Full Paper

Applied Organometallic Chemistry

Received: 27 January 2011 Revised: 13 May 2011 Accepted: 22 May 2011 Published online in Wiley Online Library: 10 August 2011

(wileyonlinelibrary.com) DOI 10.1002/aoc.1817

DNA interaction, free radical scavenging and *in-vitro* antibacterial activity of drug-based copper(II) complexes

Mohan N. Patel*, Promise A. Dosi and Bhupesh S. Bhatt

The Cu(II) complexes of type [Cu(cpf)(A^n)Cl] (A^n = terpyridines, cpf = ciprofloxacin) were synthesized and characterized using IR, mass and reflectance spectra. The free ligands and their complexes were evaluated for their *in-vitro* antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria. The complexes exhibit better or equal inhibition in comparison to free fluoroquinolones. Binding interactions of the complexes with calf thymus (CT DNA) were investigated by absorption titration, viscosity studies and DNA melting temperature experiment. The cleavage reaction on pUC19 DNA was monitored by agarose gel electrophoresis. The lower concentration of the complexes was catalysed the dismutation of superoxide radical at biological pH, which indicates that the complexes can act as a possible model for superoxide dismutase.

Supporting information may be found in the online version of this article.

Keywords: calf thymus DNA; pUC19 DNA; melting temperature; superoxide dismutase

Introduction

Transition metal complexes with their varied coordination environments and versatile redox and spectral properties offer immense scope for designing species that are suitable to bind and cleave DNA. Metal ions can play a role in biology as counter-ions for protein, DNA, RNA and in various biological organelles. The structural role is often manifested by maintenance of various biological structures, whereas a functional role brings key reactivity to a reaction centre for a protein.[1]

In recent years, there has been substantial interest in the rational design of novel transition metal complexes which bind and cleave duplex DNA with high sequential or structural selectivity.[2–4] An approach to the discovery of new metallodrugs involves binding an organic compound of known therapeutic value to a metal-containing fragment; this results in a metal–drug synergism in which the metal acts as a carrier and stabilizer for the drug until it reaches its target, while at the same time the organic drug carries and protects the metal from side reactions in its transit towards other targets of biological action.[5] Such combined effects may result in an important enhancement of the activity of the drug and opening of new mechanisms of action. The use of transition metal complexes as artificial nucleases is an area of extensive research owing to the diverse structural features and reactivity of such complexes.[6]

Quinolones, a commonly used term for the quinolonecarboxylic acids or 4-quinolones, are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton. Ciprofloxacin (CPFH) [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazine-l-yl)quinolone-3-carboxylic acid] is a synthetic fluoroquinolone derivative which has broad-spectrum activity against many pathogenic Gram-negative and Gram-positive bacteria and this antibiotic is widely used in the treatment of a variety of bacterial infections in humans and animals.[7]

A large number of mixed ligand copper(II) complexes have been shown to exhibit superoxide dismutase activity.[8] This activity depends on the Cu(II)/Cu(I) redox process, which is related to the flexibility of the geometric transformation around the metal centres.[9] Herein, we studied the mode of coordination and biological properties of Cu(II) complexes with second-generation quinolone, ciprofloxacin and tridentate ligands.

