Int. J. Pharm. Sci. Rev. Res., 26(1), May – Jun 2014; Article No. 48, Pages: 284-290 ISSN 0976 – 044X

Research Article

In vitro Antimicrobial activity and DNA cleavage studies: Synthesis and Characterization of novel M(II) complexes with tridentate [ONO] donor Schiff base ligand derived from phenylpropanehydrazide

P R Chetana[1]*, M N Somashekar[1], B S Srinatha[1], R S Policegoudra[2], S M Aradhya[3]
[1]Department of Chemistry, Central College Campus, Bangalore University, Bangalore, India.
[2]Department of Pharmaceutical Technology, Defence Research Laboratory, Tezpur, India.
[3]Department of Fruit and Vegetable Department, Central Food Technological Research Institute, Mysore, India.
***Corresponding author's E-mail:** pr.chetana@gmail.com

Accepted on: 14-03-2014; **Finalized on:** 30-04-2014.

ABSTRACT

Three new ternary complexes of general formulation [M(L)n] (1–3), where L=N`-(5-chloro-2-hydroxybenzylidine)-3-phenylpropanehydrazide; n=2;M= Cu, Ni, Zn, complexes are synthesized, characterized by various physicochemical and UV-Vis, FT-IR, ^{1}H NMR and ESI-MS spectroscopic methods. The Cyclic voltammetry show a quasi-reversible cyclic voltammetric response due to one electron Cu(II)/Cu(I) reduction near 100 mV (versus SCE) in DMF–0.1 M KCl. All the compounds were screened for their *in-vitro* antibacterial activity against Gram positive and Gram negative bacterial strains. Among them, Cu complex showed good activity against all microbes. The copper complex shows moderate chemical nuclease activity in the presence of MPA as a reducing agent.

Keywords: Antimicrobial activity, DNA studies, Metal (II) complexes, Phenyl propane hydrazides, Schiff bases.

INTRODUCTION

The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species.[1-3] Schiff base complexes of transition metals are of particular interest to inorganic chemists because of their structural, spectral, and chemical properties which are often strongly dependent on the nature of the ligand structure.[4-7] Biological activities of metal complexes differ from those of either ligands or the metal ions and increased and/or decreased biological activities are reported for several transition metal complexes, such as copper(II) and nickel(II) ions.[8] In addition, complexes of salicyl aldehyde benzoyl hydrazone were shown to be a potent inhibitor of DNA synthesis and cell growth.[9] The activity of some hydrazone complexes is very significant against Gram-positive bacteria *in vitro*. This hydrazone also has mild bacteriostatic activity and a range of analogues has been investigated as potential oral ion chelating drugs for genetic disorders such as thalassemia.[10,11]

The ability to accomplish DNA cleavage will undoubtedly allow the development of new antimicrobial drugs and chemotherapeutic agents. In addition, artificial nucleases will provide important new tools for DNA manipulation to molecular biologists. For example, bisphenanthroline copper (I) complex is used in DNA-foot printing experiments.[12] which are important for the detailed study of DNA–protein interactions.[13] 3d transition metal complexes are well suited for application as artificial nucleases, because of their cationic nature, diverse three-dimensional structural features depending on the ligand systems, and the possibility to tune their redox potential through the choice of proper ligands. The interaction of transition metals like Mn, Fe and Cu, with dioxygenin the presence of a reducing agent generates reactive oxygen species (ROS) that ultimately may cleave DNA.[14] The DNA cleavage reactions generally proceed *via* oxidative or hydrolytic cleavage pathways. The hydrolytic pathway involves phosphodiester bond hydrolysis leading to the formation of fragments that could be religated through enzymatic processes. Zn(II), being a strong Lewis acid, exchanges ligands very rapidly. Several Zn (II) complexes are well-known for their hydrolase activity.[15] The oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases. Oxidative DNA cleavage by redox-active metal complexes, like $[Fe(edta)]^{2-}$ or Cu(1,10-phenanthroline)$_2$Cl$_2$, is mediated by the production of reactive oxygen species, like $HO^{\cdot}$, through a Fenton-type mechanism.[16] These free radicals abstract the most accessible and exposed sugar hydrogens and initiate the oxidative cleavage, leading to DNA-cleavage products.

Based on the above considerations, we have synthesized Schiff base derived from hydrazides, which has more functional groups, it is an azotic ligand with lone electron pairs, and it may coordinate with many metal ions as bidentate or multidentate. Such types of ligand systems are capable to show keto and enol tautomerism which results in the coordination of ligand in deprotonated form.[17] These ligand systems have been proved to be a fruitful source to stabilize the unusual oxidation states of metal ions and to give neutral complexes.[18] Hydrazone derivatives are found to possess antimicrobial, antitubercular, anticonvulsant and anti inflammatory activities.[19-21] Particularly, the antibacterial and antifungal

properties of hydrazones and their complexes with some transition metal ions were studied and reported by Carcelli et al.[22]

Recognizing the importance of hydrazide metal complexes, we have synthesized and characterized a new hydrazide derivative, (N`-(5-chloro-2-hydroxybenzylidene)-3-phenylpropanehydrazide) (5CHPPH) and its bis[(N`-(5-chloro-2-hydroxybenzylidene)-3-phenyl propanehydrazide)] metal(II) [M= Cu(II), Ni(II), and Zn(II)]complexes. The Schiff baseL and its metal complexes were characterized by elemental analyses, IR, ^{1}H NMR, ESI-MS, UV-Vis spectral analysis, cyclic voltammetry and molar conductance studies. The antimicrobial activity and DNA interaction studies of these compounds were investigated systematically.

