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Antibacterial, SOD like and Nuclease Interaction of Fluoroquinolone Based Copper(II) Complexes

Dr. Deepen S. Gandhi
Government Science College, Sector-15, Gandhinagar, Gujarat, India

ABSTRACT

Drug–based mixed–ligand copper(II) complexes of type $[Cu(L)(A^n)Cl].5H_2O$ have been prepared with an aim to generate a database for the development of metal based therapeutic agents. Synthesized complexes were characterized using infrared spectra, electronic spectral, magnetic measurements, elemental analyses, thermal investigation and mass spectroscopy. Spectral investigations of metal complexes reveal monomeric five-coordinate square pyramidal geometry. The viscosity measurement was employed to determine the mode of binding of complexes to DNA. The DNA binding efficacy was determined using absorption titration. The binding constant (K_b) ranging from 0.846×10^4 to $2.0 \times 10^5\ M^{-1}$ pointing toward the covalent mode of binding, whereas DNA cleavage study shows better cleaving ability of the complexes compare to metal salts and standard drug exhibited via conversion of super coiled form of pUC19 DNA to linear form via circular form. From the SOD mimic study, it was found that 0.415– 1.305 µM concentrations of complexes were enough to inhibit the reduction rate of NBT by 50% (IC_{50}) in NBT/NADH/PMS system. Antibacterial activity has been assayed against selective $Gram^{(-ve)}$ and $Gram^{(+ve)}$ microorganisms using the doubling dilution technique.

Keywords : Ofloxacin, Square pyramidal, Nuclease activity, SOD mimic, Antibacterial, K_b

Abbreviations:

SOD	Superoxide Dismutase
MIC	Minimum Inhibitory Concentration
LB	Luria Broth
K_b	Intrinsic Binding Constant
NADH	Nicotinamide Adenine Di-nucleotide reduced
PMS	Phenazine Methosulphate
NBT	Nitro Blue Tetrazolium
IC_{50}	Concentration that causes 50% inhibition of Formazan

1. INTRODUCTION Four decades after the discovery of nalidixic acid; the first member of the quinolone antibacterial family, more than 7000 new analogue has been documented in the literature. Since 1977, this class of synthetic antibacterial agents has been widely used in clinics. In recent years, there has been considerable interest in the development of new fluoroquinolone agents. Heterocyclic ring systems having the piperidine-4-one nucleus have aroused great interest in the past and recent years due to their wide variety of biological properties, such as antiviral, antitumor, central nervous system, local anaesthetic, anticancer and antimicrobial activity [1–6]. The primary mechanism of the antibacterial action of these drugs is the inhibition of DNA gyrase (Topoisomerase II), an enzyme responsible for coiling the long DNA molecule into the confined space

inside the bacterial cell; inactivation of this enzyme is lethal to the microorganism [7]. The interaction of metal ions with diverse deprotonated quinolone as ligands has been thoroughly studied [8].

Superoxide dismutase (SOD) enzymes protect cells against the cytotoxic activity of the superoxide radical ($O_2^{\cdot -}$), which is a by-product of aerobic metabolism [9, 10]. One class of these enzymes, the copper–zinc SODs, occurs primarily in the cytoplasm of eukaryotes. The eukaryotic Cu–Zn SODs have been studied in some detail. The dismutation of $O_2^{\cdot -}$ proceeds via alternate reduction and oxidation of the essential Cu ion during successive encounter with the substrate to produce O_2 and H_2O_2, respectively. Both steps occur with a rate constant of 2 x $l0^9$ $M^{-1}S^{-1}$ [11–13]. The presence of bound metals greatly increases the thermal stability of the enzyme [14, 15].

In this paper, we have prepared the drug-based mixed ligand complexes with Ofloxacin(L) and neutral bidentate ligands (A^1 = pyridine-2-carboxaldehyde, A^2 = 2,2'-bipyridylamine, A^3 = thiophene-2-carboxaldehyde, A^4 = 2,9-dimethyl-1,10-phenanthroline, A^5 = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, A^6 = 4,5-diazafluoren-9-one, A^7 = 1,10-phenanthroline-5,6-dione and A^8 = 5-nitro-1,10-phenanthroline) prompting to gain an inhibitor for inhibition of DNA gyrase (Topoisomerase II). Synthesized compounds were also checked for their SOD mimic activity using nonenzymatic mode (NBT/NADH/PMS system) to measure the effect on the stabilization of the enzyme. The DNA binding and cleavage properties of the complexes have been investigated by ultraviolet spectroscopy, viscosity measurements and gel electrophoresis method. The MIC has been assayed using the doubling dilution technique.

2. Experimental

2.1Materials

All the chemicals used were of analytical grade. 2,2'-Bipyridylamine was purchased from Lancaster (Morecambe, England). Ofloxacin was purchased from Bayer AG (Wuppertal, Germany). Cupric chloride was purchased from E. Merck (India) Ltd. Mumbai. Pyridine-2-carboxaldehyde, thiophene-2-carboxaldehyde, 1,10-phenanthroline and Luria Broth were purchased from Himedia, India. 2,9-Dimethyl-1,10-phenanthroline and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline were purchased from Loba chemie PVT. LTD. (INDIA). Organic solvents were purified by standard method [16].

2.2 Instrumental details

The metal contents of the complexes were analyzed by EDTA titration after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5) [17]. The diamagnetic correction was made using Pascal's constant [18]. Infrared spectra were recorded on a FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. C, H and N elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. MIC study was carried out by means of laminar air flow cabinet, Toshiba, Delhi, India. Thermo gravimetric analyses was obtained with a model 5000/2960 SDTA, TA instrument (USA). The electronic spectra were recorded on a UV-160A UV-Vis. spectrophotometer, Shimadzu (Japan). The magnetic moments were measured by Gouy's method using mercury(II) tetrathiocyanatocobaltate(II) as the calibrant (χ_g =16.44 x 10^{-6} cgs units at 20 ºC), Citizen Balance. Mass spectra were recorded using GCMS–QP2010 having ionisation voltage of 0.90 kV, 30.0m length with a thickness of 1.0 μm with column having 0.25mm diameter.