Experimental Section

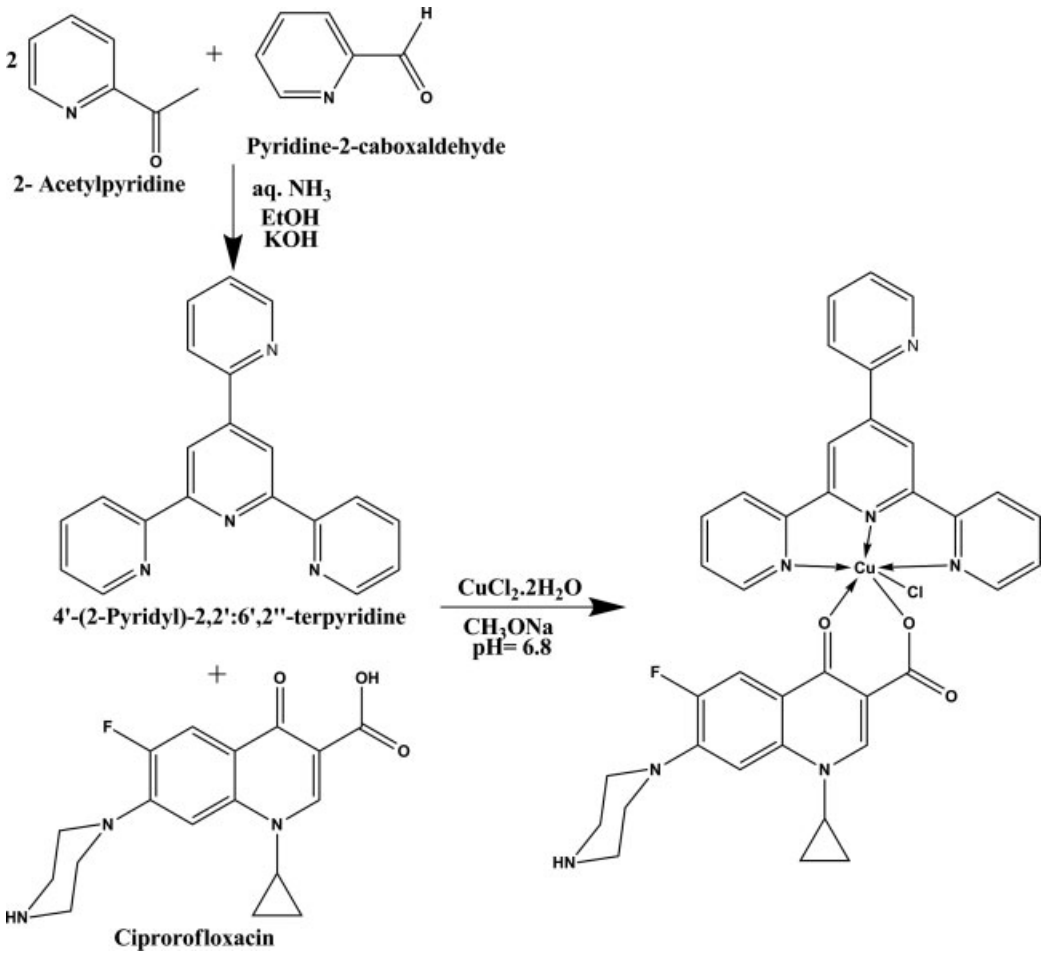
Materials and Instrumentation

All the chemicals and solvents were reagent grade and used as purchased. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). Cupric chloride dihydrate, pyridine-2-carboxaldehyde, pyridine-3-carboxaldehyde, thiophene-2-carboxaldehyde, 9-anthraldehyde and CT DNA were purchased from SD Fine Chemicals, India. Ethidium bromide (EB) and Luria Broth were purchased from Himedia, India. Acetic acid and EDTA were purchased from SD Fine Chemicals, India.

Metal contents of the complexes were analysed gravimetrically and volumetrically,[10] after decomposing the organic matter with acid mixture ($HClO_4$, H_2SO_4 and HNO_3). C, H and N elemental analyses were performed on a Perkin-Elmer 240 elemental analyser. 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 a

* *Correspondence to: Mohan N. Patel, Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India. E-mail: jeenen@gmail.com*

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India



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

Citizen Balance. The diamagnetic correction was made using Pascal's constant. IR spectra were recorded on an FT-IR Shimadzu spectrophotometer with the sample prepared as KBr pellets in the range 4000–400 cm^{-1}. The reflectance spectra were recorded on a Lambda 19 UV–vis–NIR spectrophotometer (Perkin Elmer, USA). 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 fast atomic bombardment (FAB) gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature.

Synthesis of Ligands

All tridentate ligands were synthesized using a literature procedure.[11] Aqueous KOH (10 mL, 1.5 M solution) was added to a stirred solution of 2 equiv. 2-acetylpyridine (1.20 g, 10 mmol) and 1 equiv. aldehydes (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 methanol. Ligands were characterized using ^{1}H-NMR and ^{13}C-NMR spectra.

Synthesis of Complexes

*[Cu(cpf)(A^1)Cl] [**I**]*

Complex **1** was prepared by the reaction of ciprofloxacin (1.8 g, 1.5 mmol) in CH_3OH (10 mL), which was deprotonated with CH_3ONa (0.27 g, 1.5 mmol), $CuCl_2{\bullet}2H_2O$ (0.84 g, 1.5 mmol) in CH_3OH (15 mL) and 4′-(2-pyridyl)-2,2′:6′,2″-terpyridine (1.3 g, 1.5 mmol) in CH_3OH (15 mL). The reaction mixture was refluxed for 2 h. A fine amorphous product of green colour was obtained, which was washed with ether/hexane and dried in a vacuum desiccator. The proposed reaction is shown in Scheme 1.

Yield: 64%, m.p.: 242 °C, μ_{eff}: 1.88 B.M. Anal. calcd for $C_{36}H_{31}ClFCuN_7O_3$ (727.67): C, 59.42; H, 4.29; N, 13.47 Cu, 8.73. Found: C, 59.39; H, 4.24; N, 13.34; Cu, 8.69%.

*[Cu(cpf)(A^2)Cl] [**II**]*

[Cu(cpf)(A^2)Cl] [**II**] was prepared using 4′-(3-pyridyl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 67%, m.p.: 232 °C, μ_{eff}: 1.87 B.M. Anal. calcd for $C_{36}H_{31}ClFCuN_7O_3$ (727.67): C, 59.42; H, 4.29; N, 13.47 Cu, 8.73. Found: C, 59.34; H, 4.21; N, 13.41; Cu, 8.67%.

*[Cu(cpf)(A^3)Cl] [**III**]*

[Cu(cpf)(A^3)Cl] [**III**] was prepared using 4′- phenyl-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 63%, m.p.: 222 °C, μ_{eff}: 1.91 B.M. Anal. calcd for $C_{38}H_{32}ClFCuN_6O_3$ (738.70): C, 61.79; H, 4.37; N, 11.38; Cu, 8.60. Found: C, 61.68; H, 4.24; N, 11.30; Cu, 8.53%.

*[Cu(cpf)(A^4)Cl] [**IV**]*

[Cu(cpf)(A^4)Cl] [**IV**] was prepared using 4′-(2-thiophene)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 70%, m.p.: 231 °C, μ_{eff}: 1.84 B.M. Anal. calcd for: $C_{36}H_{30}ClFCuN_6O_3S$ (743.11): C, 58.06; H, 4.06; N, 11.28; Cu, 8.53. Found: C, 58.01; H, 4.02; N, 11.21; Cu, 8.45%.

*[Cu(cpf)(A^5)Cl] [**V**]*

[Cu(cpf)(A^5)Cl] [**V**] was prepared using 4′-(anthracene-9-yl)-2,2′:6′,2″-terpyridine (1.5 mmol). Yield: 70%, m.p.: 234 °C, μ_{eff}: 1.85 B.M. Anal. calcd for $C_{46}H_{36}ClFCuN_6O_3$ (838.81): C, 65.87; H, 4.33; N, 10.02; Cu, 7.58. Found: C, 65.75; H, 4.31; N, 10.05; Cu, 7.50%.

