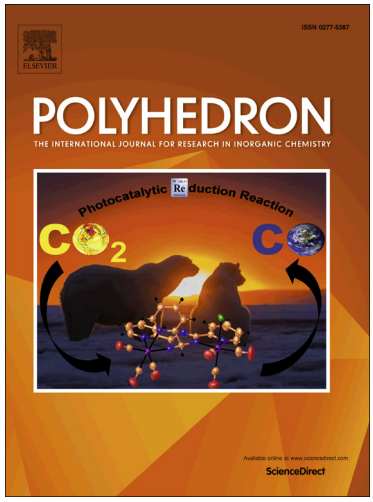


Synthesis, Crystal structure, DNA binding and cleavage studies of Copper(II) complexes with Isoxazole Schiff bases

Marri Pradeep Kumar, Somapangu Tejaswi, Aveli Rambabu, Veerendra Kumar A. Kalalbandi, Shivaraj

PII: S0277-5387(15)00407-6
DOI: http://dx.doi.org/10.1016/j.poly.2015.07.052
Reference: POLY 11438

To appear in: *Polyhedron*

Received Date: 13 April 2015
Accepted Date: 20 July 2015

Please cite this article as: M.P. Kumar, S. Tejaswi, A. Rambabu, V.K.A. Kalalbandi, Shivaraj, Synthesis, Crystal structure, DNA binding and cleavage studies of Copper(II) complexes with Isoxazole Schiff bases, *Polyhedron* (2015), doi: http://dx.doi.org/10.1016/j.poly.2015.07.052

Synthesis, Crystal structure, DNA binding and cleavage studies of Copper(II) complexes with Isoxazole Schiff bases

Marri Pradeep Kumar[a], Somapangu Tejaswi[a], Aveli Rambabu[a], Veerendra Kumar A. Kalalbandi[b] and Shivaraj[a]*

a) Department of Chemistry, Osmania University, Hyderabad, Telangana-500007, India

b) Department of Chemistry, Karnatak University, Dharwad-580003, India

Email: shivaraj_sunny@yahoo.co.in Mobile No: +91 7842572128

Abstract:

Three novel binary copper(II) complexes **1** $[Cu(L^1)_2]$, **2** $[Cu(L^2)_2]$ and **3** $[Cu(L^3)_2]$ where, L^1 (1-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)naphthalen-2-ol, $C_{16}H_{14}N_2O_2$), L^2 (2-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)-4-methoxyphenol, $C_{13}H_{14}N_2O_3$) and L^3 (2-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)-4-bromophenol, $C_{12}H_{11}BrN_2O_2$) have been synthesized. All Cu(II) complexes have been characterized by elemental analysis, FT-IR, ESI mass, UV-Visible, ESR, TG-DTA, magnetic moments and single crystal X-ray diffraction studies. Based on spectral studies square planar geometry is assigned for all Cu(II) complexes. Ligand L^1, $[Cu(L^1)_2]$ and $[Cu(L^2)_2]$ are crystallized and found to be triclinic, orthorhombic and monoclinic crystal systems respectively. The Schiff bases and their Cu(II) complexes have been screened for antibacterial activity and antifungal activity by paper disc method. It is observed that all Cu(II) complexes showed more activity than corresponding Schiff bases. DNA binding and cleavage studies of Cu(II) complexes have also been investigated. It is observed an intercalation mode of binding with CT-DNA and cleavage of supercoiled pBR322 DNA in the presence of H_2O_2 and UV light.

Key words: Schiff base, Binary Cu(II) complex, Crystal structure, Antimicrobial activity, DNA interaction.

1. Introduction

The transition metal complexes of Schiff bases have tendency to interact with DNA because of their cationic character and three dimensional structural profiles. It has been proved that azomethine linkage (C=N) of Schiff base provides an opportunity for the stupendous biological activities such as antitumor, antibacterial, antifungal and herbicidal

activities [1]. Metal complexes can bind to DNA through non-covalent interactions, such as electrostatic binding, groove binding, intercalative binding and partial intercalative binding [2]. Copper as an essential element for human beings with its bio essential activity and oxidative nature has attracted numerous inorganic chemists to address medicinal applications of Cu(II) complexes [3-8]. Copper(II) complexes containing heterocyclic bases have been extensively explored in virtue of their strong interactions with DNA via surface associations or intercalation [9-12] and potential DNA cleavage activities via hydrolytic, photolytic and oxidative mechanisms [13-18]. Square planar complexes containing an aromatic moiety can bind to DNA by intercalation and stabilize the DNA double helix [19, 20]. Synthesis, characterization, biological activity and DNA interaction of Cu(II) complexes of isoxazole Schiff bases were reported earlier from our laboratory [21].

In continuation of our earlier work, herein we report synthesis, characterisation, DNA binding and cleavage studies of copper(II) complexes **1**, **2** and **3**. The antimicrobial property of ligands and their complexes have also been assessed against selected bacteria and fungi.

2. Experimental

2.1. Materials and instrumentation

All reagents used in this study were analytical reagent grade obtained from Merck, Hi Media Ltd., and Sigma-Aldrich Chemicals. Solvents such as water, methanol, acetone, petroleum ether and chloroform were purified by standard procedures. The CT-DNA and supercoiled pBR322 DNA were purchased from Genie, Bangalore and stored at 4^{o}C. Double distilled water was used for preparing all the solutions for the DNA binding and cleavage studies. A Tris-buffer solution of CT-DNA gave a ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [22]. The DNA concentration per nucleotide in base pairs was determined spectrophotometrically by employing a molar absorptivity (6600 $M^{-1}cm^{-1}$) at 260 nm [23]. CT-DNA stock solution was prepared by diluting DNA to Tris–HCl/NaCl buffer (pH = 7.2, 5 mM Tris–HCl, 50 mM NaCl).

^{1}H-NMR and ^{13}C-NMR spectra of the Schiff bases were recorded on Bruker 400 MHz NMR instrument using TMS as internal standard. ESI mass spectra were recorded on VG AUTOSPEC mass spectrometer. Electronic spectra of all compounds were recorded on Schimadzu UV-VIS 1601 spectrophotometer. Magnetic susceptibilities of Cu(II) complexes were determined on Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard. The diamagnetic corrections of the complexes were computed using Pascal's constants. Melting

points of the ligands and decomposition temperature of complexes were determined on Polmon instrument (Model No. MP-96). IR spectra of the compounds were recorded using KBr pellets in the range 4000-400 cm^{-1} on Perkin-Elmer Infrared model 337. The percentage composition of C, H, N of the compounds were determined by using micro analytical techniques on Perkin Elmer 240C (USA) elemental analyzer. Copper content of the complexes was estimated by atomic absorption spectroscopy after decomposing the complexes with concentrated HNO_3 using GBC Avanta 1.0 AAS. The ESR spectra were recorded by using JES-FA200 ESR Spectrometer (JEOL-Japan) at liquid nitrogen temperature. The thermal studies were carried out in a dynamic nitrogen atmosphere (20ml min^{-1}) with a heating rate of 10^oC min^{-1} using a Shimadzu TGA-50H in the temperature range of 27-1000^oC. The high resolution X-ray diffraction data for compounds were collected on Bruker SMART APEX2 CCD diffractometer using Mo Kα radiation (λ=0.71073Å) at 296K. Viscosity measurements were performed using an Ostwald viscometer (Vensil).

2.2. Conventional and Microwave synthesis of Schiff bases

The synthetic route of Schiff bases L^1, L^2 and L^3 were described in **Scheme 1**. 3, 5-dimethyl-4-amino- isoxazole (1.0 mmol) was dissolved in hot methanol to which methanolic solution of 2-hydroxy naphthalene-1-carbaldehyde / 2-hydroxy-5-methoxy benzaldehyde / 2-hydroxy-5-bromo benzaldehyde (1.0 mmol) was added and the resulting mixture was refluxed with stirring at 60-80^oC for 3 hours. Microwave assisted synthesis was also carried out using few drops of methanol in a domestic oven (LG, 1300W, 28L capacity). The reaction mixture was subjected to microwave irradiation at 320W for the period of 60 to 90 sec. The dark yellow product formed was washed with petroleum ether and recrystallized from methanol. Purity of the compounds was checked by TLC. Microwave assisted synthesis brought down the reaction time from 3 hours to 90 sec and improved the yield from 70–75 to 90–95% in comparison with the conventional method. The suitable crystals for single crystal X-ray study were grown in (1:1) methanol and dichloromethane mixture.

2.2.1. ***Ligand*** *L^1*: Yield 75% and 95%. **M.P**: 170^oC. **Mol Wt**: 266. *Anal.* Calc. ($C_{16}H_{14}N_2O_2$)(%): C, 72.16; H, 5.30; N, 10.52. Found: C, 72.32; H, 5.39; N, 10.45. **IR (KBr)**(cm^{-1}): $\upsilon_{(OH)}$ 3454; $\upsilon_{(CH=N)}$ 1603; $\upsilon_{(C-O)}$ 1184. **UV ($CHCl_3$)** λ_{max}/nm(cm^{-1}): 374 (26738); 330 (30303); 246 (40650). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ = 14.65 (s, 1 H), 9.46 (s, 1 H), 8.04 (d, J = 8.4 Hz, 1 H), 7.87 (d, J = 9.0 Hz, 1 H), 7.80 (d, J = 8.1 Hz, 1 H), 7.58-7.55 (m, 1 H), 7.41-7.38 (m, 1 H), 7.20 (d, J = 9.0 Hz, 1 H), 2.56 (s, 3 H), 2.47 (s, 3 H), **Fig. S1** (see

Supplementary). **^{13}C NMR (400 MHz, $CDCl_3$)**: δ = 163.3, 159.8, 159.4, 154.8, 135.3, 132.5, 129.4, 128.1, 127.9, 125.6, 123.7, 119.8, 119.0, 109.4, 11.4, 10.8. **MS (ESI):** *m/z* = 266.9 $[M+H]^+$.

2.2.2. ***Ligand*** *L^2*: Yield 73% and 91%. **M.P**: 153°C. **Mol Wt**: 246. *Anal.* Calc. ($C_{13}H_{14}N_2O_3$)(%): C, 63.40; H, 5.73; N, 11.38. Found: C, 63.54; H, 5.69; N, 11.45. **IR (KBr)**(cm^{-1}): $\upsilon_{(OH)}$ 3443; $\upsilon_{(CH=N)}$ 1583; $\upsilon_{(C-O)}$ 1043. **UV ($CHCl_3$)** λ_{max}/nm(cm^{-1}): 368 (27174); 302 (33113); 252 (39683). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ = 12.27 (s, 1 H), 8.55 (s, 1 H), 7.02-6.94 (m, 2 H), 6.83 (d, J = 3.0 Hz, 1 H), 3.81 (s, 3 H), 2.50 (s, 3 H), 2.40 (s, 3 H), **Fig. S2** (see Supplementary). **^{13}C NMR (400 MHz, $CDCl_3$)**: δ = 162.7, 160.3, 154.9, 154.7, 152.4, 125.3, 120.5, 118.7, 118.1, 115.0, 55.9, 11.3, 10.8. **MS (ESI):** *m/z* = 247.0 $[M+H]^+$.

