

Research Article

**Synthesis and Chemical Nuclease Activities of Copper (II) and Ni (II) Complexes of 4-chloro-2-(pyridine-3-yliminomethyl) phenol Schiff Bases**P.R. Chetana*¹, S. Sahana¹, R.S.Policegoudra², Bipul Sarma³, Rajiv Khatioda³¹Department of Chemistry, Central College Campus, Bangalore University, Bangalore, India.²Department of Biotechnology, Defense Research Laboratory, Tezpur, India.³Department of Chemical Sciences, Tezpur University, Napaam, Sonitpur, Assam, India.*Corresponding author's E-mail: pr.chetana@gmail.com

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ABSTRACT

This communication describes a procedure to synthesize a novel Schiff base ligand 4-chloro-2-(pyridine-3-yliminomethyl) phenol and its transition metal complexes of Cu(II) and Ni(II) and its characterization by physicochemical techniques such as elemental, FTIR, UV-Visible, ¹H-NMR, Mass spectra and cyclic voltammetry. Further, the ligand was structurally characterized by X-ray crystallography and is found to be planar suitable for DNA studies. The complexes were screened for their *in-vitro* antimicrobial activity using various strains of gram positive and gram negative bacteria- *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The Schiff base ligand showed higher antibacterial activity than the ligand coordinated to either of the metal ion copper or nickel. The DNA cleavage studies of the complexes have been investigated and both the complexes showed extensive cleavage of DNA. However, Cu(II) complexes showed better cleavage activity compared to Ni(II) complex.

Keywords: Antibacterial activity, Chemical nuclease activity, Copper(II) complex, Nickel(II) complex, Schiff base.

INTRODUCTION

There have been considerable current interests to develop the chemistry of transition metal complexes that are capable of cleaving DNA under oxidative and/or hydrolytic conditions. There is enormous attention presently in the field of coordination chemistry of 3d transition metal ion with Schiff bases.¹ Metal complexes of Schiff bases have gained a major role in the development of coordination chemistry. Schiff bases are easily prepared by a simple one-pot condensation of active carbonyl groups and primary amines in an alcohol solvent. Hence they are considered to be privileged ligands.²⁻³ The Schiff base ligands are an important class of ligands in coordination chemistry. In recent days, Schiff base ligands have attracted much attention due to the fact that the ligands around the central metal ion are asymmetrical.⁴ Further, the literature survey reveals that Schiff base metal complexes are potentially more biologically active. Therefore extensive studies have been reported on the biological activities.⁵⁻⁶ The coordinating property of 2-amino pyridine ligand was modified to give ligand systems, formed by condensation with variety of reagents.⁷

Transition metal complexes are playing a vital role as potential drugs in different enzymatic and physiological reactions because transition metals are essential for the normal functioning of living organisms.⁸ Copper being biologically relevant element a large number of copper complexes have been synthesized and studied for biological activities.⁹⁻¹⁰ Copper(II) complexes are also attractive because Cu(II) is well-known to play an important role in naturally occurring biological systems as

well as a pharmacological agent.¹¹⁻¹³ Chakravaarty¹⁴ demonstrated that Cu(II) complexes plays a vital role in the nuclease activity on ferrocene moieties.

Nickel is extremely versatile metal in biological coordination chemistry. Biological studies showed that nickel complexes enter a cell by phagocytosis, finds way to the nucleus and cause oxidative DNA cleavage, DNA-DNA cross link and DNA-Protein cross link. Thus, interest is focused on the synthesis of Ni(II) complexes with potential applications in cancer diagnosis and treatment of tumors.¹⁵⁻¹⁷ Nickel(II) complexes are having wide potential applications in medicine and recently the complexes have been screened for cancer cell proliferation.¹⁸ Ni(II) complexes have gained significant importance as drugs and artificial nucleases.

The interaction of transition metal complexes with DNA has been studied extensively in the past decades.¹⁹⁻²⁰ Schiff bases play a vital role in bioinorganic chemistry as they possess remarkable biological activity. Transition metal complexes containing Schiff base as ligands are used as artificial nucleases and proved to be efficient DNA cleavage reagents.²¹

The cationic nature and possibility to tune the redox potential with different ligands, the interaction of transition metal complexes with mercaptopropionic acid (MPA) the reducing agent, generates reactive oxygen species that finally induce the cleavage of DNA.²² The DNA cleavage typically proceeds via oxidative or hydrolytic pathway. The hydrolytic cleavage involves phosphodiester bond hydrolysis which leads to the formation of fragments through enzymatic processes.