In-vitro Antibacterial Activity

An antibacterial activity assay was performed on *Staphylococcus aureus, Bacillus subtilis, Serratia marcescens, Pseudomonas aeruginosa* and *Escherichia coli*. The antibacterial activity for the test compounds was tested to determine the bacteriostatic concentration, i.e. minimum inhibitory concentration (MIC) in terms micromoles. The MIC value was determined by broth dilution technique.[12] A preculture of bacteria was grown in LB (Luria Broth) overnight at the most favourable temperature of each species. This culture was used as a control to examine if the growth of bacteria tested was normal. In a similar second culture, 20 µL of the bacteria as well as the tested compound at the desired concentration were added and monitored for bacterial growth by measuring turbidity of the culture after 18 h. If a certain concentration of a compound inhibited bacterial growth, half of the concentration of the compound was tested. This procedure was carried out up to the concentration which inhibited the growth of bacteria. The lowest concentration that inhibited bacterial growth was considered the MIC value. All equipments and culture media were sterilized.

Viscosity Measurement

Viscometric titrations were performed with an Ubbelohde automated viscometer. The viscometer was thermostated at 37 °C in a constant temperature bath. The concentration of DNA was 200 µM in nucleotide phosphate (NP) and the flow times were measured with an automated timer. Each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* [complex]/[DNA], where η is the viscosity of DNA in presence of complex and η_0 that of DNA alone. Viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of buffer alone (t_0), $\eta = (t - t_0)$.[13]

Absorption Titration

The UV absorbance at 260 and 280 nm of the CT DNA solution in 5 mM Tris–HCl buffer (pH 7.2) gave a ratio of 1.9, indicating that the DNA was free of protein. The concentration of CT DNA was measured from the band intensity at 260 nm with a known value (6600 M^{-1} cm^{-1}).[14] Absorption titration measurements were carried out by varying the concentration of CT DNA, keeping the metal complex concentration constant in 5 mM Tris–HCl/5 mM NaCl buffer (pH 7.2). Samples were kept for equilibrium before recording spectrum. The intrinsic binding constants (K_b) were determined from a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA] using absorption spectral titration data.

DNA Melting Experiment

Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1 °C). The melting curves were obtained by measuring the absorbance at 260 nm for solutions of CT DNA (100 µM) in the absence and presence of the Cu(II) complexes (20 µM) as a function of the temperature. The temperature was scanned from 35 to 90 °C at a speed of 5 °C/min. The melting temperature (T_m) was taken as the mid-point of hyperchromic transition.

Gel Electrophoresis

The plasmid DNA used was extracted from *E. coli* specie belonging to pUC19 genus as per the standard protocol reported by Sambrook and Russell.[15] The whole process was based on the following three steps:

(1) weakening of the bacterial cell wall by the action of lysozyme.
(2) cells being lysed by ethylenediaminetetraaccetate EDTA and detergent at high pH;
(3) insoluble cells debris consisting of genomic DNA and protein being precipitated in high salt-containing buffer, leaving behind the plasmid in solution (supernant), which is precipitated by chilled absolute alcohol and collected.

Agarose gel electrophoresis experiments were carried out on supercoiled plasmid DNA pUC19 using a horizontal gel system. The DNA cleavage profile was analysed using 1% agarose gel in a horizontal gel tank set with a running time of 2.5 h, at a constant voltage of 50 V in 1× Tris-acetate EDTA (TAE) buffer (0.04 M Tris–acetate, pH 8, 0.001 M EDTA) by loading each reaction mixture (15 µL) consisting of plasmid DNA (150 µg/mL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and 200 µM complex, which were allowed to proceed for 24 h at 37 °C and quenched by addition of 5 µL loading buffer (40% sucrose, 0.25% bromophenol blue, 0.25% xylene cyanole FF, 200 mM EDTA). The gel was stained with 0.5 µg/mL of ethidium bromide and was photographed on a UV trans-illuminator. The extent of cleavage was quantified based on the intensity of the band using AlphaDigiDoc™ RT. The degree of DNA cleavage activity was expressed in terms of the percentage of conversion of the SC-DNA to open circular (OC)-DNA according to the following equation:[16]

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

Superoxide Dismutase Activity

The superoxide dismutase (SOD) activities of the copper compounds were determined spectrophotometrically by the nitroblue tetrazolium (NBT) assay method.[17] One unit of SOD activity was defined as the concentration of the test substrate required for 50% inhibition of NBT reduction by superoxide anion (IC_{50} value).

The superoxide radial anion produced by 79 µM (nicotinamide adenine dinucleotide reduced) NADH, 30 µM phenazine methosulphate (PMS) and 75 µM NBT in phosphate buffer (pH = 7.8). The concentration of tested compounds varied from 0.25 to 3.0 µM. The amount of reduced NBT was spectrophotometrically detected by monitoring the concentration of the blue formazan form, which

Table 1. IR spectra data (cm^{-1})

Compounds	ν(C═O) pyridone	$\nu(COO)_{as}$	$\nu(COO)_s$	$\Delta\nu$	ν(M–N)	ν(M–O)
Ciprofloxacin	1708	1624	1340	284		–
Complex 1	1616	1579	1374	205	535	512
Complex 2	1615	1577	1381	196	531	505
Complex 3	1622	1584	1382	198	543	510
Complex 4	1624	1585	1382	198	538	517
Complex 5	1618	1581	1375	206	545	511

absorbs at 560 nm. The reduction rate of NBT was measured in the presence and absence of test compounds at various concentrations 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 represent 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. Concentration of the complex which causes 50% inhibition in the reduction rate of NBT is reported as the IC_{50} value of the complexes, determined by plotting the graph of percentage inhibition of NBT reduction against increase in concentration of the complex.