2.2.3. ***Ligand*** *L^3*: Yield 70% and 90%. **M.P**: 161°C. **Mol Wt**: 295. *Anal.* Calc. ($C_{16}H_{14}N_2O_2$)(%): C, 48.84; H, 3.76; N, 9.49. Found: C, 48.96; H, 3.65; N, 9.58. **IR (KBr)**(cm^{-1}): $\upsilon_{(OH)}$ 3412; $\upsilon_{(CH=N)}$ 1605; $\upsilon_{(C-O)}$ 1230. **UV ($CHCl_3$)** λ_{max}/nm(cm^{-1}): 352 (28409); 301 (33223); 248 (40323). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ = 12.71 (s, 1 H), 8.52 (s, 1 H), 7.46-7.43 (m, 2 H), 6.91 (d, J = 8.28 Hz, 1 H), 2.51 (s, 3 H), 2.41 (s, 3 H), **Fig. S3** (see Supplementary). **^{13}C NMR (400 MHz, $CDCl_3$)**: δ = 161.3, 160.9, 159.6, 154.6, 135.8, 134.0, 125.0, 120.5, 119.3, 110.8, 11.4, 11.0. **MS (ESI):** *m/z* = 297.0 $[M+2]^+$.

2.3. Synthesis of binary Cu(II) complexes

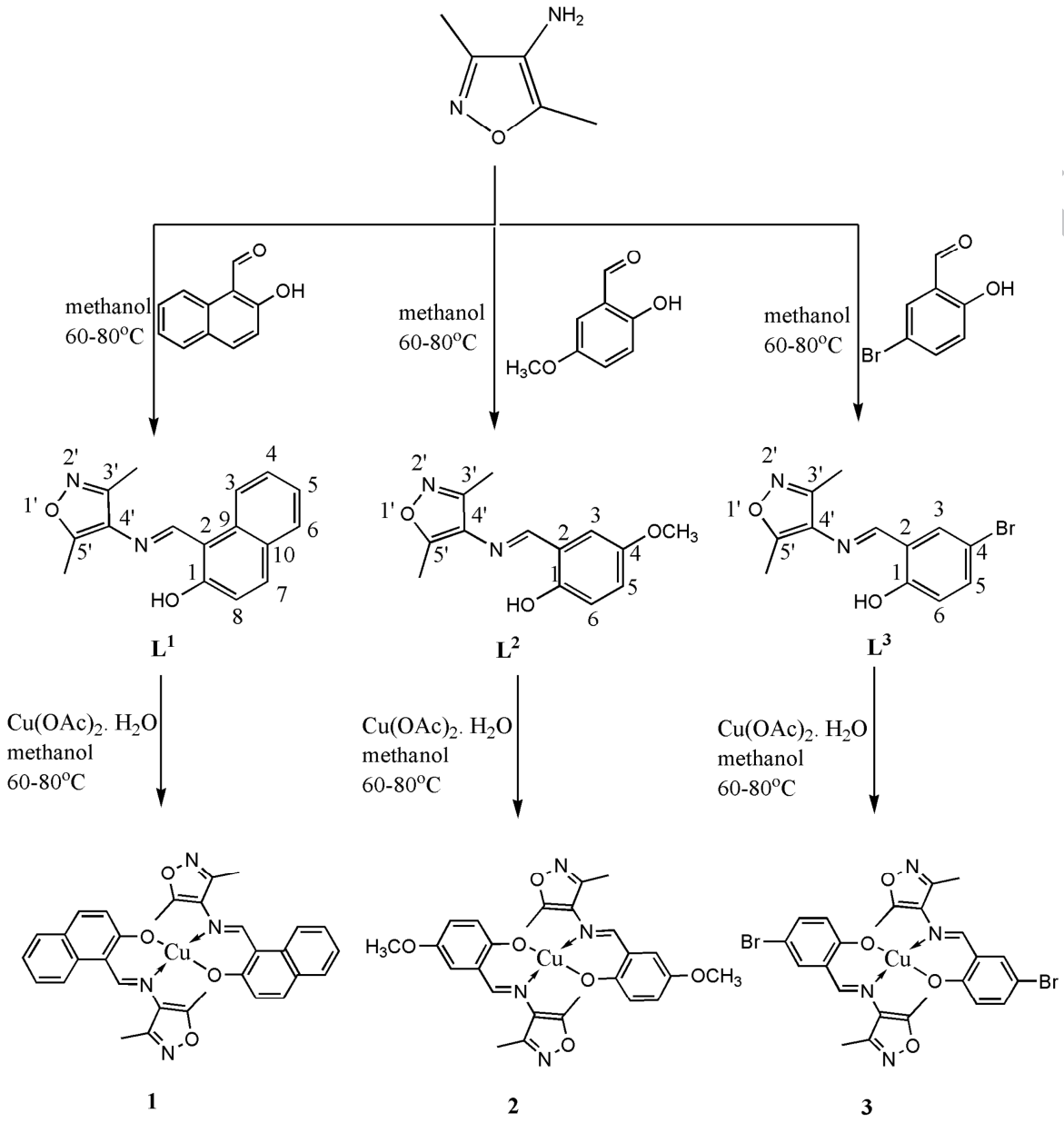
The preparation of all Cu(II) complexes **1-3** were described in **scheme. 1**.

*2.3.1. $[Cu(L^1)_2]$(**1**)*: Hot methanolic solution of $Cu(CH_3COO)_2.H_2O$ (0.5 mmol) (10 mL) was added drop wise to a solution of L^1 (1 mmol) in hot methanol (20 mL) and the reaction mixture was refluxed with stirring at 60-80°C for 5 hours. A dark colored solid product obtained was filtered and washed with cold methanol and petroleum ether, dried in vacuum desiccator over P_4O_{10}. Yield 80%. **M.P**: 208°C. **Mol Wt**: 594. *Anal.* Calc. ($C_{32}H_{26}CuN_4O_4$)(%): C, 64.69; H, 4.41; N, 9.43; Cu, 10.70. Found: C, 64.65; H, 4.44; N, 9.39; Cu, 10.67. **IR (KBr)**(cm^{-1}): $\upsilon_{(C=N)}$ 1577, $\upsilon_{(C-O)}$ 1168, $\upsilon_{(M-O)}$ 541, $\upsilon_{(M-N)}$ 464. **ESR**: $g_{\parallel}$=2.2935, $g_{\perp}$=2.0619, G=4.885. **UV-Vis (DMSO)** λ_{max}/nm(cm^{-1}): 223 (44843), 308 (32468), 416 (24038), ε ($M^{-1}cm^{-1}$) (0.013×10^6). **μ_{eff}**(BM):1.82. **Mass**(m/z): 612.0 $[M+NH_4]^+$.

*2.3.2. $[Cu(L^2)_2]$(**2**):* Following the same procedure as described in synthesis of **1** received complex **2** with L^2. Yield 78%. **M.P**: 205°C. **Mol Wt**: 554. *Anal.* Calc. ($C_{26}H_{26}CuN_4O_6$)(%):

C, 56.36; H, 4.73; N, 10.11; Cu, 11.47. Found: C, 56.32; H, 4.75; N, 10.08; Cu, 11.49. **IR (KBr)**(cm^{-1}): $\upsilon_{(C=N)}$ 1533, $\upsilon_{(C-O)}$ 1033, $\upsilon_{(M-O)}$ 559, $\upsilon_{(M-N)}$ 459. **ESR**: $g_{\parallel}$=2.2412, $g_{\perp}$=2.0532, G=4.6935. **UV-Vis (DMSO)** λ_{max}/nm(cm^{-1}): 222 (45045), 296 (33784), 356 (28090), ε ($M^{-1}cm^{-1}$) (0.015×10^{6}). μ_{eff}(BM):1.74. **Mass**(m/z)**:** 554.1 $[M]^{+}$.

*2.3.3. [Cu(L^3)$_2$](**3**):* Following the same procedure as described in synthesis of **1** received complex **3** with L^3. Yield 75%. **M.P**: 197°C. **Mol Wt**: 652. *Anal.* Calc. ($C_{24}H_{20}Br_2CuN_4O_4$)(%): C, 44.23; H, 3.09; N, 8.60; Cu, 9.75. Found: C, 44.25; H, 3.06; N, 8.66; Cu, 9.71. **IR (KBr)**(cm^{-1}): $\upsilon_{(C=N)}$ 1600, $\upsilon_{(C-O)}$ 1222, $\upsilon_{(M-O)}$ 559, $\upsilon_{(M-N)}$ 462. **ESR**: $g_{\parallel}$=2.2613, $g_{\perp}$=2.0466, G=5.8465. **UV-Vis (DMSO)** λ_{max}/nm(cm^{-1}): 224 (44643), 343 (29155), 399 (25063), ε ($M^{-1}cm^{-1}$) (0.004×10^{6}); μ_{eff}(BM):1.77. **Mass**(m/z): 652.0 $[M]^{+}$.



Scheme. 1. Synthesis of Schiff bases and their copper(II) complexes

2.4. X-ray crystallographic procedures

The single crystal of the size of 0.30x0.20x0.20 mm was selected under a polarizing microscope and it was mounted on a glass fiber for x-ray diffraction data collection. The high resolution X-ray diffraction data sets for both Schiff bases and complexes were collected on Bruker SMART APEX2 CCD diffractometer using Mo Kα radiation (λ = 0.71073 Å) at 296 K. The crystal-to-detector distance was fixed at 40 mm. The diffraction data have been scaled for absorption effect by the multi-scanning method. Data collection has been performed by

applying the apex-II Software system. Structures were solved by direct methods and refined on F^2 by weighted full-matrix least-squares. Programs SHELXS97 and SHELXL97 integrated in the WinGX v. 1.70.01 [24] software system were used to solve and refine the structure. All non-hydrogen atoms were refined anisotropically. The ORTEP, planes and packing diagrams were generated using the Mercury 3.3 software.

2.5. Antimicrobial activity

The in vitro antibacterial activity of the ligands L^1, L^2 and L^3 and their copper(II) complexes **1, 2** and **3** were tested against the gram negative bacteria *Escherichia coli (E.coli), Pseudomonas putida (P.putida)* and *Klebsiella pneumoniae (K.pneumoniae)* and gram positive bacteria *Bacillus subtilis (B.subtilis)* and *Staphylococcus aureus (S.aureus).* The antifungal activity was tested against *Aspergillus niger (A.niger)* and *Candida albicans (C.albicans)*. The paper disc technique was employed [25] for antimicrobial activity using nutrient agar as the medium. The stock solution was prepared by dissolving the 1 mg of sample in 10 mL of DMSO to give the concentration of 100µg/mL. The nutrient agar medium was inoculated with the test organisms. The sterilized blank paper discs of 6 mm diameter were impregnated with tested compounds and placed on the surface of the agar plates previously spread with 0.1 mL of overnight culture of microorganisms. The incubation was carried out for 24 hours at 30°C. During this period, the test solution diffused and the growth of the inoculated microorganisms were affected.

2.6. DNA-binding experiments

2.6.1. Absorption study

Absorption titration experiments were performed with fixed complex concentrations (10 µM) while varying the CT-DNA concentration within 0-10 µM. Because of their low solubility in the buffer solution the copper(II) complexes were dissolved in DMSO first to get a stock solution. While measuring the absorption, equal increments of CT-DNA were added to both the complex solution and the reference solution to eliminate the absorbance of CT-DNA itself.