2.3 Ligand preparation

4,5-Diazafluoren-9-one (A^6) was prepared using 1,10-phenanthroline , 1,10-phenanthroline-5,6-dione (A^7) and 5-nitro-1,10-phenanthroline (A^8) were prepared as per the reported method [19–21].

2.4. Preparation of complexes

[Cu(L)(A^n)Cl].5H_2O: An methanolic solution of $CuCl_2 \cdot 2H_2O$ (1.5 mmol) was added to methanolic solution of neutral bidentate ligand (A^n) (1.5 mmol), followed by addition of a previously prepared solution of ofloxacin (1.5 mmol) in methanol in presence of CH_3ONa (1.5 mmol). The pH was adjusted at ~ 6.2 using dilute solution of CH_3ONa. The resulting solution was refluxed for 1 h. on a steam bath, followed by concentrating it to half of its volume. A fine amorphous product of green color was obtained which was washed with ether/hexane and dried in vacuum desiccators. Physicochemical data of the synthesized complex are summarized in Table–1.

Table 1: Experimental and physical parameters of the complexes

Complexes empirical formula	Elemental analysis % found(required)				mp ^0C	% Yield	μ_{eff} BM	Formula weight (gm/mol)
	C	H	N	M				
$C_{24}H_{34}ClCuFN_4O_{10}$ (1)	43.88 (43.91)	5.19 (5.22)	8.54 (8.53)	9.66 (9.68)	206	63.7	1.75	655.12
$C_{28}H_{38}ClCuFN_6O_9$ (2)	46.62 (46.67)	5.34 (5.32)	11.63 (11.66)	8.83 (8.82)	206	62	1.69	719.18
$C_{23}H_{33}ClCuFN_3O_{10}S$ (3)	41.78 (41.76)	5.01 (5.03)	6.36 (6.35)	9.63 (9.61)	202	69.2	1.71	660.09
$C_{32}H_{41}ClCuFN_5O_9$ (4)	50.77 (50.73)	5.48 (5.45)	9.28 (9.24)	8.41 (8.39)	212	68.8	1.92	756.19
$C_{44}H_{49}ClCuFN_5O_9$ (5)	58.12 (58.08)	5.40 (5.43)	7.68 (7.70)	6.99 (6.98)	234	72.1	1.81	908.25
$C_{29}H_{35}ClCuFN_5O_{10}$ (6)	47.66 (47.61)	4.88 (4.82)	9.55 (9.57)	8.72 (8.69)	245	64.9	1.67	730.14
$C_{30}H_{35}ClCuFN_5O_{11}$ (7)	47.44 (47.43)	4.63 (4.64)	9.26 (9.22)	8.35 (8.37)	273	73.2	1.83	758.13
$C_{30}H_{36}ClCuFN_6O_{11}$ (8)	46.50 (46.51)	4.66 (4.68)	10.88 (10.85)	8.17 (8.20)	298	74.5	1.89	773.14

2.5 Biological impact of complexes

2.5.1 Test of complex against microorganism

Synthesized complexes were tested for their impact on the microorganism for which five microorganism were employed namely, Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis (B. Subtilis), Staphylococcus aureus (S. aureus), and Serratia marcescens (S. marcescens). Impact was tested in terms of minimum inhibitory concentration (MIC) using suspended Luria Broth (LB) in sterile double distilled water as a media. The compounds were dissolved in methanol. Cultured for $Gram^{(+ve)}$ and $Gram^{(-ve)}$ were incubated at 37 and 30 ^oC respectively for 24 hours. Control test with no active ingredient was also performed by adding just a solvent alone [22]. MIC was determined using double fold serial dilution in liquid media containing varying concentration of test compounds from 0.1 – 10,000 µM concentration. Bacterial growth was measured by the turbidity of the culture after 18 hour. At a particular concentration of a compound inhibited bacterial growth, half the concentration of the compound was tried. This procedure was carried on to a

concentration that bacteria grow normally. The lowest concentration that totally inhibited bacterial growth was determined as the MIC value. All equipment and culture media employed were sterile.

2.5.2 DNA–binding assay

Purity of DNA was measured by the ratio of absorption at 260 and 280 nm which was found 1.82, indicating that nucleic acid was fully free of protein [23]. The molar absorption coefficients of 6,600 $M^{-1}cm^{-1}$ were trace to determine the concentration of DNA at 260 nm by UV-Visible spectrophotometer [24]. The methodology involving interaction of complex with DNA was conducted under phosphate buffer medium (pH 7.2).

2.5.3 Absorption titration

DNA–mediated hypochromicity and bathochromicity under the influence of complex was measured via UV-Vis absorbance spectra [25–28]. With a selection of an appropriate absorbance peak by performing spectrophotometric wavelength scans of each chelating agents and their Cu(II) complexes. Absorption measurement was followed by 10 minute incubation at room temperature after addition of equivalent amount of DNA to reference cell; concentration of DNA is so set to have 10 times the test compound. DNA–mediated hypochromism (decrease in absorbance) or hyperchromism (increase in absorbance) for test compounds were calculated. It is important to note that the results in this assay were generated under the same conditions as the plasmid degradation assay. This was specifically done to enable direct comparison between the assays that was required to interpret the results obtained. The intrinsic binding constant, K_b was determine making it subject in following equation [29].

$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$

Where, [DNA] is the concentration of DNA in terms of nucleotide phosphate [NP], the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to $A_{obs.}/[M]$, the extinction coefficient for free copper complex and the extinction coefficient for free copper complex in fully bound form, respectively and K_b is the ratio of slope to the y intercept.

