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Antibacterial, DNA interaction and superoxide dismutase activity of drug based copper(II) coordination compounds

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ABSTRACT

The novel neutral mononuclear copper(II) complexes with the quinolone antibacterial drugs ciprofloxacin in the presence of the nitrogen donor heterocyclic ligand of terpyridine have been synthesized and characterized by elemental analysis, reflectance spectra, IR and mass spectroscopy. The antibacterial activities of the newly synthesized compounds were evaluated and correlated with their physicochemical properties. Results revealed that the tested compounds exhibited better inhibitory activities than the reference antibacterial quinolone drugs against Gram$^{(+ve)}$ and Gram$^{(-ve)}$ bacteria. The coordination compounds can act as catalysts for the dismutation of superoxide anion radicals ($O_2^{\cdot-}$). The detection of the rate constant of the reaction of superoxide ion with nitro blue tetrazolium (NBT) is inhibited by superoxide dismutase (SOD). The interaction of the complex with DNA was investigated using viscosity, absorption titration and DNA melting temperature techniques. The results indicate that the complexes bind to DNA with intercalative mode and they have rather high DNA-binding constants. DNA-cleavage study showed better cleaving ability of the complexes compare to metal salt and standard drugs.

1. Introduction

The interaction and reaction of transition metal complexes with DNA have been long intensively investigated in relation to applications in the fields of molecular biology, biotechnology and medicine [1–3]. DNA provides a range of binding sites and binding modes for covalent and non-covalent interactions, including intercalation, groove bindings, electrostatic forces and hydrogen bonds with metal complexes. Interest in the fashion of metal complex binding to DNA has been motivated not only by a desire to understand the basics of these interaction modes but also by the development of metal complexes into anti-inflammatory, antifungi, antibacteria or anticancer reagents [4]. Hence, much of the attention has been targeted on the design of metal-based complexes, predominantly copper(II) complexes, which can bind and cleave DNA. Copper is a bioessential element in all living systems. Recently, numerous research groups have reported novel copper(II) complexes with organic ligands showing antifungal and antibacterial properties against several pathogenic fungi and bacteria [5,6].

Superoxide dismutase (SOD) is an essential enzyme that eliminates superoxide radical anion $O_2^{\cdot-}$ and thus protects cells from damage induced by reactive oxygen species (ROS) [7,8]. The superoxide radical anion $O_2^{\cdot-}$ is formed as a byproduct of normal cellular respiration. Its decomposition produces undesired harmful species like hydroxyl radical and hydrogen peroxide. To control this, nature has created a family of enzymes which remove them from the cellular environment. One of these, superoxide dismutase (SOD), catalyzes disproportionation of superoxide to dioxygen and hydrogen peroxide, which is decomposed via catalyzes to water and dioxygen [9].

Here, we report the synthesis and characterization of drug based coordination compounds using ciprofloxacin and tridentates terpyridines. The antimicrobial efficiency of complexes has also been tested on five different microorganisms. Rate constant for the reaction of superoxide radicals was detected using nitroblue tetrazolium (NBT), which was inhibited by superoxide dismutase. The DNA-binding and cleavage properties of the complexes have been investigated using absorption titration, viscosity measurements DNA melting temperature and gel electrophoresis method.

2. Experiments

2.1. Reagent

All the chemicals and solvent used were of analytical grade. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). Pyridine, 2-acetyl pyridine, *p*-chloro benzaldehyde, *m*-chloro benzaldehyde, *p*-bromo benzaldehyde, *m*-bromo benzaldehyde and *p*-fluoro benzaldehyde were purchased from Loba Chemie Pvt. Ltd. (India). Ethidium bromide and Luria broth were purchased from Himedia, India. Acetic acid and EDTA were

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purchased from Sd fine chemicals, India. CT-DNA was purchased from Sigma Chemical Co., India.

2.2. Physical measurement

Metal contents of the complexes were analyzed by EDTA titration [10] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5). C, H and N elemental analyses were performed on a model Perkin–Elmer 240 elemental analyzer. Magnetic moments were 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 was made using Pascal's constant. IR spectra were recorded on a FT-IR Shimadzu spectrophotometer with sample prepared as KBr pellets in the range of 4000–400 cm^{-1}. The reflectance spectra were recorded on a LAMBDA 19 UV–Vis–NIR, Spectrophotometer, Perkin–Elmer (USA). The ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance (400 MHz). The FAB-mass spectra were recorded on a Jeol SX 120/Da-600 mass spectrometer/Data system using Argon/Xenon(6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature.

2.3. Synthesis of ligands

All tridentate ligands were synthesized similarly by following literature procedure [11]. Aqueous NaOH (10 mL 1.5 M solution) was added to a stirred solution of 2-acetylpyridine (1.20 g, l0 mmol) and different aldehyde (10 mmol) in ethanol (20 mL) and ammonia solution (10 mL). After stirring for 4 h at room temperature, the resultant orange precipitate was isolated by filtration and recrystallized from MeOH. Ligands were characterized using ^1H NMR and ^{13}C NMR spectra.

