DESIGNING, SYNTHESIS, SPECTRAL CHARACTERIZATION OF ANTIMICROBIAL AND DNA ACTIVE TRIDENTATE SCHIFF BASE LIGANDS AND THEIR COMPLEXES

N.RAMAN*, S.JOHNSON RAJA, J.JOSEPH, A.SAKTHIVEL AND J.DHAVEETHU RAJA

Department of Chemistry, VHNSN College, Virudhunagar- 626 001, India

(Received: November 26, 2008 - Accepted: May 10, 2008)

ABSTRACT

New neutral Schiff base tridentate ligand transition metal complexes of the composition, ML_2 , where $M = Cu(II), Ni(II), Co(II)$ and $Zn(II)$; $L =$ ligands derived from semicarbazide hydrochloride and 2,4-pentanedione/methyl acetoacetate/acetoacetanilide, were synthesized and characterized by elemental analyses, magnetic susceptibility, IR, UV-Vis., 1H -NMR, ^{13}C -NMR, CV, ESR, and FAB-mass spectral techniques. Electronic absorption spectra of the complexes indicate an octahedral geometry around the metal ion. The magnetic susceptibility measurements and low conductance data provide evidence for the monomeric and neutral natures of the complexes respectively. The electrochemical behaviour, the anodic and cathodic potential and the number of electron transfer were calculated using cyclic voltammogram. The X-band ESR spectrum of copper complex in DMSO at 300 K and 77 K was recorded and its salient features are reported. The antimicrobial activities of the ligands and their complexes have been studied by screening the compounds against the bacteria *E. coli*, *P.aeruginosa*, *P.mirabilis*, *S. typhi*, and *B.subtilis* and also the fungi *C.albicans* and *R. stolonifer* and results have been compared with the standard drug, ampicillin. The data indicate that most of the metal complexes have higher antimicrobial activity than the free ligands and the standard. The DNA cleavage experiments, performed using gel electrophoresis with the corresponding metal complexes in the presence of H_2O_2 , showed all the complexes afford a pronounced discernible DNA cleavage evidenced by the disappearance of Form I (supercoiled) of plasmid and the production of Form III (linear).

Key words: Schiff base, Metal Complexes, DNA, Antimicrobial studies

INTRODUCTION

Coordination complexes are gaining increasing importance in recent years particularly in the design of repository; slow release or long acting drugs in nutrition and in the study of metabolism¹. The metal ions are also known to accelerate drug action. The interaction of metal ions with nucleic acids and nucleic acid constituents has been actively studied in the recent years^{2,3}. The interaction of metal complexes with DNA has long been the subject of intense investigation in relation to the development of new reagents for biological and medicinal fields. DNA can be cleaved by hydrolytic or oxidative pathways. The hydrolytic process involves cleavage of the phosphodiester bond leading to the formation of fragments that could be relegated by enzymatic process⁴. Among the metal complexes so far investigated, it is well known that transition metal complexes inhibit DNA or RNA polymerase activities and induce strand scission of DNA in presence of H_2O_2 . Transition metals are essential for the normal functioning of living organisms. Therefore, it is not surprising that transition metal coordination compounds are of great interest as potential drugs⁵. A general study of the structural and bonding features of the various Schiff base complexes can help better understanding of the complex life processes. The findings of structural studies are interesting in that the Schiff base ligands can control the stereochemistry of the complex and provide us with numerous examples of unusual geometries about the central metal ion. Therefore, they can serve to illustrate the coordination flexibility of these ions.

A wide number of papers report the synthesis and characterization of many metal complexes of Schiff bases derived from aromatic aldehydes and substituted anilines. It is well known from the literature that semicarbazide compounds containing the amide moiety have a strong ability to form metal complexes⁶. Acetoacetanilide is continued to attract considerable attention from theoretical standpoints concerning the mode of bonding and its general reactivity as coordinated ligand. Keeping the above facts in mind and in continuation of our earlier work on transition metal complexes with Schiff bases⁷, the present paper describes the synthesis and characterization of $Cu(II)$, $Ni(II)$, $Co(II)$ and $Zn(II)$ complexes with the biologically important semicarbazide Schiff base.