EXPERIMENTAL

Materials

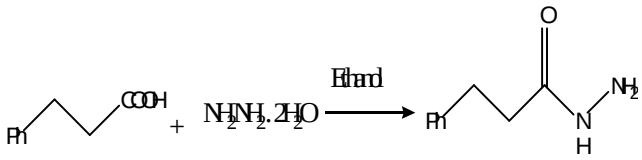
All reagents and chemicals were of AR grade and used as purchased. The 5-chlorosalcylaldehyde, benzopropionic acid and various metal salts were Merck products and used as supplied. The agarose (molecular biology grade) and ethidium bromide (EB) were obtained from Sigma. Supercoiled (SC) pUC19 DNA (cesium chloride purified) was purchased from Bangalore Genie (India). Double distilled water was used for preparing all the solutions for the DNA studies.

The elemental analyses were carried out by using vario-micro CHNS 15106062 analyzer.^{1}H NMR and ESI-MS data of the compounds were recorded at IISc, Bangalore. The IR spectra of the samples were recorded on a Shimadzu spectrophotometer from 4000 to 400 cm^{-1} using KBr pellets. The UV-Vis spectra were recorded on a Shimadzu UV-3101PC spectrophotometer using DMF as solvent. The molar conductance of the complexes was measured using Equiptronics digital conductivity meter no.EQ-660A and the melting points were checked by melting point apparatus used in laboratories. Cyclic voltammetric experiments were performed at room temperature in water:DMF under oxygen free condition created by purging pure nitrogen gas with CHI 600E electrochemical instrument. A three electrode system was used: a glassy carbon working electrode, an Ag^+/AgCl reference electrode and a Pt wire counter electrode. The working electrode was polished with 1.0, 0.3, 0.05 µm alumina prior to each experiment. Throughout the experiment oxygen-free nitrogen was bubbled through the solution for 10 min. Voltammetric experiments were performed at room temperature.

Syntheses

Synthesis of 3-phenylpropanehydrazide

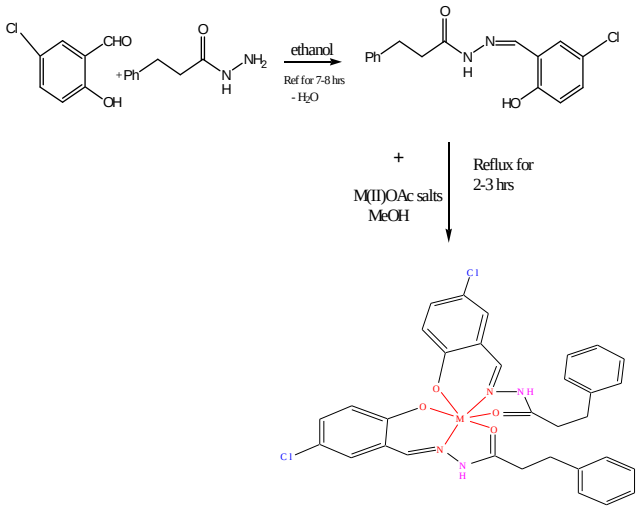
Phenyl propanehydrazide was prepared by addition of phenyl-propionic acid (0.1mol) to ethanolic solution of hydrazine hydrate (99%), (0.1mol)and the reaction mixture was refluxed on water bath for 6-7h (Scheme 1). The resulting product was poured in ice-cold water and kept overnight, light solid was crystallized out. The product was washed with ice-cold alcohol and dried in air.



Scheme 1: Preparation of 3-phenylpropane-hydrazide

Synthesis of Schiff base ligand (N`-(5-chloro-2-hydroxybenzylidene)-3-phenylpropanehydrazide) (5CHPPH)

The 5-chloro salcylaldehyde (0.1mol, 0.156g) in ethyl alcohol (20 mL) was added to an ethanolic solution (20 mL) of phenylpropane-hydrazide (0.1mol, 0.164 g). The reaction mixture was heated under reflux on an oil bath for about 7-8 h. The reaction mixture was cooled and the solid was collected by filtration. This solid was washed with cold ethanol and then with diethyl ether and then dried in vacuo. A crystalline solid was obtained by recrystallization from ethanol.



Scheme 2: Preparation of Schiff base (N`-(5-chloro-2-hydroxybenzylidene)-3-phenylpropanehydrazide) ligand and bis (N`-(5-chloro-2-hydroxybenzylidene)-3-phenylpropanehydrazide) metal(II) [M= Cu(II), Ni(II) and Zn(II)] complexes .

Preparation of bis [(N`-(5-chloro-2-hydroxybenzylidene)-3-phenylpropanehydrazide)M(II)] complexes(M= Cu, Ni, Zn)

The bis ligand metal complexes were prepared by reaction between Schiff base ligand (0.2mol) in methanol (20 mL) with the corresponding metal acetates (0.1mol) in hot methanol (10 mL). The reaction mixture was heated under reflux for 2 h on a water bath (Scheme 2). The precipitate obtained was filtered, washed with methanol and followed by diethylether. The obtained product was dissolved in DMF and on slow evaporation of the solution at room temperature yielded a crystalline material.

