Indian J Microbiol (July–Sept 2015) 55(3):302–312
DOI 10.1007/s12088-015-0525-9

ORIGINAL ARTICLE

Copper(II) Complexes with N,O-Donor Ligands and Ofloxacin Drug as Antibacterial, DNA Interacting, Cytotoxic and SOD Mimic Agent

Parag S. Karia[1] · Pankajkumar A. Vekariya[1] · Anshul P. Patidar[1] · Mohan N. Patel[1]

Received: 23 December 2014 / Accepted: 27 March 2015 / Published online: 3 April 2015

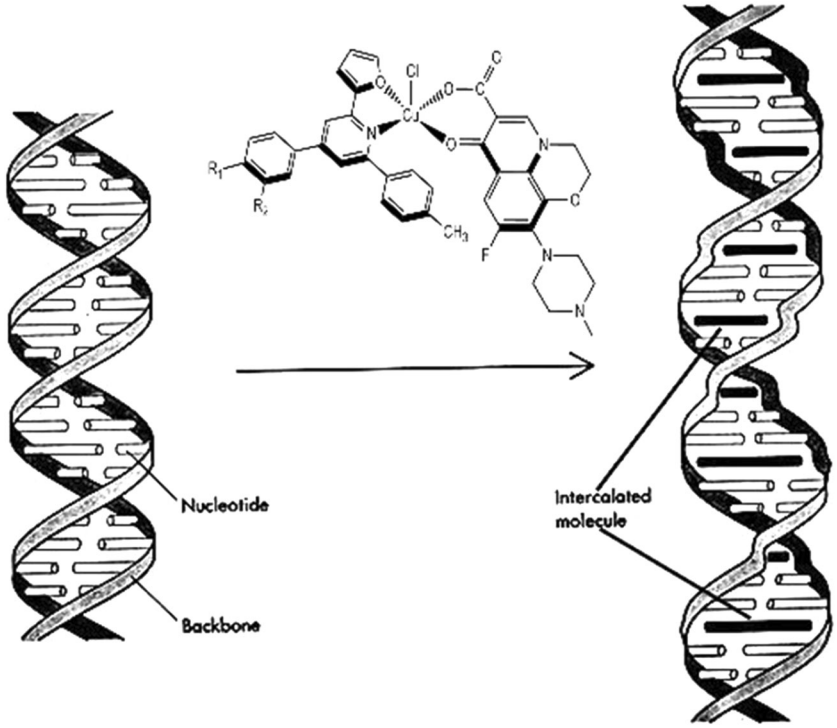
Abstract The N,O-donor bidentate ligands ($\mathbf{L^1}$–$\mathbf{L^7}$) derived from the reaction between chalcones and pyridinium salt of 2-acetyl furan were synthesized and characterized by IR and NMR spectroscopic techniques. Their complexes [**1–7**] of Cu(II) were synthesized and characterized by elemental analysis, magnetic measurements, TG analyses, IR and mass spectroscopy. Synthesized complexes were carried out for their biological elucidation using different biological experiments like minimum inhibitory concentration, DNA binding and cleavage study, cytotoxicity, and antiradical activity. Efficient cleavage of pUC19 DNA was observed for all the test complexes than the reference drug.

Graphical Abstract Increase in DNA chain length and hence the relative viscosity as the complexes binds to DNA via intercalative mode and involves a strong stacking interaction between an aromatic chromophore and the DNA base pair.

Electronic supplementary material The online version of this article (doi:10.1007/s12088-015-0525-9) contains supplementary material, which is available to authorized users.

✉ Mohan N. Patel
jeenen@gmail.com

[1] Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India



Keywords N,O-donor ligands · Transition metal compounds · Antibacterial agent · Antiradical activity · Absorption titration

Introduction

It has been mentioned by researchers, that we are more closely associated with the microbes and bacteria than our family members. In fact the body harbors more microbial cells compared to their own cells. But they are noticed only when they cause disease. *Pseudomonas* likes residing in the body and cause infective disease, whereas *Vibrios* use the human body to multiply and leave them at will through diarrheal manifestations. These bacterial infections are responsible for high mortality and morbidity in man and are a major cause of worry for Health Departments around the world. The discovery of antibiotics brought relief to many. However, microbes have been constantly evolving and becoming resistant to antibiotics: penicillin resistant *Staphylococcus* in 1940, tetracycline resistant *Shigella* emerged in the second half of the twentieth century, and erythromycin resistant *Streptococcus* in the late 1960s [1]. This had made the pharmaceutical industry to think of other approaches of curing diseases and invest in this area [2]. Nowadays keen research have been focused on the curing of diseases by interaction of small molecules with the DNA of disease causing species. In this respect, interaction of DNA with transition metal complexes of organic drug, has gained significant current interest due to its various uses in cancer research and nucleic acid chemistry, owing to their high lipophilicity compared to organic moiety alone [3]. On the other hand ofloxacin-a quinolone drug, function as an antibacterial agent by inhibiting the enzyme bacterial topoisomerase IV and DNA gyrase, which are obligatory in DNA duplication. This helps to promote breaking of DNA double stranded structure in liable bacteria. Ofloxacin has been used as successful antibiotic against various infection like chronic bronchitis, pneumonia, skin and skin structure infections, cervical gonorrhea, urinary tract infections etc. [4, 5]. Hence the combination of suitable metal and organic drug can result in a synergism of action, making it more effective against bacterial diseases. Also transition metal complexes have unique electronic and spectroscopic signatures that offer a massive amount of coordination geometries and mechanism of cytotoxic action, which is related to DNA binding affinity [6]. It can also vary consequently as the biological activity is strongly dependent on structure–activity relationship. Besides this, metal complexes also develop or create open coordination positions for DNA binding and hydrolysis generates reactive oxygen–containing species or other radicals for DNA oxidation [7]. Therefore, they can act as a metallonucleases class of compounds. Many transition metal complexes are recognized to bind with DNA via both covalent and non-covalent interactions. In covalent binding the labile ligand of the complex is replaced by nitrogen base of DNA. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding if cationic metal

complexes along of DNA helix, major or minor groove [8]. Amongst the metal ions, copper is widely distributed in the biological system and copper complexes are known to have a broad spectrum of biological action. It has been established that copper accumulates in tumors due to selective permeability of the cancer cell membranes. Because of this, a number of copper complexes have been screened for anticancer activity and some of them were found active both in vivo and in vitro [9]. In the current work we have carried out various biological activities for Cu(II) complexes with quinolone based drug and N,O-donor bidentate ligands.