Further, in continuation of our work on transition metal complexes with Schiff base ligands,²³⁻²⁴ metal complexes have been synthesized, characterized and evaluated the biological activities. In the present communication the synthesis, characterization of Schiff base metal complexes and its biological activities have been presented. A literature survey revealed that no work has been done on the condensation of 2-amino pyridine with 5-chlorosalicylaldehyde. Hence, in this communication, the synthesis, characterization, antimicrobial and DNA studies of transition metal complexes containing Schiff base derived from 2-Amino pyridine with 5-chlorosalicylaldehyde is described.

MATERIALS AND METHODS

All reagents were of AR grade and used as received. Solvents used for spectroscopic studies were purified by standard procedures.²⁵ The super coiled (SC) pUC 19 DNA (CsCl purified) was purchased from Bangalore Genei, (India). Agarose (Molecular biology Grade), ethidium bromide (EB) were obtained from Sigma (USA). Tris (hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer was prepared by doubly distilled water.

The elemental analysis were carried out using Vario-micro CHNS15106062 analyser. ¹H NMR and ESI-MS data of the compounds were recorded using Bruker DSX 300 MHz Solid-state NMR spectrometer and MicroTOF LC BrukerDaltonics spectrometer. IR spectral studies were carried out by Shimadzu spectrophotometer from 4000-400 cm⁻¹ using KBr pellets.

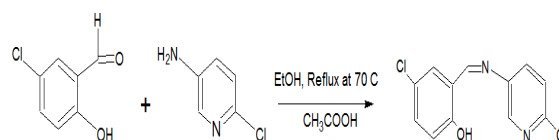
UV-Visible spectra were recorded on Shimadzu UV-3101 PC spectrophotometer using Dimethylformamide as solvent. The X-ray diffraction data were obtained using 'Bruker APEX-II CCD' diffractometer.

The molar conductance of the complexes was measured using Equiptronics digital conductivity meter no. EQ-660A and melting points were checked by melting point apparatus used in laboratories. Cyclic voltammetric experiments were performed using CHI 600E electrochemical instruments.

Synthesis

Preparation of the Schiff base 4-chloro-2-(pyridine-3-yliminomethyl)phenol-(4-CPP).

The Schiff base ligand 4-chloro-2-(pyridine-3-yliminomethyl) phenol was synthesized by the addition of 2-hydroxy-5-chlorobenzaldehyde/5-chlorosalicylaldehyde (1mmol) in ethanol to ethanolic solution of 2-chloro-5-aminopyridine (1mmol) was refluxed at 45 °C for about 7-8 hrs. The reaction was monitored by TLC and the yellowish brown needle like crystals were collected on cooling. The solid was washed with ethanol and then with diethyl ether and finally dried under vacuum. Crystals obtained were of good yield and suitable for X-ray diffraction studies.

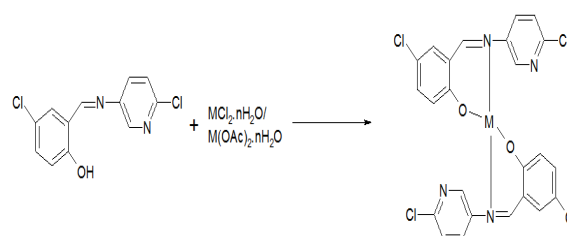


5-chlorosalicylaldehyde 2-chloro-5-aminopyridine 4-CPP

Scheme 1:(a)Preparation of Schiff base ligand 4-chloro-2-(pyridine-3-yliminomethyl) phenol (4-CPP).

Preparation of bis[4-chloro-2-(pyridine-3-yliminomethyl)phenol]Cu(II) and Ni(II) complexes (ML₂) where L is 4-CPP.

The bis ligand complexes were prepared by the reaction of methanolic solution of metal acetate (1 mmol) with 4-CPP dissolved in 1:2 ratio acetonitrile and methanol under stirred condition at room temperature and monitored by TLC. The resulting precipitate was filtered off, washed with methanol and air dried and purified by dissolution in DMF.



Scheme 1: (b) Preparation of bis[4-chloro-2-(pyridine-3-yliminomethyl) phenol] Cu(II) and Ni(II) complexes (ML₂) where L is 4-CPP.

