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Antibacterial, SOD mimic and nuclease activities of copper(II) complexes containing ofloxacin and neutral bidentate ligands

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Drug-based mixed-ligand copper(II) complexes of type $[Cu(OFL)(A^n)Cl]\cdot 5H_2O$ (OFL = ofloxacin, A^1 = pyridine-2-carbaldehyde, A^2 = 2,2′-bipyridylamine, A^3 = thiophene-2-carbaldehyde, 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) were synthesized and characterized. Spectral investigations of complexes revealed square pyramidal geometry. Viscosity measurement and absorption titration were employed to determine the mode of binding of complexes with DNA. DNA cleavage study showed better cleaving ability of the complexes compared with metal salt and standard drug by conversion of a supercoiled form of pUC19 DNA to linear via circular. From the SOD mimic study, concentration of complexes ranging from 0.415 to 1.305 μM is enough to inhibit the reduction rate of NBT by 50% (IC_{50}) in the NADH-PMS system. Antibacterial activity was assayed against selective Gram-negative and Gram-positive microorganisms using the doubling dilution technique.

Keywords: ofloxacin; nuclease activity; SOD mimic; antibacterial

Introduction

Heterocyclic ring systems having piperidine-4-one nucleus have aroused great interest in the past due to their wide variety of biological properties, such as antiviral, antitumor, local anesthetic, anticancer and antimicrobial activities.[1–6] Fluoroquinolone drugs act intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV.[7] Interaction of metal ions with diverse deprotonated quinolone as ligands has been thoroughly studied.[8] Large numbers of copper(II) complexes have been synthesized and explored for their biological activities, because of their biological relevance.[9,10] Among these copper(II) complexes, attention has been mainly focused on the copper(II) complexes with diverse drugs.[11]

Superoxide radicals ($O_2^{\bullet -}$), if not eliminated, may cause significant cellular damage such as inflammatory damage, membrane damage, DNA damage and aging.[12] To avoid such harmful consequences, all oxygen metabolizing organisms possess metalloenzymes known as superoxide dismutases (SODs), which keep the concentration of $O_2^{\bullet -}$ in controlled low limits, thus protecting biological molecules from oxidative damage.[13] Study of Cu–Zn SODs has shown that dismutation of $O_2^{\bullet -}$ proceeds via alternating reduction and oxidation of the essential Cu ion during successive encounters with the substrate to produce O_2 and H_2O_2, respectively.

In continuation of our previous work,[14,15] we synthesized copper(II) complexes with ofloxacin and neutral bidentate ligands, prompting them to gain an inhibitor for inhibition of DNA gyrase (topoisomerase II). The interaction of complexes with DNA has also been investigated using viscosity measurement, electronic absorbance spectroscopy and gel electrophoresis. Minimum concentration of the compounds required to inhibit the growth of microorganism (MIC) was obtained using the double dilution technique. The SOD mimic activity was determined using a nonenzymatic NBT–NADH–PMS system.

Experimental Section

Materials and Methods

2,2′-Bipyridylamine (A^2) was purchased from Lancaster (Morecambe, UK). Ofloxacin (OFLH) was purchased from Bayer AG (Wuppertal, Germany). Cupric chloride was purchased from E. Merck (India) Ltd, Mumbai. Pyridine-2-carbaldehyde (A^1), thiophene-2-carbaldehyde (A^3), 1,10-phenanthroline, Luria Broth, agarose, ethidium bromide, TAE (Tris–acetyl–EDTA), bromophenol blue and xylene cyanol FF were purchased from Himedia, India. 2,9-Dimethyl-1,10-phenanthroline (A^4) and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (A^5) were purchased from Loba Chemie Pvt. Ltd (India). Sperm herring DNA was purchased from Sigma Chemical Co., India.