EXPERIMENTAL

Analysis and measurements

The reagents used for the preparation of the ligand and its complexes were of Merck products. Spectroscopic grade solvents were used for spectral measurements. The carbon, hydrogen and nitrogen contents in each sample were performed at RSIC, CDRI, Lucknow. The UV-Vis. spectra of the complexes were recorded on a Shimadzu UV-1601 spectrophotometer. The IR spectra were recorded on a Perkin-Elmer 783 spectrophotometer using KBr. The molar conductivity was measured on a Systronic conductivity bridge with

a dip type cell, using 10^{-3} M solution. Metal contents of the complexes were estimated by the standard procedure. 1H -NMR spectra of the samples were recorded in $DMSO-d_6$ by employing TMS as internal standard at Madurai Kamaraj University, Madurai on a Bruker instrument. Cyclic voltammogram of the complexes was recorded in DMSO solution at 300 K using a three electrode cell comprising reference $Ag/AgCl$, auxiliary Pt and working glassy carbon electrodes. All solutions were purged with N_2 for 30 min prior to each set of experiments. The X-band ESR spectra of the complexes were recorded in DMSO at 300 K and 77 K at Pondicherry University, Pondicherry using TCNE (tetracyanoethylene) as the g-marker. The FAB mass spectra were recorded at Indian Institute of Chemical Technology, Hyderabad on Joel SX 102/DA-6000 mass spectrometer using *m*-nitrobenzylalcohol as the matrix. Magnetic susceptibility values at room temperature were determined by Gouy balance using $CuSO_4$ as calibrant and the magnetic moments were calculated making the necessary diamagnetic correction. Gel electrophoresis experiment was done by solutions of Lambda DNA in 50 mM NaCl / 5 mM tris-HCl (pH=7.0). It gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of ca. 1.8-1.9, indicating that the DNA was sufficiently free of protein contamination. The molar absorption coefficient was taken as $6600 M^{-1}cm^{-1}$. Stock solutions were kept at $4^\circ C$ and used within 4 days. Doubly distilled H_2O was used to prepare the buffer.

Synthesis of Schiff base ligands

A suspension of the semicarbazide hydrochloride (0.01 mol) in water after neutralization with 10% NaOH solution was added with 2,4-pentanedione(HL^1)/methylacetoacetate(HL^2)/acetoacetanilide(HL^3) (0.01 mol) in 90% ethanol. The resulting solution was stirred vigorously in an ice-bath for 3 h. The colourless solid separated was filtered, washed with ethanol and dried *in vacuo*. The crude samples were recrystallised in ethanol. Yield : (65-72 %).

Synthesis of complexes

The complexes of $Cu(II)$, $Co(II)$, $Ni(II)$ and $Zn(II)$ were prepared by the reaction of ethanolic solution of the ligand $HL^1/HL^2/HL^3$ (0.04 mol) and the metal chloride (0.02 mol) in 2:1 ratio with vigorous stirring for 1 h, keeping the pH of the solution at 8. The reaction mixture was kept at $0^\circ C$ for 12 h. The solid complexes formed were filtered and washed with ethanol and dried *in vacuo*. Approximately 65-75% of the yield was obtained for all the complexes.

Nuclease activity

The DNA cleavage experiment was conducted using Lambda DNA by gel electrophoresis with the corresponding metal complexes in the presence of H_2O_2 as an oxidant. The gel electrophoresis experiments were performed by incubation at $35^\circ C$ for 1.5 h as follows: Lambda DNA 10 μM , 5 μM each complex, 1 μM H_2O_2 in 50 mM tris-HCl buffer (pH=7.2) and 34 μM of double distilled water. The samples were electrophoresed for 2 h at 50 V on 1% agarose

gel using tris-acetic acid-EDTA buffer, pH = 8.3. After electrophoresis, the gel was stained using $1\mu\text{g}/\text{cm}^3$ EB and photographed under UV light. It can be seen from the figure at very low concentration (10^{-3} M).