Antibacterial assay

The antibacterial activities of the synthesized compounds were determined against clinical isolates like *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Yersinia enterocolitica, Bacillus mycoides, Bacillus subtilis*and *Staphylococcus aureus.* The test organisms were maintained on nutrient agar slants. *In vitro* antibacterial activity was determined by the agar well-diffusion method as described by Mukherjee et al.[23] The overnight bacterial culture was centrifuged at 8000 rpm for 10 min. The bacterial cells were suspended in saline to make a suspension of 10^5 CFU/mL and used for the assay. Plating was carried out by transferring the bacterial suspension to a sterile Petri plate and mixed with molten nutrient agar medium, allowing the mixture to solidify. About 75μL of the sample (2 mg/mL) was placed in the wells. Plates were incubated at 37◦C and activity was determined by measuring the diameter of the inhibition zones. The assay was carried out in triplicate.

DNA cleavage

The cleavage studies of SC pUC19 DNA by the ligand and its M(II) complexes was studied by agarose gel electrophoresis. 3- mercapto propionic acid (MPA) (5 mM) was used as the reducing agent and hydrogen peroxide (H_2O_2)was used as oxidizing agent for the chemical nuclease activity. Reactions were carried out under dark conditions. Eppendorf vials were used for experiments in a dark room at 25°C using super coiled pUC19 DNA (0.2 µg), taken in 50 mM Tris–HCl buffer (pH 7.2) containing 50 mM NaCl, was treated with the complex. The concentration of the complexes in DMF or the additives in buffer corresponded to the quantity after the dilution of the complex stock to the 20 µl final volume using Tris–HCl buffer. The SC pUC19 DNA samples were pre-incubated for one hour at 37 °C, followed by its addition to the loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol 30% glycerol (2 µl) and the solution was finally loaded on 0.8% agarose gel containing 1.0 µgml^{-1} ethidium bromide (EB). The electrophoresis was carried out in a dark room for 2 h at 45 V in TAE (Tris–-acetate–EDTA) buffer. The bands were visualized by UV light and photographed. The extent of cleavage of SC DNA was determined by measuring the intensities of the bands using a UVITECH Gel Documentation System. Due corrections were made for the low level of nicked circular (NC) form present in the original super coiled (SC) DNA sample and for the low affinity of EB binding to SC compared to NC and linear forms of DNA.[24]

RESULTS AND DISCUSSION

The analytical data for the complexes indicate MLn stoichiometry for all the complexes, where L = 5CHPPH, M= metal ions (Cu, Ni, Zn), and n = 2 (Table 1). The melting points of all complexes are above 320°C, the complexes are stable in air. The obtained crystals were not suitable for X-ray diffraction, since single crystals were not obtained. All the complexes are insoluble in common organic solvents and soluble in DMF and DMSO. The molar conductance of all complexes in DMF (10^{-3} M) solution, fall in the range of 11–14 Ohm^{-1}cm^{-2} mol^{-1}, indicating the non electrolytic nature of complexes.[25]

Table 1: Analytical and physical data of the ligand L and its complexes

Compounds (Formula)	Mol. Mass	[a]Mol cond	[b]ΔEp(V)	MP °C	N%		C%		H%	
					Exp	Obt	Exp	Obt	Exp	Obt
5CHPPH (L) ($C_{16}H_{15}ClN_2O_2$)	302.4	---	----	184	9.92	9.94	72.32	72.41	6.43	6.49
$Cu(L)_2$(1) ($C_{32}H_{28}Cl_2CuN_4O_4$)	667.3	12.00	0.446	340	8.40	8.38	57.62	57.65	4.23	4.28
$Ni(L)_2$ (2) ($C_{32}H_{28}Cl_2NiN_4O_4$)	662.3	11.45	---	338	8.86	8.98	58.04	58.10	4.26	4.28
$Zn(L)_2$(3) ($C_{32}H_{28}Cl_2N_4O_4Zn$)	668.4	12.40	-----	340	8.38	8.46	57.46	57.38	4.22	4.26

[a]Molar conductance=Λ_M (Ω^{-1} cm^2 M^{-1}) in DMF at 25°C; [b]Cyclic voltammetry; Cu(II)/Cu(I) couple in DMF-0.1M KCl, ΔEp=Ep_a-Ep_c are the anodic and cathodic peak potentials, respectively. Scan rate= 0.1 mV

Table 2: Selected ^{1}H NMRand UV-Visbands of ligand and its metal complexes

Compounds	-OH	-NH	-CH=N-	-Ph-	-CH_2 -	π→ π* (nm)	n→ π* (nm)	CT bands (nm)	d-d bands (nm)
5CHPPH(L)	10.3	9.1 (s,1H)	7.8(s,1H)	7.1-7.4(m)	2.98	282	292	332	----
$Cu(L)_2$ (**1**)	------	11.7(s,1H)	8.1(s,1H)	7.2-7.4(m)	2.91	274	319	389	630
$Ni(L)_2$ (**2**)	------	11.3(s,1H)	8.0(s, 1H)	7.2-7.4(m)	2.91	282	337	409	615
$Zn(L)_2$ (**3**)	------	11.5(s,1H)	8.0 s,1H)	7.2-7.4(m)	2.91	275	319	394	-----