Materials and Methods

Reagents and Materials

The chemicals used were of analytical grade and used as such without further purification. Ofloxacin (OFL) was generously supplied on demand by Bayer AG (Wuppertal, Germany). Cupric chloride dihydrate was purchased from E. Merck Ltd, Mumbai (India). 2-Acetylfuran, acetophenone, 4-chlorobenzaldehyde, 4-fluorobezaldehyde, 4-bromobezaldehyde, 3-fluorobenzaldehye, 4-methylbenzaldehyde, 4-methoxybenzaldehyde and 4-benzyloxybenzaldehyde were purchased from Sigma Chemical Co. (India). Ethidium bromide (EB), bromophenol blue, agarose and Luria Broth (LB) were purchased from Himedia (India). Nicotinamide adenine dinucleotide reduced (NADH), nitro blue tetrazolium (NBT) and phenazin methosulfate (PMS) were purchased from Loba Chemie Pvt. Ltd. (India).

Techniques and Measurements

Copper content for the complexes were obtained by gravimetric and volumetric techniques. A Perkin–Elmer 240 Elemental Analyzer was used to collect microanalytical data (C, H, N). Room temperature magnetic susceptibility was measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20 °C), on Citizen Balance. The diamagnetic correction has been made using Pascal's constant. FTIR data were collected with the help of FT-IR ABB Bomen MB 3000 spectrophotometer. UV–Vis spectra of the complex were recorded on UV-160A UV–Vis spectrophotometer, Shimadzu (Japan) and corrected for background due to solvent absorption. The percentage of pUC19 DNA cleavage was quantified by AlphaDigiDoc^{TM} RT. Version V.4.0.0 PC-Image software.

Synthesis of Ligands

Literature procedure [10, 11] has been used for the synthesis of different N,O-donor bidentate ligands.

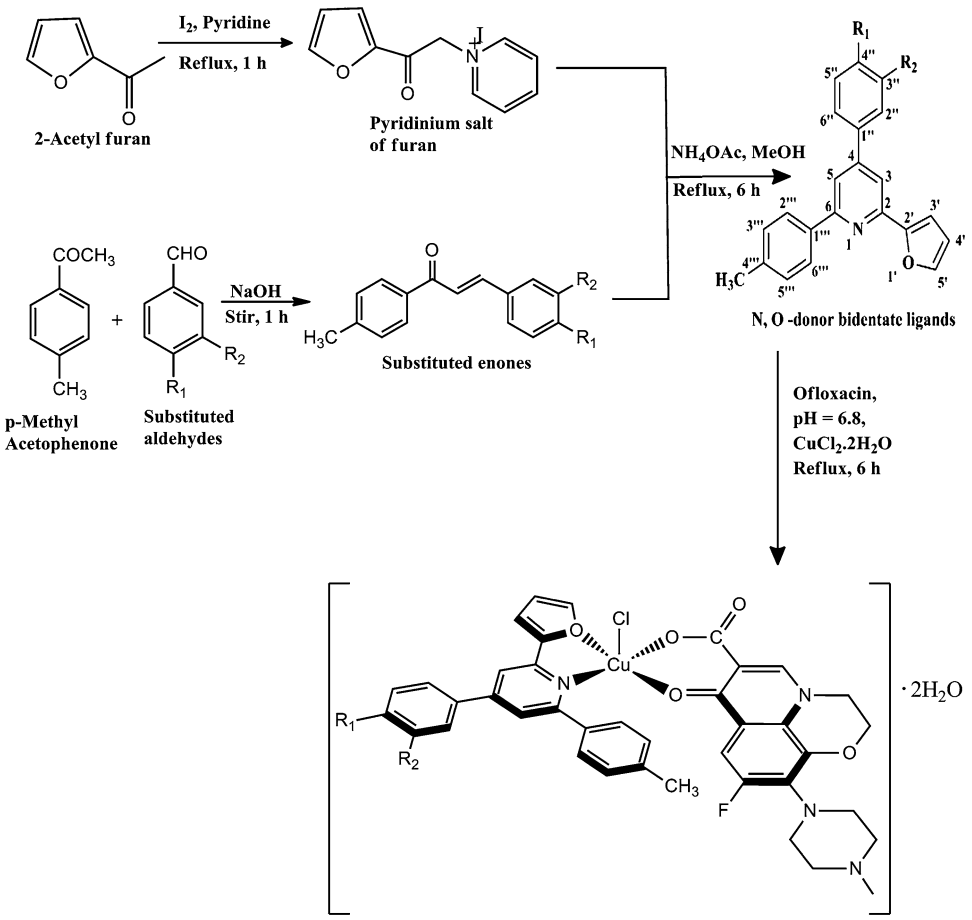
*Synthesis of 4-(4-Fluorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine (**L^1**)*

Substituted enones synthesized by the reaction of *p*-fluorobenzaldehyde and *p*-methylacetophenone was refluxed with pyridinium salt of 2-acetylfuran in presence of ammonium acetate for 6 h. The reaction mixture was allowed to cool at room temperature. The solid product was filtered and recrystallized from hexane (Scheme 1). Similar procedure was employed for the synthesis of ligands **L^2**–**L^7**. Yield: 53 %, m.p.: 118 °C, Anal. Calc. for $C_{22}H_{16}FNO$ (329.37): Calc. (%): C, 80.23; H, 4.90; N, 4.25. Found (%): C, 80.21; H, 4.93; N, 4.27. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.068–8.064 (d-poor resolved, 2H, $H_{2'',6''}$), 7.823–7.819 (d, 1H, H_3), 7.765–7.730 (complex, 3H, $H_{3'',5'',5}$), 7.590–7.584 (dd-poor resolved, 1H, $H_{5'}$), 7.345–7.325 (d, 2H, $H_{3''',5'''}$), 7.284–7.209 (complex, 3H, $H_{2''',6''',4'}$), 6.606–6.593 (dd, 1H, $H_{3'}$), 2.456 (s, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ/ppm: 157.19 ($C_{4''}$), 155.83 (C_2), 149.96 ($C_{2',6}$), 143.04 (C_4), 139.37 ($C_{5'}$), 136.88 ($C_{1''}$), 131.48 ($C_{1'''}$), 129.44 ($C_{2'',6'',4'''}$), 128.14 ($C_{3''',5'''}$), 124.12 ($C_{3,2''',6'''}$), 116.41 ($C_{3'}$), 114.78 ($C_{3'',5''}$), 112.25 (C_5), 109.07 ($C_{4'}$), 20.53 $-CH_3$. IR (KBr, 4000–400 cm^{-1}): 3032 ν(C–H)$_{ar}$; 1543 ν(C=C); 1497 ν(C=N); 1220 ν(C–F); 1304 pyridine skeleton band; 740 ν(C–H); 918, 818 (*p*-substituted ring).