Infrared spectra were recorded on a FT–IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The electronic spectra were recorded on a UV–160A UV–vis spectrophotometer, Shimadzu (Japan). Mass spectra were recorded using GCMS–QP2010 with an ionization voltage of 0.90 kV, 30.0 m length and a thickness of 1.0 μm with a column having 0.25 mm diameter. The magnetic moment was measured by Gouy's method using mercury(II) tetrathiocyanatocobaltate(II) as the calibrant

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($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20 °C), Citizen Balance. The metal contents of 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).[16] Percentages of C, H and N were determine using a model 240 Perkin Elmer elemental analyzer. The thermogravimetric curve was obtained with a model 5000/2960 SDTA, TA instrument (USA).

Synthesis of Ligands

4,5-Diazafluoren-9-one (A^6), 1,10-phenanthroline-5,6-dione (A^7) and 5-nitro-1,10-phenanthroline (A^8) were prepared as per the reported methods.[17–19]

General Synthesis of Complexes

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 methanolic solution of ofloxacin (1.5 mmol) in the presence of CH_3ONa (1.5 mmol). The pH of the reaction mixture was adjusted to ~6.4 using a 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 obtained was washed with ether–hexane and dried in vacuum desiccators.

*[Cu(OFL)(A^1)Cl]·5H_2O (**1**)*

Yield: 64%, m.p. 206 °C, mol. wt 655.12, $\mu_{eff.}$ = 1.75 B.M. Calcd for $C_{24}H_{34}ClCuFN_4O_{10}$: C, 43.91; H, 5.22; N, 8.53. Found: C, 43.78; H, 5.39; N, 8.38. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1633 (vs); $\nu(COO)_{asym}$, 1576 (vs); $\nu(COO)_{sym}$, 1381 (vs); ν(M–O), 515; ν(M–N), 535.

*[Cu(OFL)(A^2)Cl]·5H_2O (**2**)*

Yield: 62%, m.p. 206 °C, mol. wt 719.18, $\mu_{eff.}$ = 1.69 B.M. Calcd for $C_{28}H_{38}ClCuFN_6O_9$: C, 46.67; H, 5.32; N, 11.66. Found: C, 46.53; H, 5.52; N, 11.41. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1619 (vs); $\nu(COO)_{asym}$, 1578 (vs); $\nu(COO)_{sym}$, 1377 (vs); ν(M–O), 504; ν(M–N), 542.

*[Cu(OFL)(A^3)Cl]·5H_2O (**3**)*

Yield: 69%, m.p. 202 °C, mol. wt 660.09, $\mu_{eff.}$ = 1.71 B.M. Calcd for $C_{23}H_{33}ClCuFN_3O_{10}S$: C, 41.76; H, 5.03; N, 6.35. Found: C, 41.63; H, 5.26; N, 6.48. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1626 (vs); $\nu(COO)_{asym}$, 1579 (vs); $\nu(COO)_{sym}$, 1376 (vs); ν(M–O), 510; ν(M–S), 428.

*[Cu(OFL)(A^4)Cl]·5H_2O (**4**)*

Yield: 68%, m.p. 212 °C, mol. wt 756.19, $\mu_{eff.}$ = 1.92 B.M. Calcd for $C_{32}H_{41}ClCuFN_5O_9$: C, 50.73; H, 5.45; N, 9.24. Found: C, 50.85; H, 5.63; N, 9.05. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1622 (vs); $\nu(COO)_{asym}$, 1568 (vs); $\nu(COO)_{sym}$, 1371 (vs); ν(M–O), 508; ν(M–N), 540.

*[Cu(OFL)(A^5)Cl]·5H_2O (**5**)*

Yield: 72%, m.p. 234 °C, mol. wt 908.25, $\mu_{eff.}$ = 1.81 B.M. Calcd for $C_{44}H_{49}ClCuFN_5O_9$: C, 58.08; H, 5.43; N, 7.70. Found: C, 58.24; H, 5.31; N, 7.83. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1620 (vs); $\nu(COO)_{asym}$, 1566 (vs); $\nu(COO)_{sym}$, 1343 (vs); ν(M–O), 511; ν(M–N), 542.