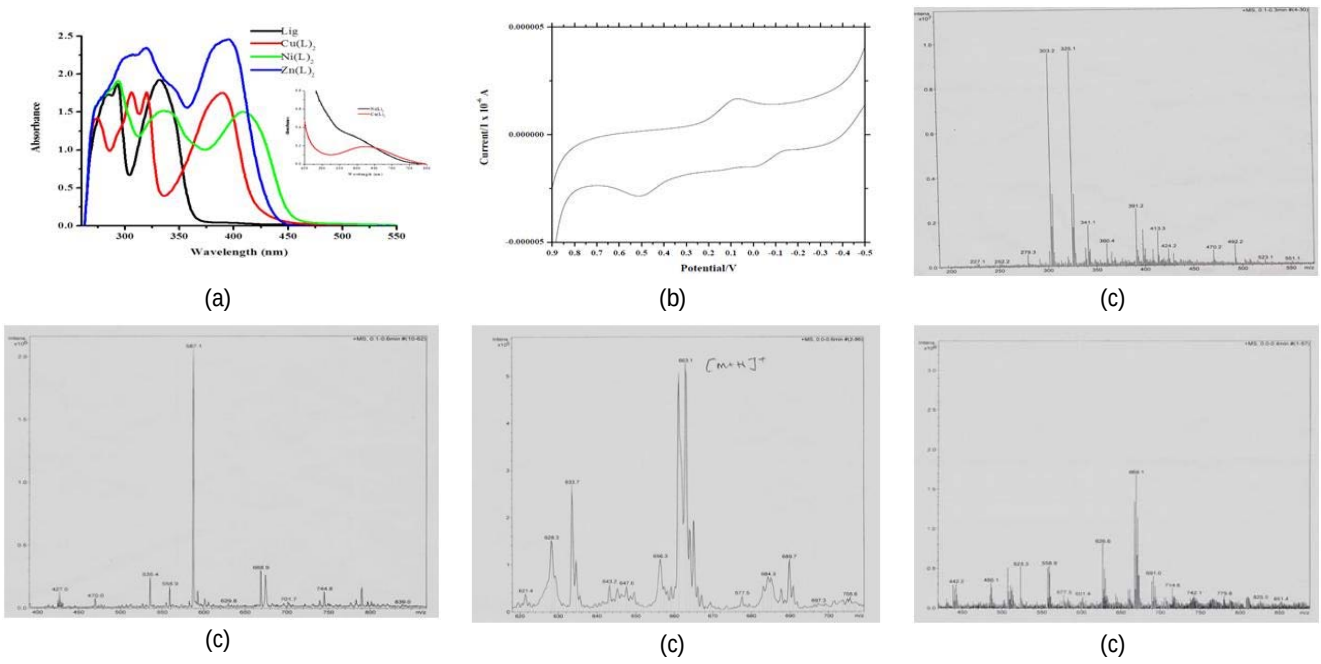


Figure1: (a) The Electronic spectrum of ligand L and its Cu(II), Ni(II), Zn(II) complexes. Inset: The d-d bands of complexes 1 and 2; (b) Cyclic voltammogram of $Cu(L)_2$ with scan rate 0.1V/s, (c) Mass spectrum of ligand L and its complexes 1, 2, 3.

Table 3: The values of zone inhibition (mm)of microorganisms for theL and its metal complexes

	Bacteria	L	1	2	3
Gram -ve	*Klebsiella pneumoniae*(MTCC 109)	--	16	--	--
	Proteus mirabilis(MTCC 743)	--	15	--	--
	Pseudomonas aeruginosa(MTCC 741)	--	18	--	--
	Yersinia enterocolitica(MTCC 4848)	--	11	--	--
Gram +ve	*Bacillus mycoides*(MTCC 645)	--	16	--	--
	Bacillus subtilis(MTCC 441)	--	16	--	--
	Staphylococcus aureus(MTCC 3160)	--	15	--	--

Table 4: Selected cleavage data of SC pUC19 by ligand and its complexes 1, 2, 3

Lane No.	Complex	[a]NC %	Lane No.	Complex	[a]NC %
1	DNA control	0	9	DNA + MPA+ **2**(60µM)	45.1
2	DNA + L (60 µM)	0.4	10	DNA + H_2O_2 + **2**(60µM)	16.2
3	DNA + MPA + L (60 µM)	42.4	11	DNA + **3**(60µM)	5.93
4	DNA + H_2O_2 + L(60 µM)	7.0	12	DNA + MPA+ **3**(60µM)	37.8
5	DNA + **1** (60 µM)	4.6	13	DNA + H_2O_2 + **3**(60µM)	11.2
6	DNA + MPA + **1**(60 µM)	52.4	14	DNA + **1**(100µM)	11.0
7	DNA + H_2O_2 + **1**(60µM)	28.6	15	DNA + MPA	2.5
8	DNA + 2(60µM)	0.9	16	DNA + H_2O_2	2.2

Electronic spectra

The UV-Vis electronic spectra of the free ligand L and complexes measured in DMF at room temperature over 200-800nm range (Figure 1(a)) and the selected bands are given in Table 2. Hydrazide Schiff base ligand exhibited three main bands at 282, 292 and 332 nm. Ligand exhibits a band around 282 nm which is due to the intra ligand π-π* transition, which is unaltered in spectra of complexes. The peak at 292 nm is assigned for n-π* transition of imine group and the transitions occurred around 332nm are due to n-π* transitions of carbonyl group.[26] The second and third bands were attributed to imino (C=N) π-π* and n-π* transition of carbonyl group, which were slightly affected by chelation. In the UV spectra of the complexes (**1-3**), the appearance of two new bands at ~395 nm and at ~600 nm regions showed the metal ligand coordination and d-d transitions in all the complexes.