2.6.2. Viscosity measurements

Viscosity experiments were carried out by using an Ostwald capillary viscometer immersed in a thermostatic water bath with the temperature setting at 30±0.1°C. In this

experiment DNA concentration was kept constant and concentration of complexes solution increased gradually. The flow time of the solution through the capillary was determined by a digital stop-watch. This flow time measurement was repeated three times for the same complexes solution to get the average flow time. To study the effect of DNA binding on the viscosity of the complex, a plot was made between $(\eta/\eta_o)^{1/3}$ and the ratio of the concentration of the complexes to CT-DNA, where η is the viscosity of CT-DNA in the presence of the complex and η_o is the viscosity of CT-DNA alone. Viscosity values were calculated from the experimental flow time of CT-DNA containing solutions corrected from the flow time of buffer alone.

2.7. DNA cleavage experiments

Interactions between all Cu(II) complexes and supercoiled pBR322 DNA were studied using agarose-gel electrophoresis at pH 7.5 in Tris–HCl buffer solution. Oxidative DNA cleavage in the presence of H_2O_2 and photolytic cleavage in the presence of UV light were monitored by treating with supercoiled pBR322 DNA by varying concentration of Cu(II) complexes and followed by dilution with 5mM Tris-HCl / 5mM NaCl buffer to a total volume of 16µL. The samples were incubated at 37°C for 2 hours. DNA cleavage activity was evaluated by monitoring the conversion of supercoiled pBR322 DNA (Form I) to nicked circular DNA (Form II) and linear DNA (Form III).

3. Results and discussion

All the Cu(II) complexes are coloured, stable at room temperature and non-hygroscopic. On heating they decompose at high temperatures. These complexes are insoluble in water, alcohol and chloroform but soluble in acetonitrile, DMF and DMSO. The analytical data obtained for all complexes are consistent with the formation of mononuclear copper complexes and the metal to ligand ratio is 1:2.

3.1. X-ray diffraction analysis

Single crystals of ligand L^1 and complexes **1** and **2** were grown by slow evaporation of methanol and dichloromethane mixture of solvents and their crystal structures were solved and refined by SHELXS-97 [24]. The ORTEP diagrams of the L^1, the mononuclear unit of complexes **1** and **2** are presented in **Fig. 1**. The crystal refinement data is given in **Table. 1**. The L^1 is almost planar as the dimethyl isoxazole ring is not making any significant dihedral

angle with naphthalenol moiety. All the bond lengths and bond angles are observed to be in normal ranges. There is hydrogen bonding between hydrogen atom attached to O1 and N1 of Schiff base. In crystal packing of L^1 it is observed that two molecules were packed in the unit cell.

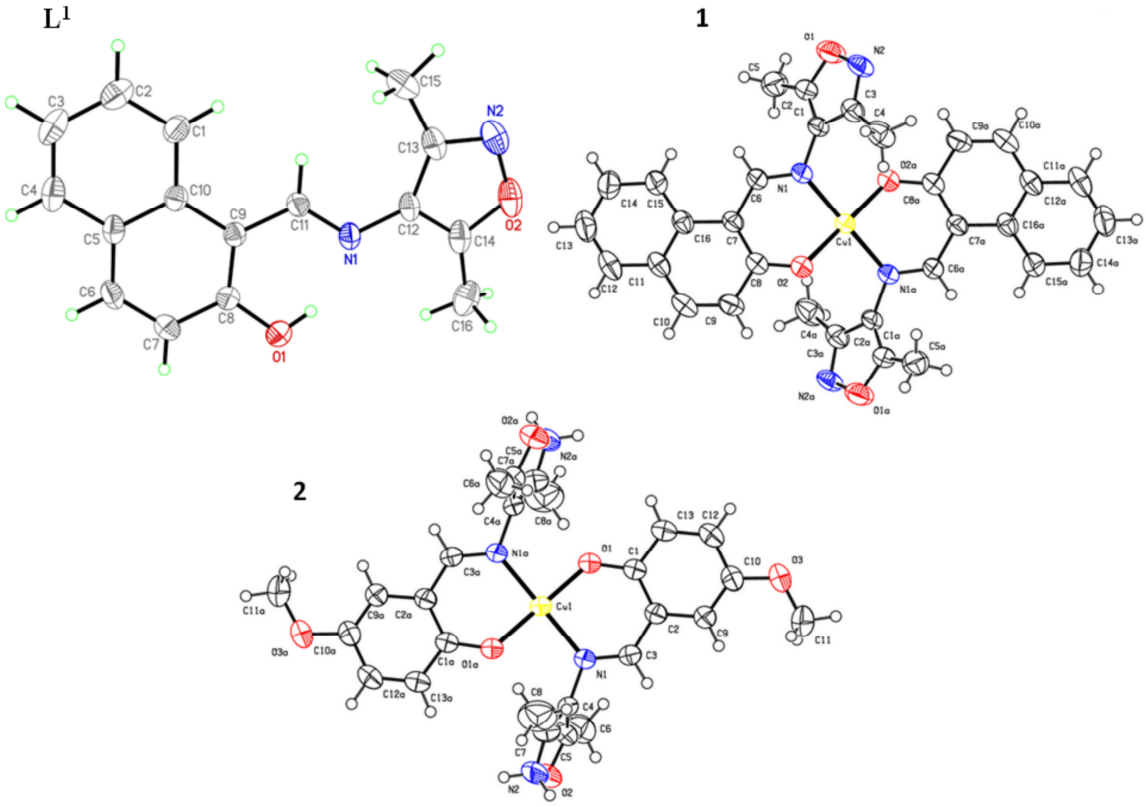


Fig. 1. Thermal ellipsoidal plot of L^1, complexes **1** and **2** with atom labeling scheme. Displacement ellipsoids are drawn at 50% probability level except for the H atoms, which are shown as circles of arbitrary radius.

Table. 1. Crystal structure refinement data of ligand L^1, complexes **1** and **2**.

Parameter	L^1	**1**	**2**
Empirical formula	$C_{16}H_{14}N_2O_2$	$C_{32}H_{26}CuN_4O_4$	$C_{26}H_{26}CuN_4O_6$
Formula weight	266.29	594.12	554.05
Temperature	296K	296K	296K
Crystal size	0.42x0.22x0.18mm	0.30x0.20x0.20 mm	0.30x0.20x0.20 mm
Crystal system	Triclinic	Orthorhombic	Monoclinic

Space group	$P\bar{1}$	Pbca	$P2_1/n$
A	7.5360 (8)(Å)	10.5512 (3)(Å)	10.6396 (7) (Å)
B	9.0395 (11) (Å)	15.2811 (5) (Å)	10.6082 (7) (Å)
C	10.8864 (11) (Å)	16.8042 (6) (Å)	11.9303 (8) (Å)
α	74.071 (10)˚	90˚	90˚
β	86.689 (9)˚	90˚	104.258 (4)˚
γ	69.227 (11)˚	90˚	90˚
Limiting indices	-8<=h<=8, -9<=k<=10, -12<=l<=11	-13<=h<=13, -16<=k<=18, -8<=l<=21	-13<=h<=13, -13<=k<=13, -14<=l<=14
Volume	666.12 (13) ($Å^3$)	2709.41 (15) $Å^3$	1305.06 (15) $Å^3$
Z, Calculated density	2, 1.328 mg/m^3	4, 1.466 mg/m^3	2, 1.425 mg/m^3
Measured reflections	4114	12575	9270
No. of independent reflections	2335	3006	2588
θ range for data collection	θ_{max} = 24.99°, θ_{min} = 2.72°	θ_{max} = 27.31°, θ_{min} = 2.42°	θ_{max} = 27.31°, θ_{min} = 2.42°
Final R indices [I> 2σ(I)]	R1 = 0.0525, wR2 = 0.1246	R1 = 0.0360, wR2 = 0.1052	R1 = 0.0389, wR2 = 0.1036
R indices (all data)	R1 = 0.0964, wR2 = 0.1619	R1 = 0.0530, wR2 = 0.1163	R1 = 0.0542, wR2 = 0.1117
Data / restraints / parameters	2335 / 0 / 184	3006 / 0 / 189	2588 / 0 / 172
Structure Determination	SHELXS-97	SHELXS-97	SHELXS-97
Refinement	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Difference electron density Min/max/rms	-0.189/0.138/0.038	-0.504/0.238/0.050	-0.471/0.232/0.052
CCDC number	CCDC-936590	CCDC 1005830	CCDC 1005831

The crystal units of complexes **1** and **2** have crystallographic inversion centre located through the Cu atom. In both complexes, Cu(II) ion is surrounded by two molecules of respective ligands and each ligand acts as a mono negative bidentate chelating agent. The structures of complexes **1** and **2** reveal that Cu(II) is in a square planar environment having *trans*-N_2O_2 donor set. However, the complex **1** is observed to be slightly distorted from

square planar when compared to that of complex **2** as evidenced by bond angles [N1-Cu1-O2 89.95(7)º (θ_1), O2-Cu1-N1 90.05(7)º (θ_2) for complex **1** and N1-Cu1-O1 88.11(8)º (θ_1), O1-Cu1-N1a 91.89(8)º (θ_2) for complex **2**]. Further, *trans* L-M-L angles θ_1 and θ_2 are equal to 180º in both complexes [26]. The Cu···N distances in complexes **1** and **2** are 1.969(17) Å and 1.988(19) Å respectively while the Cu···O distances in complexes **1** and **2** are 1.895(15) Å and 1.870(17) Å respectively. Upon examination of metal-ligand distances showed that the Cu···N distances in complex **1** are shorter than that in complex **2**, whereas the Cu···O distances in complex **1** are longer than that observed in complex **2**. Further, the Cu···N distances are longer than Cu···O distances in both complexes. Typical Cu···N distances are ranging from 1.939 to 2.019 Å while the Cu···O distances ranging from 1.879 to 1.893 Å [27-30].

From the crystal structure of the ligand L^1 it is evident that the ligand molecule is almost planar, but during complexation the five membered dimethyl isoxazole ring [O1 N2 C2 C3 C1] forms a dihedral angle of 41.64º with the mean plane of naphthalenol moiety [C11 C12 C13 C14 C15 C16] to stabilize the complex structure **Fig. 2**., while in complex **2** both the central metal ion and phenol rings are in the same plane whereas the five membered dimethyl isoxazole ring forms a dihedral angle of 79.20º with the mean plane of the phenol ring to stabilize the complex structure **Fig. 2**. Further, no classical hydrogen bonding is observed in both complexes. However, the molecules of both complexes are stabilized by several weak intermolecular interactions in the solid state, **Table. S1** (see Supplementary). The crystal packing structures of complexes **1** and **2** are shown in **Fig. S4** (see Supplementary).