*[Cu(OFL)(A^6)Cl]·5H_2O (**6**)*

Yield: 65%, m.p. 245 °C, mol. wt 730.14, $\mu_{eff.}$ = 1.67 B.M. Calcd for $C_{29}H_{35}ClCuFN_5O_{10}$: C, 47.61; H, 4.82; N, 9.57. Found: C, 47.49; H, 4.73; N, 9.38. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1624 (vs); $\nu(COO)_{asym}$, 1571 (vs); $\nu(COO)_{sym}$, 1372 (vs); ν(M–O), 502; ν(M–N), 537.

*[Cu(OFL)(A^7)Cl]·5H_2O (**7**)*

Yield: 73%, m.p. 273 °C, mol. wt 758.13, $\mu_{eff.}$ = 1.83 B.M. Calcd for $C_{30}H_{35}ClCuFN_5O_{11}$: C, 47.43; H, 4.64; N, 9.22. Found: C, 47.57; H, 4.53; N, 9.36. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1621 (vs); $\nu(COO)_{asym}$, 1569 (vs); $\nu(COO)_{sym}$, 1364 (vs); ν(M–O), 506; ν(M–N), 541.

*[Cu(OFL)(A^8)Cl]·5H_2O (**8**)*

Yield: 74%, m.p. 298 °C, mol. wt 773.14, $\mu_{eff.}$ = 1.89 B.M. Calcd for $C_{30}H_{36}ClCuFN_6O_{11}$: C, 46.51; H, 4.68; N, 10.58. Found: C, 46.36; H, 4.79; N, 10.72. IR (KBr pellet, cm^{-1}): $\nu(C{=}O)_p$, 1623 (vs); $\nu(COO)_{asym}$, 1564 (vs); $\nu(COO)_{sym}$, 1372 (vs); ν(M–O), 507; ν(M–N), 544.

Antibacterial Activity (Minimum Inhibitory Concentration)

Synthesized complexes were tested for their impact on the microorganism, namely *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Serratia marcescens*. Impact was tested in terms of minimum inhibitory concentration (MIC) using suspended Luria Broth (LB) in sterile double-distilled water as a medium. Gram-positive and Gram-negative cultures were incubated for 24 h at 37 and 30 °C, respectively. A control test with no active ingredient was also performed by adding just an equivalent amount of solvent.[20] MIC was determined using double-fold serial dilution in liquid media containing varying concentrations of test compounds from 0.1 to 10 000 μM. Bacterial growth was measured by the turbidity of the culture after 18 h. If particular concentration of compound inhibits bacterial growth, half the concentration of the compound was tested. This procedure was carried on to a concentration at which bacteria grow normally. The lowest concentration that inhibits the bacterial growth totally was determined as MIC value. All equipment and culture media employed during the process were sterile.

DNA Interaction Study

Absorption titration

DNA-mediated hypochromic and bathochromic shift under the influence of complexes were measured via UV–vis absorbance spectra.[21–24] The concentration of DNA was so set to have 1.5 times the concentration of test compound. After addition of equivalent amount of DNA to reference cell, it was incubated for 10 min at room temperature, followed by absorbance measurement. DNA-mediated hypochromism (decrease in absorbance) or hyperchromism (increase in absorbance) for test compounds was 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 determined, making it the subject in following equation:[25]

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

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.

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.[26] Flow time was measured with a digital stopwatch with an accuracy of 0.01 s. Each sample was measured three times with a precision of 0.1 s and an average flow time was calculated. Data were represented graphically as $(\eta/\eta_0)^{1/3}$ vs concentration ratio ([Complex]/[DNA]),[27] where η is the viscosity of DNA in the presence of complex 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), $\eta = t - t_0$.

DNA Cleavage Study

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out in TAE buffer (0.04 M Tris–acetate, pH 8, 0.001 M EDTA) with 15 µl of reaction mixture containing plasmid DNA in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and 200 µM complex. Reactions were allowed to proceed for 3 h at 37 °C. All reactions were quenched by addition of 5 µl loading buffer (0.25% bromophenol blue, 40% sucrose, 0.25% xylene cyanole and 200 mM EDTA). The aliquots were loaded directly on to 1% agarose gel and electrophoresed at 50 V in 1X TAE buffer. Gel was stained with 0.5 µg ml^{-1} ethidium bromide and was photographed on a UV illuminator. The percentage of each form of DNA was determined using AlphaDigiDoc™ RT version 4.0.0 PC–image software.

