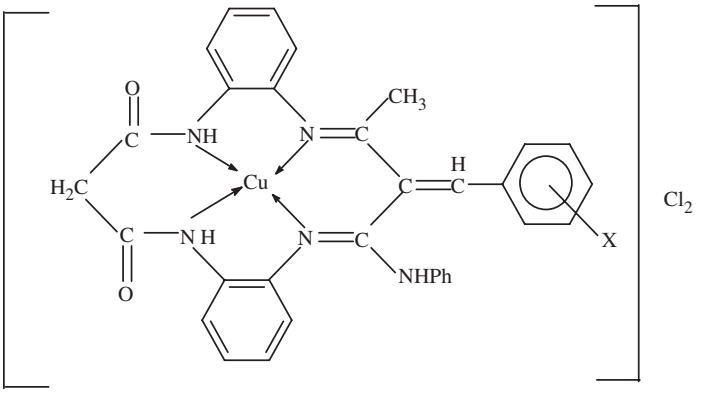


Figure 2. Structure of macrocyclic complexes.

Table 3. Minimum inhibitory concentration of the synthesized compounds against the growth of bacteria ($\mu g\,mL^{-1}$).

Compound	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. aeruginosa*	*S. aureus*
L^1	52	54	58	60	63
L^2	56	56	62	64	68
L^3	60	60	66	66	72
L^4	66	64	68	70	75
$[CuL^1]Cl_2$	40	36	28	25	34
$[CuL^2]Cl_2$	30	32	24	20	30
$[CuL^3]Cl_2$	24	30	18	15	26
$[CuL^4]Cl_2$	18	24	14	12	20
Streptomycin	10	15	6	12	4

3.5. *Electrochemical behavior*

Redox potentials of Schiff-base complexes markedly depend on the ligand, solvent and geometry of the complexes [27]. Electron-withdrawing substituents on the ligands shift the redox potential positively, whereas electron-donating groups have the opposite effect. Oxidation potentials are also dependent on the imine bridge of the Schiff base, with more positive potentials observed for complexes with aromatic bridges attributed to (i) an increase in unsaturation eases electron-density delocalization from the metal to the ligand or (ii) higher rigidity of the ligand, imparted by the aromatic bridges hinders contraction of the hole cavity.

Redox potentials show a cathodic shift with an increase in the donor capacity of the solvent. The cathodic shift in the redox potentials ongoing from DMF to DMSO is attributed to the stronger axial bonds that are established between the metal(III) center and the better donor stabilizing the higher oxidation state of the metal [28]. The size of the coordination cavity in the complexes and the geometric requirements and the size of the metal ions in different oxidation states also affect the redox potential [29].

The cyclic voltammogram of $[CuL^1]Cl_2$ in DMSO at 300 K in the potential range +0.8 to −0.4 V shows a well-defined redox process corresponding to formation of the quasi-reversible copper(II)/copper(III) couple. The anodic peak at $E_{pa} = 0.56$ V versus Ag/AgCl and the associated cathodic peak at $E_{pc} = 0.30$ V correspond to the copper(II)/copper(III). The $[Cu(L^1)]Cl_2$ complex exhibits a quasi-reversible behavior as indicated by the non-equivalent current intensity of cathodic and anodic peaks and also the large peak separation indicates quasi-reversible behavior.

3.6. *Antimicrobial activity*

The *in vitro* biological screening effects of the compounds against bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and fungi such as *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* were tested by the serial dilution method. The minimum inhibitory concentration (MIC) values of the compounds are summarized in tables 3 and 4. 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 3 and 4), $[Cu(L^4)]Cl_2$ was more potent than the other investigated complexes and standard.

Table 4. Minimum inhibition concentration of the synthesized compounds against the growth of fungi ($\mu g\,mL^{-1}$).

Compound	*A. niger*	*R. stolinifer*	*A. flavus*	*R. bataicola*	*C. albicans*
L^1	60	55	84	45	42
L^2	42	44	68	40	38
L^3	35	34	54	36	36
L^4	24	20	28	34	30
$[CuL^1]Cl_2$	28	30	34	38	32
$[CuL^2]Cl_2$	24	25	24	30	25
$[CuL^3]Cl_2$	19	20	20	25	22
$[CuL^4]Cl_2$	15	14	18	22	20
Nystatin	10	16	8	14	12

The enhanced activity of the complexes can be explained on the basis of their solubility, fitness of the particles, size of the metal ion and the presence of the bulkier organic moieties. The biological activity involves inhibition of DNA synthesis [30] by creating lesions in DNA strands by oxidative rupture [31] and by binding the nitrogen bases of DNA or RNA, hindering or blocking base replication. By changing the ligand environment around copper, the redox potential of the couple can be significantly changed. The O_2^- and H_2O_2 produced by such redox reactions cause cell toxicity by their potential oxidizing effect on vital cell components such as lipoic 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. Further studies are required to explore these complexes as drugs.

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