RESULTS AND DISCUSSION

All the complexes are air stable, insoluble in water and soluble in DMSO, CHCl_3 and CH_2Cl_2 . They were identified by their physical and analytical data. The analytical data (Table 1) are in good agreement with the general formula ML_2 . The monomeric nature of the complexes was evidenced from their magnetic susceptibility values. Study of magnetic and electronic spectral data is quite informative in characterizing the geometry of the complexes. These complexes were non-electrolytic⁸ due to the low conductivity values.

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

S.No	Compound	Content (Found/calc.)%				λ_M mho cm^2 $\text{mol}^{-1} \times 10^{-3}$	Magnetic moment $\mu_{\text{eff}}(\text{BM})$
		M	C	H	N		
1.	$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_2$	--	45.9/46.1	6.3/6.4	26.7/26.9	--	--
2.	$\text{Cu} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	16.5/16.9	38.0/38.3	5.3/5.3	26.8/26.9	0.3	1.05
3.	$\text{Ni} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	15.7/15.8	38.2/38.8	5.3/5.4	22.4/22.7	5.2	3.27
4.	$\text{Co} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	16.1/15.9	38.5/38.8	5.4/5.4	22.7/22.6	1.5	4.0
5.	$\text{Zn} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	17.1/17.3	38.0/38.2	5.2/5.3	22.1/22.3	0.15	--
6.	$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_3$	--	42.0/41.8	5.6/5.8	24.1/24.4	--	--
7.	$\text{Cu} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	15.5/15.6	35.1/35.3	4.8/4.9	20.5/20.6	6.5	1.90
8.	$\text{Ni} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	14.2/14.6	35.9/35.8	5.0/5.0	20.6/20.9	10.0	3.50
9.	$\text{Co} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	14.3/14.6	35.4/35.7	4.9/5.0	20.9/20.8	2.8	4.00
10.	$\text{Zn} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	15.5/15.9	35.0/35.1	4.8/4.9	20.2/20.5	9.8	--
11.	$\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2$	--	56.7/56.6	5.4/5.6	24.2/24.0	--	--
12.	$\text{Cu} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	11.9/12.0	49.6/49.8	8.8/8.7	21.3/21.1	6.2	1.80
13.	$\text{Ni} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	11.0/11.2	50.1/50.3	8.6/8.8	21.4/21.3	8.0	3.70
14.	$\text{Co} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	11.0/11.2	50.2/50.3	8.5/8.7	21.5/21.3	3.9	4.50
15.	$\text{Zn} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	12.1/12.3	49.2/49.7	8.2/8.6	21.2/21.0	9.0	--

Mass spectra

The FAB mass spectra of the Schiff bases and their cobalt complexes are used to compare their stoichiometric composition. The Schiff base HL^1 shows a molecular ion peak M^+ at $m/z = 157$. The molecular ion peak for the cobalt complex was observed at $m/z = 372$. Similarly HL^2 and HL^3 and their cobalt complexes show the molecular ion peaks at 173, 234, 404, 526 respectively, confirming the stoichiometry of the metal chelates as ML_2 .

Electronic spectral studies

The spectra of the ligands $\text{HL}^1/\text{HL}^2/\text{HL}^3$ and their metal complexes were recorded in DMSO solution at room temperature. From the data (Table 2), it is observed that all the complexes exhibit octahedral geometry around the metal ions. No transitions were observed in the visible region for $\text{Zn}(\text{II})$ complex consistent with the d^{10} configuration of the $\text{Zn}(\text{II})$ ion. This complex is also found to be diamagnetic as expected for d^{10} configuration.