Results and Discussion

Reflectance Spectra and Magnetism

The diffuse reflectance spectra of Cu(II)–ciprofloxacin complexes consisted of one asymmetric broad band around 15 000 cm^{-1}. These spectra suggest an octahedral geometry of the ligands donor atoms around the cupric ion.[18]

The magnetic moments of the copper(II) complexes lie in the 1.84–1.91 B.M. 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 owing to the spin–orbit coupling contribution.[19]

IR Spectra

The IR spectra of ligands are quite complex owing to the presence of numerous functional groups in the molecules. The characterization of metal complexes can be achieved by studying the most typical vibrations that are characteristic of the coordination sites of ligands. The determination of the coordinating atoms was made on the basis of the comparison of the IR spectra of the ligands and complexes Significant wave numbers are given in Table 1.

(1) The ν(C═O) stretching vibration band appeared at 1708 cm^{-1} for ciprofloxacin, whereas for complexes it appeared at 1615–1624 cm^{-1}. This shift towards lower energy suggests that coordination occurs through carbonyl oxygen of pyridine ring.

(2) Strong absorption band at 1624 and 1340 cm^{-1} in ciprofloxacin could be assigned to ν(COO) asymmetric and symmetric vibration, respectively, while in metal complexes these bands were observed at 1577–1585 and 1374–1382 cm^{-1}. The difference $\Delta\nu = \nu_{as}(COO) - \nu_s(COO)$ is useful for determining the coordination mode of ligands. The $\Delta\nu$ values were greater than 200 cm^{-1}, indicating the monodentate coordination mode of carboxylato group.[20–24] These data are further supported by ν(M–O) which appears at 505–517 cm^{-1} for complexes.

(3) In investigated complexes the ν(C═N) band of terpyridines appeared at ~1584 cm^{-1}. This band shifted to a higher frequency at ~1626 cm^{-1}[25,26] in complexes, indicating tridentate N–N coordination of the ligand. The N → M bonding was supported by the ν(M–N) band[27] at ~531–545 cm^{-1} for complexes.

Mass Spectra

Figure 1 represents the FAB – mass spectrum of complex **I**, that is [Cu(cpf)(A^1)Cl], obtained using *m*-nitro benzyl alcohol as matrix.

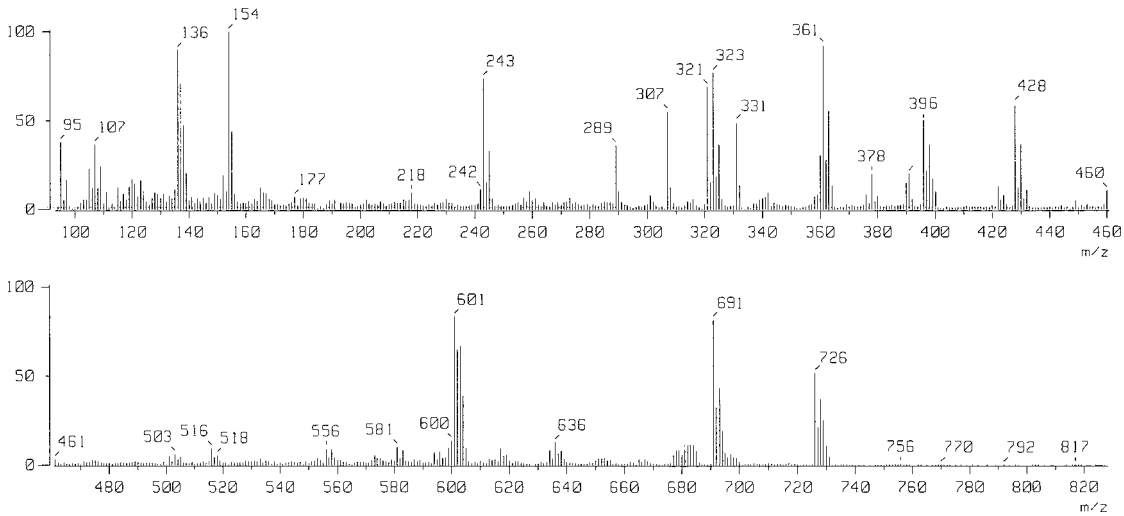


Figure 1. FAB-mass spectrum of complex 1, that is [Cu(cpf)(A^1)Cl], obtained using *m*-nitro benzyl alcohol.

Table 2. Minimum inhibitory concentration 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
Ligand 1 (A^1)	550	500	550	525	550
Ligand 2(A^2)	575	575	575	550	550
Ligand 3 (A^3)	700	750	750	725	750
Ligand 4 (A^4)	550	575	650	650	650
Ligand 5 (A^5)	650	650	750	725	750
Ligand 1 + ciprofloxacin	2.8	2.3	2.7	2.7	2.7
Ligand 2 + ciprofloxacin	2.9	2.4	2.9	2.7	2.8
Ligand 3 + ciprofloxacin	3.5	2.9	3.3	3.2	3.3
Ligand 4 + ciprofloxacin	2.9	2.4	3.2	3.1	3.1
Ligand 5 + ciprofloxacin	3.3	2.9	3.3	3.0	3.3
[Cu(cpf)(A^1)Cl] (1)	1.1	0.9	0.4	0.4	1.2
[Cu(cpf)(A^2)Cl] (2)	0.7	0.9	1.8	0.9	1.5
[Cu(cpf)(A^3)Cl] (3)	1.2	1.1	1.7	1.4	1.1
[Cu(cpf)(A^4)Cl] (4)	0.8	1.0	0.6	1.1	1.0
[Cu(cpf)(A^5)Cl] (5)	1.2	0.7	1.7	1.2	1.0