Antimicrobial studies

The antimicrobial activity of the synthesized complexes and ligands were evaluated against pathogenic bacterial strains like *Bacillus mycoides*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The test organisms were maintained on nutrient Agar slants. The reported procedure²⁶⁻²⁸ of agar well diffusion method was followed to determine the antibacterial activity. The bacterial culture was centrifuged at 8000 rpm for 10 mins and suspended in saline to obtain a suspension of 10⁵ CFU per ml and used for the assay. The bacterial suspension was transferred to sterile petri plate and mixed with molten nutrient agar medium and allowed to solidify. About 2mg/ml concentrated solution of the sample (75 μl) was added to well and incubated at 37 °C. The diameter of the inhibition zone determines the antibacterial activity.

DNA Cleavage Studies

The cleavage of SC pUC19 DNA by the ligand and its complexes (ML₂) were studied by agarose gel electrophoresis. 3-mercaptopropionic acid (MPA) (5 mmol) served as the reducing agent for the chemical nuclease activity. The reactions were carried out under dark conditions at 25 °C. Agarose gel electrophoresis was done to determine the extent of cleavage of SCDNA (0.2μg) in 50 mmol Tris-HCl buffer (pH 7.2) containing 50

mmolNaCl treated with the ligand and metal complexes. The concentrations of the ligands/complexes and the additives in the buffer were diluted to the final volume of 2 μ l using Tris-HCl buffer. The SC pUC19 DNA samples were incubated at 37 °C for duration of 1 hr followed by addition of loading buffer containing 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (2 μ l) and solution was finally loaded on to agarose gel (0.8%) containing ethidium bromide(1 μ g/ml). Electrophoresis was carried out in a dark room for 2 hrs at 60V inTris-acetate EDTA buffer (TAE). UV light was used for visualization of the bands that were photographed. The extent of DNA cleavage was measured from the intensities of the bands using the UVTEC gel Documentation system. Due corrections were made for the low level of the 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.²⁹

Studies on DNA interaction

The UV absorbance at 260 and 280 nm of the CT-DNA solutions in 5 mM Tris-HCl buffer (pH 7.2) gave a ratio of 1.9, indicating that the DNA was free from protein.³⁰ The concentration of CT-DNA was measured from band intensity at 260 nm with a known ϵ value (6600 cm⁻¹).³¹ Absorption titration measurements were done by varying the concentrations of the CT DNA, keeping the metal complex concentration constant in 5 mM Tris-HCl/5mM NaCl buffer. Samples were kept for equilibrium before recording each spectrum.

The intrinsic binding constant (K_b) for the interaction of the complexes with CT DNA were obtained from the absorption spectral titration data using the following equation:

$$(\epsilon_a - \epsilon_f) / (\epsilon_a - \epsilon_f) = (b - (b^2 - 2K_b^2 C_t [DNA] / s)^{1/2}) / 2K_b C_t (1)$$

Where $b = 1 + K_b C_t + K_b [DNA] / 2S$; ϵ_a the extinction coefficient observed for the charge transfer absorption band at a given DNA concentration; ϵ_f , the extinction coefficient of the complex free in solution; ϵ_b , the extinction coefficient of the complex when fully bound to DNA; K_b , the equilibrium binding constant; C_t , the total metal complex concentration; $[DNA]$, the DNA concentration in nucleotides and s , the binding site size in base pairs.³²

Cyclic voltammetric studies

Cyclic voltammetric experiments were performed at room temperature in water: DMF under oxygen free conditions created by purging pure nitrogen gas with CHI 600E electrochemical instruments. A three electrode system was used: a glassy carbon working electrode, an Ag⁺/AgCl reference electrode and Pt wire counter electrode. The working electrode was polished with 1.0, 0.3, 0.05 μ m alumina prior to each experiment. Through the experiment oxygen-free nitrogen was bubbled through the solution for ten minutes. Voltammetric experiments were performed at room temperature.