2.5.4 Viscosity study

Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature of 27.0 (±0.1) °C in a thermostatic jacket. DNA samples with an approximate average length of 200 base pairs were prepared by sonication in order to minimize complexities arising from DNA flexibility [30]. Flow time was measured with a digital stopwatch with an accuracy of 0.01 second. Each sample was measured three times with a precision of 0.1 second and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus concentration ratio ([Complex]/[DNA]) [31], where as the viscosity of DNA in the presence of complex η and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions ($t > 100$ s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

2.5.5 DNA cleavage study

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out with 15 µL reaction mixture containing 300 µg/mL plasmid DNA (10 mM Tris, 1 mM EDTA, pH 8.0) and 200 µM complex in TE buffer. Reactions were allowed to proceed for 3 h at 37 °C. All reactions were quenched by addition of 5 µL loading buffer (40% sucrose, 0.2% bromophenol blue). The aliquots were loaded directly on to 1% agarose gel and electrophoresed at 50 V in 1X TAE buffer. The gel was stained with 0.5 µg/mL ethidium bromide and was photographed on a UV illuminator. The percentage of each form of DNA was quantities using AlphaDigiDoc™ RT. Version V.4.1.0 PC–Image software.

2.5.6 SOD like activity

SOD-like activity of the complex was determined by NBT/NADH/PMS system [32]. The superoxide radial produce by 79 μM NADH, 30 μM PMS, system containing 75 μM NBT, phosphate buffer (pH = 7.8), and 0.25 to 5.0 μM tested compound. The amount of reduced NBT was spectrophotometrically detected by monitoring the concentration of blue formazan form which absorbs at 560 nm. The NBT reduction rate was measured in the presence and absence of test compounds at various concentration of complex in the system. All measurements were carried out at room temperature. The % inhibition (η) of NBT reduction was calculated using following equation

η (% inhibition of NBT reduction) =(1–k'/k)X 100%

Where k' and k present the slopes of the straight line of absorbance values as a function of time in the presence and absence of SOD mimic or a model compound, respectively. The IC_{50} of the complex was determined by plotting the graph of percentage of inhibiting NBT reduction against the increase in the concentration of the complex. The concentration of the complex which causes 50% inhibition of NBT reduction is reported as IC_{50}.

3. Result and discussion

3.1 Complex characterization

Electronic spectra, magnetic measurements, TGA, IR, and GCMS were used to configure the structure of synthesized complexes. The elemental analysis data are in good concurrence with the proposed formulation and theoretical expectation i.e. 1:1:1 ratio of metal:L:A^n.

3.1.1 IR spectra

The IR spectra of the Cu(II) complexes shows major changes as compared to the free ligands which are comprise in Table 2. The absorption bands observed in case of ofloxacin at 1620 and 1332 cm^{-1} are assign to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$ respectively, where as in case of complexes these bands are observed between 1564–1579 and 1343–1381 cm^{-1}. The frequency of separation ($\Delta\nu = \nu COO_{asy} - \nu COO_{sym}$) in investigated complexes is ~200 cm^{-1}, suggest unidentate nature for the carboxylato group [33, 34]. The sharp band at 3520 cm^{-1} is due to hydrogen bonding for the case of quinolone moiety [35]; which results from free hydroxyl stretching vibration. The complete disappearance of this band says that deprotonation of carboxylic acid group of ofloxacin. The peak at 1728 cm^{-1} responsible for ν(C=O) stretching vibration band in ofloxacin is found to be observed between 1619 – 1633 cm^{-1} in case of complexes; this shift in band towards lower energy suggest that coordination occurs through the pyridone oxygen atom [36]. These data are further supported by ν(M–O) [37] which appear at ~512 cm^{-1}. In the investigated complexes the ν(C=N) band of ligand A^2 appears at 1580 cm^{-1}. N→M bonding was supported by ν(M–N) band [38] at ~530 cm^{-1}.

Table 2: Infrared spectral data

Compounds	ν(C=O) cm^{-1} pyridone	$\nu(COO)_{asy}$ cm^{-1}	$\nu(COO)_{sym}$ cm^{-1}	Δν cm^{-1}	ν(M-N) cm^{-1}	ν(M-O) cm^{-1}	ν(M-S) cm^{-1}
Ofloxacin	1728	1620	1332	288	-	-	-
1	1633	1576	1381	195	535	515	-
2	1619	1578	1377	201	542	504	-
3	1626	1579	1376	203	-	510	428

4	1622	1568	1371	197	540	508	-
5	1620	1566	1343	223	542	511	-
6	1624	1571	1372	199	537	502	-
7	1621	1569	1364	205	541	506	-
8	1623	1564	1372	192	544	507	-

3.1.2 Reflectance spectra and magnetic behaviour

Large width of absorption band observed for the copper complexes i.e. d^9 system for the simplest the ligand at low temperature make them very difficult to interpret. Copper complexes are well known for their various coordination numbers resulting in varieties of geometries in there structure. The Cu(II) complexes exhibit a broad band at ~15300 cm^{-1} [39–41]. These bands are characteristic of Cu(II) d–d transition in tetragonal field in which Cu(II) atom is in distorted square pyramidal coordination environment.

The magnetic moments for any geometry in case of Cu(II) is generally about 1.8 B.M. which is very close to spin–only value i.e. 1.73 B.M. The observed values in our case are very close to the spin–only values(Table 1) expected for S = ½ system (1.73 B.M.) which lead to a path to conclude that metal center in synthesized complexes posses five coordination number with of one unpaired electron responsible for S = ½ system [42, 43].