In the electronic spectra of Cu (II) complex displays one broad band at ~ 625 nm (16,000 cm^{-1}), corresponds to $^2Eg \rightarrow {}^2T_2g$ transition under a distorted octahedral environment. The width of the band provides evidence for distortion,[27] and the electronic spectrum of Ni(II) complex displays shoulder bands at ~620 nm (16,129 cm^{-1}) and ~410nm (24,390 cm^{-1}). These bands may be assigned to $^3A_2g(F) \rightarrow {}^3Tg(F)$ and $^3A_2g(F) \rightarrow {}^3T_1g(P)$ transitions, respectively. It suggests octahedral geometry of Ni(II) complex.[28] The electronic spectrum of zinc complex, the d-d was not observed may be the diamagnetic property of zinc ion.

IR spectra

IR spectra usually provide a lot of valuable information on coordination reactions. The IR spectra for our studied complexes give information about the coordination of ligand to metal. The IR spectra of all complexes indicate that the ν (C=N) bands of the ligand at 1616 cm^{-1} are due to the azomethine linkage which were shifted towards lower frequency 1606 cm^{-1}, indicating that the ligands coordinate to metal ions via the azomethine nitrogen. The peak exhibited at 1665 cm^{-1} due to ν (C=O) vibration of the free ligand were shifted to lower frequencies 1529–1516 cm^{-1} in the complexes. This shift confirms that, the group loses its original characteristics and forms coordinative bonds with metal. The absence of band due to phenolic OH group at 3410 cm^{-1} and increase in frequency of phenolic C–O vibration from 1269 cm^{-1} of ligand to 1301–1304 cm^{-1} in the spectra of all metal complexes suggest the coordination of ligand to the metal via deprotonation,[29,30] which infers that azomethine–nitrogen, phenolic-oxygen and carbonyl-oxygen as the coordination sites of the monobasic tridentate ligand. Besides, two non-ligand peaks at 565–552 cm^{-1} and 420–465 cm^{-1} of complexes were assigned to ν (M–O) and ν (M–N) stretching vibrations respectively.

^{1}H NMR spectra

Further evidence for the coordinating mode of the ligand is obtained by ^{1}H NMR spectral studies. The ^{1}H NMR spectral data given in Table 2, recorded in $CDCl_3$ and DMSO-d6. The ligand is characterized by five signals at 10.86(singlet), 8.44(singlet), 7.69(singlet), 7.27-6.8 (multiplet) and 3.07-2.99(two doublets) ppm, which are assigned to the protons associated with –OH, -N=CH, -CONH, aromatic ring protons and $–CH_2-CH_2-$, respectively. The presence of CH=N proton signal at d= 8.44 ppm in the ligand (L) confirmed its formation by the condensation of the 5-chlorosalcylaldehyde and hydrazide. In the ^{1}H NMR spectra of complexes (**1-3**), shows a new signal at 10.89 - 11.89 ppm is due to the free NH group of ligand, which is not involved in the coordination to metal ion in complexes. The absence of proton at 11-11.5 indicating that phenolic proton is absent in complexes. This information suggests the adjustment of electronic current upon coordination of >C=O group to the metal ion.

Mass spectra

The ESI-MS of Schiff base ligand and their complexes showed molecular ion peaks which were in agreement with their molecular formula. The molecular ion peak for the ligand HL (C16H15ClN2O2) corresponds to m/z 302.2 and its complexes $Cu(L)_2$(C32H28Cl2CuN4O4), $Ni(L)_2$ (C32H28Cl2NiN4O4) and $Zn(L)_2$(C32H28Cl2N4O4Zn)are at m/z 663.1, 667.4 and 669.1 respectively (Figure 1(C)).

Cyclic Voltammetry

The copper complex (0.001 M in DMF) was scanned in the potential range of -1.0 V to 1.0 V in deareated condition with scan rate 0.1V/s. The voltammogram with scan rate 0.1 V/s is given in Figure 1(b) and numerical results are represented in Table 1. A cathodic peak observed in the voltammograms in the range Epc = 0.15 to 0.07 V evidences the reduction of metallic species, $Cu^{II} \rightarrow Cu^{I}$. The reverse scan shows two anodic peaks with potentials in the range, Epa_1 = -0.1 to -0.5 V and Epa_2 = 0.4 to 0.68 V corresponding to the oxidation reactions, $Cu^{I} \rightarrow Cu^{II}$ and $Cu^{II} \rightarrow CuIII$.[31] The high value of ΔEp, separation between the cathodic and anodic peak potentials (Epa–Epc) for the couple Cu^{I}/Cu^{II} which is greater than 60 mVindicate the quasi-reversible nature of the redox process.[32]

Antibacterial activity

The *in-vitro* antibacterial activity of the Schiff bases, solvent (DMSO) and their Cu(II), Ni(II) and Zn(II)complexes were evaluated against three gram positive, *S. aureusand B. subtilis*, *Bacillus mycoides,* and four gram negative bacteria, *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Yersinia enterocolitica,*Table 3 illustrate the antimicrobial activity of the synthesized compounds. DMSO (blank) and streptomycin was used as controls. In general, the activity against gram negative bacteria is higher than those of gram positive bacteria this may be due to the greater lipophilic nature[33] of the Schiff bases than their metal complexes but for our synthesized compounds the activity is more or less similar for both. Among the synthesized compounds only copper complex exhibitedmoderate activity towards the all the bacterial strains.