2.4. Synthesis of complexes

2.4.1. [Cu(cpf)(A^1)Cl]

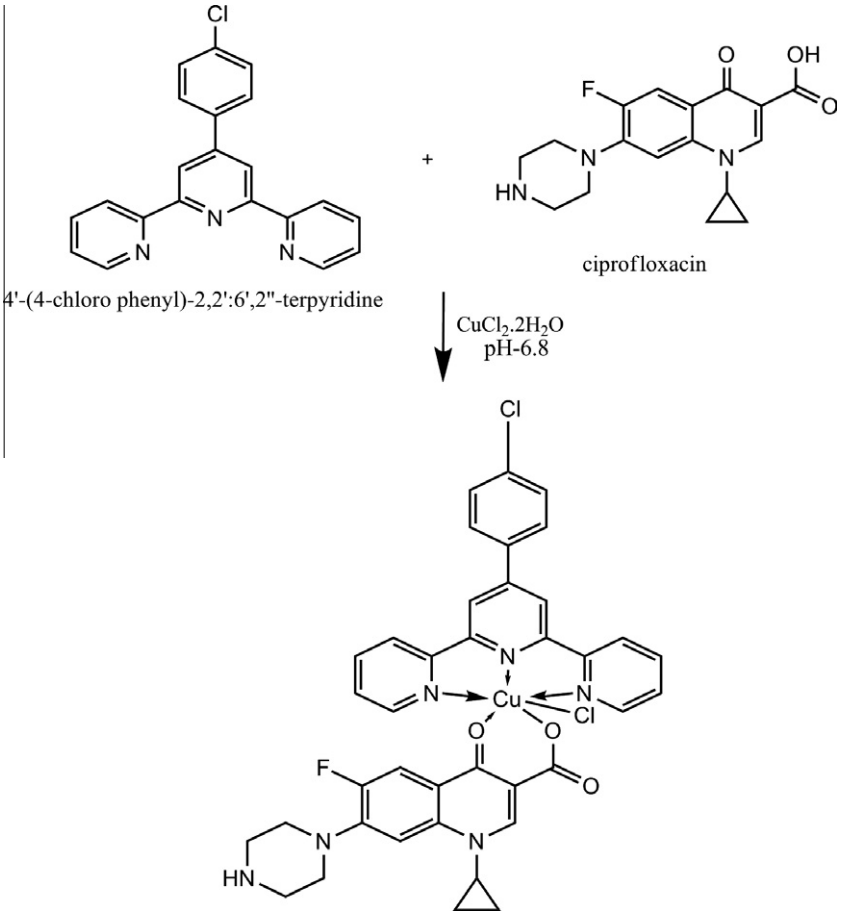
Methanolic solution of $CuCl_2{\cdot}2H_2O$ (1.5 mmol) was added to methanolic solution of 4′-(4-chloro phenyl)-2,2′:6′,2″-terpyridine (1.5 mmol), followed by addition of a previously prepared solution of ciprofloxacin (1.5 mmol) in methanol in presence of CH_3ONa (1.5 mmol). The pH was adjusted at ~6.8 using dilute solution of CH_3ONa. Resulting solution was refluxed for 2 h on water 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. The proposed reaction is shown in Scheme 1.

Yield: 64.7%, m.p.: 230 °C, μ_{eff}: 1.89 B.M. *Anal.* Calc. for $C_{38}H_{31}Cl_2CuFN_6O_3$ (733.14): C, 59.03; H, 4.04; N, 10.87; Cu, 8.22. Found: C, 58.94; H, 3.97; N, 10.96; Cu, 8.46%.

In similar way, complexes **2**–**5** were prepared with the use of corresponding ligands.

2.4.2. [Cu(cpf)(A^2)Cl]

It was prepared using 4′-(3-chloro phenyl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 65.6%, m.p.: 228 °C, μ_{eff}: 1.92 B.M. *Anal.* Calc. for $C_{38}H_{31}Cl_2CuFN_6O_3$ (733.14): C, 59.03; H, 4.04; N, 10.87; Cu, 8.22. Found: C, 59.24; H, 3.78; N, 10.76; Cu, 8.15%.



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

2.4.3. [Cu(cpf)(A^3)Cl]

It was prepared using 4′-(4-bromo phenyl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 67.8%, m.p.: 238 °C, μ_{eff}: 1.89 B.M. *Anal.* Calc. for $C_{38}H_{31}BrClCuFN_6O_3$ (817.59): C, 55.82; H, 3.82; N, 10.28; Cu, 7.77. Found: C, 55.62; H, 4.06; N, 10.11; Cu, 7.65%.

2.4.4. [Cu(cpf)(A^4)Cl]

It was prepared using 4′-(3-bromo phenyl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 67.8%, m.p.: 234 °C, μ_{eff}: 1.97 B.M. *Anal.* Calc. for $C_{38}H_{31}BrClCuFN_6O_3$ (817.59): C, 55.82; H, 3.82; N, 10.28; Cu, 7.77. Found: C, 55.72; H, 3.71; N, 10.15; Cu, 7.94%.