RESULTS AND DISCUSSION

Synthesis and General Aspects

A set of two different novel Cu(II) and Ni(II) complexes were prepared with high yield and the analytical data for the complexes indicate ML_n stoichiometry for all the complexes where L=4-CPP, M= Cu and Ni and n=2. The melting points of all complexes are above 300 °C, the complexes are stable in air. All the complexes are insoluble in common organic solvents and soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO). The molar conductance of Cu complex in DMF (10⁻³M) solution, fall in the range of 11 ohm⁻¹cm²mol⁻¹, indicating the non-electrolytic nature of complex, i.e. the anions are coordinated to the metal ions.³⁴

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

Compounds (Formula)	Mol. Mass	^a Mol Cond	^b ΔE_p (V)	MP °C	N%		C%		H%	
					Exp	Obt	Exp	Obt	Exp	Obt
4-CPP (L) (C ₁₂ H ₈ Cl ₂ N ₂ O)	267.1	---	---	150	10.49	10.85	53.96	53.80	3.02	3.37
CuL ₂ (1) (C ₂₄ H ₁₈ Cl ₄ N ₄ O ₂ Cu)	599.8	11.56	0.386	306	9.34	9.28	48.06	48.3	3.02	3.19
NiL ₂ (2) (C ₂₄ H ₁₈ Cl ₄ N ₄ O ₂ Ni)	594.9	---	---	326	9.32	9.24	48.45	48.26	3.05	3.24

^aMoar Conductance = Λ_M (Ω^{-1} cm²M⁻¹) in DMF at 25 OC, ^bCyclicvoltametry, Cu(II)/Cu(I) couple in DMF-0.1M KCl, $\Delta E_p = E_{p_a} - E_{p_c}$ are the anodic and cathodic peak potentials, respectively. Scan rate = 0.1 mV.

IR Spectra

IR spectra usually provide a lot of information on coordination reactions.

The IR spectra for our studied complexes give information about the coordination of the ligand to metal.

The IR spectrum of the ligand indicate that the $\nu(C=N)$ band of ligand at 1616 cm⁻¹ is due to the azomethine

linkage which were shifted towards lower frequency 1604 cm⁻¹, indicating that the ligand coordinate to metal ions via the azomethine nitrogen.

The absence of peak due to the phenolic OH group at 3410cm⁻¹ suggests the coordination of the ligand to the metal via deprotonation which infers that azomethine-nitrogen and phenolic oxygen are the coordination sites of the bidentate ligand.



Electronic spectra

The UV-Vis electronic spectra of the free ligand L and the complexes were measured in DMF at room temperature over 200-800nm range and presented in Figure 1 (a) and (b). The peaks appear at 251 and 273nm attributed to $\pi-\pi^*$ of phenyl ring transitions and a peak attributed to the azomethine group transition is observed at 330 nm. In the spectra of the complexes, the bands of the azomethine chromophore bands are shifted and indicated that the imine nitrogen atom is involved in coordination to the metal ion.

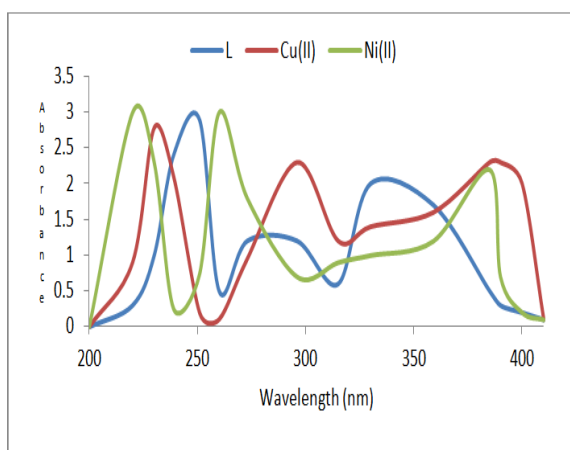


Figure 1(a): The Electronic spectrum of ligand L and its Cu(II), Ni(II) complexes.

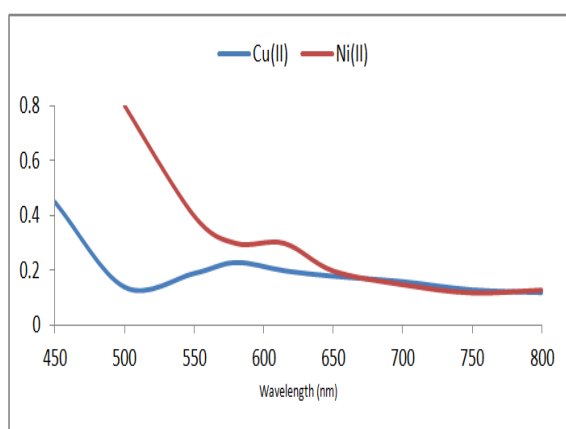


Figure 1(b): The d-d bands of complexes Cu(II) and Ni(II) complexes.