The antimicrobial activity of bis ligand copper complexes exhibited promising results than the ligand, nickel and zinc complexes against all the test bacterial strains. It was evident that overall potency of the ligand was enhanced on coordination with the metal ions. This enhancement in the activity may be rationalized on the basis that ligands mainly possess C=N bond. It has been suggested that the ligands with nitrogen and oxygen donor atoms inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover coordination reduces the polarity of the metal ion essentially because of the partial sharing of its positive charge with the donor groups with the chelate ring system formed during coordination.[34,35] This process in turn increases the lipophilic nature of the central metal

atom, which favors its permeation more effectively through the lipid layer of microorganism, thus destroys them more aggressively.[36]

DNA Cleavage activity by Gel Electrophoresis method

Gel electrophoresis is an extensively studied technique for the binding of compounds with nucleic acids; in this method segregation of the molecules will be on the basis of their relative rate of movement through a gel under the influence of an electric field. DNA is negatively charged and when it is placed in an electric field, it migrates towards the anode; the extent of migration of DNA is decided by the strength of electric field, buffer, density of agarose gel and size of the DNA. Generally it is seen that mobility of DNA is inversely proportional to its size. Gel electrophoresis photograph in Figure 2 shows the bands with different bandwidth and brightness compared to the control. The difference observed in the intensity and the band width is the criterion for the evaluation of cleavage ability of ligand and its transition metal complexes with DNA. The gel electrophoresis clearly revealed that the difference in migration of the lanes of ligand and complexes are due to the effect of reducing agent MPA. Control experiments using only SCpUC19 DNA (lane 1), MPA (500µM, lane 15), H_2O_2(200µM, lane 16) or the complexes (lane. 2, 5, 8, 11) alone do not show any apparent cleavage of SC DNA under similar reaction conditions (Table.4). The ligand L (60 μM) and its$Cu(L)_2$, $Ni(L)_2$and Zn(L) complexes shows moderate "chemical nuclease" activity. The maximum cleavage was exhibited by $Cu(L)_2$ at 60 μM concentration. The DNA cleavage reaction of complexes in the presence of MPA probably proceeds through the hydroxyl radical pathway in a similar way as proposed by sigman.[37]

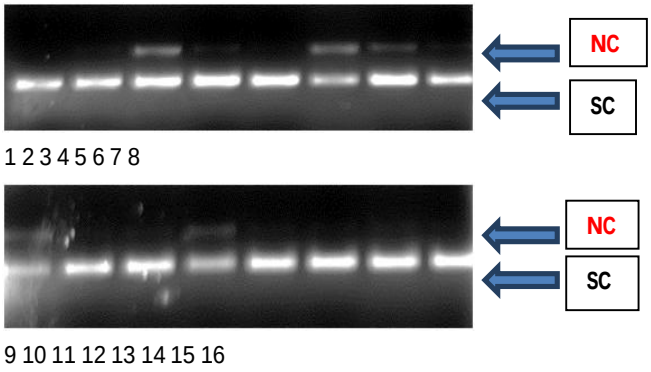


Figure 2: Gel electrophoresis diagram showing the cleavage of SC pUC19DNA (0.2 μg, 33.3 μM) by ligand and its complexes (30 μM) in 50 mM Tris–HCl/50 mM NaCl buffer (pH 7.2) in the presence of MPA (500µM): Lane1, DNA control; Lane 2, DNA + L(60 μM); Lane 3, DNA + MPA + L (60 µM); Lane 4, DNA + H_2O_2 + L(60 µM); Lane 5, DNA + $Cu(L)_2$ (60 µM); Lane6, DNA + MPA + $Cu(L)_2$ (60 µM); Lane 7, DNA + H_2O_2 + $Cu(L)_2$ (60µM); Lane 8, DNA + $Ni(L)_2$ (60µM); Lane 9,DNA + MPA+ $Ni(L)_2$ (60µM); Lane 10, DNA + H_2O_2 + $Ni(L)_2$ (60µM); Lane 11, DNA + $Zn(L)_2$ (60µM); Lane 12, DNA + MPA+ $Zn(L)_2$ (60µM); Lane 13, DNA + H_2O_2 + Zn $(L)_2$ (60µM); Lane 14, DNA + Cu $(L)_2$ (100µM); Lane 15, DNA + MPA control; Lane 16, DNA + H_2O_2control

CONCLUSION

We report the synthesis of six coordinated Cu(II), Ni(II) and Zn(II) complexes of Schiff bases prepared by the 2:1 condensation process. The analytical and spectral data provides the ML_2type complexes with an ONO coordination sphere, the ligand coordinates to metal ions in proposed octahedral fashion to give the stable complexes. Analytical data correspond to the monomeric composition of the complexes. The antibacterial examination of the compounds led to the conclusion that the copper metal complex exhibited moderate activity compared to free ligand, nickel and zinc complexes. The influences of DNA cleavage property of the complexes were analyzed by agarose gel electrophoresis method in which the copper complex showed moderate activity. The maximum cleavage was exhibited by Cu $(L)_2$ at 60 μM concentration.