2.4.5. [Cu(cpf)(A^5)Cl]

It was prepared using 4′-(4-fluoro phenyl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 65.0%, m.p.: 218 °C, μ_{eff}: 1.87 B.M. *Anal.* Calc. for $C_{38}H_31ClCuF_2N_6O_3$ (756.69): C, 60.32; H, 4.13; N, 11.11; Cu, 8.40. Found: C, 60.11; H, 4.34; N, 10.98; Cu, 8.31%.

2.5. Antibacterial activity

Antibacterial activity of the compounds (metal salts, standard drugs and complexes) was screened against two Gram$^{(+ve)}$ *Staphylococcus aureus*, *Bacillus subtilis*, and three Gram$^{(-ve)}$ *Serratia marcescens, Escherichia coli* and *Pseudomonas aeruginosa*. Screening was performed by determining the minimum inhibitory concentration (MIC). All cultures were incubated at 37 °C. Control tests with no active ingredients were also performed. The MIC was determined using twofold serial dilutions in liquid media of the compound being tested. The solvent used was DMSO.

A preculture of bacteria was grown in LB (Luria Broth) overnight at the most favorable temperature of each species. This culture was used as a control to examine if the growth of bacteria tested is normal. In a similar second culture, 20 μL of the bacteria as well as the tested compound at the desired concentration were added. We monitored bacterial growth by measuring turbidity of the culture after 18 h. If a certain concentration of a compound inhibited bacterial growth, half the concentration of the compound was tested. This procedure was carried on to a concentration that bacteria grow normally. The lowest concentration that inhibited bacterial growth was determined as the MIC value. All equipment and culture media were sterilised.

The bactericidal action of all compounds was evaluated against three Gram$^{(-ve)}$ and two Gram$^{(+ve)}$ bacteria. The inoculum was prepared by diluting an overnight culture, grown in Luria broth, to obtain 10^6 viable bacteria/mL, confirmed in each experiment by colony counts. Bacteria were exposed to concentrations of 0.25–1.75 μg/mL of compounds. The final volume was 1 mL. Cultures were incubated at 37 °C for 2 h. The 100 μL bacterial culture from above was taken and spread over previously prepared agar plate. These were incubated for 24 h at 37 °C and the visual colonies were calculated in order to check biocidal activity of metal complexes, yielding 30–250 colonies.

2.6. DNA interaction activity

2.6.1. Absorption titration

The DNA-binding experiments were performed in Tris–HCl/NaCl buffer (50 mM Tris–HCl/1 mM NaCl buffer, pH 7.5). The concentration of calf thymus (CT) DNA was determined measuring absorption intensity at 260 nm with a ε value [12] of 6600 M^{-1} cm^{-1}. Absorption titration experiments were made using different concentrations of CT-DNA, keeping the concentration of the complexes constant, with due correction for the absorbance of the CT-DNA itself. Samples were equilibrated before recording each spectrum.

To compare quantitatively the affinity of the complexes bound to DNA, the intrinsic-binding constants K_b of the complexes were obtained by monitoring the changes in absorbance with increasing concentration of DNA using the following Eq. (1) [13].

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

where, [DNA] is the concentration of DNA in base pair, ε_a, ε_f and ε_b correspond to the apparent absorption coefficient A_{obs}/[Cu], the extinction coefficient for the free complex and the extinction coefficient for the complex in the fully bound form, respectively.

2.6.2. Viscosity measurements

Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 27.0 ± 0.1 °C in a thermostatic bath. DNA samples approximately 200 base pairs in average length were prepared by sonicating it to minimize complexities arising from DNA flexibility [14]. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. The rate of flow of sodium phosphate buffer (pH ~7.2), DNA (100 μM) and DNA with the copper complexes at various concentrations were measured. Data are presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio [15], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

2.6.3. DNA melting temperature

Thermal denaturation studies were performed with a Perkin–Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1 °C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (100 μM) in the absence and the presence of complex (20 μM). The temperature of the solution was increased by 1 °C min^{-1}.

2.6.4. DNA cleavage study

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out in TE buffer within 15 μL reaction mixture containing 300 μg/mL plasmid DNA (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 (40% sucrose, 0.2% bromophenol blue). The aliquots were loaded on to 1% agarose gel and electrophoresed at 50 V in 1X TAE buffer. Gel was stained with 0.5 μg/mL ethidium bromide

Table 1
IR spectra data.

Compounds	ν(C=O) cm^{-1} pyridone	$\nu(COO)_{as}$ cm^{-1}	$\nu(COO)_s$ cm^{-1}	$\Delta\nu$ (cm^{-1})	ν(M–N) cm^{-1}	ν(M–O) cm^{-1}
Ciprofloxacin	1708	1624	1340	284		
Complex-**1**	1622	1564	1364	200	535	515
Complex-**2**	1617	1578	1377	201	542	504
Complex-**3**	1624	1579	1378	203	540	510
Complex-**4**	1619	1564	1374	197	541	508
Complex-**5**	1617	1562	1343	223	542	511

and was photographed on UV illuminator. The percentage of each form of DNA was quantitised using AlphaDigiDoc™ RT. Version V.4.0.0 PC-IMAGE software. The degree of DNA cleavage activity was expressed in terms of the percentage of cleavage of the SC-DNA according to the following equation [16]:

$$\%\,\text{DNA cleavage activity} = \{[(\%\ \text{of SC-DNA})_{\text{control}} - (\%\ \text{of SC-DNA})_{\text{sample}}]/(\%\,\text{of SC-DNA})_{\text{control}}\} \times 100$$