Table-2. Electronic absorption spectral data of the complexes in CHCl_3 Solution

Compound	Solvent	Absorption (cm^{-1})	Band assignment	Geometry
$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_2$	CHCl_3	42735	INCT	—
$\text{Cu} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	CHCl_3	33898, 24450	INCT ${}^2\text{E}_g \rightarrow {}^2\text{E}_g$	Distorted octahedral
$\text{Co} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	CHCl_3	15873, 9259 44584, 12077, 20747	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$ ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$ INCT ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$ ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$	octahedral octahedral
$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_3$	CHCl_3	38735	INCT	—
$\text{Cu} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	CHCl_3	22779 12531	${}^2\text{E}_{1g} \rightarrow {}^2\text{T}_{2g}$ ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$	Distorted octahedral
$\text{Co} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	CHCl_3	14836 16447	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{F})$ ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$	Octahedral
$\text{Ni} [\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	CHCl_3	22749	${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$	Octahedral
$\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2$	CHCl_3	40155	INCT	—
$\text{Cu} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	CHCl_3	22500 11800	${}^2\text{E}_{1g} \rightarrow {}^2\text{T}_{2g}$ ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$	Distorted octahedral
$\text{Co} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	CHCl_3	13890 15450	${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^3\text{T}_{2g}(\text{F})$ ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$	Octahedral
$\text{Ni} [\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	CHCl_3	20950	${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$	Octahedral

INCT-Intraligand Charge Transfer

IR spectra

It is well documented⁹ that the semicarbazone derivatives can coordinate to the central metal ion through O and N^1 of semicarbazone moiety only.

The broad absorption bands observed around 3200 cm^{-1} , assigned to the OH group of the β -diketone moiety, disappeared in all the complexes due to the deprotonation. This indicates that the oxygen coordinates to the metal ion. The two bands observed in the range $1738\text{--}1760\text{ cm}^{-1}$ and $1590\text{--}1660\text{ cm}^{-1}$ can be assigned to $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ respectively. In spectra of all the metal complexes, these bands show red shifts indicating the coordination of metal ions through carbonyl oxygen as well as imine nitrogen of semicarbazone moiety. The metal complexes show new bands around $490\text{--}500\text{ cm}^{-1}$ and $380\text{--}390\text{ cm}^{-1}$ which are due to formation of M-N and M-O bands respectively. In the complexes derived from acetoacetanilide, a medium intensity absorption band observed in the region $1400\text{--}1500\text{ cm}^{-1}$, assigned to the phenyl ring.

$^1\text{H-NMR}$ spectra

The $^1\text{H-NMR}$ spectra of the ligands and their zinc complexes recorded in CDCl_3 at room temperature are given in Table 3. In the spectra of the complexes the enolic proton signal observed at 12.0δ , is found to be absent, confirming deprotonation of the ligand and its subsequent involvement in coordination to the metal ion.

Table -3. The ^1H -NMR of the ligand and their zinc complexes in CDCl_3

S.No	Compound	Solvent	$-\text{CH}_3$	$-\text{CH}_2$	$-\text{NH}$	$-\text{NH}_2$	$-\text{OH}$
1.	$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_2$	CDCl_3	1.6	2.1	7.7	8.9	12.1
2.	$\text{C}_6\text{H}_{11}\text{N}_3\text{O}_3$	CDCl_3	1.8	2.2	6.3	8.4	11.5
3.	$\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2$	CDCl_3	1.9	2.0	7.1	9.1	12.0
4.	$\text{Zn}[\text{C}_6\text{H}_{10}\text{N}_3\text{O}_2]_2$	CDCl_3	1.6	2.2	7.3	8.6	---
5.	$\text{Zn}[\text{C}_6\text{H}_{10}\text{N}_3\text{O}_3]_2$	CDCl_3	1.7	2.3	7.2	8.3	---
6.	$\text{Zn}[\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2]_2$	CDCl_3	1.8	2.1	6.1	9.0	---

 ^{13}C -NMR spectra

The number of signals in the ^{13}C -NMR spectra corresponds to the number of magnetically non-equivalent carbon atoms in the Schiff base complexes. The ^{13}C -NMR spectral data of the zinc complexes are assigned and given in Tables 4(a), 4(b) and 4(c).