*Synthesis of 4-(4-Chlorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine (**L^2**)*

It has been synthesized using the chalcone prepared from the reaction between *p*-chlorobenzaldehyde and *p*-methylacetophenone. Yield: 59 %, m.p.: 110 °C, Anal. Calc. for $C_{22}H_{16}ClNO$ (345.82): Calc. (%): C, 76.41; H, 4.66; N, 4.05. Found (%): C, 76.44; H, 4.67; N, 4.08. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.064–8.044 (dd, 2H, $H_{2'',6''}$), 7.823–7.821 (dd, 1H, $H_{5'}$), 7.757 (s, 1H, H_3), 7.714–7.693 (d, 2H, $H_{3''',5'''}$,), 7.588 (s, 1H, H_5), 7.524–7.503 (d, 2H, $H_{3'',5''}$), 7.345–7.325 (d, 2H, $H_{2''',6'''}$), 7.284–7.271 (dd, 1H, $H_{4'}$), 6.606–6.594 (dd, 1H, $H_{3'}$), 2.456 (s, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 400 MHz) δ/ppm: 156.52 ($C_{4''}$), 154.13 (C_2), 150.83 ($C_{2'}$), 144.66 (C_6), 139.89 (C_4), 138.54 ($C_{5'}$), 136.11 ($C_{1'',1'''}$), 132.60 ($C_{2'',6'',4'''}$), 130.22 ($C_{3,3''',5'''}$), 128.61 ($C_{2''',6'''}$), 126.40 ($C_{3'',5''}$), 124.08 (C_5), 117.52 ($C_{4'}$), 115.91 ($C_{3'}$), 20.66 $-CH_3$. IR (KBr, 4000–400 cm^{-1}): 3063 ν(C–H)$_{ar}$; 1543 ν(C=C); 1489 ν(C=N); 1040 ν(C–Cl); 1327 pyridine skeleton band; 733 ν(C–H); 987, 810 (*p*-substituted ring).

Scheme 1 General reaction scheme for the synthesis of ligands and complexes



Complex	R_1	R_2
1	F	H
2	Cl	H
3	Br	H
4	CH3	H
5	OCH3	H
6	OCH2PH	H
7	H	F

*Synthesis of 4-(4-Bromophenyl)-2-(furan-2-yl)-6-p-tolylpyridine (**L**^{3})*

It has been synthesized using the chalcone prepared from the reaction between *p*-bromobenzaldehyde and *p*-methylacetophenone. Yield: 49 %, m.p.: 130 °C, Anal. Calc. for $C_{22}H_{16}BrNO$ (390.27): Calc. (%): C, 67.71; H, 4.13; N, 3.59. Found (%): C, 67.74; H, 4.17; N, 3.61. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.071–8.051 (d, 2H, $H_{2'',6''}$), 7.968–7.948 (dd, 1, $H_{5'}$), 7.831 (s, 1H, H_3), 7.792 (s, 1H, H_5), 7.580–7.539 (t, 3H, $_{2''',6''',3'''}$), 7.500 (s poor resolved, 1H, $H_{5'''}$), 7.335–7.240 (complex, 3H, $H_{3'',5'',4'}$), 6.607–6.597 (dd, 1H, $H_{3'}$), 2.420 (s, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 157.64 ($C_{4''}$), 155.01 (C_2), 150.05 ($C_{2'}$), 148.01 (C_6), 143.01 (C_4), 140.76 ($C_{5'}$), 139.50 ($C_{1''}$), 136.51 ($C_{1'''}$), 135.17 ($C_{2''}$), 130.14 ($C_{6''}$), 129.88 ($C_{3''',4''',5'''}$), 126.88 ($C_{3,3'',5''}$), 124.90 ($C_{2'''}$), 117.08 ($C_{6'''}$), 115.11 (C_5), 112.52 ($C_{4'}$), 108.37 ($C_{3'}$), 20.54 $-CH_3$. IR (KBr, 4000–400 cm^{-1}): 3044 ν(C–H)$_{ar}$; 1543 ν(C=C); 1427 ν(C=N); 1050 ν(C–Br); 1373 pyridine skeleton band; 771 ν(C–H); 920, 818(*p*-substituted ring).

*Synthesis of 2-(furan-2-yl)-4,6-di-p-tolylpyridine (**L**^{4})*

It has been synthesized using the chalcone prepared from the reaction between *p*-methylbenzaldehyde and *p*-methylacetophenone. Yield: 34 %, m.p.: 90 °C, Anal. Calc. for: $C_{23}H_{19}NO$ (325.40): Calc. (%): C, 84.89; H, 5.89; N, 4.30. Found (%): C, 84.85; H, 5.89; N, 4.33. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.071–8.051 (d, 2H, $H_{2'',6''}$),

7.866–7.863 (d, 1H, H_3), 7.803–7.799 (d, 1H, H_5), 7.693–7.637 (d, 2H, $H_{3''',5'''}$), 7.589–7.583 (dd, 1H, $H_{5'}$), 7.360–7.322 (t, 4H, $H_{3'',5'',2''',6'''}$), 7.262–7.252 (dd, 1H, $H_{4'}$), 6.600–6.587 (dd, 1H, $H_{3'}$), 2.465 (s, 3H, $CH_{3\ acetophenone}$), 2.454 (s, 3H,–$CH_{3\ benzaldehyde}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 157.50 ($C_{4''}$), 154.83 (C_2), 149.86 ($C_{2'}$), 143.11 (C_6), 139.01 (C_4), 137.07 ($C_{5'}$), 136.06 ($C_{1''}$), 129.90 ($C_{2'',6'',1''',3''',4''',5'''}$), 127.04 ($C_{3,2''',6'''}$), 117.22 ($C_5$), 114.22 ($C_{4'}$) 112.36 ($C_{3'',5''}$), 108.37 ($C_{3'}$), 21.32 $(-CH_3)_{2\ acetophenone,\ benzadldehye}$, IR (KBr, 4000–400 cm^{-1}): 3032 ν(C-H)$_{ar}$; 1543 ν(C=C); 1412 ν(C=N); 1350 pyridine skeleton band; 733 ν(C–H); 918, 810 (*p*-substituted ring).