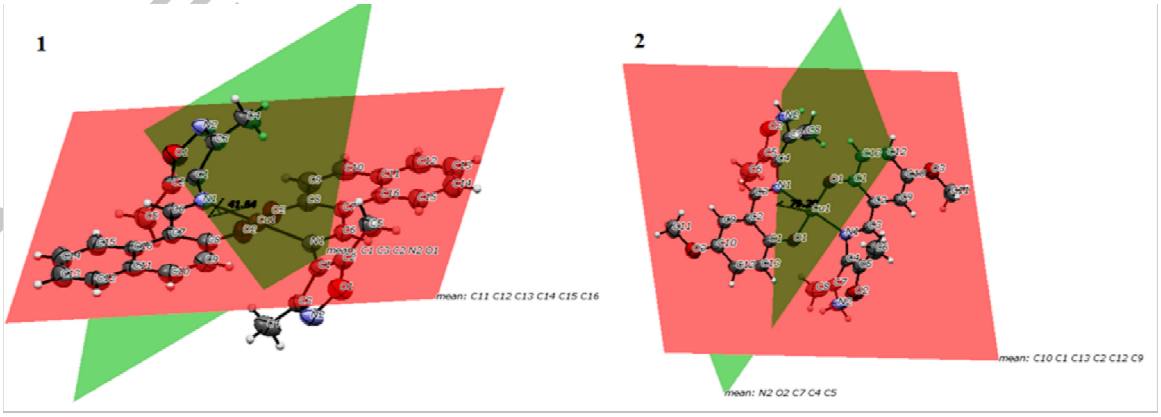


Fig. 2. Dihedral angles formed in complexes **1** and **2**.

3.2. Spectral characterization

3.2.1. FT-IR spectra

The coordination mode and sites of the ligands to the metal ions have been investigated by comparing the infrared spectra of the free ligands with their corresponding Cu(II) complexes. There are some significant shifts in stretching frequencies of Cu(II) complexes when compared to free ligand upon chelation as expected. The IR data is presented in **Table. 2**. The Schiff bases exhibit a characteristic strong band around 1605–1583 cm^{-1} due to the υ(C=N). This band is shifted by 5–50 cm^{-1} to a lower wave number in complexes indicating coordination of azomethine nitrogen atom of Schiff bases [31-34]. A broad band in the range of 3412-3454 cm^{-1} is due to phenolic -OH group of Schiff base disappeared in their copper(II) complexes confirming the participation of oxygen of phenolic group in coordination to the metal ion. This is also confirmed by the shift in the υ(C–O) band at 1043-1230 cm^{-1} to lower frequency to the extent of 8–16 cm^{-1} in respective Cu(II) complexes [35]. The coordination of the azomethine nitrogen and phenolic oxygen are further supported by the appearance of two non ligand bands at 541–559 cm^{-1} and 459–464 cm^{-1} due to υ(Cu-O) and υ(Cu-N) respectively [36, 37], **Figs. S5** and **S6** (see Supplementary).

Table. 2. Some important IR Absorption frequencies (cm^{-1}) of Schiff bases and their Cu(II) complexes.

COMPOUND	*ν*(OH)	*ν*(HC=N)	*ν* (C-O)	*ν* (M-O)	*ν* (M-N)
L^1	3454	1603	1184	-	-
$[Cu(L^1)_2]$	-	1577	1168	541	464
L^2	3443	1583	1043	-	-
$[Cu(L^2)_2]$	-	1533	1033	559	459
L^3	3412	1605	1230	-	-
$[Cu(L^3)_2]$	-	1600	1222	559	462

3.2.2. Electronic spectra and magnetic susceptibility

The magnetic susceptibility values (μ_{eff}) of all the Cu(II) complexes are in the range of 1.74–1.82 BM which is consistent with the presence of a single unpaired electron [38, 39]. Three absorption bands for L^1, L^2 and L^3 Schiff base ligands are observed at 252–246 nm, 330–301 nm and 374–352 nm respectively, **Figs. S7** and **S8** (see Supplementary). The bands at higher energies (252–246 nm) and (330–301 nm) are attributed to the π-π* transitions of the benzene ring while the other low energy bands (374–352) are assigned to the π-π* transitions of the azomethine chromophore respectively [40, 41]. These absorptions are also present in the spectra of copper(II) complexes but they undergone bathochromic shift. This shift in the spectra of the complexes supported the coordination of the ligands to Cu(II) ion. In addition to these bands all the Cu(II) complexes exhibit an additional and much important characteristic broad d-d transition band at 416-356 nm due to $^2B_{1g} \rightarrow {}^2E_g$ transition. From the electronic spectral data and magnetic susceptibility values a square planar geometry is assigned to all Cu(II) complexes [42].

3.2.3. ESR spectra

The ESR spectra of Cu(II) complexes provide information about the extent of the delocalization of unpaired electron. The X-band ESR spectra of Cu(II) complexes were recorded in liquid state at liquid nitrogen temperature (77K), their $g_{\parallel}$, $g_{\perp}$, Δg, g_{av} and G have been calculated. ESR spectra of complexes **1**, **2** and **3** are given in **Fig. 3**. The values of ESR parameters $g_{\parallel}$, $g_{\perp}$, g_{av}, Δg and G for complex **1** are found to be 2.2935, 2.0619, 2.1391, 0.2316 and 4.885 respectively, for complex **2** are 2.2412, 2.0532, 2.116, 0.188, and 4.6935 respectively and for complex **3** are 2.2613, 2.0466, 2.118, 0.2147 and 5.8465 respectively. The values $g_{\parallel} > g_{\perp} > g_e(2.0023)$ revealed that the unpaired electron is localized in $d_x^2\text{-}_y^2$ orbital with $^2B_{1g}$ ground state of Cu(II) square planar complex [43]. The $g_{\parallel}$ values for these complexes are found to be less than 2.3 suggesting covalent environment [44]. In all Cu(II) complexes the G values are found to be within the range of 4.6935-5.8465 indicating negligible exchange interaction of Cu-Cu in the complex according to Hathaway [45].

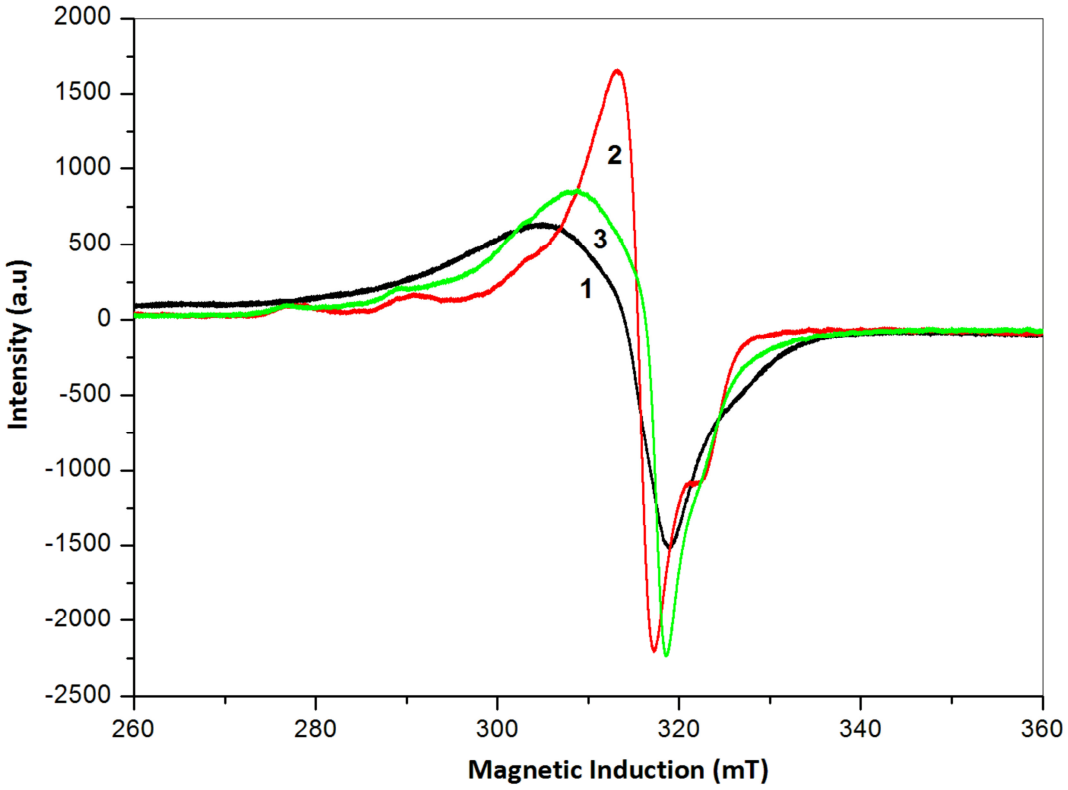


Fig. 3. ESR spectra of complexes **1**, **2** and **3.**

3.2.4. ESI mass spectral studies

The mass spectra of ligands L^1, $L^{2,}$ L^3 and complexes **1**, **2** and **3** are recorded in positive mode exhibited peaks at ESI mass: 266.9, 247.0, 297.0, 612.0, 554.1 and 652.0 respectively, **Figs. S9-S14** (see Supplementary) indicating the presence of $[M+H]^+$, $[M+H]^+$, $[M+2]^+$, $[M+NH_4]^+$, $[M]^+$ and $[M]^+$ ions respectively. These molecular ions of complexes confirm the stoichiometry of copper complex as $[Cu(L)_2]$ type.

3.2.5. Thermal analysis

Thermo gravimetric (TG) and differential thermal analysis (DTA) are useful techniques to determine the thermal stability of the metal complexes. In the present study heating rate is suitably controlled at 10°C/min under nitrogen atmosphere and the loss in weight is measured up to 1000°C. Thermo grams of complexes **1**, **2** and **3** are shown in **Fig. 4**. The results showed that all the Cu(II) complexes have a similar decomposition process mainly in two steps. These complexes exhibit thermal stability up to 200°C which confirms

that these complexes are free from coordinated water molecules. In the first step, these complexes showed weight loss around 215–350°C associated with the partial loss of ligand. In the second decomposition step mass loss is appeared at the temperature range 409–790°C corresponding to the complete thermal decomposition of the complexes and the loss of their organic portion resulting CuO as final products. This process is accompanied by an exothermic process in the range of 311°C to 349°C in DTA curves of the complexes [46], **Fig. S15** (see Supplementary).