Table-4(a). ^{13}C -NMR spectral data of the $\text{Zn}(\text{L}^1)_2$ complex

	Values (ppm)	Assignments
175.3	C ₁
	145.2	C ₂
	15.8	C ₃
	31.6	C ₄
	150.7	C ₅
	12.8	C ₆

Table-4(b). ^{13}C -NMR spectral data of the $\text{Zn}(\text{L}^2)_2$ complex

	Values (ppm)	Assignments
170.3	C ₁
	157.2	C ₂
	15.8	C ₃
	35.6	C ₄
	168.7	C ₅
	51.8	C ₆

Cyclic voltammetry

The cyclic voltammetry is the most versatile electroanalytical technique for the study of electroactive species. The cyclic voltammogram of CuL^1 was recorded in CH_3COCH_3 using LiClO_4 as supporting electrolyte in the potential range +2 V to -2 V at scan rate 100 mVs^{-1} . The cyclic voltammogram of the pure ligand shows an irreversible cathodic peak at -1.54 V, which fortuitously, is outside the range of potential studied for the complex. It shows a well defined redox process corresponding to the formation of the Cu(I)/Cu(II) at $E_p = -0.87 \text{ V}$ and the cathodic peak at $E_p = 1.55 \text{ V}$ corresponding to the ligand. The CuL^2 complex recorded over a potential range +1.2 V to -1.2 V and exhibits one irreversible, oxidative peak (response) at -0.32 V due to the formation of Cu(0)/Cu(I) and reductive peak at 0.65 V due to Cu(II)/Cu(I) . NiL^2 shows two irreversible peaks, one at cathodic direction and another at anodic direction. The irreversible reduction peak at 1.01 V is due to the formation of Ni(III)/Ni(II) and another irreversible oxidation peak at -0.88 V is due to the formation of Ni(I)/Ni(II) .

The cyclic voltammogram of the CuL^3 complex in DMSO solution recorded over a potential range +2.0 V to -2.0 V shows two irreversible peaks, one at cathodic direction and another at anodic direction. The irreversible reduction peak at -0.6 V is due to the formation of Cu(I)/Cu(0) and another irreversible oxidation peak at -0.85 V is due to the formation of Cu(I)/Cu(II) .

ESR spectra

The ESR spectrum of the copper complex in DMSO at 300 K and 77 K were recorded (Fig. 1). The spectrum of the complex at 300 K shows one intense absorption band in the high field region and is isotropic due to tumbling motion of the molecules. The ESR spectrum of the copper complex at 77 K indicates a poorly resolved nitrogen super hyperfine structure in the perpendicular region due to the interaction of the Cu(II) odd electron with nitrogen atoms. The magnetic susceptibility value reveals that the copper complex has a magnetic moment, 1.8 B.M., corresponding to the one unpaired electron, indicating that the complex is mononuclear. This fact was also evident from the absence of a half field signal, observed in the spectrum at 1600 G due to the $\Delta m_s = \pm 2$ transitions, ruling out any Cu-Cu interaction.

Figure 1. The X-band ESR spectrum of the copper complex in DMSO at 300 K (a) and 77 K (b)

The g-tensor value of the copper complex can be used to derive the ground state. In octahedral complexes, the unpaired electron lies in the $d_{x^2-y^2}$ orbital. In our copper complex, the observed g-tensor values are $g_{\parallel} (2.36) > g_{\perp} (2.08) > g_e (2.00)$ which suggest that this complex has octahedral geometry. The ESR parameters of the complex coincide well with related systems which suggest that the complexes have distorted octahedral geometry and the systems are axially symmetric¹⁰. In the axial spectra, the g-values are related with exchange interaction coupling constant (G) by the expression,

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

According to Hathaway¹¹, if the G value is larger than four, the exchange interaction is negligible because the local tetragonal axes are aligned parallel or are 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, it is 4.5 which suggests that the local tetragonal axes are aligned parallel or are slightly misaligned and consistent with a $d_{x^2-y^2}$ ground state.