2.7. Determination of SOD-like activity

SOD–like activity of the complexes was determined using NBT/NADH/PMS system [17]. The superoxide radical produce by 79 μM NADH, 30 μM PMS, system containing 75 μM NBT, phosphate buffer (pH 7.8), and 0.25–3.0 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 reduction rate of NBT was measured in presence and absence of test compounds at various concentrations of complexes in the system. All measurements were carried out at room temperature. IC_{50} value of the complexes was determined by plotting the graph of percentage inhibition of NBT reduction against increase in concentration of complexes. Concentration of complexes which causes 50% inhibition of NBT reduction is reported as IC_{50}.

3. Result and discussion

3.1. Reflectance spectra and magnetism

The magnetic moments of the copper(II) complexes lie in the 1.87–1.97 BM range. These values are typical of mononuclear copper(II) compounds with d^9 electronic configuration. The observed magnetic moments of all the complexes correspond to typical high-spin octahedral complexes. However, the values are slightly higher than the expected spin-only values due to spin–orbit-coupling contribution [18].

Copper(II) complexes with octahedral geometry are mock to demonstrate the absorption spectra in visible region and only one broad band about 660 nm [19]. Here in our case same results are obtained, that is, the only λ_{max} at about 615 nm points toward octahedral geometry.

Table 2
MIC data of the compounds (μM).

	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
Complex-**1**	1.16	1.12	0.59	0.68	1.13
Complex-**2**	1.21	1.19	0.73	0.52	1.19
Complex-**3**	1.07	0.88	1.21	0.87	1.05
Complex-**4**	1.54	1.08	1.67	2.01	1.97
Complex-**5**	1.04	0.57	0.33	0.34	1.26

3.2. IR spectra

The determination of the coordinating atoms was made on the basis of the comparison of the IR spectra of the ciprofloxacin and of the complexes. Significant wave numbers are given in Table 1. The ν(C=O) stretching vibration band appears at 1708 cm^{-1} for ciprofloxacin, where as for complexes it appear at 1617–1624 cm^{-1}, this shift towards lower energy suggests that coordination occurs through carbonyl oxygen of pyridine ring. Strong absorption band at 1624 and 1340 cm^{-1} in ciprofloxacin could be assigned for ν(COO) asymmetric and symmetric vibration, respectively, while in metal complexes these bands were observed at 1564 and 1364 cm^{-1}. The difference $\Delta = \nu_{as}(COO) - \nu_s(COO)$ is useful for determining the coordination mode of ligands. The Δ values are greater than 200 cm^{-1}, indicating monodentate coordination mode of carboxylato group [20–24] of the ligands. These data are further supported by ν(M–O) which appear at 504–515 cm^{-1} for complexes.

In investigated complexes the ν(C=N) band of terpyridine appears at 1584 cm^{-1}. This band shift to higher frequency at ~1626 cm^{-1} [25,26] in complexes indicates bidentate N–N