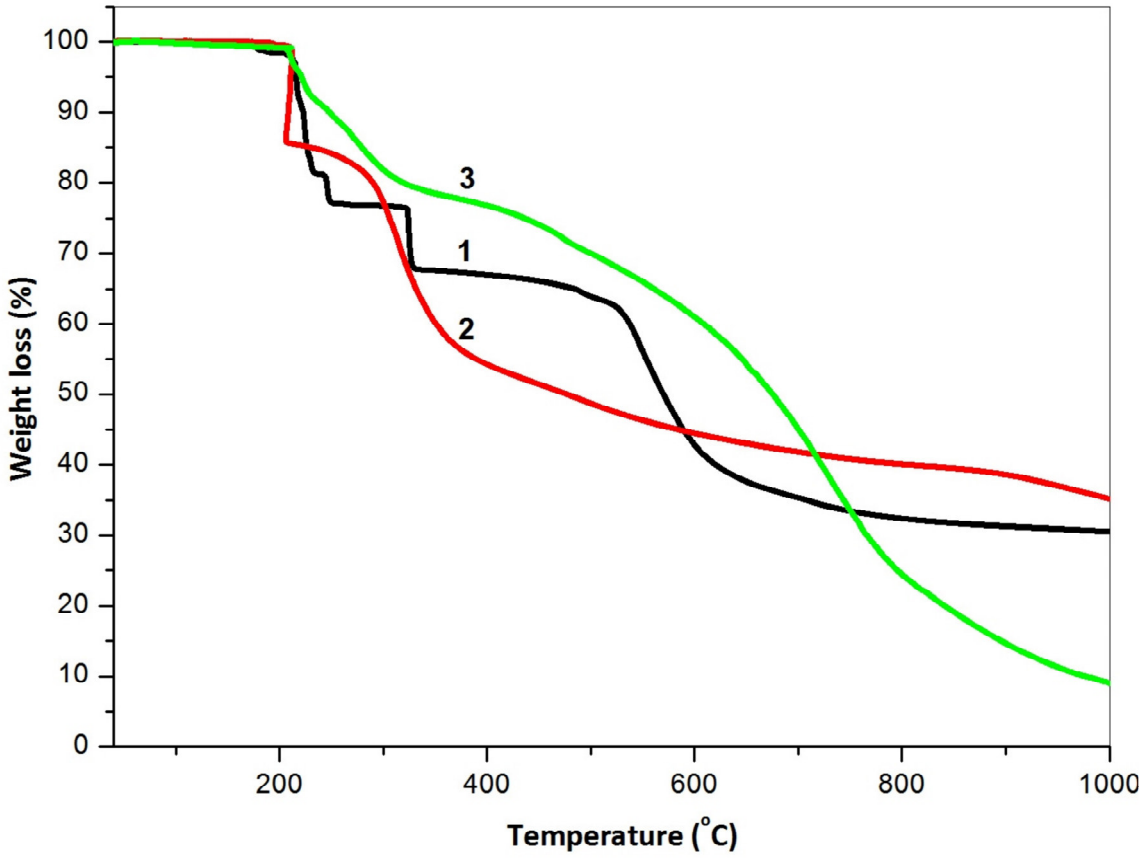


Fig. 4. Thermal analysis curves of complexes **1**, **2** and **3**.

3.3. Antimicrobial activity

In vitro biological screening effects of the ligands L^1 , L^2, L^3 and their Cu(II) complexes **1**, **2** and **3** are tested against the bacterial species such as *E. coli, P. putida, K. pneumoniae, B. subtilis and S. aureus* as well as fungal species such as *A. niger and C. albicans* by the paper disc method. The inhibition zone values (mm) of the compounds are summarized in **Table. 3**. A comparative study of the ligands and their complexes indicates that complexes exhibited higher antimicrobial activity than the free ligands. The increase in

antimicrobial activity of the complexes can be explained on the basis of Overtone's concept [47] and Tweedy's Chelation theory [48, 49]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid soluble materials makes which liposolubility as an important factor which controls the antimicrobial activity. Moreover, coordination reduces the polarity of the metal ion essentially because of the partial sharing of its positive charge with the donor groups [50] within the chelate ring system formed during coordination. 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 [51] thus destroying them more aggressively.

Table. 3. Antimicrobial activity results of Schiff bases and their Cu(II) complexes at 100µg/mL concentration.

Compound	Bacterium(mm)					Fungi(mm)	
	Gram-negative bacteria			Gram-positive bacteria			
	E.coli	P.putida	K.pneumoniae	B.subtilis	S.aureus	A.niger	C.albicans
L^1	10	9	11	8	10	11	9
L^2	12	11	9	10	7	10	7
L^3	11	8	9	11	9	10	8
1	22	20	20	21	23	25	24
2	23	24	21	22	20	23	24
3	24	21	19	20	20	24	25
$Cu(NO_3)_2.3H_2O$	2	-	3	-	-	2	-
Ampicillin	30	31	30	33	31	-	-
Ketoconazole	-	-	-	-	-	32	31

3.4. DNA binding studies

3.4.1. UV–Vis spectroscopic studies

The binding activities of DNA–metal complexes have been a clue of paramount importance for understanding the mechanism of effective metal based chemotherapeutic drugs [52]. Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal complexes [53, 54]. The binding behavior of the metal complexes to DNA helix is often investigated by the changes in the absorbance and shift in the wavelength. A complex bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength due to the intercalative mode involving a stacking interaction between the aromatic chromophore and the DNA base pairs. Metallo-intercalators are metal complexes bearing planar aromatic side groups of the coordinating ligands. As the name suggests, these ligands, oriented parallel to the base pairs and protruding away from the metal center, can readily π-stack in the DNA duplex. Often, in such complexes the metal ion is part of the planar portion [55]. After the complexes intercalate to the base pair of DNA, the π * orbital of the intercalated ligand on the complexes can couple with π orbital of the base pairs, thus decreasing the π-π * transition energies. On the other hand, the coupling π * orbital are partially filled by electrons, thus decreasing the transition probabilities [56, 57]. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction [58]. In the present investigation, the Cu(II) complexes show a band in the range of 416–356 nm attributed to a d-d transition band. On addition of DNA, the intensity of the d-d transition band decreases by 31–73% (hypochromism) with a red shift of 2–16 nm in the absorption spectra. The absorption spectra of complexes **1**, **2** and **3** in absence and presence of CT-DNA are shown in **Figs. 5, 6** and **7.**

The intrinsic binding constant K_b is determined from a plot of [DNA]/(ε_a-ε_f) vs [DNA] using the equation: [DNA]/(ε_a-ε_f) = [DNA]/(ε_b-ε_f) +1/K_b(ε_b-ε_f), where [DNA] is the concentration of DNA in base pairs, and the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obs}/[complex], the extinction coefficient for the free compound and for the compound in the fully bound form respectively [59]. K_b is given by the ratio of slope to the intercept. From the absorption data, the binding constant K_b for complex **1** is $7.43\pm0.02\times10^6$ M^{-1}, complex **2** is $3.38\pm0.03\times10^5$ M^{-1} and complex **3** is $2.92\pm0.02\times10^5$ M^{-1}. The K_b values are comparable with the potential intercalators like ethidium bromide (EB) ($K_b=7\times10^7$ M^{-1}) [60]. These results show that the strength of interaction of the complexes with CT-DNA.

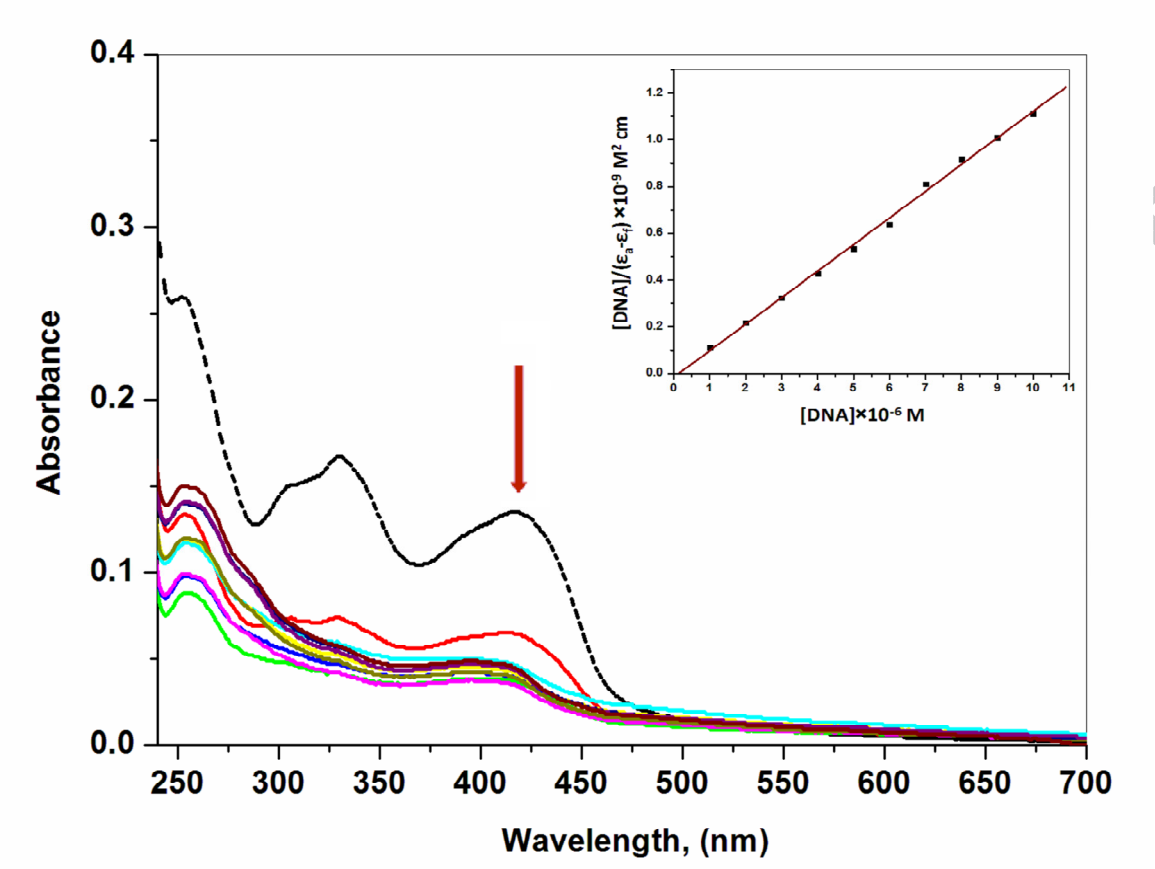


Fig. 5. Absorption spectra of **1** in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA in Tris HCl buffer (pH 7.2). Conditions: [**1**] =10 µM, [DNA] = 0–10 µM. Arrow (↓) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant, K_b.

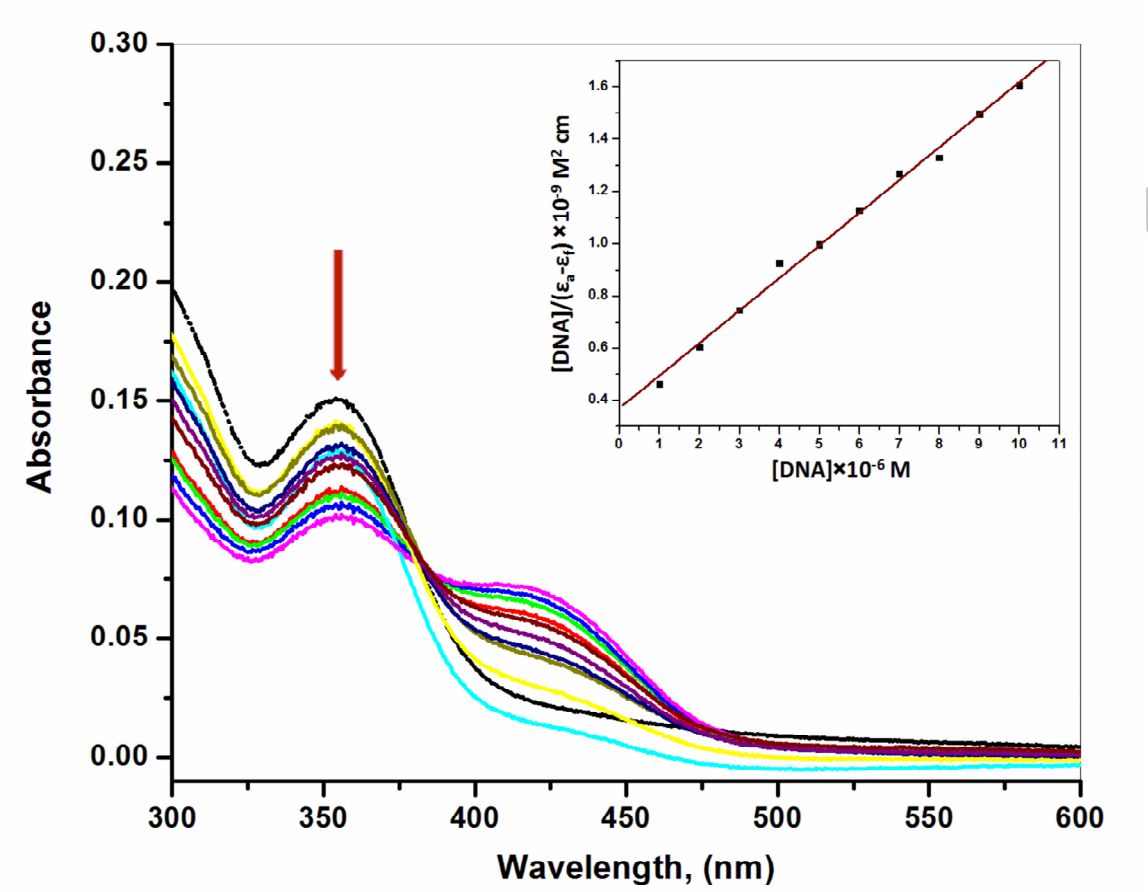


Fig. 6. Absorption spectra of **2** in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA in Tris HCl buffer (pH 7.2). Conditions: [**2**] =10 µM, [DNA] = 0–10 µM. Arrow (↓) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant, K_b.