3.1.3 Thermal analysis

Compositional difference and associated water molecules of the complexes are determine using thermal gravimetric analyses of the complexes in N_2 atmosphere using 5000/2960 SDTA, TA instrument (USA) operating at a heating rate of 10 °C per minute in the range of 20–800 °C. The TGA data indicate that all of the complexes decompose in three steps [44]. The clear interpretation made from the TGA curve shows that loss occurring during first step i.e. 50–120 °C is due to loss of to five molecules of water of crystallization, whereas weight loss during second step i.e. 180–420 °C corresponds to loss of neutral ligand and the loss of weight during final step i.e. 440–690 °C is due to the loss of ofloxacin leaving behind the CuO as a residue. Suggested structure of complexes from above analytical facts is as shown in given Figure 1.

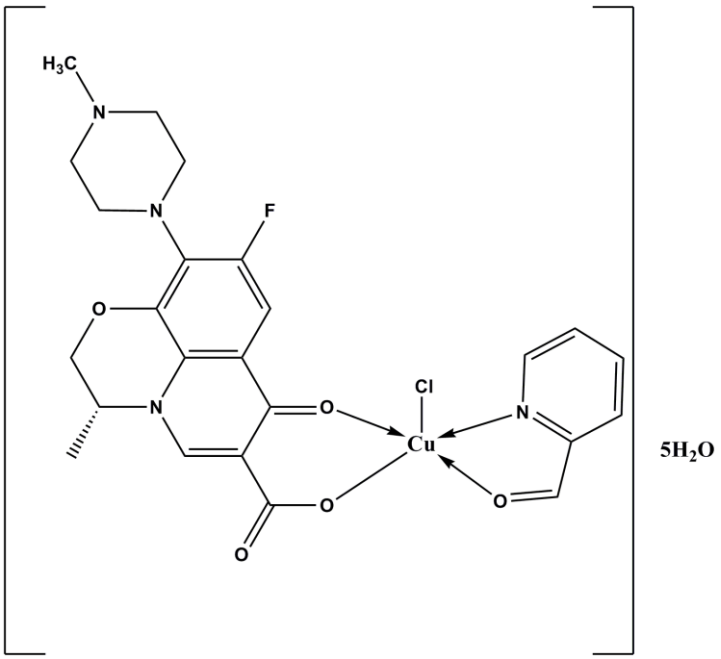

Figure 1: Structure of the title complex [Cu(L)(A^1)Cl].5H_2O.

3.1.4 GC– Mass spectra

Mass spectra of the complexes were obtained using methanol as a solvent with concentration (1 mg/mL). The injecting temperature was maintained at 200 °C. The oven temperature was maintain at 200 °C and was programmed at heating rate of 20 °C min^{-1} and final temperature of oven was 350 °C. The mass spectrum of the complex 1 is shown in Figure 2. Obtained spectra in this mode did not show a molecular ion [M^+] at m/z= 655 [45]. The highest peak was observed at m/z= 245 followed by a peak at m/z= 217. Several other peak at m/z= 205, 170, 107 and 78 were observed from the fragments from pyridine-2-carboxaldehyde.

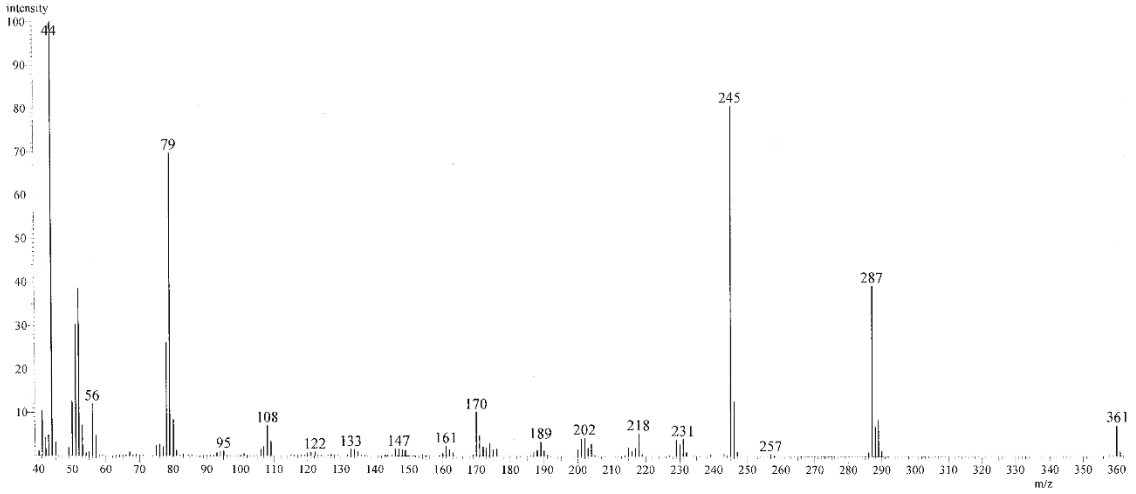

Figure 2: GCMS of [Cu(L)(A^1)Cl].5H_2O using methanol as a solvent at 200 °C injecting and oven temperature at a heating rate of 20 °C per min.

3.2 Biological impact of complexes

3.2.1 Test of complex against microorganism

The complexes were screened for in vitro activity against three Gram$^{(-ve)}$ i.e. S. marcescens, E. coli and P. aeruginosa and two Gram$^{(+ve)}$ i.e. S. aureus, B. subtilis microorganisms using MIC method, Table 3 comprise of the MIC data. Neutral bidentate ligands exhibit a little antimicrobial activity. It is clear from the data that the complexation of drug and ligand with metal make a far difference in the antibacterial activity. In case of B. subtilis, complex–4 has highest potency among all complexes and active compare to gatifloxacin, norfloxacin, enrofloxacin and pefloxacin. In case of S. aureus complex–1, 4 and 5 were active compare to all standard drugs. Similarly for the case of S. marcescens complex–5, 6, 7 and 8 were active. Complexes–1, 6, 7 and 8 were active against P. aeruginosa. Again complexes–6, 7 and 8 were found active against E. Coli. Out of all the complexes, complex–2 has lower potency compare to tested standard drugs.