It is usual to determine the covalent bonding parameter for the Cu(II) ion in various ligand field environments. We adopted the simplified molecular orbital theory¹² to calculate the bonding coefficients such as in-plane π -bonding (β^2), out-of-plane π -bonding (γ^2) and in-plane σ -bonding (α^2).

The in-plane σ -bonding parameters, α^2 are related to $g_{\parallel}$ and $g_{\perp}$ according to the following equation

$$\alpha^2 = A_0/p + (g_{\parallel} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04$$

If the α^2 value = 0.5, it indicates a complete covalent bonding, while the value of $\alpha^2 = 1.0$ suggests a complete ionic bonding. The observed value (0.892) indicates that the complexes have some covalent character. The out-of-plane π -bonding (γ^2) and in-plane π -bonding (β^2) parameters are calculated from the following expressions:

$$\beta^2 = (g_{\parallel} - 2.0023) E / 8\lambda\alpha^2$$

$$\gamma^2 = (g_{\perp} - 2.0023) E / 2\lambda\alpha^2$$

The optical and ESR data are correlated and the individual spin-orbital coupling parameter (λ) is calculated using the formula¹³

$$g_{\text{ave}} = 2.0023 - 8\lambda/\nabla$$

The value of λ obtained is 403 cm^{-1} , while the free copper(II) ion the value is 828 cm^{-1} . This reduction in λ from the free ion value 828 cm^{-1} is an evidence of covalence in the M-L bond. The lower value of λ as compared to free ion value indicates that there is a considerable mixing of ground and excited state terms. The observed β^2 (0.9894) and α^2 (0.6211) values indicate that there is interaction in the out-of-plane π -bonding, whereas the in-plane π -bonding is completely ionic. Thus, the ESR study of the copper complex has provided supporting evidence for the optical results.

Based on the spectral and analytical data, the proposed structure of the complexes is given below (Fig. 2).

Fig. 2. Structure of the complexes

DNA study

All the complexes afford a pronounced discernible DNA cleavage in the presence of oxidant as evidenced by the disappearance of Form I of plasmid and the production of Form III⁴. This may be attributed to the Fenton-type reaction¹⁵ as shown below:

where $\text{L} = \text{L}^1, \text{L}^2, \text{L}^3$

Three hydroxyl free radicals participate in the oxidation of deoxyribose moiety, followed by the sugar phosphate backbone¹⁶. Thus, the above proposed mechanism reinforces the conclusions drawn from the cyclic voltammetric study.

The more pronounced nuclease activity of these adducts in the presence of oxidant are shown in the following figures. The Fig. 3a shows two out of four complexes exhibit fair nuclease activity. Two complexes, nickel complex (lane 4) and zinc complex (lane 5) cleave supercoiled form (Form I) completely to give rise to linear form (Form III). Other two complexes cleave supercoiled form to slightly linear form.

Figure 3(a). Gel diagram of $M(\text{L}^1)_2$ complexes

Lane 1- Lambda DNA alone

Lane 2- DNA + Cu complex

Lane 3- DNA + Co complex

Lane 4- DNA + Ni complex

Lane 5- DNA + Zn complex

Lane

1 2 3 4 5

Figure 3(b). Gel diagram for $M(\text{L}^2)_2$ complexes

Lane 1- Lambda DNA alone

Lane 2- DNA + Cu complex

Lane 3- DNA + Co complex

Lane 4- DNA + Ni complex

Lane 5- DNA + Zn complex

Lane 1 2 3 4 5

Figure 3(c). Gel diagram for $M(L^3)_2$ complexes

Lane 1- Lambda DNA alone

Lane 4- DNA + Ni complex

Lane 2- DNA + Cu complex

Lane 5- DNA + Zn complex

Lane 3- DNA + Co complex

In Fig. 3b, nickel complex (lane 3) was completely degraded. This shows that a slight increase in concentration over the optimal value (*i.e.*, the value at which 100% cleavage efficiency was observed) led to extensive degradations, resulting in disappearance of bands on agarose gel. The cobalt and zinc complex integrate DNA efficiently in super coiled form (Form I) which cleaves completely and gives rise to linear form (Form III) [lane 4 and lane 5] while copper complex (lane 2) does not cleave DNA in the presence of oxidant (H_2O_2).