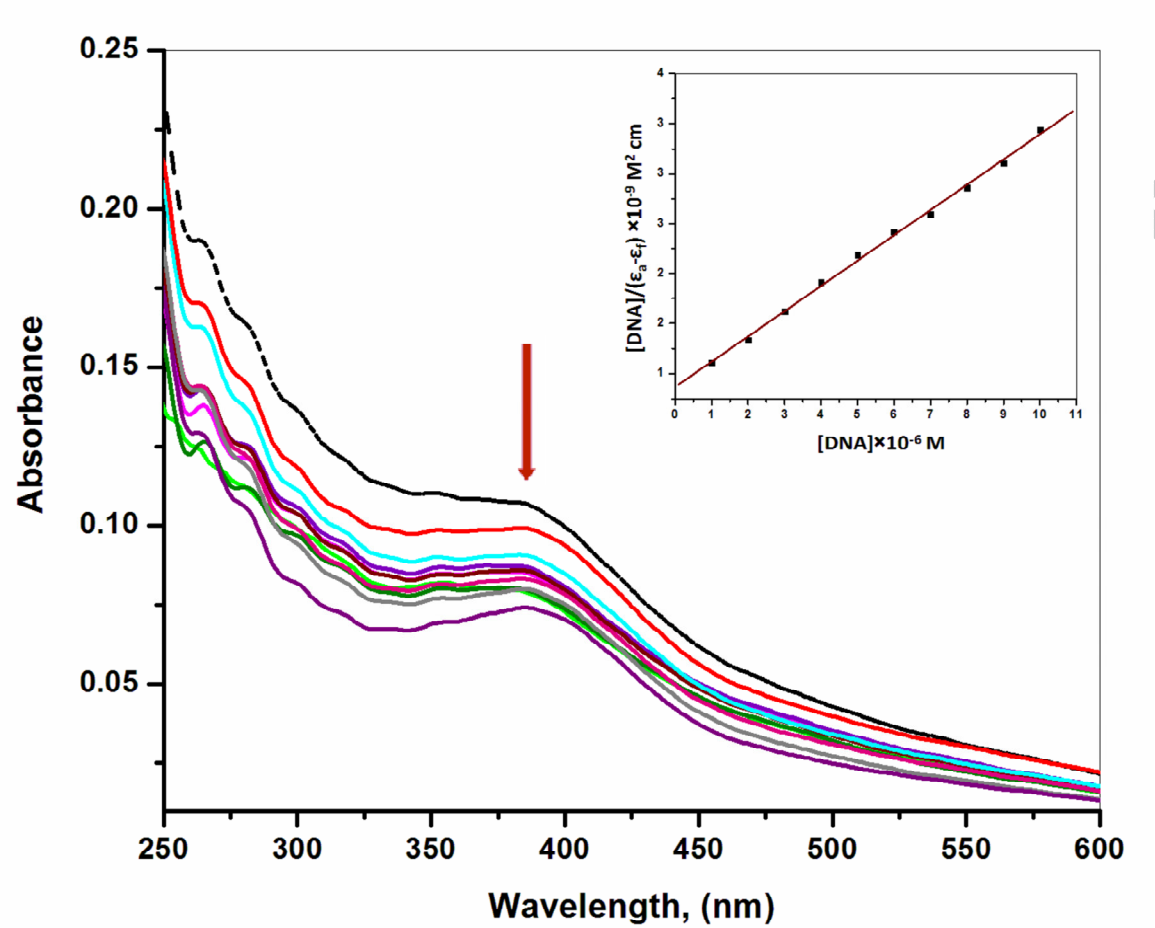


Fig. 7. Absorption spectra of **3** in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA in Tris HCl buffer (pH 7.2). Conditions: [**3**] =10 µM, [DNA] = 0–10 µM. Arrow (↓) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant, K_b.

3.4.2. Viscosity measurements

The viscometric measurement is also an important tool to find the nature of binding of metal complexes to the DNA. Hydrodynamic measurements (i.e., viscosity and sedimentation) sensitive to length changes are the most critical and least ambiguous tests of binding in solution without crystallographic data [61, 62]. A classical intercalation molecule, such as EB, results in the lengthening of the DNA helix. This leads to an increase in DNA viscosity, which is caused by an increase in the separation of base pairs at the interaction site and an increase in overall double helix length. By contrast, a partial or non classical intercalation of the complex results in bending of the DNA helix, which reduces the effective length of DNA, as well as the DNA viscosities. The relative specific viscosity of DNA is determined by varying the concentration of the added metal complexes. The effects of all the

synthesized Cu(II) complexes on the viscosity of DNA at 30 ± 0.1°C are shown in **Fig. 8**. The experimental results clearly showed that all the complexes can intercalate between adjacent DNA base pairs as also evidenced by UV-Vis spectroscopic results. By increasing the complex concentration to DNA causing an extension in the helix and thus increase the viscosity of DNA [63]. The complexes can intercalate strongly leading to a greater increase in viscosity of the DNA with an increasing concentration of complexes. The increase in the degree of viscosity follows the order of complex **1> 2>3.** This may be due to affinity of the complex towards DNA.

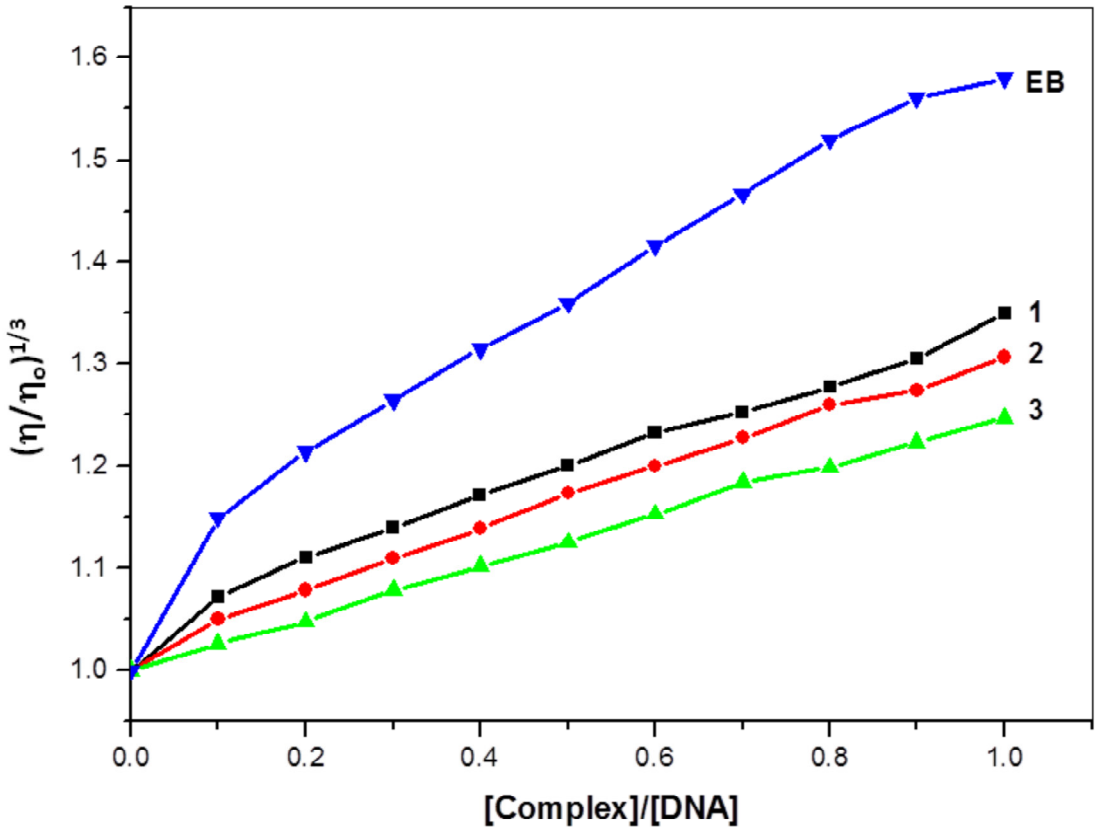


Fig. 8. Effect of increasing amounts of EB, complexes **1**, **2** and **3** on the relative viscosity of CT-DNA at 30±0.1°C.

3.5. DNA cleavage

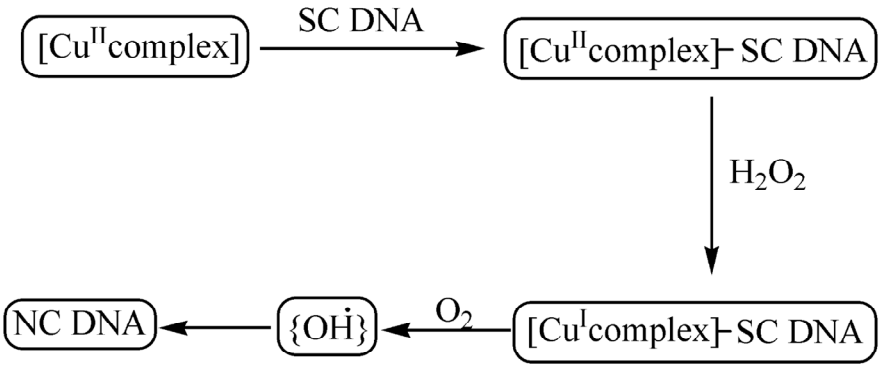
The DNA cleavage of super coiled pBR322 DNA is investigated by agarose gel electrophoresis method with synthesized Cu(II) complexes at different concentrations in presence of H_2O_2 and UV light. The results are shown in **Figs. 9**, **10** and **Figs. S16**, **17** (see

Supplementary). The ability of the complexes in DNA cleavage is estimated by the conversion of DNA from Form I to Form II and Form III. The fastest migration is detected in the supercoiled form (Form I). If only one strand is cleaved, the super coils relax to convert into a slower-moving nicked form (Form II). If both strands are cleaved, a linear form (Form III) is produced which migrates in between Form I and Form II [64].