Peaks at 136, 137, 154, 289 and 307 *m/z* value were due to the usage of matrix. Peaks at 726 and 728 in spectra were assigned to (M) and (M + 2) of the complex molecule associated with four H^+ ions in the absence of lattice water. There also existed a doublet at $m/z = 564$ for a fragment of the complex ($m/z = 428$) rid of the neutral ligand associated with the matrix ($m/z = 136$). The doublet nature observed in case of the fragment suggests the presence of one Cl atom. Loss of the chlorine atom gave a fragment ion peak at $m/z = 691$, which also confirms that the chlorine atom attached to the metal ion with a covalent bond. Several other fragments at 601, 396, 361, 331 and 323 *m/z* value were observed. The isotopic pattern owing to the presence of chlorine and copper appeared in the spectrum.

In-vitro Antibacterial Activity

The efficiency of the ligands and the complexes was tested against three Gram-negative (*E. coli*, *S. marcescens* and *P. aeruginosa*) and two Gram-positive (*S. aureus* and *B. subtilis*) microorganisms. The results of the minimum inhibitory concentration (MIC), expressed in micromoles, are presented in Table 2.

In case of *S. aureus*, all compounds showed good activity compared with reference drugs. In case of *B. subtilis*, all compounds exhibited good activity compare to reference drugs. In case of *S. marcescens*, compounds **I** and **IV** were found to be more potent than the reference drugs. In the case of *P. aeruginosa*, compounds **I, II, IV** and **V** exhibited good activity compared with the reference drugs. In the case of *E. coli*, compounds **I** and **III**–**V** showed more potency than the reference drugs. The complexes showed better antimicrobial activity than the free ligands and ciprofloxacin. The higher antimicrobial activity can be mainly attributed to the existence of the quinolone in the complexes. The antibacterial activity of all the complexes is even higher than the antibacterial activity of complexes, which were previously reported by our group.[28]

It has also been suggested[29,30] that the ligands with nitrogen donor systems might inhibit enzyme production, since the enzymes that require these groups for their activity appear to be especially susceptible to deactivation by the metal ions upon chelation. Chelation reduces the polarity[29,30] 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 increases the lipophilic nature of the central metal atom, which in turn favours its permeation through the lipoid layer of the membrane, increasing the hydrophobic character and liposolubility of the molecule in crossing the cell membrane of the microorganism, and hence enhances the biological utilization ratio and activity of the testing drugs/compounds.

Electronic Absorption Titration

DNA can provide three distinctive binding sites for quinolone-metal complexes, namely groove, phosphate group and intercalation.[31,32] This behavior is of great importance with regard to the relevant biological role of quinolone antibiotics in the body.[33–35]

Electronic absorption spectra are universally employed to determine the binding of complexes with DNA. Complex bound to DNA through intercalation usually results in hypochromism and red shift (bathchromism). The extent of the hypochromism is commonly consistent with the strength of intercalative interaction.[36]

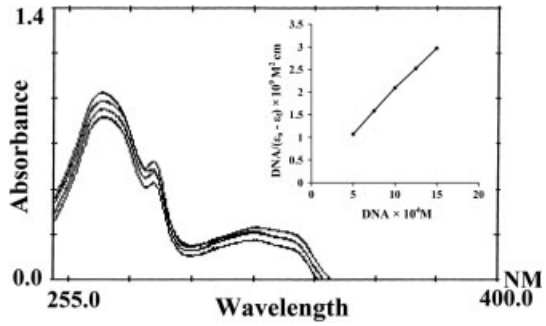


Figure 2. Electronic absorption titration curve of [Cu(cpf)(A[1])Cl] in absence and in presence of increasing amount of DNA; 50–150 μM in phosphate buffer in 5 mM Tris-HCl buffer (pH 7.2), [complex] = 15 μM, with incubation period of 30 min. at 37 °C, Inset: Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA].

The concentration of CT DNA was determined from the absorption intensity at 260 nm with an ε value of 6600 M^{-1} cm^{-1}. From Fig. 2 absorption titration experiments were made using different concentrations of CT DNA, while keeping the complex concentration constant. Due correction was made for the absorbance of the CT DNA itself. Samples were equilibrated before recording each spectrum. For complexes, the intrinsic binding constant, K_b was been determined from spectral titration data using following equation:[37]

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

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.

The binding constants obtained for complexes **I**–**V** were 3.33×10^4, 3.12×10^4, 2.33×10^4, 2.71×10^4 and 2.54×10^4 M^{-1}, respectively. The K_b values of complexes were similar to that of $[Ru(bpy)_2ppd]^{2+}$ (2.18×10^4 M^{-1}) complex.[38] The K_b value of **I** and **II** is higher than that for most of the previously reported complexes of type [Cu(cpf)(A^n)Cl].[28] These spectral characteristics are consistent with a mode of interaction that involves a stacking interaction between the complex and the base pairs of DNA, which means that the complexes can intercalate into the double helix structure of DNA.