The Fig. 3c shows that copper complex cleaves more effectively (supercoiled form to linear form). The other complexes are slightly give rise to linear form compared to copper complex.

Microbial activity

Antimicrobial screening test of the ligands $HL^1/HL^2/HL^3$ and their metal chelates against the microorganisms has been carried out employing disc diffusion plate method¹⁷. The Tables 5(a) and 5(b) show the inhibition zone of the complexes at minimum inhibition concentration (MIC) values against the growth of bacteria and fungi. It is much larger for the metal chelates than the ligands and ampicillin (standard). Such increased activity of the metal complexes can be explained on the basis of chelation theory¹⁸. Chelation reduces the polarity of the metal ion mainly due to the partial sharing of its positive charge with the donor groups and possible π -electron delocalization over the whole chelate ring and enhances the lipophilicity of the complexes.¹⁹ This increased lipophilicity leads to breakdown of the permeability barrier of the cell and thus retards the normal cell processes²⁰⁻²².

Table-5. Antimicrobial study of the investigated compounds ($MIC \times 10^2$ M).

S.No	Compound	E. coli	S. typhi	S. aureus	K. pneumoniae	B. subtilis	C. albicans	R. stolonifer
1.	$C_6H_{11}N_3O_2$	1.5	1.1	2.0	1.9	2.8	0.9	1.0
2.	$Cu [C_6H_{10}N_3O_2]_2$	1.4	1.5	1.2	1.3	2.0	1.3	1.2
3.	$Ni [C_6H_{10}N_3O_2]_2$	1.0	1.6	1.4	1.6	1.5	1.5	1.4
4.	$Co [C_6H_{10}N_3O_2]_2$	1.5	1.0	1.3	1.1	0.9	1.9	1.3
5.	$Zn [C_6H_{10}N_3O_2]_2$	1.2	1.2	1.1	1.0	2.4	0.9	1.1
6.	$C_6H_{11}N_3O_3$	1.6	2.5	2.9	2.1	1.6	2.9	3.8
7.	$Cu [C_6H_{10}N_3O_3]_2$	1.3	2.4	1.8	2.2	2.0	1.1	1.5
8.	$Ni [C_6H_{10}N_3O_3]_2$	1.4	0.7	1.1	0.9	1.5	1.9	1.5
9.	$Co [C_6H_{10}N_3O_3]_2$	2.2	1.3	1.6	1.4	1.8	2.0	1.8
10.	$Zn [C_6H_{10}N_3O_3]_2$	1.5	1.2	1.8	2.1	2.4	0.9	1.2
11.	$C_{11}H_{14}N_4O_2$	2.0	2.1	2.0	1.9	2.7	3.0	3.3
12.	$Cu [C_{11}H_{13}N_4O_2]_2$	0.9	1.1	1.0	1.5	1.4	1.1	1.4
13.	$Ni [C_{11}H_{13}N_4O_2]_2$	2.8	2.9	2.4	2.0	1.5	1.3	1.7
14.	$Co [C_{11}H_{13}N_4O_2]_2$	1.5	2.0	1.7	1.6	1.9	1.4	1.5
15.	$Zn [C_{11}H_{13}N_4O_2]_2$	1.1	1.0	1.5	1.8	1.6	0.9	1.0
16.	Ampicillin	1.6	1.9	2.4	2.8	2.5	0.9	1.1