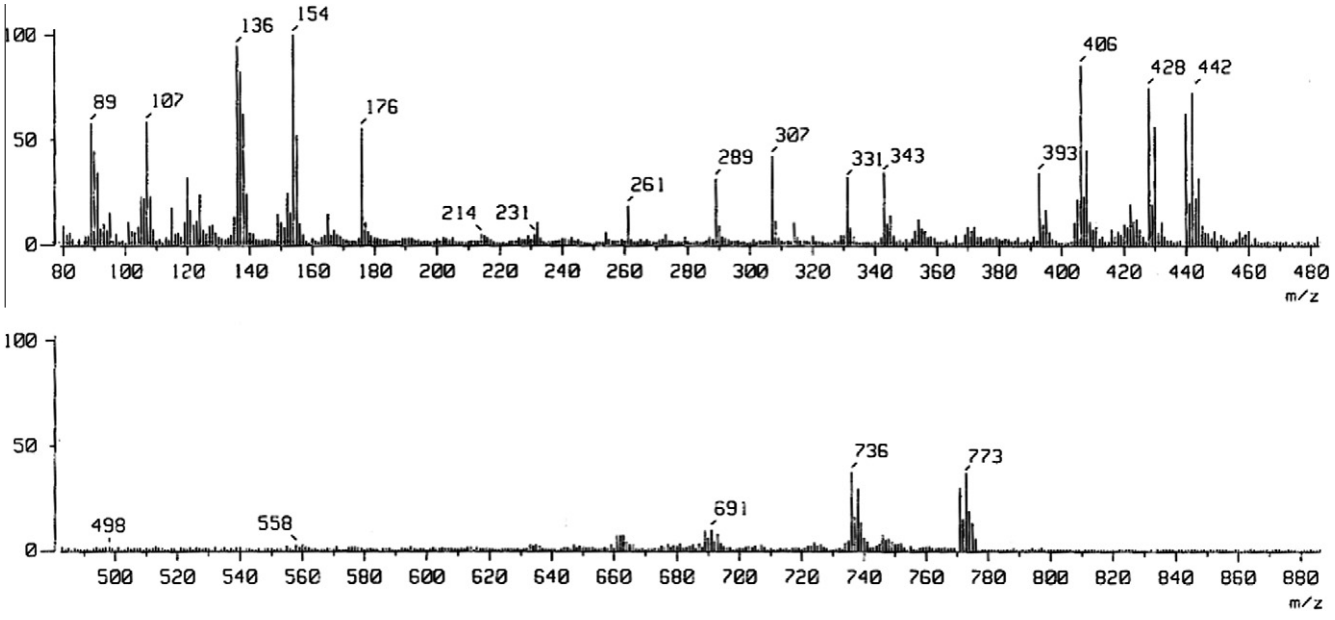


Fig. 1. FAB-mass spectrum of complex **1**, that is [Cu(cpf)(A^1)Cl], obtained using *m*-nitro benzyl alcohol.

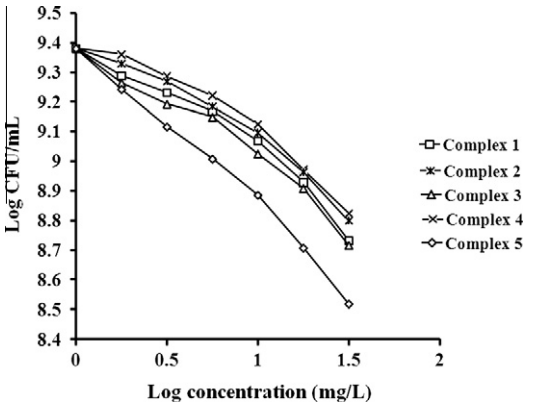


Fig. 2. Relationship between concentration and bactericidal activity of all complexes against *S. aureus*.

coordination of the ligand. N→M bonding was supported by ν(M–N) band [27] at ~535–542 cm^{-1} for complexes.

3.3. Mass spectra

Fig. 1 represents the FAB-mass spectrum of complex **1**, that is [Cu(cpf)(A^1)Cl], obtained using *m*-nitro benzyl alcohol as matrix. Peaks at 136, 137, 154, 289 and 307 *m/z* are due to usage of matrix. The molecular ion peak is observed at *m/z* = 773 which is equivalent of its molecular weight. Loss of chlorine atom gave a fragment ion peak at *m/z* = 736, which confirm that chlorine atom attached to metal ion with covalent bond. The highest peak was observed at *m/z* = 406. Other fragment corresponds to peaks at 661, 442, 393, 343 and 331 *m/z* value.

3.4. Antibacterial activity

A comparative study of MIC values of the fluoroquinolones and its mixed ligand complexes indicate that the metal complexes have better antibacterial activity than fluoroquinolones (Table 2). The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal centre as well as bulkiness of the ligands. This may support the argument that some type of molecular binding to the metal ions or intercalation or electrostatic interactions causing the inhibition of biological synthesis and preventing the organisms from reproducing. The increased activity of copper complexes compared to that of the free ligands may be explained in terms of chelation theory [28]. According to this theory, formation of the chelate ring enhanced the lipophilicity of complexes, which breaks down the permeability barrier of the cell retarding the normal cell processes.

Efficiencies of ligands and complexes have been tested against three Gram$^{(-ve)}$, *E. coli*, *S. marcescens* and *P. aeruginosa* and two Gram$^{(+ve)}$, *S. aureus* and *B. subtilis* microorganisms. In case of *S. aureus*, all compounds shows good activity compare to reference drugs. In case of *B. subtilis*, all compounds exhibit good activity compare to fluoroquinolones. In case of *S. marcescens*, compounds **I**, II, III and **V** found more potent than reference drugs. In case of *P. aeruginosa*, compounds **I**, **II**, III and **V** exhibit good activity compare to fluoroquinolones. In case of *E. coli*, compounds **I**, **II**, III and **IV** shows more potency than reference drugs.

In general, antimicrobial activity of the metal complexes depends on following five factors [29]: (i) the chelate effect, that is, ligands that are bound to metal ions in a bidentate and tridentate fashion, such as the quinolones and the N-donor ligands (terpy), show higher antimicrobial efficiency towards complexes than unidentate N-donor ligands, e.g., pyridine; (ii) the nature of the ligands, there are two different type of ligands and both ligands are responsible for higher antimicrobial activity; (iii) the total charge of the complex; generally the antimicrobial efficiency decreases in the order cationic > neutral > anionic complex; (iv) the nature of the ion neutralizing the ionic complex; (v) the nuclearity of the metal center in the complex; dinuclear centers are usually more active than mononuclear ones. The first two of the five above-mentioned factors may be responsible for higher antimicrobial activity; that is the chelate effect provided by both the ciprofloxacin ligand and the N-donor ligand and the nature of the ligands.

This is probably one of the main reasons for the diverse antibacterial activities shown by the complexes. The significant improvement of the activity of ciprofloxacin when coordinated to the copper complex is simply an evidence of the role of the coordinated metal ion [30].