Viscosity Measurement

The binding mode of complexes to DNA was explained by viscosity measurement. Hydrodynamic measurements, which are sensitive to the increase in length of DNA, are regarded as the least ambiguous and the most critical tests in absence of crystallographic structure data.[39,40] A classical intercalation leads to lengthening of DNA helix because of the separation of base pairs to accommodate the ligand, and thus the viscosity of DNA increases while a partial nonclassical intercalation could bend the DNA helix and thus reduce its effective length along with its viscosity.[41]

The viscosity of DNA is increased with increasing amounts of complexes **I**–**V** (Fig. 3). Upon increasing the amounts of complexes, the relative viscosity of DNA increases steadily, less than the classical intercalator EB. The increased degree of viscosity, which may depend on the affinity for DNA, follows the order EB > **I** > **II** > **IV** > **V** > **III** > ciprofloxacin. The viscosity results show that all complexes intercalate through classical intercalation mode.

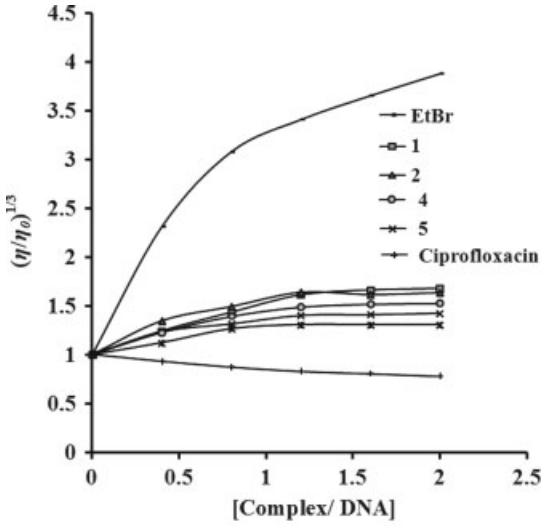


Figure 3. Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27 ± 0.1 °C in 5 mM Tris-HCl buffer (pH 7.2) as a medium.

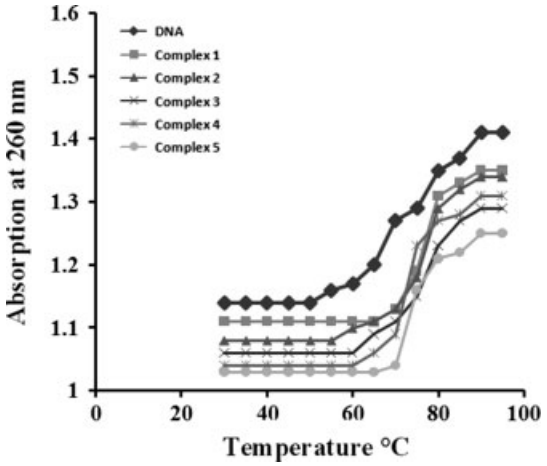


Figure 4. Melting curves of CT DNA in the absence and presence of complexes 1–5.

DNA Melting Experiment

The melting temperature (T_m) of double-stranded DNA changes with different binding modes. Generally, the melting temperature increases when metal complexes bind to DNA by intercalation, as intercalation of the complexes into DNA base pairs causes stabilization of base stacking and hence raises the melting temperature of the double-stranded DNA. DNA melting experiments are useful in establishing the extent of intercalation.[42] A large change in the T_m of DNA is indicative of a strong interaction with DNA. The effect of complexes on the melting temperature of CT DNA in buffer is shown in Fig. 4. At the melting temperature, the double helix denatures into single-stranded DNA. The thermal denaturation experiment carried out for DNA in the absence of complex gave a T_m74.2 ± 1 °C under our experimental conditions. The observed melting temperatures in the presence of complex were 79.0 ± 1 °C, 78.5 ± 1 °C and 78.4 ± 1 °C for complexes **I, II** and **IV**, respectively, and for complexes **III** and **V** the T_m increased to 77.0 ± 1 and 78.2 ± 1 °C respectively. The ΔT_m (3.8–4.6 °C) values of the DNA increased in the presence of the complexes and were comparable

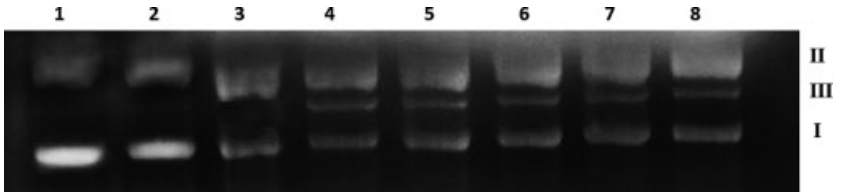


Figure 5. Photogenic view of interaction of pUC19 DNA (450 μg/mL) with series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μL, for 3 h. at 37 °C.: Lane 1, DNA control; Lane 2, $CuCl_2 \cdot 2H_2O$; 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].

Table 3. Gel electrophoresis data

Compounds	% SC	% OC	% L	% DNA cleavage
DNA control	76	24	–	–
DNA + metal salt	71	29	–	6.57
DNA + ciprofloxacin	65	35	–	14.47
DNA + I	21	47	32	72.36
DNA + II	23	43	34	69.73
DNA + III	28	45	27	63.15
DNA + IV	24	43	34	68.4
DNA + V	27	45	28	64.47

to those observed for classical intercalator,[43] suggesting the large DNA-binding affinity of the complexes.

Gel Electrophoresis

DNA cleavage accelerated by transition metal complexes is the centre of interest.[44,45] The cleavage of plasmid pUC19 DNA was monitored by gel electrophoresis to investigate the ability of the copper(II) complexes to serve as metallonucleases. The naturally occurring supercoiled form (form I), when nicked, gives rise to an open circular relaxed form (form II) and further cleaves to a linear form (form III). From Fig. 5, when subjected to gel electrophoresis, form I shows the fastest migration compared with forms II and III. Form II migrates very slowly prior to its relaxed structure, whereas form III migrates somewhere between the positions of form I and form II. These clearly show that the relative binding efficacy of complexes to DNA is much higher than the binding efficacy of metal salt or ciprofloxacin (Table 3). The difference in DNA–cleavage efficiency of complexes was due to the difference in binding affinity of complexes to DNA.