Acknowledgement: Mr. Somashekar M N sincerely thanks to the Department ofScience and Technology (DST) PURSE programmer, Bangalore University, Bangalore, for providing the Fellowship.

REFERENCES

1. Jayabalakrishnan C, Natarajan K, Synthesis, Characterization and biological activities of Ruthenium (II) carbony complexes containing bifunctional tridentate schiff bases, Reactivity in Inorganic Metal-Organic Chemistry, 31, 2001, 983-995.
2. Dharmaraj N, Viswanathamurthi P, Natarajan K, Ruthenium (II) complexes containing bidentate Schiff bases and their antifungal activity, Transition Metal Chemistry, 26, 2001, 105-109.
3. Jeeworth T, WalH LK, Bhowon MG, Ghoorhoo D, Babooram K, Synthesis and anti-bacterial/catalytic properties of schiff bases and schiff base metal complexes derived from 2,3-diaminopyridine, synthesis Reactivity Inorganic Metal-organic Chemistry, 30, 2000, 1023.
4. NejoA A, Kolawole GA, NejoA O, Synthesis, characterization, antibacterial, and thermal studies of unsymmetrical Schiff-base complexes of cobalt(II), Journal of Coordination Chemistry, 63, 2010, 4398-4410.
5. Vafazadeh R, Kashfi M, Synthesis and Characterization of Cobalt (III) Octahedral Complexes with Flexible Salpn Schiff Base in Solution. Structural Dependence of the Complexes on the Nature of Schiff Base and Axial Ligands, Bulletin Korean Chemical Society, 28, 2007, 1227- 1230.
6. Raman N, Dhaveethuraja J, Sakthivel A, Synthesis, spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies, Journal of Chemical Science, 119, 2007, 303-310.
7. Nathan L C, Koehne J E, Gilmore J M, Hannibal K A, Dewhirst W E, Mai T D, The X-ray structures of a series of copper(II) complexes with tetradentate Schiff base ligands derived from salicylaldehyde and polymethylene diamines of varying chain length, Polyhedron, 22, 2003, 887-894.
8. Krishnamoorthy P, Sathyadevi P, Cowley A H, Butorac R R, Dharmaraj N, Evaluation of DNA binding, DNA cleavage, protein binding and *in vitro* cytotoxic activities of bivalent transition metal hydrazone complexes, Europian Journal Medicinal Chemistry, 46, 2011, 3376–3387.
9. Johnson D K, Murphy T B, Rose N J, Goodwin W H, Pickart L, Cytotoxic chelators and chelates 1, Inhibition of DNA synthesis in cultured rodent and human cells by aroylhydrazones and by a

copper(II) complex of salicylaldehyde benzoyl hydrazone, Inorganic Chimica Acta, 67, 1982, 159-165.

10. RanfordJ D, VittalJ J, Wang Y M, Dicopper (II) Complexes of the Antitumor Analogues Acylbis (salicylaldehyde hydrazones) and Crystal Structures of Monomeric [Cu_2(1,3-propanedioyl bis(salicylaldehyde hydrazone))$(H_2O)_2$]·$(ClO_4)_2$·$3H_2O$ and Polymeric [{Cu_2(1,6-hexanedioyl bis(salicylaldehyde hydrazone))$(C_2H_5OH)_2$}$_m$]·$(ClO_4)_{2m}$·$m(C_2H_5OH)$, Inorganic Chemistry, 37, 1998, 1226-1231.

11. BussJ L, GreeneB T, TurnerJ, TortiF M, TortiS V, Iron chelators in cancer chemotherapy, Current Top Medicinal Chemistry, 4, 2004, 1623-163.

12. Sigman DS, Mazumder A, Perrin DM, Chemical Nucleases, Chemical Reviews, 93, 1993, 2295-2316.

13. PogozelskiW K, TulliusT D, Oxidative Strand Scission of Nucleic Acids: Routes Initiated by Hydrogen Abstraction from the Sugar Moiety Chemical Reviews, 98, 1998, 1089-1107.

14. Tullius TD, Greenbaum JA, Mapping nucleic acid structure by hydroxyl radical cleavage, Current Opinion Chemical Biology, 9, 2005, 127-134.

15. Boseggia E, Gatos M, Lucatello L, Mancin F, Moro S, Palumbo M, Sissi C, Tecilla P, Tonellato U, Zagotto G, Toward Efficient Zn(II)-Based Artificial Nucleases, Journal of American chemical society, 126, 2004, 4543-4549.

16. Bowen WS, Hill WE, Lodmell JS, Comparison of rRNA cleavage by complementary 1,10-phenanthroline-Cu(II)- and EDTA-Fe(II)-derivatized oligonucleotides, Methods, 25, 2001, 344-350.

17. Bakir M, Hassan I, Johnson T, X-ray crystallographic, electrochemical and spectroscopic properties of 2-pyridinio 2-pyridyl ketone phenyl hydrazone chloride hydrate, Journal of Molecular Structure, 688, 1–3, 2004, 213–222.

18. Stadler AM, Harrofield J, Bis-acyl-/aroyl-hydrazones as multidentate ligands, Inorganica Chimica Acta, 362, 2009, 4298-4314.

19. Vicini P, Zani F, Cozzini P, Doytchinoval, Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations, Europian Journal of Medicinal Chemistry, 37, 2002, 553-564.

20. Kocyigit-Kaymakcioglu B, Rollas S, Synthesis, characterization and evaluation of anti tuberculosis activity of some hydrazones, Farmaco, 57, 2002, 595-599.