After incubation of supercoiled pBR322 DNA with the Cu(II) complexes in presence of H_2O_2(oxidative cleavage) and UV irradiation at 345 nm (photo cleavage) [13], for control experiments no DNA cleavage is observed as there is no complex(lane 1). With increasing concentrations of the Cu(II) complexes, the percentage of super coiled DNA (Form I) diminished gradually where as nicked circular DNA (Form II) and linear DNA (Form III) increased. These results indicate that the Cu(II) complexes can degrade supercoiled pBR322 DNA. Further it is observed that complex **1** promote the cleavage of supercoiled pBR322 DNA more efficiently than complexes **2** and **3**.

3.5.1. Mechanism for Oxidative Cleavage:

On the basis of above observation, the mechanism of DNA cleavage mediated by **1**-**3** may be proposed as follows: DNA cleavage is redox-mediated. The complexes would first interact with DNA by intercalation to form a $Cu^{II} \cdots$ DNA adduct species, followed by its reduction by the external agent (H_2O_2) to a $Cu^{I} \cdots$ DNA adduct, which then generates $HO^{\bullet}$ radicals on reaction with O_2. These $HO^{\bullet}$ radicals would then attack DNA, causing strand scission (**Scheme 2**). A similar pathway was proposed by Sigman for the oxidative cleavage reaction of the bis(phen)copper complex [64].



Scheme 2. A Possible Mechanism for the Oxidative Cleavage of DNA by complexes **1**–**3** in the Presence of H_2O_2.

3.5.2. Mechanism for Photolytic Cleavage:

Observation of DNA photolytic cleavage mediated by **1**-**3** indicates the involvement of singlet oxygen ($^1O_2$) as the reactive species. As suggested by Toshima *et al*., the triplet states resulting from the n-p* and π-π* photo-excitation in the UV radiation could activate oxygen to form $^1O_2$ that cleaves DNA [65].

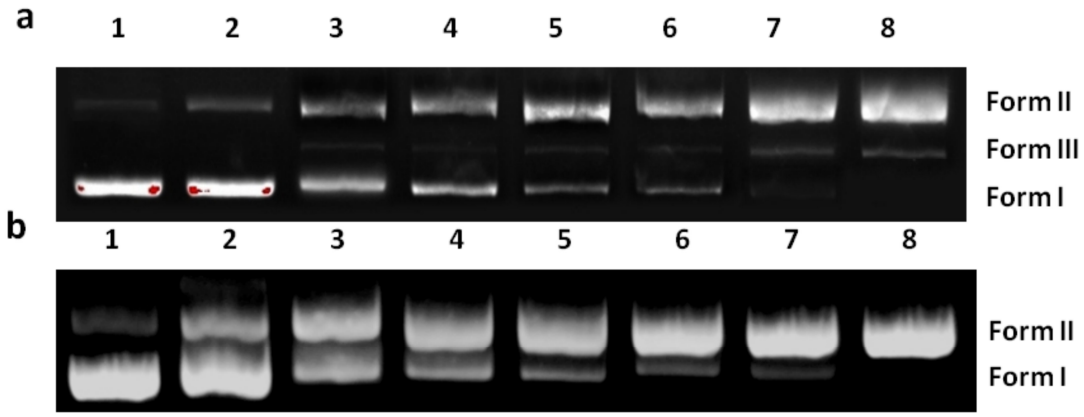


Fig. 9. Oxidative cleavage of supercoiled pBR322 DNA(0.2 μg, 33.3 μM) at 37 °C in 5mM Tris. HCl/5 mM NaCl buffer by the Cu(II) complexes **1** and **3**. **(a)** Lane 1, DNA control; Lane 2, DNA + H_2O_2 (1mM); Lane 3–8, DNA + H_2O_2 (1mM) + complex **1**, [complex] = 20, 30, 40, 50, 60 and 70 μM, respectively. **(b)** Lane 1, DNA control; Lane 2, DNA + H_2O_2 (1mM); Lane 3–8, DNA + H_2O_2 (1mM) + complex **3**, [complex] = 20, 30, 40, 50, 60 and 70 μM, respectively.

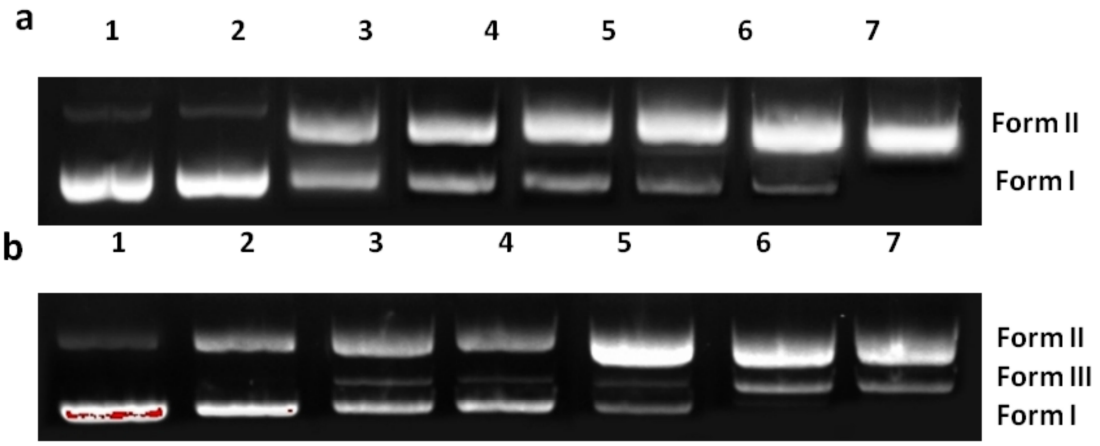


Fig. 10. Photoactivated cleavage of supercoiled pBR322 DNA (0.2 μg, 33.3 μM) at 37 °C in 5mM Tris. HCl/5 mM NaCl buffer by the Cu(II) complexes **1** and **2** using UV irradiation of wavelength 345 nm. **(a)** Lane 1, DNA control; Lane 2–7, DNA + complex **1**, [complex] = 20,

30, 40, 50, 60 and 70 μM, respectively. **(b)** Lane 1, DNA control; Lane 2–7, DNA + complex **2**, [complex] = 20, 30, 40, 50, 60 and 70 μM, respectively.

Conclusion

The synthesized Schiff bases and their binary Cu(II) complexes have been characterized by analytical and spectral analysis. The crystal structures of ligand L^1, complexes **1** and **2** have also been determined by X-ray diffraction studies. From the data it is observed that in solid state all Cu(II) complexes adopt a square planar geometry. In vitro antibacterial and antifungal activities of Schiff base ligands and their Cu(II) complexes have been screened. It is observed that all the Cu(II) complexes exhibit better activity than the corresponding Schiff base ligands and also some complexes showed comparable activity with the standard drug. The interaction between the Cu(II) complexes and CT-DNA have been investigated by UV-Vis spectra and viscosity measurements. The respective results of the binding studies revealed that an intercalative mode of binding. The DNA cleavage studies of Cu(II) complexes revealed that these complexes can effectively cleave supercoiled pBR322 DNA in the presence of H_2O_2 and also in the presence of UV light. Further it is observed that among the complexes, complex **1** exhibits good antimicrobial, DNA binding and cleaving properties.

Acknowledgements

We express our sincere thanks to the Head, Department of the Chemistry for providing the necessary facilities. We are thankful to the Director, CFRD, Osmania University, Hyderabad, and the Director, IICT, Hyderabad, and the SAIF, IIT Bombay for providing spectral and analytical data. We are thankful to the Department of Chemistry, Karnatak University, Dharwad for providing single crystal X-ray diffraction analysis. We are also thankful to CSIR, New Delhi, DST-SERB and UGC-UPE (FAR) for providing financial assistance.

Appendix A. Supplementary material

CCDC 936590, 1005830 and 1005831 contain the supplementary crystallographic data for L^1, complex **1** and **2**, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic

Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

References

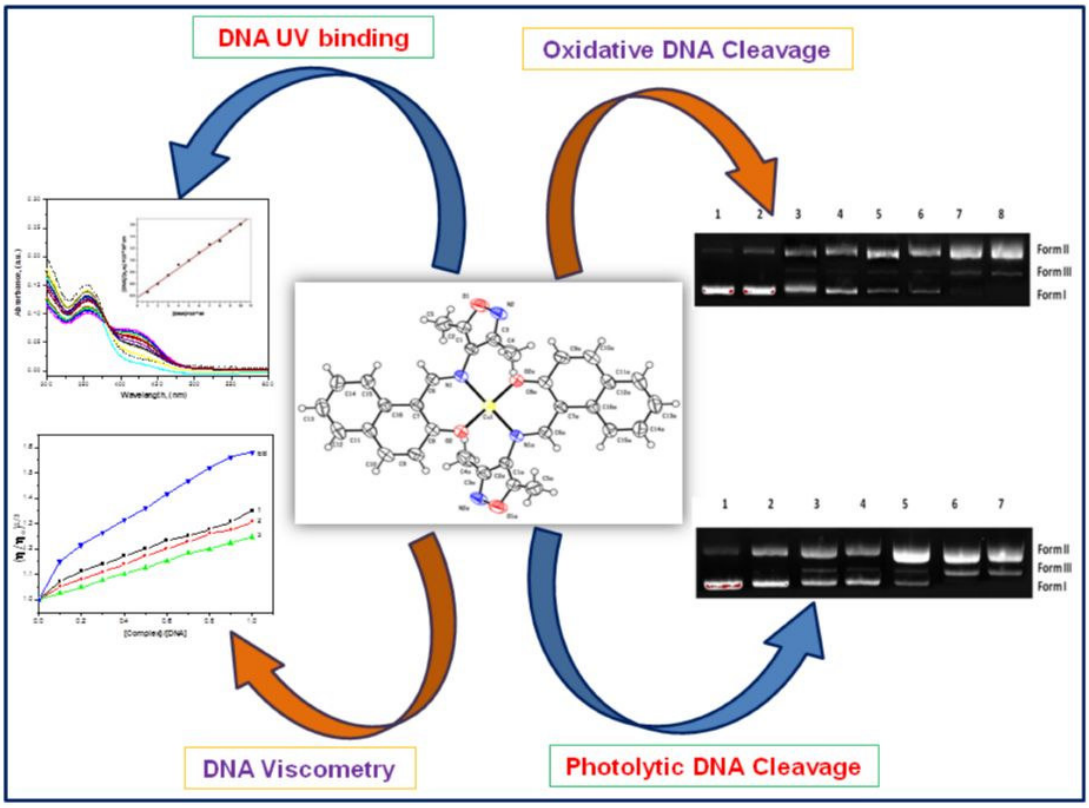
[1] N. Raman, A. Selvan, S. Sudharsan, Spectrochim. Acta A, 79 (2011) 873-883.
[2] S. Delaney, M. Pascaly, P.K. Bhattacharya, K. Han, J.K. Barton, Inorg. Chem., 41 (2002) 1966-1974.
[3] D.R. Green, J.C. Reed, Science, 281 (1998) 1309-1312.
[4] Y. Ma, L. Cao, T. Kawabata, T. Yoshino, B.B. Yang, S. Okada, Free Radic. Biol. Med., 25 (1998) 568-575.
[5] F. Liang, C. Wu, H. Lin, T. Li, D. Gao, Z. Li, J. Wei, C. Zheng, M. Sun, Bioorg. Med. Chem. Lett., 13 (2003) 2469-2472.
[6] J. Easmon, G. Pürstinger, G. Heinisch, T. Roth, H.H. Fiebig, W. Holzer, W. Jäger, M. Jenny, J. Hofmann, J. Med. Chem., 44 (2001) 2164-2171.
[7] S. Tabassum, S. Amir, F. Arjmand, C. Pettinari, F. Marchetti, N. Masciocchi, G. Lupidi, R. Pettinari, Eur. J. Med. Chem., 60 (2013) 216-232.
[8] V.M. Manikandamathavan, V. Rajapandian, A.J. Freddy, T. Weyhermuller, V. Subramanian, B.U. Nair, Eur. J. Med. Chem., 57 (2012) 449-458.
[9] D.K. Chand, H.J. Schneider, A. Bencini, A. Bianchi, C. Giorgi, S. Ciattini, B. Valtancoli, Chem. Eur. J., 6 (2000) 4001-4008.
[10] E.L. Hegg, J.N. Burstyn, Coord. Chem. Rev., 173 (1998) 133-165.
[11] S. Dhar, D. Senapati, P.K. Das, P. Chattopadhyay, M. Nethaji, A.R. Chakravarty, J. Am. Chem. Soc., 125 (2003) 12118-12124.
[12] S. Dhar, P.A.N. Reddy, M. Nethaji, S. Mahadevan, M.K. Saha, A.R. Chakravarty, Inorg. Chem., 41 (2002) 3469-3476.
[13] X. Li, C.F. Bi, Y.H. Fan, X. Zhang, X.D. Wei, X.M. Meng, Transition Met. Chem., 39 (2014) 577-584.
[14] B.K. Santra, P.A.N. Reddy, G. Neelakanta, S. Mahadevan, M. Nethaji, A.R. Chakravarty, J. Inorg. Biochem., 89 (2002) 191-196.
[15] S.Y. Tsang, S.C. Tam, I. Bremner, M.J. Burkitt, Biochem. J., 317 (1996) 13-16.
[16] J. Bernadou, G. Pratviel, F. Bennis, M. Girardet, B. Meunier, Biochemistry, 28 (1989) 7268-7275.
[17] F.B. Tamboura, M. Gaye, A.S. Sall, A.H. Barry, T. Jouini, Inorg. Chem. Commun., 5 (2002) 235-238.
[18] Z. K. Khoramdareh, S. A. Hosseini-Yazdi, B. Spingler, A. A. Khandar, Inorg. Chim. Acta, 415 (2014) 7-13.
[19] K.R. Barnes, S.J. Lippard, Metal Ions in Biological Systems, 42 (2004) 143-177.
[20] L.S. Lerman, J. Mol. Biol., 3 (1961) 18-30.
[21] Y. Prashanthi, K. Kiranmai, N.J.P. Subhashini, Shivaraj Spectrochim. Acta A, 70 (2008) 30-35.
[22] J. Marmur, J. Mol. Biol., 3 (1961) 208-218.
[23] M.E. Reichmann, S.A. Rice, C.A. Thomas, P. Doty, J. Am. Chem. Soc., 76 (1954) 3047-3053.

[24] G.M. Sheldrick, Acta Cryst., A64 (2008) 112-122.
[25] N. Pravin, N. Raman, Inorg. Chem. Commun., 36 (2013) 45-50.
[26] P. Zabierowski, J. Szklarzewicz, K. Kurpiewska, K. Lewinski, W. Nitek, Polyhedron, 49 (2013) 74-83.
[27] T. Hokelek, N. Gunduz, Z. Hayvali, Z. Kilic, Acta Cryst., C51 (1995) 880-884.
[28] J.M. Fernandez-G, M.J. Rosales-Hoz, M.F. Rubio-Arroyo, R. Salcedo, R.A. Toscano, A. Vela, Inorg. Chem., 26 (1987) 349-357.
[29] J.M. Fernandez, M.J. Rosales, R.A. Toscano, R.G. Tapia-T, Acta Cryst., C42 (1986) 1313-1316.
[30] T. Hokelek, N. Gunduz, Z. Hayvali, Z. Kilic, J. Chem. Crystallogr., 25 (1995) 831-836.
[31] D. Maity, M.G.B. Drew, J.F. Godsell, S. Roy, G. Mukhopadhyay, Transition Met. Chem., 35 (2010) 197-204.
[32] S. Bhunora, J. Mugo, A. Bhaw-Luximon, S. Mapolie, J. Van Wyk, J. Darkwa, E. Nordlander, Appl. Organometal. Chem., 25 (2011) 133-145.
[33] N.S. Youssef, E. El-Zahany, A.M.A. El-Seidy, A. Caselli, S. Fantauzzi, S. Cenini, Inorg. Chim. Acta, 362 (2009) 2006-2014.
[34] G.C. Percy, D.A. Thornton, J. Inorg. Nucl. Chem., 35 (1973) 2319-2327.
[35] S. Saydam, E. Yilmaz, Spectrochim. Acta A, 63 (2006) 506-510.
[36] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, fifth ed., Wiley-Interscience, New York, (1997).
[37] L.J. Bellamy, The Infrared Spectra of Complex Molecules, second ed., Chapmann and Hall, London, (1980).
[38] F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry, third ed., Interscience Publisher, New York, (1972).
[39] A.B.P. Lever, Inorganic Electronic Spectroscopy, second ed., Elsevier, Amsterdam, (1984).
[40] Y. Zhou, X. Ye, F. Xin, X. Xin, Transition Met. Chem., 24 (1999) 118-120.
[41] R. Ramesh and S. Maheswaran, J. Inorg. Biochem., 96 (2003) 457-462.
[42] H. Unver, Z. Hayvali, Spectrochim. Acta A, 75 (2010) 782-788.
[43] R.N. Patel, N. Singh, K.K. Shukla, U.K. Chauhan, J. Nicols -Gutierrez, A. Castineiras, Inorg. Chim. Acta, 357 (2004) 2469-2476.
[44] D. Kivelson, R. Neiman, J. Chem. Phys., 35 (1961) 149-155.
[45] B.J. Hathaway, D.E. Billing, Coord. Chem. Rev., 5 (1970) 143-207.
[46] V.A. Joseph, K.M. Vyas, J.H. Pandya, V.K. Gupta, R.N. Jadeja, J. Coord. Chem., 66 (2013) 1094-1106.
[47] Y. Anjaneyulu and R. P. Rao, Synth. React. Inorg. Met. –Org. Chem., 16 (1986) 257-272.
[48] J.W. Searl, R.C. Smith, S.J. Wyard, Proc. Phys. Soc., 78 (1961) 1174-1176.
[49] B.G. Tweedy, Phytopathology, 55 (1964) 910-914.
[50] A.K. Sharma, S. Chandra, Spectrochim. Acta A, 78 (2011) 337-342.
[51] Z.H. Chohan, Synth. React. Inorg. Met. Org. Chem., 34 (2004) 833-846.
[52] S. Tabassum, A. Asim, F. Arjmand, M. Afzal, V. Bagchi, Eur. J. Med. Chem., 58 (2012) 308-316.

[53] J.K. Barton, A. T. Danishefsky, J.M. Goldberg, J. Am. Chem. Soc., 106 (1984) 2172-2176.
[54] S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas, J. Phys. Chem., 97 (1993) 1707-1711.
[55] H.-K. Liu, P.J. Sadler, Acc. Chem. Res. 44 (2011) 349-359.
[56] M.A. Pyle, P.J. Rehmann, R. Meshoyrer, J.N. Kumar, J.N. Turro, K.J. Barton, J. Am. Chem. Soc. 111 (1989) 3053–3063.
[57] T.R. Li, Z.Y. Yang, B.D.Wang, D.D. Qin, Eur. J. Med. Chem. 43 (2008) 1688–1695.
[58] C.N. Sudhamani, H.S. Bhojya Naik, T.R. Ravikumar Naik, M.C. Prabhakara, Spectrochim. Acta A, 72 (2009) 643-647.
[59] K. Sampath, S. Sathiyaraj, C. Jayabalakrishnan, Spectrochim. Acta A, 105 (2013) 582-592.
[60] M.J. Waring, J. Mol. Biol., 13 (1965) 269-282.
[61] M. Tarui, M. Doi, T. Ishida, M. Inoue, S. Nakaike, K. Kitamura, Biochem. J. 304 (1994) 271-279.
[62] H. Guo, J. Lu, Z. Ruan, Y. Zhang, Y. Liu, L. Zang, J. Jiang, J. Huang, J. Coord. Chem. 65 (2012) 191-204.
[63] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Biochemistry, 32 (1993) 2573-2584.
[64] D.S. Sigman, Acc. Chem. Res., 19 (1986) 180-186.
[65] K. Toshima, R. Takano, T. Ozawa and S. Matsumura, *Chem. Commun.*, 2002, 212-213.

Abstract:

Three novel binary copper(II) complexes **1** [$Cu(L^1)_2$], **2** [$Cu(L^2)_2$] and **3** [$Cu(L^3)_2$] where, L^1 (1-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)naphthalen-2-ol, $C_{16}H_{14}N_2O_2$), L^2 (2-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)-4-methoxyphenol, $C_{13}H_{14}N_2O_3$) and L^3 (2-((E)-(3,5-dimethylisoxazol-4-ylimino)methyl)-4-bromophenol, $C_{12}H_{11}BrN_2O_2$) have been synthesized. All Cu(II) complexes have been characterized by elemental analysis, FT-IR, ESI mass, UV-Visible, ESR, TG-DTA, magnetic moments and single crystal X-ray diffraction studies. Based on spectral studies square planar geometry is assigned for all Cu(II) complexes. Ligand L^1, [$Cu(L^1)_2$] and [$Cu(L^2)_2$] are crystallized and found to be triclinic, orthorhombic and monoclinic crystal systems respectively. The Schiff bases and their Cu(II) complexes have been screened for antibacterial activity and antifungal activity by paper disc method. It is observed that all Cu(II) complexes showed more activity than corresponding Schiff bases. DNA binding and cleavage studies of Cu(II) complexes have also been investigated. It is observed an intercalation mode of binding with CT-DNA and cleavage of supercoiled pBR322 DNA in the presence of H_2O_2 and UV light.



Few of the ligands (Schiff bases) and their Cu(II) complexes are characterized by single crystal X-ray diffraction analysis. And we have also done DNA interaction studies of all Cu(II) complexes by using CT-DNA for binding and supercoiled pBR322 DNA for cleavage studies.

Highlights

- Three novel Cu(II) complexes of Isoxazole Schiff bases are synthesized and characterized by different spectroscopic techniques and single crystal X-ray diffraction analysis.
- All Cu(II) complexes are biologically more active than free ligands and few are comparable with standard drug.
- Binding studies of Synthesized Cu(II) complexes with CT-DNA suggest intercalation mode and cleave supercoiled pBR322 DNA effectively.