SOD-like Activity

The SOD-like activity of the complex was determined by NBT/NADH/PMS system.[28] The superoxide radicals were produced by 79 µM NADH, 30 µM PMS and 75 µM NBT in phosphate buffer (pH = 7.8). The concentration of the tested compounds varied from 0.25 to 3.0 µM. The amount of reduced NBT was spectrophotometrically detected by monitoring the concentration of blue formazan form, which absorbs at 560 nm. The reduction rate of NBT 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 percentage inhibition (η) of NBT reduction was calculated using the following equation:

$$\eta(\%\ \text{inhibition of NBT reduction}) = (1 - k'/k) \times 100\%$$

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} value of the complex was determined by plotting the graph of percentage inhibition of NBT reduction against increase in concentration of the complex. The concentration of the complex to cause 50% inhibition of NBT reduction is reported as IC_{50}.

Results and Discussion

IR Spectra

In the IR spectrum, absorption bands observed for ofloxacin at 1620 and 1332 cm^{-1} were assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$ respectively. On complexation, these bands appeared between 1564–1579 and 1343–1381 cm^{-1}. The frequency of separation ($\Delta\nu = \nu COO_{asym} - \nu COO_{sym}$) in the investigated complexes was ~200 cm^{-1}, suggest a unidentate nature for the carboxylato group.[29,30] The sharp band at 3520 cm^{-1}, due to the stretching vibration of free hydroxyl in the quinolone moiety,[31] completely disappeared in the spectra of the complexes. The band at 1728 cm^{-1} responsible for ν(C=O) in ofloxacin was observed between 1619 and 1633 cm^{-1} for the complexes. This shift in band towards lower energy suggests that the coordination occurs through the pyridone oxygen atom.[32] These data were further supported by ν(M–O) which appeared at ~512 cm^{-1}.[33] N → M bonding was supported by ν(M–N) band at ~530 cm^{-1}.[34]

Reflectance Spectra and Magnetic Behavior

Visible emission spectra of the copper(II) complexes, i.e. d^9 system, were recorded in DMSO. Complexes exhibited only broad λ_{max} between 663 to 695 nm, which were attributed to the d–d transition for Cu(II) atom in a distorted square pyramidal environment.[35–38] The possibility of trigonal bipyrimidal geometry at the metal center was ruled out because the pattern of $\lambda_{max} > 800$ nm along with the shoulder at ~660 nm was not observed in the case of synthesized complexes.[39,40]

The magnetic moment for any geometry in case of Cu(II) is generally about 1.8 B.M. Magnetic moment values of complexes range from 1.67 to 1.92 B.M., very close to the spin–only values expected in the $S = 1/2$ system (1.73 B.M.). These values indicate that copper(II) in synthesized complexes possesses one unpaired electron responsible for the $S = 1/2$ system.[41,42]

Thermal Analysis

The TG data indicate that all of the complexes decompose in three steps.[43] The clear interpretation made from the TG curve shows that loss occurring during first step, i.e. 50–120 °C, is due to the loss of five molecules of crystallization water, whereas weight loss during the second step, i.e. 180–420 °C corresponds to the loss of the neutral ligand, and the loss of weight during the final step, i.e. 440–690 °C is due to loss of ofloxacin, leaving behind CuO as a residue. The suggested structure of complex **1** from above analytical facts is as shown in Fig. 1.

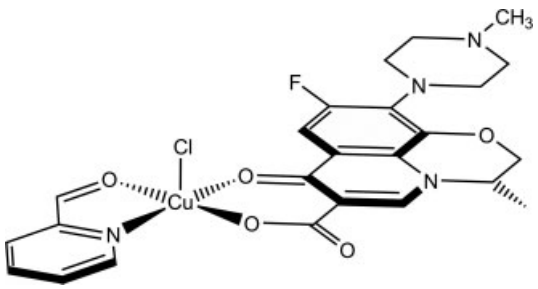

Figure 1. Structure of the title complex [Cu(OFL)(A^1)Cl]·5H_2O.