¹H-NMR spectra

Further, evidence for the coordinating mode of the ligand is obtained by the ¹H-NMR studies. ¹H-NMR spectra data is recorded in CDCl₃. The ligand is characterized by 8 signals at 12.51(S), 8.56(S), 8.34 (S), 6.92-7.9 (M) which are assigned to -OH, -N=CH-, =N-CH- and aromatic protons respectively (Figure 1 (c)). The presence of -N=CH- proton signal at 8.56 in the ligand (L) confirms the formation of condensation of 5-chlorosalicylaldehyde with 2-chloro-5-aminopyridine. In the ¹H NMR spectra of complexes (1-2), the absence of proton at 12.51 indicating that phenolic proton is absent in complexes. This information suggest the adjustment of electronic

current upon coordination of >C=O group to the metal ions.

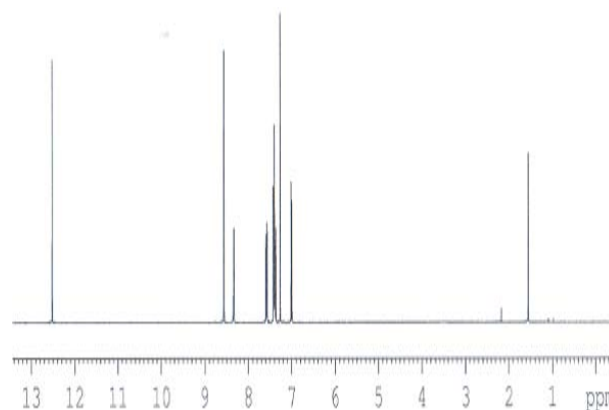


Figure 1(c): ¹H -NMR spectrum of 4-CPP.

Mass Spectra

The ESI-MS of Schiff base ligand and their complexes showed molecular ion peaks which were in perfect agreement with their molecular formula. The molecular ion peak for the ligand 4-CPP(C₁₂H₈Cl₂N₂O) with the monoisotopic mass corresponds to 267.8 m/z in +ve mode of ESI (Figure 1(d)) and its complexes CuCl₂(C₂₄H₁₈C₁₄N₄O₂Cu) and NiL₂(C₂₄H₁₈C₁₄N₄O₂Ni) are at 599 and 594 m/z in ESI -ve mode, respectively.

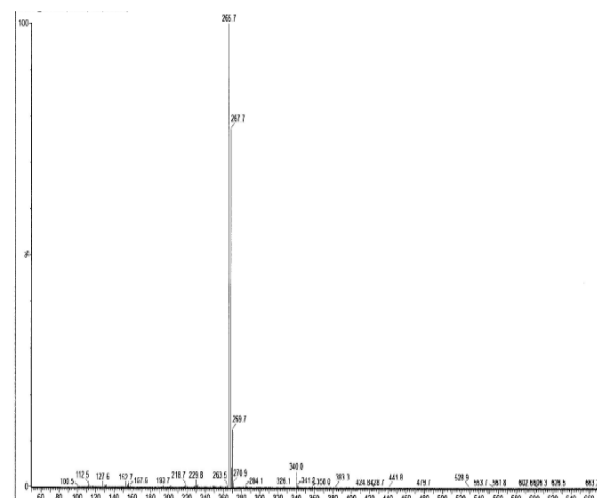


Figure 1(d): Mass spectrum of 4-CPP.

Crystal studies of 4-CPP

The ligand 4-CPP was structurally characterized using single crystal X-ray diffraction technique. The ORTEP diagram is presented in Figure 2. Crystallographic data are presented in Table 2.

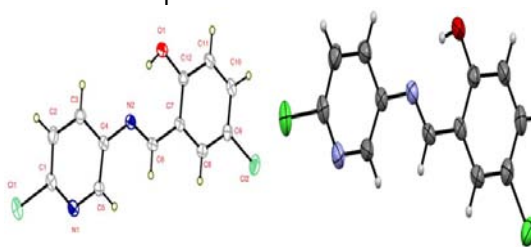


Figure 2: The ORTEP and ball and stick view of the ligand.