Table 3: MIC data of the compounds (μM)

Compounds	Gram positive		Gram negative		
	S. aureus	B. subtilis	S. marcescens	P. aeruginosa	E. coli
$CuCl_2{\cdot}2H_2O$	2698.00	2815.00	2756.00	2404.00	3402.00
Ciprofloxacin	1.6	1.1	1.6	1.4	1.4
Gatifloxacin	5.1	4.0	2.9	1.0	2.9
Norfloxacin	2.5	2.5	4.1	3.8	2.8
Enrofloxacin	1.9	3.9	1.7	1.4	1.4
Pefloxacin	2.1	2.4	5.1	5.7	2.7

Levofloxacin	1.7	2.2	1.7	1.7	1.0
Sparfloxacin	1.3	2.0	1.5	1.5	1.3
Ofloxacin	1.9	1.4	1.7	2.2	1.4
A^1	3821.0	3414.0	3333.0	2902.0	3739.0
A^2	3212.0	3271.0	3212.0	3183.0	3154.0
A^3	>10000.0	>10000.0	>10000.0	>10000.0	>10000.0
A^4	130.0	250.0	506.0	154.0	129.0
A^5	194.0	169.0	272.0	255.0	278.0
A^6	631.0	670.0	604.0	725.0	758.0
A^7	829.0	733.0	771.0	738.0	762.0
A^8	578.0	631.0	609.0	658.0	591.0
1	0.9	3.5	2.6	0.9	3.5
2	11.4	12.3	14.0	13.1	7.0
3	4.3	12.1	17.3	13.8	1.2
4	0.9	2.2	1.8	3.5	1.8
5	0.8	2.9	0.8	1.3	1.3
6	4.7	4.5	0.2	0.2	0.2
7	7.4	8.3	1.2	0.7	0.2
8	6.2	5.2	0.7	0.6	0.2

The overall conclusion from the MIC data can be made that the planarity of the bidentate ligand is responsible for more profound effect. This increase in biocidal activity may be due to light of Overtone's concept [36], chelation theory [46] or may be due to the effect of the metal ion on the normal cell process.

3.2.2 Complex DNA interaction

3.2.2.1 Absorption titration

Absorption titration methodology is extensively used to determine the binding affinity of coordination compounds toward DNA. The basic principle of methodology is the change in spectral transition of central metal ion of coordination compounds on interaction with DNA. Due to varying concentration ratio of complex and DNA there occurs a fractional changes in absorbance of complexes and a titration curve is generated as a function of DNA concentration. With increase in DNA to complex ratio hypochromism and a red shift is observed in UV region, indicates coordination through N7 position of guanine (Figure 3). The extent of hypochromism also reveals nature of binding affinity and the hypochromism observed for this complex implies that it does not intercalate with DNA base pairs. Titration curves exhibiting red shift lead to path of decision covalent/coordinate linkage. The extent of the binding strength of complexes is quantitatively determined by measuring the intrinsic binding constants K_b of the complexes by monitoring the changes of absorbance at 274 nm with varying concentration of DNA. From the plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ vs. [DNA], (Inset figure 3) the K_b value of complexes were determine and were found in the power of four (Table 4). Which is much lower than the K_b value of classical intercalations (ethidium bromide) thus there is a possibility of intercalation in the complexes. Also, these values are closely comparable to some known complexes exhibiting covalent mode of binding [47], which also suggest that copper(II) ion prefers to bind to the N7 position of guanine [48, 49]. Therefore, the

above results indicate that complexes may first bind with the phosphate group of DNA, neutralize the negative charge of DNA phosphate group, and cause the contraction and conformational change of DNA.

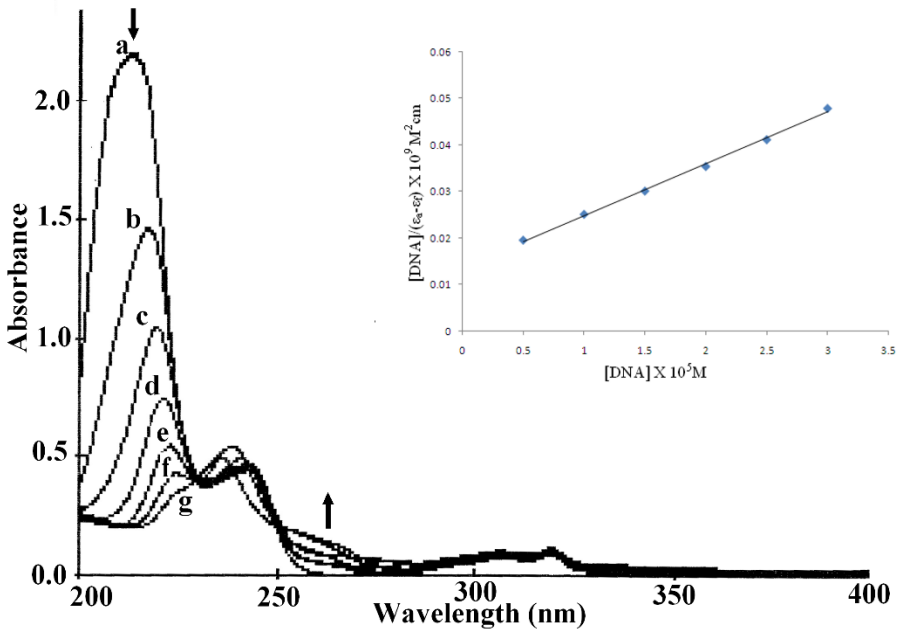

Figure 3: Electronic absorption spectra of [Cu(L)(A[1])Cl].5H2O in absence and in presence of increasing amount of DNA; a, 0 µM; d –g, 5-30 µM in phosphate buffer(Na_2HPO_4 /NaH_2PO_4, pH 7.2), [complex]= 3µM, [DNA]= 0 – 30 µM with incubation period of 30 min. at 37 °C, Inset: Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA]. Arrow shows the absorbance change upon increasing DNA concentrations.