GC-Mass Spectra

The mass spectrum of complex **1** did not show a molecular ion [M^+] at $m/z = 565$.[44] The highest peak was observed at $m/z = 361$ corresponding to ofloxacin. Peaks at $m/z = 245$ and 217 corresponded to the fragments of ofloxacin. The peak at $m/z = 170$ corresponded to a fragment of pyridine-2-carbaldehyde with copper. Other peaks at $m/z = 107$ and 79 were observed from the fragments of pyridine-2-carbaldehyde.

Test of Complex Against Microorganisms

MIC data suggests that complexation of drug and ligand with metal ion makes a large difference to the antibacterial activity. The MIC data are given in Table 1. For *S. aureus*, complexes **1, 4** and **5** were more active than drug. For *B. subtilis*, the potency of all complexes decreased compared with ofloxacin. For *S. marcescens*, complexes **5**–**8** were active compared with ofloxacin. Complexes **1** and **5**–**8** were active against *P. aeruginosa* compared with ofloxacin. Again complexes **3** and **5**–**8** were found to be more active against *E. coli* than ofloxacin. Out of all complexes, complex **2** had lower potency compared with the tested standard drug. An overall conclusion from the MIC data can be made that the planarity of bidentate ligand is responsible for the greater effect. This increase in antimicrobial activity may be due to Overtone's concept,[32] chelation theory[45] or the effect of the metal ion on the normal cell process.

Complex–DNA Interaction

Absorption titration

The basic principle of absorption titration is a change in spectral transition of coordination compounds on interaction with DNA. With increase in DNA-to-complex ratio, hypochromism and red shift are observed (Fig. 2). The extent of the binding strength of complexes is quantitatively determined by measuring an intrinsic binding constants K_b (Table 2). This is much lower than the K_b value of the classical intercalator ethidium bromide. Thus, there is a possibility of intercalation of complexes. The low K_b value of complexes **1, 4** and **5** is due to the nonplanarity and steric constraint of methyl groups near the Cu(II) core in the ancillary ligand.[46,47] The highest binding constant of complex **8** is due to the electron-withdrawing group present on the ancillary ligand.[48]

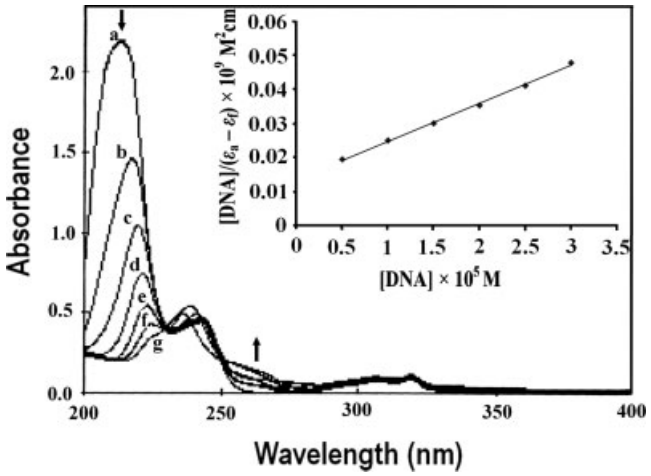

Figure 2. Electronic absorption spectra of [Cu(OFL)(A^1)Cl]·$5H_2O$ in the absence and presence of increasing amounts of DNA: (a) 0 μM; (d–g) 5–30 μM in phosphate buffer (Na_2HPO_4–NaH_2PO_4, pH 7.2), [complex] = 20 μM, [DNA] = 0–30 μM with an incubation period of 30 min at 37 °C. Inset: plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA]. The arrow shows the absorbance change upon increasing DNA concentrations.