It has also been suggested [31,32] that the ligands with nitrogen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation. Chelation reduces the polarity [31,32] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalization within the whole chelate ring system thus formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn favors its permeation through the lipoid layer of the membrane. This in turn is responsible for increasing the hydrophobic character and liposolubility of the molecule in crossing cell membrane of the microorganism and hence enhances the biological utilization ratio and activity of the testing drug/compound.

In addition, our study regarding bacteriocidal activity in terms of CFU/mL of above metal complexes against same microorganisms (three Gram$^{(-ve)}$ and two Gram$^{(+ve)}$) revealed decrease in number of colonies with increasing the concentration of compounds. The results are shown in Fig. 2 for all the complexes against *S. aureus*. The maximum concentration of all the complexes was 1.75 CFU/mL which gave lesser number of colonies. The number of colonies counted in this technique was 30–250. Complexes **1**, 2 and **5** had lesser number of colonies than complexes **3** and **4**. Results for microorganism *B. subtilis*, *S. marcescens*, *P. aeruginosa* and *E. coli* are kept in Supplementary data.

3.5. Metal DNA interaction

3.5.1. Photophysical measurement

A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the

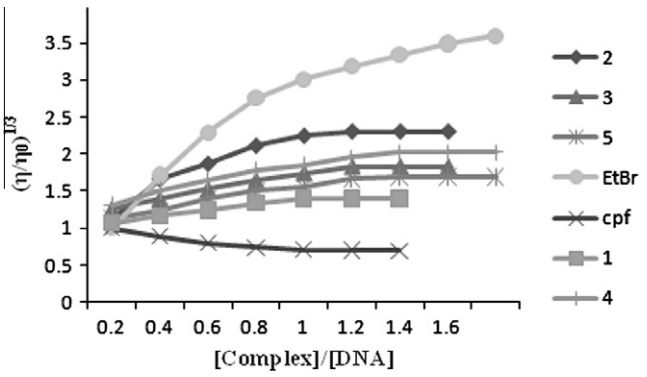


Fig. 3. Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27 ± 0.1 °C in phosphate buffer as a medium.

binding ligand, leading to an increase of the viscosity of the DNA solution [33]. Binding modes of the complexes were further investigated by viscosity measurements. Though photophysical experiments, give necessary information about binding modes of metal complexes with DNA, they do not provide conclusive evidences for the exact mode of binding. Hydrodynamic measurements such as viscosity which is sensitive to length changes are regarded as the least ambiguous and the most critical tests of binding modes in solution [34]. Viscosity of DNA is increased in case of classical intercalators due to increase in the length of DNA helix, as base pairs are separated to accommodate the intercalator. In contrast, decrease in DNA viscosity is noted in partial or non-classical intercalator, because it may bend (or kink) DNA helix, thereby decreasing its effective length. From Fig. 3 as increasing the amounts of complexes, viscosity of DNA increases steadily, which is similar to that of the classical intercalative complex $[Ru(phen)_2(DPPZ)]^{2+}$. Amongst all, complexes **1**, 2 and **5** bind more strongly than **3** and **4**. The increased degree of viscosity, which may depend on the binding affinity to DNA, follows the order of increasing viscosity is EB > [Cu(cpf)(A^n)Cl] > Ciprofloxacin. These results suggest that the all title complexes intercalate between the base pairs of DNA and the binding affinity of complexes is higher than ciprofloxacin but less than ethidium bromide.

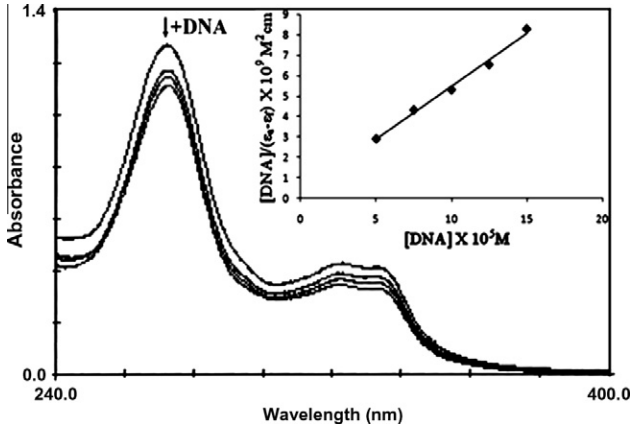


Fig. 4. Electronic absorption titration curve of [Cu(cpf)(A^1)Cl]·5H_2O in absence and in presence of increasing amount of DNA; 50–150 μM in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2), [complex] = 15 μM, [DNA] = 50–150 μM with incubation period of 30 min at 37 °C, Inset: Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA].

Table 3
Binding constant and IC_{50} values of copper(II) complexes.