*Synthesis of 2-(furan-2-yl)-4-(4-methoxyphenyl)-6-p-tolylpyridine (**L^5**)*

It has been synthesized using the chalcone prepared from the reaction between *p*-methoxybenzaldehyde and *p*-methylacetophenone. Yield: 57 %, m.p.:123 °C, Anal. Calc. for: $C_{23}H_{19}NO_2$ (341.40): Calc. (%): C, 80.92; H, 5.61; N, 4.10. Found (%): C, 80.85; H, 5.63; N, 4.15, ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.071–8.050 (d, 2H, $H_{2'',6''}$), 7.845–7.842 (d, 1H, H_3), 7.779–7.725 (complex, 3H, $H_{2''',6''',\ 4'}$), 7.587 (s, 1H, H_5), 7.342–7.322 (d, 2H, $H_{3''',5'''}$),7.284–7.252 (dd, 1H, $H_{5'}$), 7.076–7.055 (d, 2H, $H_{3'',5''}$), 6.601–6.588 (dd, 1H, $H_{3'}$), 3.910 (s, 3H, –OCH_3), 2.454 (s, 3H, –CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 161.01 ($C_{4''}$), 157.09 (C_2), 154.82 ($C_{2'}$), 149.11 ($C_{4,6}$), 143.67 ($C_{5'}$), 138.56 ($C_{1''}$), 137.06 ($C_{1'''}$), 131.44 ($C_{4'''}$), 129.58 ($C_{2'',6''}$), 128.08 ($C_{3''',5'''}$), 126.57 ($C_{2''',6'''}$), 116.46 (C_3), 114.47 ($C_{5,3'',5''}$), 112.61 ($C_{4'}$), 108.88 ($C_{3'}$), 56.14 –OCH_3, 20.52 –CH_3. IR (KBr, 4000–400 cm^{-1}): 3032 ν(C–H)$_{ar}$; 1512 ν(C=C); 1427 ν(C=N); 1389 pyridine skeleton band; 741 ν(C–H); 926, 879 (*p*-substituted ring).

*Synthesis of 4-(4-(Benzyloxy)phenyl)-2-(furan-2-yl)-6-p-tolylpyridine (**L^6**)*

It has been synthesized using the chalcone prepared from the reaction between *p*-benzyloxybenzaldehyde and *p*-methylacetophenone. Yield: 49 %, m.p.: 136 °C, Anal. Calc. for: $C_{29}H_{23}FNO_2$ (417.50): Calc. (%): C, 83.43; H, 5.55; N, 3.35. Found (%): C, 83.40; H, 5.58; N, 3.33. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.065–8.044 (d, 2H, $H_{2'',6''}$), 7.842–7.838 (d, 1H, H_3), 7.777–7.773 (complex, 3H, $H_{3''',5''',\ 5'}$), 7.755–7.723 (d, 1H, H_5), 7.583–7.579 (d, 2H, $H_{2''',6'''}$), 7.506–7.488 (d, 2H, $H_{3'',5''}$), 7.458–7.376 (d, 1H, $H_{4'}$), 7.338–7.318 (d, 2H, $H_{2''',6'''}$), 7.254–7.245 (dd, 1H, $H_{4'}$), 7.149–7.127 (dd, 2H, $H_{3''',5'''}$), 6.597–6.585 (dd, 1H, $H_{3'}$), 5.177 (s, 2H, –OCH_2), 2.452 (s, 3H, –CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 157.84 ($C_{4''}$), 155.12 (C_2), 150.07 ($C_{2'}$), 148.00 (C_6), 144.12 (C_4), 143.15 ($C_{5',1''}$), 139.14 ($C_{1''',4'''}$), 137.66 ($C_{2''}$), 136.50 ($C_{6''}$), 133.47 (C_3), 134.88 ($C_{3'}$), 129.48 ($C_{3''',5'''}$) 128.45 ($C_{3'',5''}$), 127.11 ($C_{2''',6'''}$),122.5 ($C_{5,4'}$), 116.45 ($c_{2,6\ ph}$), 112.10 ($C_{3,5\ ph}$), 109.02 ($C_{1,6\ ph}$), 58.70 –OCH_2, 21.22 –CH_3. IR (KBr, 4000–400 cm^{-1}): 3063 ν(C–H)$_{ar}$; 1589 ν(C=C); 1489 ν(C=N); 1335 pyridine skeleton band; 733 ν(C–H); 918, 810 (*p*-substituted ring).

*Synthesis of 4-(3-Fluorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine (**L^7**)*

It has been synthesized using the chalcone prepared from the reaction between *m*-fluorobenzaldehyde and *p*-methylacetophenone. Yield: 59 %, m.p.: 108 °C, Anal. Calc. for: $C_{22}H_{16}FNO$ (329.37): Calc. (%): C, 80.23; H, 4.90; N, 4.25. Found (%): C, 80.24; H, 4.88; N, 4.27. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.075–8.054 (d, 2H, $H_{2'',6''}$), 7.839–7.836 (d, 1H, H_3), 7.774–7.771 (d, 1H, H_5), 7.597–7.593 (d, 1H, $H_{5'''}$), 7.566–7.454 (complex, 3H, $H_{2''',6''',4'''}$), 7.350–7.330(d, 2H, $H_{3'',5''}$), 7.283–7.274 (d-poor resolved, 1H, $H_{5'}$), 7.211–7.164 (t-poor resolved, 1H, $H_{4'}$), 6.610–6.592 (dd, 1H, $H_{3'}$), 2.459 (s, 3H, –CH_3). 13C NMR (CDCl3, 100 MHz) δ/ppm: 157.11 ($C_{3''}$), 154.1 (C_2), 149.96 ($C_{2'}$), 148.51 (C_6), 143.88 (C_4,), 139.07 ($C_{1''}$), 137.05 ($C_{5'}$), 130.98 ($C_{1'''}$), 129.43 ($C_{3''',5'''}$), 128.86 ($C_{4'''}$), 127.08 ($C_{5'',2''',6'''}$), 123.32 ($C_{6''}$), 116.45 ($C_{3,5}$), 114.06 ($C_{4'}$), 112.67 ($C_{2'',4''}$), 109.07 ($C_{3'}$), 20.67 –CH_3. IR (KBr, 4000–400 cm^{-1}): 3063 ν(C–H)$_{ar}$; 1551 ν(C=C); 1489 ν(C=N); 1222 ν(C–F); 1381 pyridine skeleton band; 748 ν(C–H); 926, 864 (*p*-substituted ring).

Synthesis of Complexes

*Synthesis of [Cu(OFL)**L^1**Cl]·3H$_2$O [1]*

Solution of 4-(4-fluorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine [**L^1**] (1.5 mmol) in methanol and methanolic solution of $CuCl_2{\cdot}2H_2O$ (1.5 mmol) was refluxed for 30 min followed by addition of methanolic solution of OFL (1.5 mmol) in presence of CH_3ONa (1.5 mmol). The pH was adjusted to ~6.8 using dilute solution of CH_3ONa. Resulting solution has been refluxed for 6 h on water bath and concentrated to half of its volume. The green color amorphous product was obtained which was then washed with $CHCl_3$. General reaction scheme for the synthesis of complexes is shown in Scheme 1. Yield: 57.9 %, m.p.: 264.10 °C, μ_{eff}: 1.79 B.M. Anal. Calc. for: $C_{40}H_{41}ClCuF_2N_4O_8$ (842.77): Calc. (%): C, 57.01; H, 4.90; N, 6.65; Cu, 7.54. Found (%): C, 57.04; H, 4.94; N, 6.60; Cu, 7.51.