Viscosity measurement

In the absence of crystallographic study, it was found that relative viscosity measurement is the most critical test for determining the interaction properties between complexes and DNA in the solution state. Figure 3 shows that the binding ability of classical

Table 1. MIC data of the compounds (μM)

Compounds	Gram positive		Gram negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$CuCl_2 \cdot 2H_2O$	2698.0	2815.0	2756.0	2404.0	3402.0
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	>10 000.0	>10 000.0	>10 000.0	>10 000.0	>10 000.0
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

Table 2. The binding constants (K_b) of Cu(II) complexes with DNA in phosphate buffer, pH 7.2

Complexes	K_b (M^{-1})
[Cu(OFL)(A^1)Cl]·5H_2O (**1**)	0.846×10^4
[Cu(OFL)(A^2)Cl]·5H_2O (**2**)	5.34×10^4
[Cu(OFL)(A^3)Cl]·5H_2O (**3**)	6.66×10^4
[Cu(OFL)(A^4)Cl]·5H_2O (**4**)	2.26×10^4
[Cu(OFL)(A^5)Cl]·5H_2O (**5**)	1.09×10^4
[Cu(OFL)(A^6)Cl]·5H_2O (**6**)	2.33×10^4
[Cu(OFL)(A^7)Cl]·5H_2O (**7**)	4.22×10^4
[Cu(OFL)(A^8)Cl]·5H_2O (**8**)	6.74×10^4

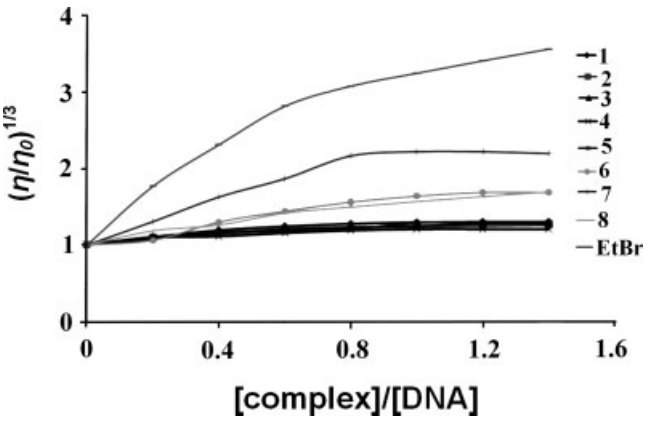

Figure 3. Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27 ± 0.1 °C in phosphate buffer (Na_2HPO_4–NaH_2PO_4, pH 7.2) as a medium.

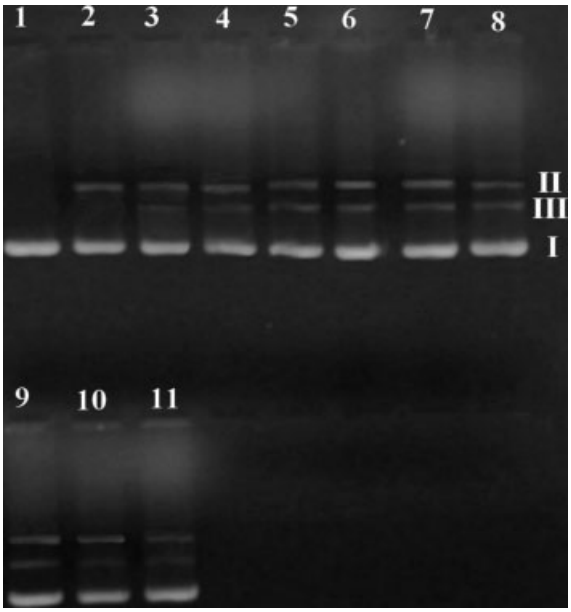

Figure 4. Photogenic view of interaction of pUC19 DNA with series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg ml^{-1} ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μl, for 3 h at 37 °C: lane 1, DNA control; lane 2, $CuCl_2 \cdot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

intercalator ethidium bromide is greater compared with the other complexes. However, among all the complexes, **7** exhibited the most intense effect on the relative viscosity of DNA compared with the other classical intercalators reported herein. The increase in DNA viscosity observed in the complexes, which was different from the interaction of $[Ru(phen)_3]^{2+}$ with DNA,[49,50] suggests a classical intercalative mode and/or covalent binding with DNA.[51]