Table 2: Single crystal data parameter for 4-CPP

Crystal Data	4-CPP
Formula unit	C ₁₂ H ₈ Cl ₂ N ₂ O
Formula wt.	267.12
Crystal system	Monoclinic
T [K]	296
a [Å]	21.2826(9)
b [Å]	4.5534(2)
c [Å]	11.9954(6)
α [°]	90.00
β [°]	103.086(3)
γ [°]	90.00
Volume [Å ³]	1132.27
Space group	P2 ₁ /c
Z	4
D _{calc} [g cm ⁻³]	1.5668
μ/mm ⁻¹	0.555
Reflns. Collected	8794
Unique reflns.	2235
Observed reflns.	1712
R ₁ [I>2σ(I)], wR ₂	0.0371; 0.0774
GOF	1.037
Instrument	Bruker APEX-II
X-ray	MoKα; λ=0.71073
CCDC Reference No.	1413619

Cyclic voltammetry

The copper complex (0.001M in DMF) was scanned in the potential range of -1.0 V to 1.0V in deaerated condition with scan rate 0.1V/s. The numerical results with scan rate 0.1v/s are given in Table 1. A cathodic peak observed in the voltammograms in the range E_{pc} = 0.15 to 0.07 V evidences the reduction of metallic species, Cu(II) to Cu(I). The reverse scan shows two anodic peaks with potentials in the range E_{pa1} = -0.1 to -0.5V and E_{pa2} = 0.35 to 0.68V corresponding to the oxidation reactions, Cu(I) to Cu(II) and Cu(II) to Cu(III). The high value of ΔE_p, separation

between the cathodic and anodic peak potentials for the couple Cu(I)/Cu(II) which is greater than 60mV indicate the quasi-reversible nature of the redox process.

Antibacterial activity

The *in-vitro* antibacterial activity of the Schiff base, solvent (DMSO) and their Cu(II) and Ni(II) complexes were evaluated against the Gram positive bacteria's-*Bacillus mycoides*, *Bacillus subtilis*, *Micrococcus luteus*, *Proteus mirabilis* and gram negative bacteria's *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. *Escherichia coli* and Table 3 illustrates the antibacterial activity of the compounds. DMSO (blank) and Ampicillin were used as controls. In general, the activity against gram negative bacteria is higher than those of gram positive bacteria. This could be attributed to the greater lipophilic nature³⁵ of the Schiff bases than their metal complexes. Cu(II) complex exhibited antibacterial activity against, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* and whereas Ni(II) complex showed the activity against *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. The factors that govern antibacterial activities are strongly dependent on the central metal ion and the coordination numbers and also due to the presence of nitrogen and sulfur donor groups.³⁶⁻³⁷

DNA Cleavage Studies

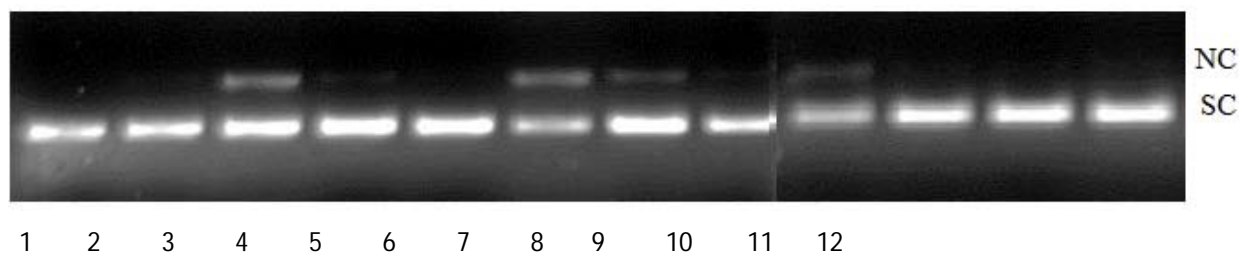
The oxidative DNA cleavage activity of the complexes in the presence of reducing agent 3-mercaptopropionic acid (MPA, 5mM) is investigated by agarose gel electrophoresis using super coiled (SC) plasmid pUC19 DNA (0.2μg, 3.33μM NP) in 50 mMTris-HCl/50 mMNaCl buffer (pH, 7.2.)³⁸ Selected DNA cleavage data are given in the Table 4. The complexes Cu(II) and Ni(II) shows efficient "chemical nuclease" activity. Control experiments with MPA or the complex alone do not show any apparent conversion of SC to its nicked-circular (NC) form. The maximum cleavage was exhibited by Cu(II) complex at 40 μm concentration (Figure 3). The DNA cleavage of complexes in the presence of MPA probably proceeds through the hydroxyl radical pathway as proposed by Sigman.³⁹