21. RagavendranJ V, Sriram D, Patel SK, Reddy IV, Bharathwajan N, Stables J, Yogeeswari P, Design and synthesis of anticonvulsants from a combined phthalimide eGABAeanilide and hydrazone pharmacophore, European Journal of Medicinal Chemistry, 42, 2007, 146-151.

22. Carcelli M, Mazza P, Pelizi C, Zani F, Antimicrobial and genotoxic activity of 2,6-diacetylpyridine bis(acylhydrazones) and their complexes with some first transition series metal ions. X-ray crystal structure of a dinuclear copper (II) complex, Journal of Inorganic Biochemistry, 57, 1995, 43-62.

23. Mukherjee PK, Balaslubramanian R, Saha K, Saha BP, Pal M, Antibacterial efficiency of Nelumbo nucifera(nymphaeaceae) rhizomes extract, Indian drugs, 32, 1995, 274-276.

24. Bernadou J, Pratviel G, Bennis F, Girardet M, Meunier B, Potassium monopersulfate and a water-soluble manganese porphyrin complex, [Mn(TMPyP)](OAc)5, as an efficient reagent for the oxidative cleavage of DNA, Biochemistry, 28, 1989, 7268-7275.

25. Chetana PR, Somashekar MN, Srinatha BS, Policegoudra RS, Aradhya SM, Ramakrishna Rao, Synthesis, Crystal Structure, Antioxidant, Antimicrobial, and Mutagenic Activities and DNA Interaction Studies of Ni(II) Schiff Base 4-Methoxy-3-benzyloxybenzaldehyde Thiosemicarbazide Complexes, ISRN Inorganic Chemistry, 2013, 2013, 11.

26. HalliM B, Shashidhar, Qureshi ZS, Synthesis, Spectral Studies, and Biological Activity of Metal Complexes of Benzofuran Thio semicarbazides, Synthesis and Reactivity In Inorganic And Metal-Organic Chemistry, 34, 10, 2004, 1755–1768.

27. Liu Z-C, Yang Z-Y, Li T-R, Wang B-D, Li Y, Wang M-F, DNA-binding, antioxidant activity and solid-state fluorescence studies of copper(II), zinc(II) and nickel(II) complexes with a Schiff base derived from 2-oxo-quinoline-3-carbaldehyde, Transition Metal Chemistry, 36, 2011, 489–489.

28. Patel RN, Vishnu P. Sondhiya, Dinessh K Patel, Shukla KK, Singh Y, Synthesis, crystal structure, spectroscopic and superoxide dismutase activity of copper(II) and nickel(II) complex of N'-[phenyl(pyridine-2-yl)methylidene] benzohydrazone, Indian Journal of Chemistry, 51A, 2012, 1695-1700.

29. Singh PK, Kumar DN, Spectral studies on cobalt (II), nickel(II) and copper(II) complexes of naphthaldehyde substituted aroylhydrazones, Spectrochim. Acta Part A, 64, 2006, 853–858.

30. Richardson DR, Bernhardt PV, Crystal and molecular structure of 2-hydroxy-1-naphthaldehydeisonicotinoyl hydrazone (NIH) and its iron(III) complex: an iron chelatorwith anti-tumour activity, Journal of biological inorganic chemistry, 4, 1999, 266–273.

31. Naveen V. Kulkarni, Anupama Kamath, Srinivasa Budagumpi, Vidyanand K. Revankar, Pyrazole bridged binuclear transition metal complexes: Synthesis, characterization, antimicrobial activity and DNA binding/cleavage studies, Journal of Molecular Structure, 1006, 2011, 580–588.

32. Bailey CL, Bereman RD, Rillema DP, Redox and Spectral Properties of Cobalt (II) and Copper (II) Tetraazaannulene Complexes: {H2[Me4(RBzo)2[14]tetraeneN4]] (R = H, CO,CH,). Evidence for Superoxide Ligation and Reduction, Inorganic Chemistry, 25, 1986, 3149–3153.

33. Selvarani V, Annara jB, Neelakantan MA, Sundaramoorthy S, Velmurugan D, Synthesis, characterization and crystal structures of copper (II) and nickel(II) complexes of propargyl arm containing N_2O_2 ligands: Antimicrobial activity and DNA binding, Polyhedron, 54, 2013, 74–83.

34. Halli MB, Sumathi RB, Mallikarjun Kinni, Synthesis, spectroscopic characterization and biological evaluation studies of Schiff's base derived from naphthofuran-2-carbohydrazide with 8-formyl-7-hydroxy-4-methyl coumarin and its metal complexes, Spectrochimica Acta A: Molecular and Biomolecular spectroscopy, 99, 2012, 46-56.

35. Sharma AK, Chandra S, Complexation of nitrogen and sulphur donor Schiff's base ligand to Cr(III) and Ni(II) metal ions: Synthesis, spectroscopic and antipathogenic studies, Spectrochimica Acta A, 78, 2011, 337-342.

36. Chohan ZH, Synthesis and Biological Properties of Cu(II)Complexes with 1,10-Disubstituted Ferrocenes, Synthetic reactivity Inorganic Metal Organic Chemistry, 34, 2004, 833-846.

37. Sigman DS, Chemical nucleases, Biochemistry, 29, 39, 1990, 9097–9105.

Source of Support: Nil, **Conflict of Interest:** None.