DNA cleavage study

DNA cleavage accelerated by transition metal complexes is the center of interest.[52,53] Figure 4 shows the electrophoretic separation of pUC19 DNA reacted with complexes under aerobic conditions. When the plasmid DNA was subjected to electrophoresis upon reacting with complexes, the fastest migration was observed for the supercoiled (SC) form (form I), the slowest moving open circular (OC) form (form II) produced upon relaxing SC, and the intermediate moving linear (LC) form (form III), generated upon cleavage of the circular form. Data for the cleavage study are presented in Fig. 5. The difference in the DNA–cleavage efficiency of complexes was due to the difference in the binding affinity of the complexes to DNA.

SOD-like Activity

The NBT/NADH/PMS system was used to check the SOD-like activity of the synthesized complexes. The percentage inhibition of formazan formation at various concentrations of complexes as a function of time was measured by measuring the absorbance at 560 nm and plotted on a straight line (Fig. 6). With the increase in the concentration of tested compounds, a decrease in slope (m) was observed. Percentage inhibition of the reduction of NBT was plotted against concentration of the complex (Fig. 7). Compounds exhibited SOD-like activity at biological pH with their IC_{50} values ranging from 0.425 to 1.305 μM. The superoxide scavenging data (Table 3) suggest that complexes **4**–**7** were found to be active compared with the complexes reported by Casanova *et al*.[54,55] The higher IC_{50} can only be accredited to the vacant coordination site, which facilitates the binding of superoxide anion, electrons of aromatic ligands that stabilize Cu–$O_2^{\bullet-}$ interaction and not only to the partial dissociation of complex in solution.

Table 3. The IC_{50} values taken from reports on SOD-like activities of copper(II) complexes

Complexes	IC_{50} (μM)	References
[Cu(OFL)(A^1)Cl]·5H_2O (**1**)	1.305	This work
[Cu(OFL)(A^2)Cl]·5H_2O (**2**)	1.305	This work
[Cu(OFL)(A^3)Cl]·5H_2O (**3**)	1.015	This work
[Cu(OFL)(A^4)Cl]·5H_2O (**4**)	0.500	This work
[Cu(OFL)(A^5)Cl]·5H_2O (**5**)	0.900	This work
[Cu(OFL)(A^6)Cl]·5H_2O (**6**)	0.425	This work
[Cu(OFL)(A^7)Cl]·5H_2O (**7**)	0.625	This work
[Cu(OFL)(A^8)Cl]·5H_2O (**8**)	1.000	This work
[Cu(stz)$(py)_3$Cl]	1.310	54
[Cu(Hstz)$(MeOH)Cl_2$]	5.170	55
[Cu$(stz)_2$(1,2-dmHim)$_2$]	1.03	55
[CuL_2(4-mHim)$_2$]	5.86	55