Superoxide Dismutase Activity

The SOD activity of the complexes was investigated by NBT assay. Several complexes containing transition metals,[46] including copper, are known to give good SOD activity, although their structures are totally unrelated to the native enzyme.[47] Herein, the SOD activity was measured at pH 7.8. The concentration required to yield 50% inhibition of the reduction rate of NBT (IC_{50}) was higher. The observed higher values may be due to the presence of α-triimine ligands (terpyridine) and greater ligand field crowding over the central metal ion.

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 to a straight line (Fig. 6). As the concentration of tested complexes increased,

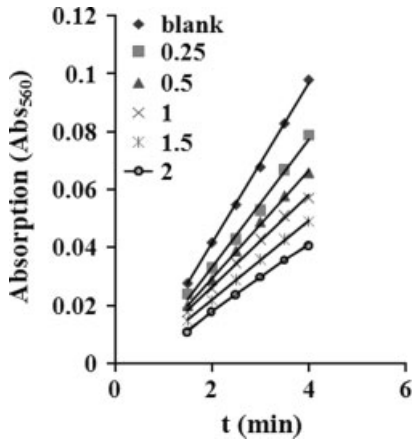


Figure 6. plot of absorbance values (Abs_{560}) against time (t).

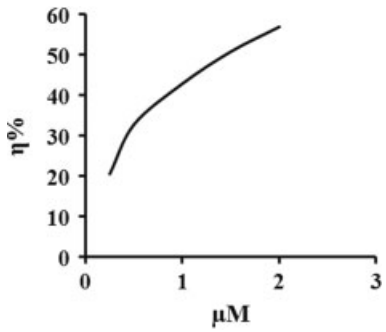


Figure 7. Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex 1.

Table 4. IC_{50} values of copper(II) complexes

Complexes	IC_{50} (μM)
[Cu(cpf)(A^1)Cl] (1)	0.5
[Cu(cpf)(A^2)Cl] (2)	0.75
[Cu(cpf)(A^3)Cl] (3)	1.25
[Cu(cpf)(A^4)Cl] (4)	1.0
[Cu(cpf)(A^5)Cl] (5)	1.05

the slope (m) decreased. Percentage inhibition of the reduction of NBT was plotted against the concentration of the complex (Fig. 7). Compounds exhibited SOD-like activity at biological pH with the IC_{50} values in the range 0.5–1.25 μM, which is very similar for the complexes of same type reported previously by our group.[28] The superoxide scavenging data are shown in Table 4.

The results show that complexes exhibit greater scavenging activity towards superoxide radicals, which may be accredited to the redox potential of Cu(II) complex, which depends on the geometry at the metal centre. The mechanism for scavenging of ROS may move forward via an unstable octahedral adduct under influence of the Jahn–Teller effect.[48] Hence, there is a possibility of rapid interconversion of Cu(II) and Cu(I) via electron transfer between copper and reactive oxygen radical anions following the principle of electroneutrality.[49,50]

Conclusion

The binding behaviours of the copper complexes of terpyridines and ciprofloxacin were found to be subtly but distinctly different, depending on the structure of terpyridines. The increasing order for the interacting behaviour of synthesized complexes was **III** < **V** < **IV** < **II** < **I** on the bases of K_b values, change in relative viscosity and plasmid cleavage study. The ligand can facilitate the stabilization of bonding between metal centre and the oxygen radical anion, favouring the enhancement of enzymatic behaviour.

Acknowledgements

The authors thank the Head of the Department of Chemistry, Sardar Patel University, India for making it convenient to work in the laboratory and UGC for providing financial support under ''UGC Research Fellowship for Meritorious Students'' scheme.

Supporting information

Supporting information may be found in the online version of this article.