CONCLUSIONS

The Cu(II), Ni(II), Co(II) and Zn(II) complexes were synthesized using the Schiff bases formed by the condensation of semicarbazide hydrochloride and 2,4-pentanedione/methylacetoacetate/acetoacetanilide. They were characterized by the usual spectral and analytical data. From the available data, it is concluded that all the complexes exhibit six-coordinated octahedral geometry. The complexes were found to be active against microorganisms. The antimicrobial activity of the ligand is enhanced by the chelation with metal(II) ions. The DNA cleavage experiments showed all the complexes afford a pronounced discernible DNA cleavage evidenced by the disappearance of Form I of plasmid and the production of Form III.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Head of the Department of Chemistry, Principal and the Managing Board of VHNSN College, Virudhunagar for their constant encouragement and providing research facilities. SJ thanks the Tamilnadu Government for financial assistance.

REFERENCES

1. N. Bharti, R.M. Maurya, F. Naqvi, A. Bhattacharya, S. Bhattacharya, A. Azam, *Eur. J. Med. Chem.* **35**, 481, (2000).
2. U. Kohn, M. Schulz, M. Gohl, E. Anders, *Tetrahedron; Asymmetry* **16**, 2125, (2005).
3. R.G.S. Berlinck, M.H. Kossuga, A. M. Nascimento, *Sci. Synth.* **18**, 1077, (2005).
4. T. Gupta, A. K. Patra, S. Dhar, M. Nethaji and A. R. Chakravarty, *J. Chem. Sci.* **117**, 123, (2005).
5. E. Malhotra, N.K. Kaushik, H. S. Malhotra, *Indian J. of Chem. Sec(A)*, **45A**, 370 (2006).
6. A.N.M. Kasim, D. Venkappayya, G.V. Prabhu, *J. Indian Chem. Soc.* **76**, 67, (1999).
7. N. Raman, A. Kulandaisamy, C. Thangaraja, *Transition Met. Chem.* **29**, 129, (2004).
8. F. A. Kettle, *Coordination Compounds*, (Thomas Nelson & Sons) 1975.
9. A. Gingras, R. L. Somarjai, G.S. Bayley, *Can. J. Chem.*, **30**, 1865, (1968).
10. B. T Thaker, P. K. Tandel, A.S. Patel, C.J. Vyas, M.S. Jesani, D.M. Patel, *Indian J. Chem. Sec(A)* **44A**, 265, (2005).
11. B. J. Hathaway, A. A.G. Tomlinson, *Coord. Chem. Rev.* **5**, 1, (1970).
12. R. Seangprasertkij, T. L. Riechel, *Inorg. Chem.* **23**, 991, (1984).
13. R. Srinivasan, I. Sougandi, R. Venkatesan, P. Sambasivarao, *Proc. Indian Acad. Sci. (Chem Sci)* **115**, 91, (2003).
14. K. Hussain Reddy, P. Sambasiva Reddy, P. Ravindra Babu, *J. Biochem.*, **77**, 169, (1999).

15. B. Macias, I.García, M.V. Villa, J. Borrás, M.G.Alvarez, A. Castineiras, *J. Inorg. BioChem.* **96**, 367, (2003).
16. P. Marfey, E. Robinson, *Mutat. Res.* **86**, 1585, (1981).
17. J. C. Vincent, H.W. Vincent, *Pros. Soc. Exptl. Biol. Medi.*, **55**, 162, (1944).
18. R. S. Srivastava, *Inorg. Chim. Acta.* **56**, 65, (1944).
19. N. Gupta, R. Swaroop, R.V. Singh, *Main Group Met. Chem.* **114**, 387, (1997).
20. L. Mishra, V.K. Singh, *Indian J. of Chem. Sec(A)* **32A**, 446, (1997).
21. R. Malhotra, S. Kumar, K.S. Dhindsa, *Indian J. Chem. Sec(A)* **32A**, 457, (1993).
22. W. Levinson, E. Jawetz, *Medical microbiology and immunology*, (4th Ed., Stamford), 1996.