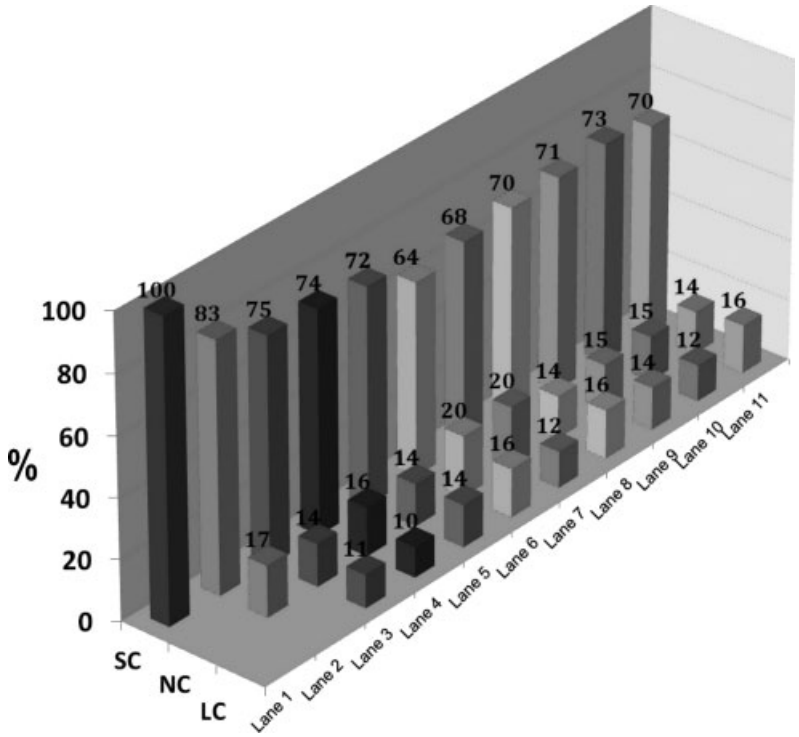

Figure 5. Gel eletrophoretic data for DNA cleavage study.

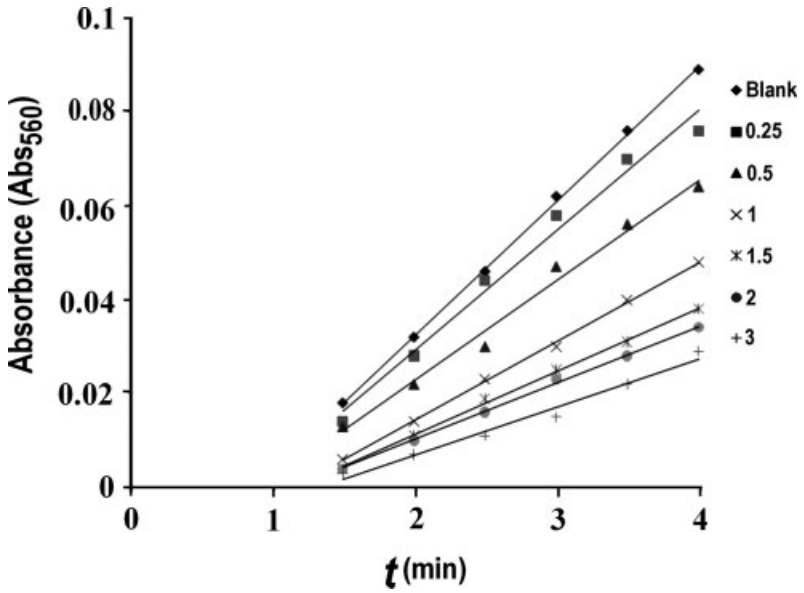

Figure 6. Absorbance values (Abs_{560}) as a function of time (t) plotted for varying concentrations of complex **1** from 0.25 to 3 μM for which a good straight line is observed.

Conclusion

Here in this work, we have synthesized eight Cu(II) metallointercalators with different neutral bidentate ligands and ofloxacin. The antibacterial activity of ofloxacin is changed upon coordination with the metal. Hypochromism and bathochromism of the band in absorption titration and the increase in relative viscosity of DNA suggest that all complexes bind with DNA via the classical intercalative mode. Complexation of drug and metal enhances their DNA cleavage ability. The data from SOD mimic activity suggest that activity is due to the vacant coordination site at the central metal ion. Thus, from the above studies, it can be concluded that the presence of planer heterocyclic ligand in Cu(II) drug-based mixed-ligand complex changes the interaction of complex in biological systems.

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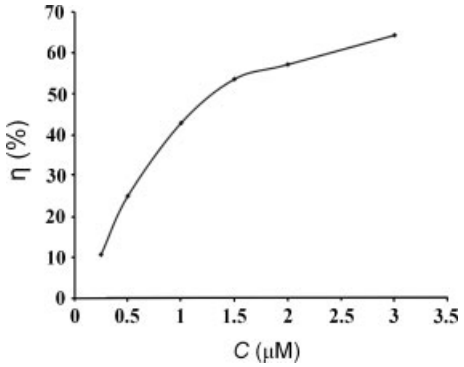

Figure 7. Plot of percentage of inhibiting NBT reduction with increasing the concentration of complex **1**.

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