References

[1] D. C. Crans, J. J. Smee, E. Gaidamauskas, L. Yang, *Chem. Rev.* **2004**, *104*, 849.
[2] B. H. Geierstanger, M. Marksich, P. B. Dervan, D. E. Wemmer, *Science* **1994**, *266*, 646.
[3] C. Liu, J. Zhou, Q. Li, L. Wang, Z. Liao, H. Xu, *J. Inorg. Biochem.* **1999**, *75*, 233.
[4] G. Prativiel, J. Bernadou, B. Meunier, *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746.
[5] R. A. Sánchez-Delgado, A. Anzelloti, *Mini Rev. Med. Chem.* **2004**, *4*, 23.
[6] D. S. Sigman, A. Mazumder, M. D. Perrin, *Chem. Rev.* **1993**, *93*, 2295.
[7] A. I. Darkopoulos, P. C. Ioannou, *Anal. Chem.* **1997**, *354*, 197.
[8] A. L. Abuhijleh, *J. Inorg. Biochem.* **1997**, *68*, 167.
[9] J. C. Casanova, G. Alzuci, J. Borras, J. Latorre, M. Sanau, S. G. Granda, *J. Inorg. Biochem.* **1995**, *60*, 219.
[10] A. I. Vogel, *Textbook of Quantitative Inorganic Analysis*, 4th edn, ELBS and Longman: London, **1978**.
[11] G. S. Hanan, J. Wang, *Synlett.* **2005**, 1251.
[12] D. Sinha, A. K. Tiwari, S. Singh, G. Shukla, P. Mishra, H. Chandra, A. K. Mishra, *Eur. J. Med. Chem.* **2008**, *43*, 160.
[13] G. Cohen, H. Eisenberg, *Biopolymers* **1969**, *8*, 45.
[14] R. Rao, A. K. Patra, P. R. Chetana, *Polyhedron* **2008**, *27*, 1343.
[15] J. Sambrook, D. W. Russell, *Preparation of Plasmid DNA by Alkaline Lysis with SDS: Minipreparation, Molecular Cloning, A Laboratory Manual*, 3rd edn, Vol. 1, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY.
[16] J. Yang, R. N. S. Wong, M. S. Yang, *Chem.-Biol. Interact.* **2000**, *125*, 221.
[17] V. Ponti, M. V. Dianzaini, K. J. Cheesoman, T. F. Stater, *Chem.-Biol. Interact.* **1978**, *23*, 281.
[18] B. N. Figgis, J. Lewis, in *Modern Coordination Chemistry: Principles and Methods* (Eds.: J. Lewis, R. G. Wilkins), Interscience: New York, **1960**, p. 400.
[19] F. A. Cotton, G. Wilkinson *Advanced Inorganic Chemistry*, 5th edn, Wiley: New York, **1988**.
[20] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 4th edn, Wiley Interscience: New York, **1986**.
[21] J. R. Anacona, I. Rodriguez, *J. Coord. Chem.* **2004**, *57*, 1263.
[22] G. B. Deacon, R. Philips, *J. Coord. Chem. Rev.* **1980**, *33*, 227.
[23] Z. H. Chohan, C. T. Suparan, A. Scozzafava, *J. Enz. Inhib. Med. Chem.* **2005**, *23*, 303.
[24] N. H. Patel, P. K. Panchal, P. B. Pansuriya, M. N. Patel, *J. Macro. Sci.: Part A Pure Appl. Chem.* **2006**, *43*, 1083.
[25] P. B. Pansuriya, P. Dhnadhukia, V. Thakkar, M. N. Patel, *J. Enz. Inhib. Med. Chem.* **2007**, *22*, 477.
[26] P. K. Panchal, M. N. Patel, *Synth. React. Inorg. Met.-Chem.* **2004**, *34*, 1277.
[27] H. H. Freedman, *J. Am. Chem. Soc.* **1961**, *83*, 2900.
[28] M. N. Patel, P. A. Dosi, B. S. Bhatt, *Polyhedron* **2010**, *29*, 3238–3245.
[29] Z. H. Chohan, *App. Organomet. Chem.* **2006**, *20*, 112.
[30] Z. H. Chohan, M. Arif, Z. Shafiq, M. Yaqub, C. T. Supuran, *J. Enz. Inhib. Med. Chem.* **2006**, *21*, 95.
[31] A. Rodger, B. Norden (Eds.), *Circular Dichroism and Linear Dichroism*, Oxford University Press: Oxford, **1997**.
[32] B. M. Zeglis, V. C. Pierre, J. K. Barton, *Chem. Commun.* **2007**, 4565.
[33] G. Song, Y. He, Z. Cai, *J. Fluoresc.* **2004**, *14*, 705.
[34] P. Drevensek, I. Turel, N. P. Ulrih, *J. Inorg. Biochem.* **2003**, *96*, 407.
[35] G. S. Son, J.-A. Yeo, M.-S. Kim, S. K. Kim, A. Holmen, B. Akerman, B. Norden, *J. Am. Chem. Soc.* **1998**, *120*, 6451.
[36] T. M. Kelly, A. B. Tossi, D. J. McConnell, T. C. Strekas, *Nucl. Acids Res.* **1985**, *13*, 6017.
[37] M. T. Carter, M. Rodriguez, A. Bard, *J. Am. Chem. Soc.* **1989**, *111*, 8901.
[38] B. Peng, H. Chao, B. Sun, H. Li, F. Gao, L. N. Ji, *J. Inorg. Biochem.* **2006**, *100*, 1487.
[39] J. K. Barton, A. T. Danishefsky, J. M. Goldberg, *J. Am. Chem. Soc.* **1984**, *106*, 2172.
[40] A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro, J. K. Barton, *J. Am. Chem. Soc.* **1989**, *111*, 3051.
[41] H. Deng, J. Li, K. C. Zheng, Y. Yang, H. Chao, L. N. Ji, *Inorg. Chim. Acta* **2005**, *358*, 3430.
[42] J. M. Kelly, A. B. Tossi, D. J. McConnell, C. Ohuigin, *Nucl. Acids Res.* **1985**, *13*, 6017.
[43] M. J. Han, L. H. Gao, Y. X. Lu K. Z. Wang, *J. Phys. Chem. B*, **2006**, *110*, 2364.
[44] R. P. Hertzberg, P. B. Dervan, *J. Am. Chem. Soc.* **1982**, *104*, 313.
[45] D. S. Sigman, D. R. Graham, L. E. Marshall, K. A. Reich, *J. Am. Chem. Soc.* **1980**, *102*, 5419.
[46] R. Rajan, R. Rajaram, B. U. Nair, T. Ramasami, S. K. Mandal, *J. Chem. Soc., Dalton Trans.* **1996**, 2019.
[47] U. Weser, L. M. Schubotz, E. Lengfelder, *J. Mol. Catal.* **1981**, *13*, 249.
[48] F. Cotton, G. Wilkinson, *Advanced Inorganic Chemistry*, Wiley: New York, **1972**.
[49] J. A. Fee, *Metallons in Biological Systems* (Ed.: H. Siegel), Vol. 13, Marcel Dekker: New York, **1981**.
[50] J. E. Huheey, *Inorganic Chemistry Principles of Structure and Reactivity*, 3rd edn, Harper international: London, **1983**.