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

	Bacteria	4-CPP	CuL ₂	NiL ₂
Gram Positive	<i>Bacillus mycoides</i> (MTCC 645)	12	--	--
	<i>Bacillus subtilis</i> (MTCC 441)	13	10	--
	<i>Micrococcus luteus</i> (MTCC 106)	12	--	--
	<i>Proteus mirabilis</i> (MTCC 743)	13	10	11
Gram Negative	<i>Pseudomonas aeruginosa</i> (MTCC 741)	17	18	15
	<i>Yersinia enterocolitica</i> (MTCC 4848)	15	11	14
	<i>Escherichia coli</i> (MTCC 443)	14	10	14

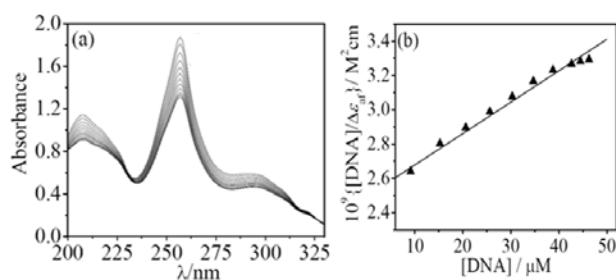
Table 4: Selected cleavage data of SC pUC19 by ligand and its Cu (II) and Ni (II) complexes.

Lane no	Complex	^a NC%	Lane no	Complex	^a NC%
1	DNA control	0	7	DNA+ H ₂ O ₂ + Cu(II) complex (40μm)	27
2	DNA+ L (40μm)	7	8	DNA+Ni(II) complex(40μm)	4
3	DNA+MPA+L(40μm)	35	9	DNA+MPA+Ni(II) complex(40μm)	32
4	DNA+H ₂ O ₂ +L(40μm)	6	10	DNA+ H ₂ O ₂ +Ni(II) complex (40μm)	9
5	DNA+Cu(II) complex (40μm)	5	11	DNA+MPA	2
6	DNA+MPA+ Cu(II) complex (40μm)	61	12	DNA+ H ₂ O ₂	3

**Figure 3:** Gel electrophoresis diagram showing the cleavage of SC pUC DNA (0.2 μg, 3.33μM) by ligand and its complexes (30 μM) in 50 mMNaCl buffer (pH 7.2) in the presence of MPA (500 μM). Reaction details are given in Table 5 in which entry number corresponds to the lane number of the Figure.

DNA Binding Studies

DNA binding mode and affinity are influenced by factors such as the metal ion type and its flexible valency⁴⁰ the coordination geometry, the ligand donor atom type⁴¹ and planarity of ligands.⁴² The UV binding of the complexes to the calf thymus (CT) DNA has been studied by the electronic absorption spectral techniques. The intrinsic binding constant (K_b) value was $3.80 (\pm 0.04) \times 10^6 \text{ M}^{-1}$ for Cu(II) complex (Figure 4(b)). The Ni(II) complex didn't show binding affinity to calf thymus (CT) DNA due to the change in metal ion. The experimental value of K_b indicates that the Cu (II) complex binds to DNA via intercalative mode.

**Figure 4:** (a) Absorption spectral traces on addition of CT-DNA to the solution of Cu(L)₂

(b) The best least squares fit of $\Delta\epsilon_{af}/\Delta\epsilon_{bf}$ vs [DNA]

CONCLUSION

We have described the preparation, characterization and biological activities of novel asymmetrical Schiff base ligands and their Cu(II) and Ni(II) metal complexes. The structural properties of ligands and their metal complexes were proposed based on the elemental analysis, FTIR, UV-Vis, ¹H NMR and ESI mass spectra the ligand was structurally characterized by X-ray crystallography. The outcome of the results confirms the stoichiometry of the

complexes to be 1:2 (M:L) as indicated by elemental analysis. This has been further confirmed by Mass spectra studies.

The complexes were found to have biological activities compared to the respective ligand. The DNA interactions of the prepared complexes were evaluated by absorption method and results showed the binding affinity of the complexes to DNA.

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