*Synthesis of [Cu(OFL)(**L^2**)Cl]·3H$_2$O [2]*

It has been synthesized using 4-(4-chlorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine as ligand [**L^2**]. Yield: 53.3 %, m.p.:

262.20 °C, μ_{eff}: 1.83 B.M. Anal. Calc. for: $C_{40}H_{41}Cl_2CuFN_4O_8$ (857.16): Calc. (%): C, 55.91; H, 4.81; N, 6.52; Cu, 7.40. Found (%): C, 55.81; H, 4.78; N, 6.59; Cu, 7.34.

Synthesis of [Cu(OFL)(L^3)Cl]·3H_2O [3]

It has been synthesized using 4-(4-bromophenyl)-2-(furan-2-yl)-6-p-tolylpyridine as ligand [L^3]. Yield: 53.4 %, m.p.: 263.20 °C, μ_{eff}: 1.89 B.M. Anal. Calc. for: $C_{40}H_{41}BrClCuFN_4O_8$ (903.68): Calc. (%): C, 53.16; H, 4.57; N, 6.20; Cu, 7.03. Found (%): C, 53.18; H, 4.55; N, 6.24; Cu, 7.02.

Synthesis of [Cu(OFL)(L^4)Cl]·3H_2O [4]

It has been synthesized using 2-(furan-2-yl)-4,6-dip-tolylpyridine as ligand [L^4]. Yield: 52.1 %, m.p.: 243.00 °C, μ_{eff}: 1.83 B.M. Anal. Calc. for: $C_{41}H_{44}ClCuFN_4O_8$ (838.81): Calc. (%): C, 58.71; H, 5.29; N, 6.68; Cu, 7.58. Found (%): C, 58.71; H, 5.25; N, 6.27; Cu, 7.55.

Synthesis of [Cu(OFL)(L^5)Cl]·3H_2O [5]

It has been synthesized using 2-(furan-2-yl)-4-(4-methoxyphenyl)-6-p-tolylpyridine as ligand [L^5]. Yield: 59.12 %, m.p.: 249.40 °C, μ_{eff}: 1.84 B.M. Anal. Calc. for: $C_{41}H_{44}ClCuFN_4O_9$ (854.81): Calc. (%): C, 57.61; H, 5.19; N, 6.55; Cu, 7.43. Found (%): C, 57.63; H, 5.18; N, 6.54; Cu, 7.40.

Synthesis of [Cu(OFL)(L^6)Cl]·3H_2O [6]

It has been synthesized using 4-(4-(benzyloxy)phenyl)-2-(furan-2-yl)-6-p-tolylpyridine as ligand [L^6]. Yield: 51.70 %, m.p.: 251 °C, μ_{eff}: 1.88 B.M. Anal. Calc. for: $C_{47}H_{48}ClCuFN_4O_9$ (930.90): Calc. (%): C, 60.64; H, 5.20; N, 6.02; Cu, 6.83. Found (%): C, 60.61; H, 5.23; N, 6.04; Cu, 6.85.

Synthesis of [Cu(OFL)(L^7)Cl]·3H_2O [7]

It has been synthesized using 4-(3-fluorophenyl)-2-(furan-2-yl)-6-p-tolylpyridine as ligand [L^7]. Yield: 54.14 %, m.p.: 210.00 °C, μ_{eff}: 1.81 B.M. Anal. Calc. for: $C_{40}H_{41}ClCuF_2N_4O_8$ (842.77): Calc. (%): C, 57.01; H, 4.90; N, 6.65; Cu, 7.54. Found (%): C, 57.12; H, 4.87; N, 6.65; Cu, 7.58.

In Vitro-Antibacterial Broth Dilution Screening

Antibacterial activity for the test compound was performed against suspected pathogens viz. Two gram$^{(+ve)}$ *(Bacillus subtilis* and *Serratia marcescens)* and three gram$^{(-ve)}$ (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). To elucidate bactericidal effect of the synthesized complexes, minimum inhibitory concentration was found out by broth dilution technique in terms of μM. From a preculture of pathogens, bacterial culture in Luria Broth (LB) solution was prepared in sugar tube and serially two-fold diluted amount of complexes was added to each bacterial culture and incubated overnight at 37 °C. The concentration of the respective complexes in the tube with highest dilution for disappearance of turbidity is reported at MIC value.

Relative Affinity and DNA Binding Mode Estimation

UV–Vis Spectrophotometric Quantization of DNA Binding Mode

Compounds may bind to DNA either by covalent or non-covalent interaction. For compounds that bind to DNA non-covalently, intercalation, electrostatic interaction and groove binding are the most likely binding modes [12–14]. Possible criteria to be used in inferring the binding mode have been reviewed. Groove binding typically results in only subtle changes in structure. In contrast, intercalation, in which a planar ligand moiety is inserted between adjacent base pairs, results in a substantial change in DNA structure. It may cause distortions, unstacking and unwinding of the helix. A strong interaction between an aromatic chromophore of metal complexes and the base pairs of DNA, hypochromism and hyperchromism are generally observed [15]. DNA binding study was carried out by absorption titration method. The concentration of complex was kept constant and DNA concentration was varied by successive addition of its 100 μL aliquots. Absorbance spectra were recorded for consecutive addition of DNA.

Hydrodynamic Chain Length Study

The structural changes of DNA by binding with agents have been the most stringent indication of the binding mode. Viscosity measurements to examine the change in hydrodynamic chain length may be used to monitor such DNA structural changes by the length changes of rod-like DNA [16]. The different concentrations of fixed solution of complexes 1–7 and DNA in phosphate buffer media are used, i.e. [Complex]/[DNA] = 0.04, 0.08, 0.12, 0.16, 0.20. Before testing, keep them at 27 ± 0.1 °C for 1 h in thermostatic water bath. The viscosity is related to time as:

$$\eta \propto t - t_0,$$

where t is the time for the solution upon the addition of DNA and complex and t_0 is the time for DNA solution alone. Viscosity curves were obtained by a picture with $(\eta/\eta_0)^{1/3}$ as Y-axis and with ([Complex]/[DNA]) as X-axis.

In Vitro Cytotoxicity: Brine Shrimp Lethality Assay

Cytotoxicity assay are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. In order to determine anticancer properties of the complexes, in vitro cytotoxicity tests were screened for all the synthesized complexes against brine shrimp. Brine shrimp eggs were sprinkled and incubated for 48 h in a sea salt solution in presence of light. 10 nauplii were taken in test tube and complexes in the concentration range of 2, 4, 8, 12, 16 and 20 mg mL^{-1} (in DMSO) were added to it and number of dead nauplii was calculated. Three sets for each aliquot of complexes and control containing only DMSO were monitored under same experimental condition.