Complexes	K_b (M^{-1})	IC_{50}
[Cu(cpf)(A^1)Cl] (**1**)	2.66×10^4	0.750
[Cu(cpf)(A^2)Cl] (**2**)	2.62×10^4	1.25
[Cu(cpf)(A^3)Cl] (**3**)	1.67×10^4	1.35
[Cu(cpf)(A^4)Cl] (**4**)	1.27×10^4	1.5
[Cu(cpf)(A^5)Cl] (**5**)	4.73×10^4	0.5

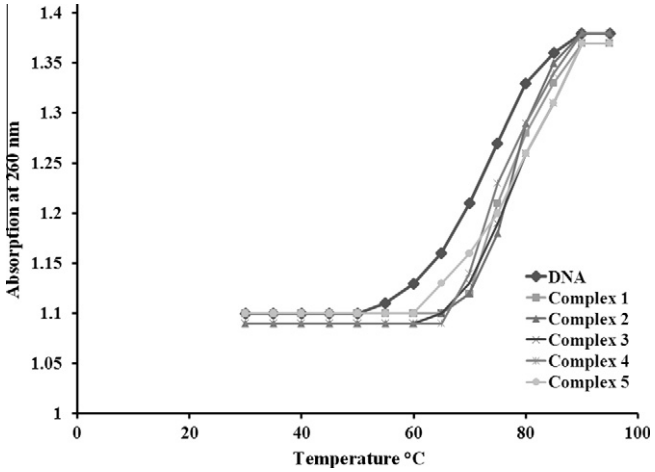


Fig. 5. Melting curves of CT-DNA in the absence and presence of complexes **1**–**5**.

3.5.2. UV–Vis absorption spectra

DNA can provide three distinctive binding sites for the quinolone complexes; namely, groove binding, binding to phosphate groups, and intercalation. This behavior is of great importance with regard to the biological role of fluoroquinolone antibiotics in the human body [35].

Interaction of metal complexes with DNA can be monitored by absorption spectral titration [36]. In Fig. 4, with increasing concentration of CT-DNA, absorption bands of the complexes are affected, resulting in hypochromism with bathochromic shift. The change in absorbance values were used to evaluate intrinsic-binding constant K_b for the complexes (Table 3). Binding constants of the parent complexes are sufficiently high due to their octahedral nature [37]. To compare binding strength of complexes quantitatively, the intrinsic binding constants K_b of complexes were determined by monitoring the changes of absorbance at 274 nm with increasing concentration of DNA. From the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], K_b value of all complexes (Table 3) were found to be in range of 1.2×10^4–4.73×10^4 M^{-1}.

3.5.3. Thermal denaturation study

Thermal behaviors of DNA in the presence of complexes can give insight in to their conformational changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the temperature in the solution increases, the double stranded DNA gradually dissociates to single strands and generates a hyperchromic effect on the absorption spectra of DNA bases (λ_{max} = 260 nm). In order to identify this transition process, the melting temperature T_m, which is defined as the temperature where half of the total base pairs are unbounded, is usually introduced. According to the literature the interaction of metallointercalators generally results in a

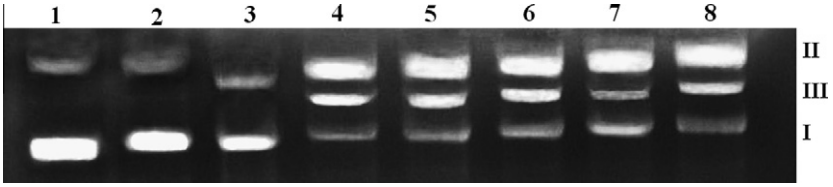


Fig. 6. Photogenic view of interaction of pUC19 DNA (450 μg/mL) with series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μL, for 3 h at 37 °C. Lane 1, DNA control; Lane 2, $CuCl_2·2H_2O$; Lane 3, ciprofloxacin; Lane 4, [Cu(cpf)(A^1)Cl]; Lane 5, [Cu(cpf)(A^2)Cl]; Lane 6, [Cu(L)(cpf)Cl]; Lane 7, [Cu(cpf)(A^4)Cl]; Lane 8[Cu(cpf)(A^5)Cl].

considerable increase in melting temperature (T_m). DNA (100 μM) melting experiments revealed that T_m of CT-DNA is 74.2 ± 1 °C in the absence of the complex (Fig. 5). However, with addition of complexes **1**, **2** and **5** the T_m increased to 78.9 ± 1 °C, 78.8 ± 1 °C and 79.0 ± 1 °C, respectively and for complexes **3** and **4** the T_m increased to 78.3 ± 1 °C and 78.1 ± 1 °C, respectively. The increased T_m (3.9–4.8 °C) value of the DNA after addition of the complexes is comparable to that observed for classical intercalators [38].

3.5.4. Gel electrophoresis

Fig. 6 illustrates gel electrophoretic separations showing cleavage of pUC19 DNA induced by the complexes under aerobic conditions [39]. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for supercoiled form-I (SC). If one strand is cleaved, the supercoiled will relax to produce a slower-moving open circular form-II (OC). If both strands are cleaved, linear form-III (LC) will be generated that migrates in between. This clearly shows that the relative binding efficacy of complexes to DNA is much higher than the binding efficacy of metal salt or ciprofloxacin (Table 4). Different DNA-cleavage efficiency of the complexes was due to different binding affinity of complexes to DNA, which has been observed in other cases.