Table 4: Binding constants (K_b) of Cu(II) complexes with DNA in Phosphate buffer pH 7.2

Complexes	K_b (M^{-1})
[Cu(L)(A[1])Cl].5H2O (1)	0.846 x 10^4
[Cu(L)(A[2])Cl].5H2O (2)	8.00 x 10^4
[Cu(L)(A[3])Cl].5H2O (3)	6.66 x 10^4
[Cu(L)(A[4])Cl].5H2O (4)	2.00 x 10^5
[Cu(L)(A[5])Cl].5H2O (5)	1.00 x 10^4
[Cu(L)(A[6])Cl].5H2O (6)	2.33 x 10^4
[Cu(L)(A[7])Cl].5H2O (7)	4.22 x 10^4
[Cu(L)(A[8])Cl].5H2O (8)	6.74 x 10^4

3.2.2.2 Viscosity measurement

In absence of crystallographic study it is found that relative viscosity measurement study is the most critical tests for exploding the interaction properties between the complexes and the DNA in solution state. In order to determine the said one, relative viscosity is measured by varying the concentrations of added complexes and results are interpreted from the plot of $[\eta/\eta_0]^{1/3}$ versus [Complex]/[DNA] ratio; presented in Figure 4. Figure shows that the binding ability of classical intercalator ethidium bromide is more compare to all complexes.

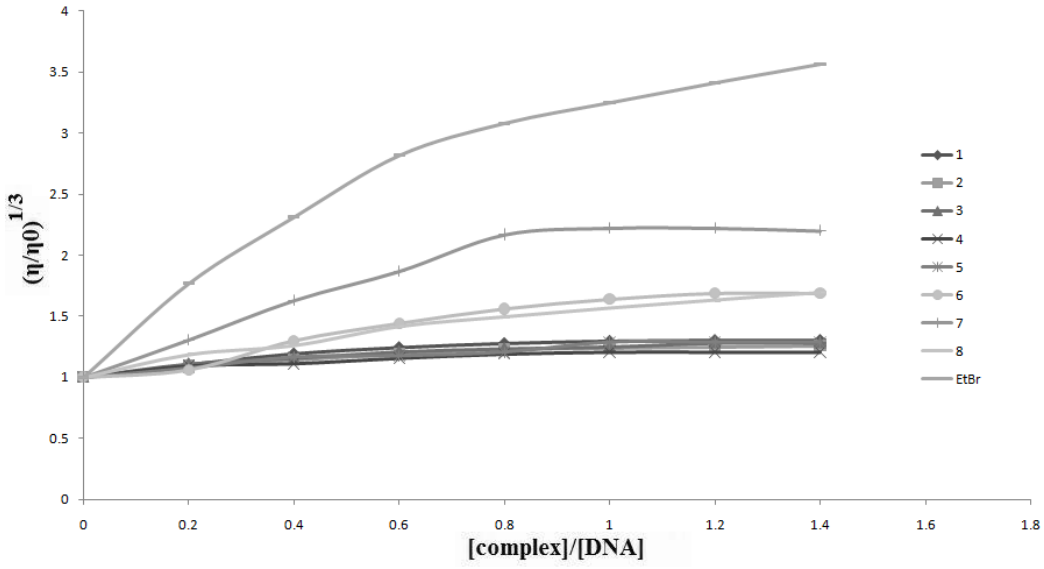

Figure 4: Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27±0.1 °C in phosphate buffer (Na_2HPO_4 /NaH_2PO_4, pH 7.2), as a medium.

But among all complexes, complex–7 exhibit intense effect on relative viscosity of DNA compare to other classical intercalator reported herein. Photophysical experiments provide necessary but not sufficient clues to support a binding mode. The increase in DNA viscosity observed in the complexes which is different from the interaction of Δ–$[Ru(phen)_3]^{2+}$ with DNA [30, 50] suggest a classical intercalative mode [51] and/or covalent binding with DNA.

3.2.2.3 DNA cleavage study

DNA cleavage accelerated by transition metal complexes is the center of interest [52, 53]. Figure 5 shows the electrophoresis separation of pUC19 DNA reacted upon complexes under aerobic condition.