Antiradical Activity

Superoxide radicals are produced inside the body which cause various diseases like ageing, heart problem etc. The control of this reactive oxygen species (ROS) is essential to prevent diseases. Naturally occuring Superoxide Dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to them by superoxide, the toxic free radical in the body. However the natural SOD's present inside the body has very high molecular weight. Hence the development of the molecule with low molecular weight which could inhibit the reactive oxygen species plays a crucial role in the discovery of new drug. In order to determine the oxygen scavenging ability of the synthesized complexes, superoxide dismutase assay was carried out using NBT/NADH/PMS system. Nonenzymatic (PMS/NADH) systems was used to generate Superoxide anion $(O_2^{\bullet -})$ in the presence or absence of test compounds. The generated $O_2^{\bullet -}$ was than scavenged by NBT. Reduction in rate of NBT to monoformazan dye formation was determined by measuring absorbance at 560 nm and the graph of percentage inhibition was plotted against concentration of the complex.

Nuclease Activity: Gel Electrophoresis Study

The DNA cleavage could occur by two major pathways, viz. hydrolytic and oxidative pathways. Hydrolytic DNA cleavage involves cleavage of phosphoester bond to generate fragments those could be subsequently relegated. Cleavage of plasmid DNA was probed by using agarose gel electrophoresis. The pUC19 DNA taken in Tris–EDTA buffer was treated with the complexes and incubated for 24 h at 37 °C. The reaction was quenched by addition of bromophenol blue. This system was then loaded into the well and electrophoresis was carried out for 2 h at 100 V in tris–acetate–EDTA (TAE) buffer. Bands were visualized by UV light and photographed for analysis. The extent of cleavage was measured from the intensities of the bands using AlphaDigiDocTM RT. Version V.4.1.0 PC-Image software; gel documentation system.

Results and Discussion

Electronic Spectra and Magnetism

The one-electron paramagnetic complexes display intense charge transfer (CT) band in the range of 200–320 nm that can be attributed to the π–π* transition of the coordinated N,O-donor ligand. The d–d band is observed around 600 nm in DMSO. Distorted square pyramidal geometry for copper(II) complexes has been proved by a broad band at around 600 nm [17]. This band is a characteristic of d–d transition in tetragonal field. The magnetic moment of all the complexes lie in the range 1.80–1.89 BM, which is slightly higher than the spin—only value of 1.73 BM which bring to a close that metal centre in synthesized complexes possess one unpaired electron responsible for s = ½ system.

IR Spectra

Heteroatom coordinating to the metal atom gives explicit bands in the IR spectra. Thus, IR spectra can be used to confirm whether heteroatom has been coordinated to the metal atom or not. The data obtained from the IR spectra are represented in supplementary material 1. The IR spectra of ofloxacin exhibited carboxylic stretch $\nu(C{=}O)_{carb}$ at 1760 cm^{-1} and the pyridone stretch $\nu(C{=}O)_p$ at 1646 cm^{-1}. After complexation disappearance of $\nu(C{=}O)_{carb}$ and shifting of $\nu(C{=}O)_p$ to lower values in the complexes (1619–1638) is the indication of coordination occurring through carbonyl oxygen of pyridine ring. The peaks corresponding to the ring stretching frequencies of ν(C=N) of ligands were shifted to higher frequencies upon complexation indicating the coordination of the heterocyclic nitrogen atoms to the metal ion [17]. Bands are observed in the range 1560–1588 cm^{-1} and 1352–1379 cm^{-1} assigned as ν(O–C–O) asymmetric and symmetric stretching vibrations, respectively. Also ν(M–N) band at ~540–562 cm^{-1} is assigned to N → M bonding of complexes [18].

Thermal Decomposition Study

The TG analyses for complex-[**1**] carried out at a heating rate of 10 °C per minute from ambient to 900 °C under N_2 atmosphere. In the TG curve three distinct mass losses are observed (Supplementary material 2). The first mass loss occurs between 70 and 130 °C, which is attributed to loss of water of crystallization, second between 180 and 450 °C corresponding to removal of chlorine atom and decomposition of neutral bidentate ligand, and finally between 600

and 800 °C corresponds to decomposition of OFL and leaving behind the CuO as a residue.

Mass Spectra

The mass spectrum of complex-[**1**] (Supplementary material 3) shows molecular ion peak at $m/z = 787.00$ and 789.09 is assigned (M) and (M + 2) peak due to the presence of one chlorine atom and it also confirms that chlorine atom is attached to metal atom through covalent bond. The peak observed at $m/z = 752.06$ is due to loss of one chlorine atom. Several other fragments at 458.03, 427.03, 392.07, 361.11 and 329.08 *m/z* value are observed. The proposed fragmentation pattern of complex-[**1**] is shown in (Supplementary material 4).

In Vitro-Antibacterial Broth Dilution Screening

It is used to determine the minimum inhibitory concentration (MIC) of antibacterial agent. This is the lowest concentration of antibacterial agent at which no visible growth or turbidity in the dilution tube is observed. The result obtained in μM is presented in the Table 1. The results of antibacterial screening data reveals that all the test complexes show good antibacterial potency in comparison with $CuCl_2{\cdot}2H_2O$, free ligands and the reference drug ofloxacin. The lower MIC value of complexes compared to ligand can be attributed to the increased polarity of complexes compared to free ofloxacin and ligands and thus makes the complex more lipophillic as compared to the ofloxacin drug. Due to higher lipophilicity of the complexes compared to free ligands, complexes can easily penetrate into the bacterial cell and hence cause the rapid attack in comparison to the free ligands.

Relative Affinity and DNA Binding Mode Estimation

UV–Vis Spectrophotometric Quantization of DNA Binding Mode

In order to investigate the binding mode of the synthesized complexes absorption titration was carried out. The absorption spectra were recorded in the absence and presence of the synthesized complexes in reference and sample cell respectively. Concentration of HS–DNA was kept increasing. The binding constants (K_b) are calculated from the plot of [DNA]/(ε_a–ε_f) versus [DNA] (Fig. 1) using following equation.

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

where, ε_a, ε_f, and ε_b, correspond to A_{obsd}/[Cu(II) complex], the extinction coefficient for the free complex, and the extinction coefficient for complex in fully bound form, respectively. With the addition of DNA, hypochromicity for CT transition without bathochromic shift showed interaction between the surface of DNA and copper complexes [19]. The obtained K_b values for the test compounds are represented in the Table 2. The K_b values for the synthesized complexes are found in the ranges of 1.44×10^5 to 2.8368×10^5 M^{-1}, which are lower than those of classical intercalator (ethidium bromide) and higher than [Cu(Phimp)$_2$] (1.99×10^4 M^{-1}) [20], indicating that the complexes bind more efficiently to DNA.