3.6. Free radical scavenger activity

The superoxide radicals ($O_2^{\cdot -}$) were generated in vitro using a non-enzymatic (NBT/NADH/PMS) system and determined spectrophotometrically. 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. The SOD activities of complexes were investigated by NBT assay. SOD data for the complexes have been compiled in Table 3 along with SOD activity value of other similar complexes and containing copper metal ion [40].

Table 4
Gel electrophoresis data.

Compounds	%SC	%OC	%LC	% Cleavage
DNA control	75	25		
DNA + metal salt	72	28		4.00
DNA + CPFH	67	33		10.66
DNA + **I**	16	52	32	78.66
DNA + **II**	15	52	33	80.00
DNA + **III**	20	50	30	73.33
DNA + **IV**	29	56	15	61.33
DNA + **V**	14	56	30	81.33

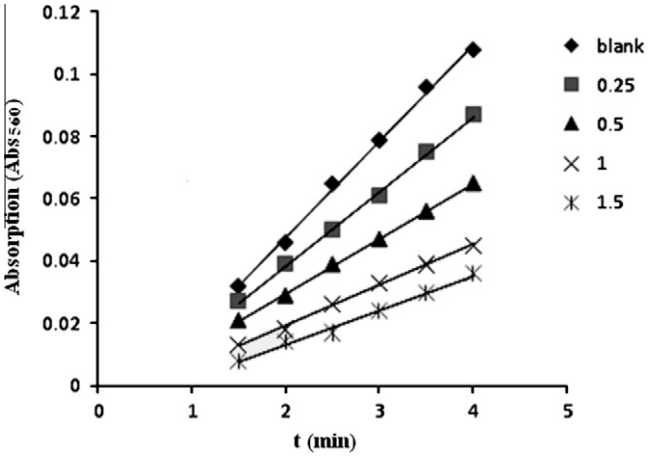


Fig. 7. Plot of absorbance values (A_{obs560}) against time (t).

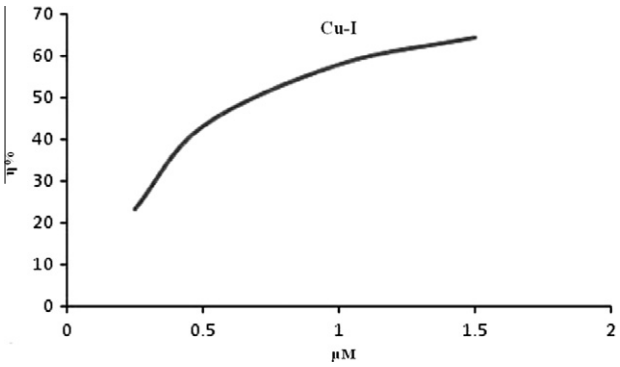


Fig. 8. Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex **1**.

Copper gives good SOD activity, although it structure is totally unrelated with native enzyme. The ping-pong mechanism of SOD activity is given in Eqs. (2) and (3).

$$O_2^- + Cu^{II} \rightarrow O_2 + Cu^{I} \quad (2)$$

$$O_2^- + Cu^{I} + 2H^+ \rightarrow H_2O_2 + Cu^{II} \quad (3)$$

Fig. 7 represents plot of absorbance values (A_{obs560}) against time (t) with varying concentration of complex **1** from 0.25 to 5 μM. Fig. 8 illustrate that chromophore requires to yield 50% inhibition of the reduction of NBT (IC_{50}) value of complexes are higher than exhibited by the copper salt. The copper(II) complexes showed SOD-like activity which was evaluated by the scavenger concentration causes 50% inhibition in the detector formation, IC_{50}. Compounds exhibit SOD-like activity at biological pH with IC_{50} values ranging from 0.5 to 1.5 μM. Complexes **1** and **5** were found even more active compare to **2**–**4**.

3.7. Structure–activity relationship

The quinolone/naphthyridone ring system can be modified at various sites and consequently, over the years, hundreds of analogs have been investigated in an attempt to increase efficacy and reduce adverse effects. Features that enhance the effectiveness of the quinolones most are a halogen (F or Cl) at C8, which improves oral absorption and activity against anaerobes; alkylated pyrrolidine or piperazine at C7, which increases serum half-life and activity against gram-positive microorganisms; and a cyclopropyl group at N1 and an amino substituent at C5, which improve overall potency [41].

The electron-withdrawing substituent on the intercalative ligand can improve the DNA interaction affinity, free radical scavenging activity and antibacterial activity of the original complex, whereas the electron-pushing substituent is reverse. Such a trend suggests that the DNA interaction affinity, free radical scavenging activity and antibacterial activity of the complex can be effectively controlled by the substituents. In our case there are three different substituents and all are electron withdrawing in nature. Increasing ability of electron-withdrawing power of substituents in order of F > Cl > Br. In case of antibacterial activity, DNA interaction and superoxide dismutase, compound containing electron-withdrawing substituents (F) has better activity then compound containing substituents Cl and Br. The ascending order of complexes towards the biological activities is **V** > **I** > **II** > **III** > **IV**.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.poly.2010.08.037.

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