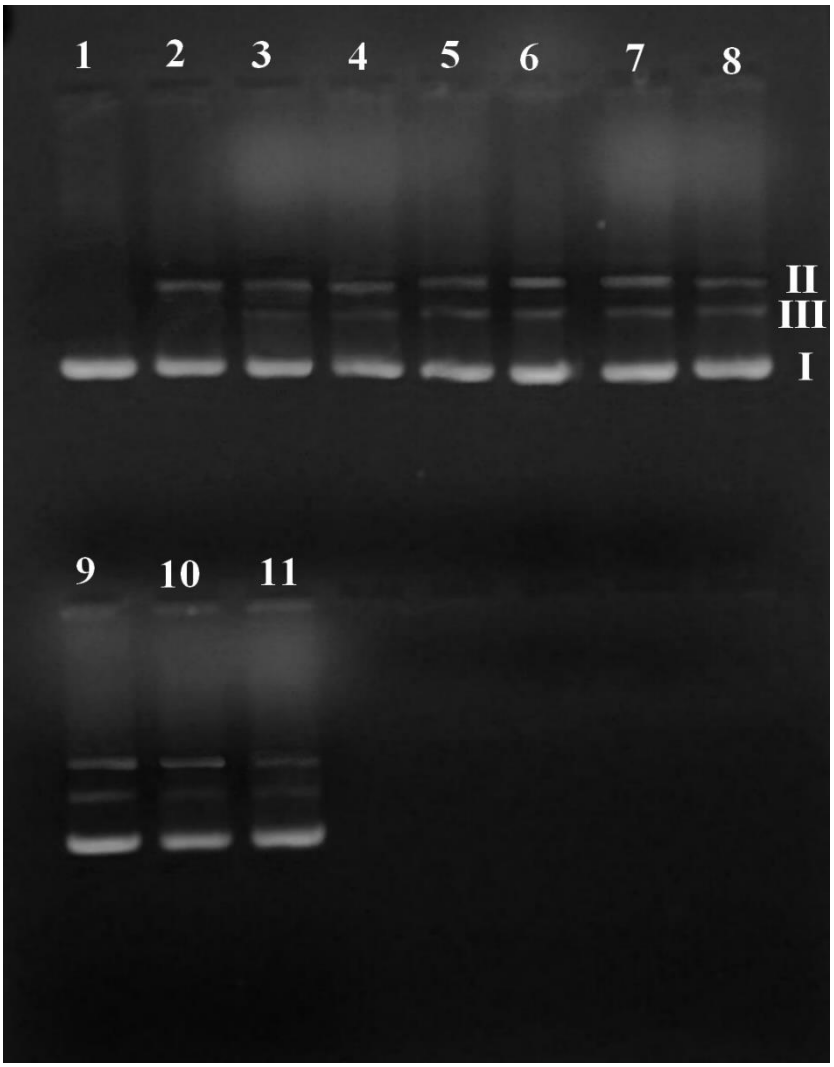

Figure 5: Photogenic view of interaction of pUC19 DNA (300 µg/mL) with series of copper(II) complexes (200 µM) using 1% agarose gel containing 0.5 µg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 µL, for 3 h. at 37 °C. : Lane 1, DNA control; Lane 2, $CuCl_2{\cdot}2H_2O$; Lane 3, Ofloxacin; Lane 4, $[Cu(L)(A^1)Cl].5H_2O$; Lane 5, $[Cu(L)(A^2)Cl].5H_2O$; Lane 6, $[Cu(L)(A^3)Cl].5H_2O$; Lane 7, $[Cu(L)(A^4)Cl].5H_2O$; Lane 8, $[Cu(L)(A^5)Cl].5H_2O$; Lane 9, $[Cu(L)(A^6)Cl].5H_2O$; Lane 10, $[Cu(L)(A^7)Cl].5H_2O$; Lane 11, $[Cu(L)(A^8)Cl].5H_2O$.

When the plasmid DNA was subjected to electrophoresis upon reaction with complexes the fastest migration was observed for super coiled (SC) Form I, the slowest moving open circular (OC) form (Form II) will produce upon relaxing of SC, the intermediate moving is the linear form (Form III) generated on cleavage of circular form. The data of the cleavage are presented in Table 5. The different DNA-cleavage efficiency of the complexes was due to the difference in binding affinity of the complexes to DNA.

Table 5: Gel eletrophoretic data for DNA cleavage study

Lane No.	Compound	Form I	Form II	Form III
1	Control	100	-	-
2	$CuCl_2{\cdot}2H_2O$	83	17	-

3	Ofloxacin	75	14	11
4	[Cu(L)(A^1)Cl].5H_2O (1)	74	16	10
5	[Cu(L)(A^2)Cl].5H_2O (2)	72	14	14
6	[Cu(L)(A^3)Cl].5H_2O (3)	64	20	16
7	[Cu(L)(A^4)Cl].5H_2O (4)	68	20	12
8	[Cu(L)(A^5)Cl].5H_2O (5)	70	14	16
9	[Cu(L)(A^6)Cl].5H_2O (6)	71	15	14
10	[Cu(L)(A^7)Cl].5H_2O (7)	73	15	12
11	[Cu(L)(A^8)Cl].5H_2O (8)	70	14	16

3.2.2.4 SOD–like activity

The system used as a source of superoxide radical generator was NBT/ NADH/PMS system in order to check SOD like activity of the synthesized complexes. Absorbance at a function of time was plotted to have a straight line obeying equation Y = mX + C(Figure 6); with increase in concentration of tested complexes deterioration in slope (m) is observed. Figure 7 shows percentage inhibition of the reduction of nitro blue tetrazolium (NBT) plotted against the concentration of the complex–1. Compounds exhibit SOD–like activity at biological pH with their IC_{50} values ranging from 0.425 to 1.305 μM. The superoxide scavenging data (Table 6) suggest that all the complexes are active compare to the complexes reported by Chao et al. [54] but in comparisons to the complexes reported by Casanova et al. complexes–4, 6 and 7 were found even more active [55]. The higher IC_{50} can only be accredited to the vacant coordination which facilitates the binding of superoxide anion, electrons of aromatic ligands that stabilize Cu–$O_2^{\cdot -}$ interaction and not only to the partial dissociation of complex in solution.

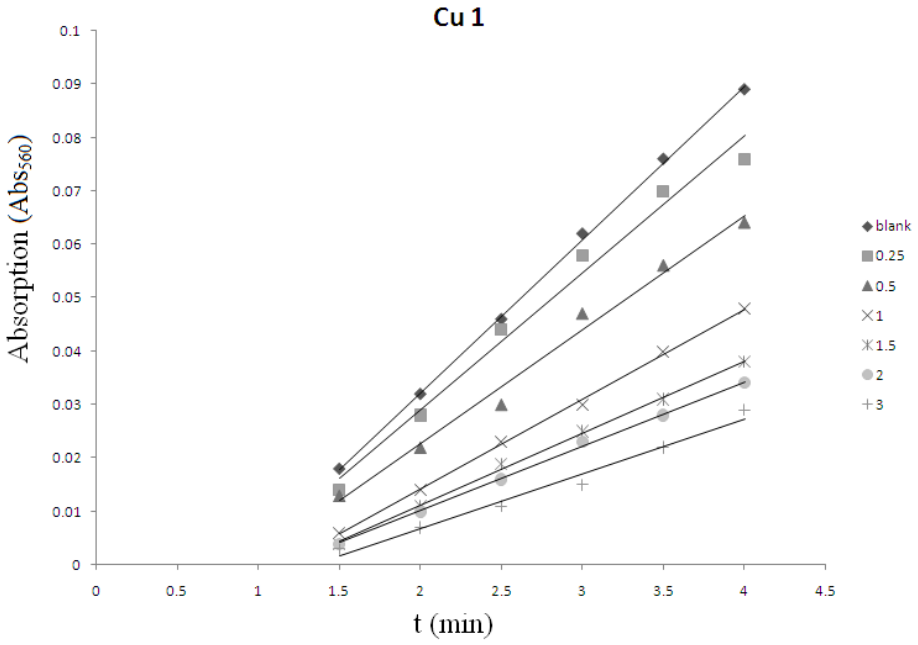

Figure 6: Absorbance values(Abs_{560}) as a function of time (t) plotted for varying concentration of complex 1 from 0.25 μM to 3 μM for which a good straight line are observed.