Table 1 MIC in terms of μM

Compounds	Gram positive		Gram negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
Ofloxacin	1.7	1.24	1.52	2.0	1.24
(**L**^{1})	225	230	225	325	180
(**L**^{2})	250	255	270	375	225
(**L**^{3})	275	295	310	500	240
(**L**^{4})	290	460	445	440	370
(**L**^{5})	440	365	425	430	270
(**L**^{6})	480	355	340	390	375
(**L**^{7})	230	235	225	360	225
[Cu(OFL)**L**^{1}Cl] [**1**]	1.6	0.32	0.30	0.60	0.42
[Cu(OFL)**L**^{2}Cl] [**2**]	0.45	0.35	0.40	1.6	0.45
[Cu(OFL)**L**^{3}Cl] [**3**]	1.2	0.40	0.45	0.80	0.48
[Cu(OFL)**L**^{4}Cl] [**4**]	0.9	0.42	0.40	0.75	0.70
[Cu(OFL)**L**^{5}Cl] [**5**]	0.8	0.42	0.40	0.80	0.80
[Cu(OFL)**L**^{6}Cl] [**6**]	0.7	0.45	0.32	0.90	0.45
[Cu(OFL)**L**^{7}Cl] [**7**]	1.4	0.30	0.30	0.80	0.45

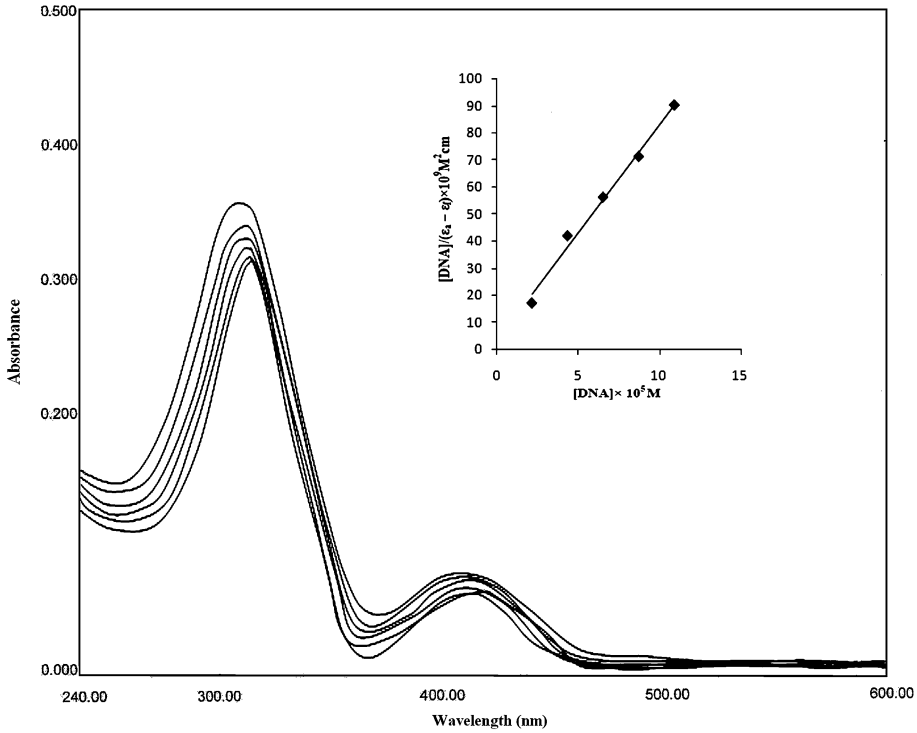


Fig. 1 Electronic absorption spectra of complex-[**1**] with increasing concentration of Herring Sperm DNA (HS–DNA) in phosphate buffer *Inset*: Plots of [DNA]/(ε_a–ε_f) versus [DNA] for the titration of DNA with copper(II) complexes

Table 2 K_b, LC_{50} and IC_{50} values of complexes

Complexes	K_b ($\times 10^5$)	LC_{50} (μM)	IC_{50} (μg/mL)
[Cu(OFL)$\mathbf{L^1}$Cl] [**1**]	3.38	5.63	0.534
[Cu(OFL)$\mathbf{L^2}$Cl] [**2**]	2.63	6.06	0.624
[Cu(OFL)$\mathbf{L^3}$Cl] [**3**]	2.52	7.96	0.994
[Cu(OFL)$\mathbf{L^4}$Cl] [**4**]	1.51	11.0	1.06
[Cu(OFL)$\mathbf{L^5}$Cl] [**5**]	1.57	11.6	1.15
[Cu(OFL)$\mathbf{L^6}$Cl] [**6**]	2.80	11.6	1.33
[Cu(OFL)$\mathbf{L^7}$Cl] [**7**]	1.44	7.79	1.01

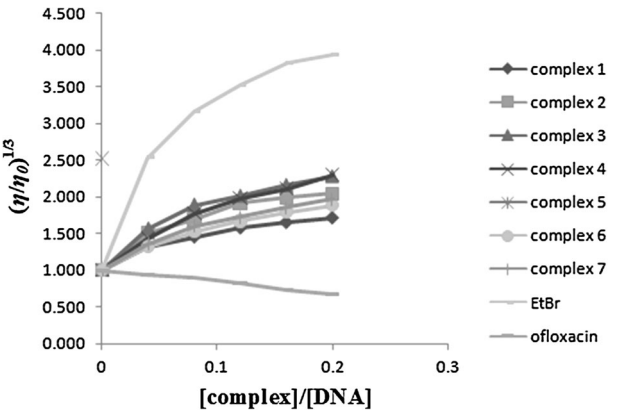


Fig. 2 Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 37 ± 0.1 °C in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2)

Hydrodynamic Chain Length Study

An increase in the viscosity of DNA containing solution upon increasing the complex: DNA concentration, suggest the intercalation mode of binding [21]. Viscosity measurements are sensitive to change in length of DNA as viscosity is proportional to length^3. If circular plasmid is used and concentration of test compound is increased, viscosity first increases and then decreases indicative of the DNA changing from a negative- supercoiled to a relaxed and finally a positive-supercoiled state [22]. From the Fig. 2, we can see that the relative viscosity of DNA increases steadily on increasing the amount of Cu(II) complex, demonstrating that the compounds bind to DNA by intercalation.

In Vitro Cytotoxicity: Brine Shrimp Lethality Assay

The assay was based on the ability of extracts/pure compounds to kill laboratory cultured *Artemia* nauplii brine shrimp. Assay was carried out in accordance with protocol reported by Meyer et al. Log of concentration of samples were plotted against percent of mortality of nauplii, which showed a linear correlation (Supplementary material 5). Results are represented in Table 2.

All the complexes showed better toxicity (5.63–11.6 μg/mL) than the reference standard (LC_{50} value of potassium dichromate is 32 μg/mL) used in the bioassay. All the

complexes exhibit potential toxicity profile after 24 h of exposure. 100 % mortality was observed after 48 h of exposure.

Antiradical Activity

Concentration of blue formazan is measured spectrophotometrically at 560 nm. The plot of absorption of mono formazan as a function of time for different concentrations of complex-[**1**] is shown in Fig. 3. The rate of absorption change was determined and the concentration required to produce 50 % inhibition (IC_{50}) can be obtained by graphing the rate of NBT reduction (along Y-axis) versus the concentration of the test solution (along X axis) as shown in Fig. 4. The % inhibition of NBT reduction is calculated using following equation:

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

where, k' and k represent slopes of the straight line of absorbance values as a function of time in the presence and absence of SOD mimic compounds, respectively. The antiradical (superoxide) scavenging data are shown in Table 2.

All the complexes exhibit antiradical activity in the range of 0.534–1.33 μM, which is better than clinical requirement value (20 μM) [23]. The complex-[**1**] exhibits IC_{50} value (0.534 μM), which is close to the value of native bovine Cu–Zn superoxide dismutase (0.48 μM) [24].

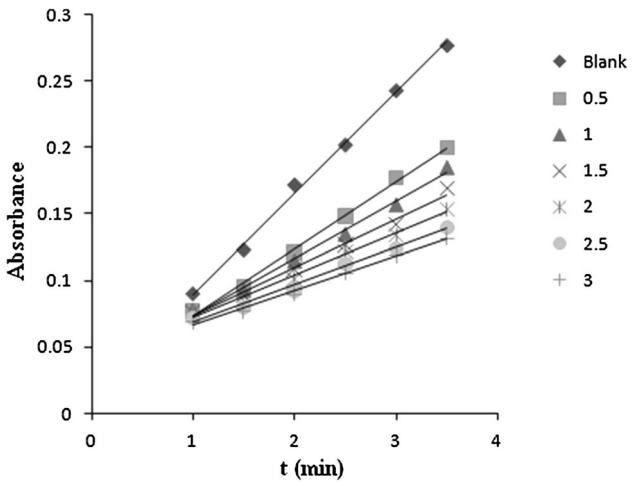


Fig. 3 Absorbance values (Abs_{560}) as a function of time (t) plotted for varying concentration of complex-[**1**] from 0.5 to 3 μM for which *good straight lines* are observed

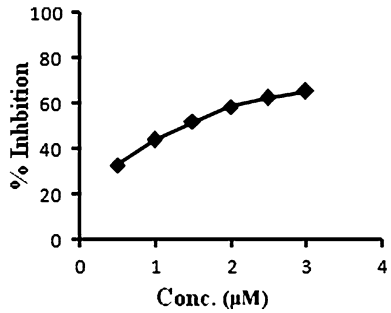


Fig. 4 Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex-[**1**]

Nuclease Activity: Gel Electrophoresis Study

From the Fig. 5, it is shown that when plasmid DNA is subjected to electrophoresis after interaction upon illumination of gel, pUC19-DNA in presence of reference compounds, $CuCl_2{\cdot}2H_2O$ (lane-1) and ofloxacin (lane-2) respectively show only two bands suggesting cleavage of pUC19-DNA into only two forms Open Circular (OC) and Super Coiled (SC). Upon addition of complexes (lane 3–10), three forms are observed suggesting that pUC19-DNA cleaves into OC, SC and an additional Linear form (L), which migrates in between OC and SC indicating that complexes show significant nuclease activity than the reference compounds $CuCl_2{\cdot}2H_2O$ and ofloxacin, respectively. Quantification of DNA cleavage is represented in the Table 3. From the data, percentage of SC form is highest

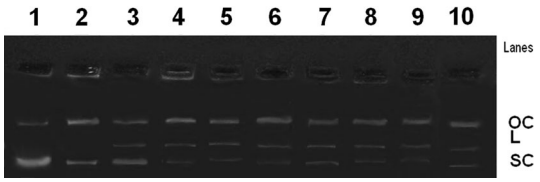


Fig. 5 Cleavage of pUC19 plasmid DNA modified by [Cu($\mathbf{L}^n$)(OFL)Cl]. *Lane 1*, DNA control; *Lane 2*, $CuCl_2{\cdot}2H_2O$; *Lane 3*, Ofloxacin; *Lane 4*, [Cu(OFL)($\mathbf{L}^1$)Cl]; *Lane 5*, [Cu(OFL)($\mathbf{L}^2$)Cl]; *Lane 6*, [Cu(OFL)($\mathbf{L}^3$)Cl]; *Lane 7*, [Cu(OFL)($\mathbf{L}^4$)Cl]; *Lane 8*, [Cu(OFL)($\mathbf{L}^5$)Cl]; *Lane 9*, [Cu(OFL)($\mathbf{L}^6$)Cl]; *Lane 10*, [Cu(OFL)($\mathbf{L}^7$)Cl]

Table 3 Complex mediated DNA cleavage data by gel electrophoresis

Lane	Compound	Form I (SC)	Form II (OC)	Form III (LC)	% Cleavage
1	Control	87.3	12.7	–	–
2	$CuCl_2{\cdot}2H_2O$	64.0	36.0	–	26.6
3	Ofloxacin	52.8	30.5	19.7	39.5
4	[Cu(OFL)$\mathbf{L}^1$Cl] [**1**]	7.0	68.4	24.5	91.8
5	[Cu(OFL)$\mathbf{L}^2$Cl] [**2**]	14.0	63.1	22.9	83.9
6	[Cu(OFL)$\mathbf{L}^3$Cl] [**3**]	10.4	69.6	20.0	88.0
7	[Cu(OFL)$\mathbf{L}^4$Cl] [**4**]	22.8	54.8	22.4	64.5
8	[Cu(OFL)$\mathbf{L}^5$Cl] [**5**]	10.3	61.5	28.2	88.2
9	[Cu(OFL)$\mathbf{L}^6$Cl] [**6**]	19.4	67.4	13.2	77.7
10	[Cu(OFL)$\mathbf{L}^7$Cl] [**7**]	17.2	62.9	19.9	80.2

for the complex-[**1**] (lane-3) deducing that complex-[**1**] cleaves the DNA more efficiently than rest of others.

Conclusion

MIC values suggest that complexes are more bacteriostatic than the reference drug. The DNA binding experiment through absorption titration studies revealed that the complex-**1** binds to the DNA significantly (3.38×10^5 M^{-1}) and viscosity measurements reveal that complexes bind to DNA via classical intercalation mode. Cytotoxic studies of metal complexes shows good potency against brine shrimp. The DNA cleavage study of pUC19 shows that all the complexes exhibit higher cleavage ability compared to the metal salt and ofloxacin. SOD—like activity suggests that the synthesized complexes have ability to scavenge free radical anion of molecular oxygen.

Acknowledgments We are thankful to the Head of Chemistry Department and UGC RFSMS Scheme for providing financial support.

Conflict of interest The authors report no conflicts of interest.

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