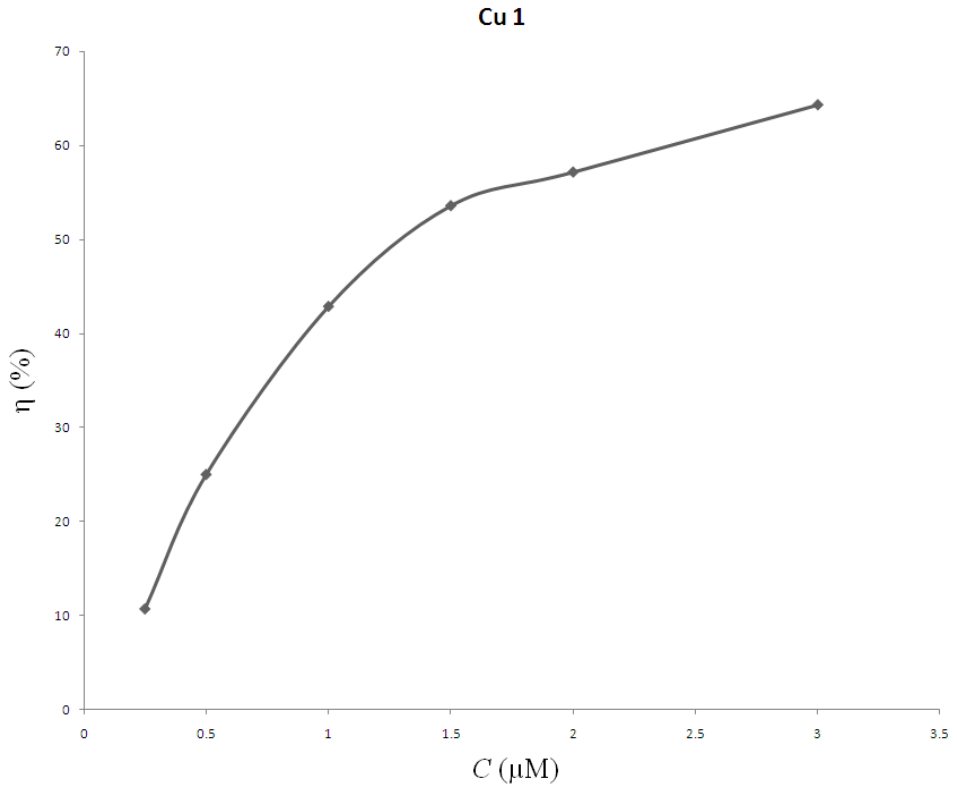

Figure 7: Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex 1.

Table 6: The IC_{50} values taken from reports on SOD-like activities of copper(II) complexes

Complexes	IC_{50} (μM)	References
[Cu(L)(A^1)Cl].5H_2O (1)	1.305	This work
[Cu(L)(A^2)Cl].5H_2O (2)	1.305	This work
[Cu(L)(A^3)Cl].5H_2O (3)	1.015	This work
[Cu(L)(A^4)Cl].5H_2O (4)	0.500	This work
[Cu(L)(A^5)Cl].5H_2O (5)	0.900	This work
[Cu(L)(A^6)Cl].5H_2O (6)	0.425	This work
[Cu(L)(A^7)Cl].5H_2O (7)	0.625	This work
[Cu(L)(A^8)Cl].5H_2O (8)	1.000	This work
[Cu(stz)$(py)_3$Cl]	1.310	54
[Cu(Hstz)(MeOH)Cl_2]	2.510	54
[Cu(Hstz)(MeOH)Cl_2]	5.170	55
[Cu$(stz)_2$(4,4-dmHim)$_2$]	0.742	55
[Cu$(stz)_2$(1,2-dmHim)$_2$]	1.03	55
[Cu$(stz)_2$(4-mHim)$_2$]	0.586	55

4 Conclusions

Here in this work group we have prepared 8 different Cu(II) metallointercalators with different NN donor ligands and ofloxacin as a uninegative bidentate ligand. From the MIC technique it is clear that the complexes derived from phenanthroline derivatives are more active against tested specie compare to the complexes derived from A^1, A^2 & A^3. Relative viscosity curve reveals that the complex 6, 7 & 8 bind to DNA more strongly via classical intercalative mode compare to other, which is further supported by the data from absorption titration where it is clear that the binding constant k_b for complexes 6, 7 & 8 is more compare to rest of complexes. DNA cleavage study result shows that the cleavage ability of all the complexes is in good accordance with the ability of drug. 0.415–1.305 μM concentration of complexes is enough to inhibit the reduction rate of NBT by 50% (IC_{50}) in NBT/NADH/PMS system. The results from the Table 6 again shows that complex 6, 7 & 8 posses higher SOD mimic activity compare to other. Thus from above all study it can be concluded that the presence of planer heterocyclic ligand in Cu(II) drug based mixed ligand complex increases the interaction of complexes in biological system. Our group is currently examining a range of biological interactions that these metallointercalators may undergo inside the cell to better understand their biochemistry and mechanism of action.

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