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Mohan N. Patel

Chintan R. Patel

Hardik N. Joshi
Sheridan College, hardik.joshi@sheridancollege.ca

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Interaction of drug based copper(II) complexes with Herring Sperm DNA and their biological activities

Mohan N. Patel *, Chintan R. Patel, Hardik N. Joshi

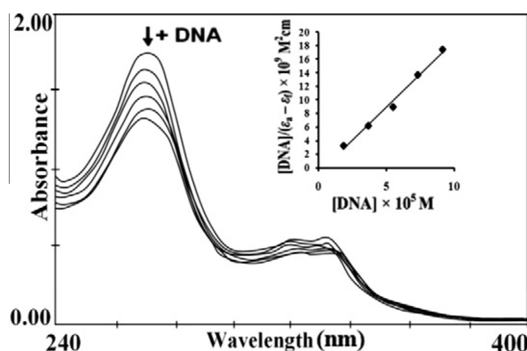
Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India

HIGHLIGHTS

- ▶ Synthesis of substituted NS donor ligand and their Cu(II) complexes.
- ▶ Characterization by elemental analysis, IR, UV–visible, LC–MS spectrometry.
- ▶ All Cu(II) complexes have tested for their biological studies.
- ▶ The interaction of DNA with complexes has been performed.

GRAPHICAL ABSTRACT

The DNA binding ability of the complexes with Herring Sperm DNA has been performed using absorption titration. With increase in DNA to complex ratio, hypochromism and red shift was observed. It suggests that complexes bind via interaction mode.



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ABSTRACT

Square pyramidal Cu(II) complexes with NS donor ligand and ciprofloxacin have been synthesized and characterized using analytical and spectral techniques. The synthesized complexes have been tested for their antimicrobial activity using double dilution technique in terms of minimum inhibitory concentration (MIC) and colony forming unit (CFU). The DNA binding ability of the complexes with Sperm Herring DNA has been performed using absorption titration and viscosity measurement. The nuclease activity of complexes with plasmid DNA (pUC19) has been carried out using agarose gel electrophoresis technique. Synthesized complexes have been tested for their SOD mimic activity using NBT/NADH/PMS system. The cytotoxic properties of metal complexes have been evaluated using brine shrimp lethality bioassay.

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Introduction

Quinolones are a large group of synthetic antibacterial agents used in a clinical practice for the treatment of variety of bacterial infections [1]. Ciprofloxacin is the drug of choice for treating victims infected by anthrax, urinary, skin and respiratory infections [2,3]. Quinolones are chelating agents for a variety of metal ions including alkaline earth and transition metal ions. Although reports indicate that the coordination of quinolones to metal ions such as Cu(II), Mg(II) and Ca(II) appear to be important for the activity of the quinolone antibiotics [4], it has a detrimental effect on their absorption [5]. Mixed ligand copper–quinolones complexes using different NN donor ligand have been synthesized

* Corresponding author. Tel.: +91 (2692) 226856x218.
E-mail address: jeenen@gmail.com (M.N. Patel).

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and explored for their biological activities [6–8]. The complexes of vanadium(IV), copper(II), magnesium(II), uranium(VI), manganese(II), iron(III), cobalt(II), nickel(II), molybdenum(II) and europium(III) with ciprofloxacin have been synthesized and explored for their biological activities, because of its biological relevance [9–16].

Metal-containing drugs have for long been of interest, but the interest of the scientific community for medicinal aspects of metal compounds has rapidly grown in the last few decades [17,18]. Complexation with the metal protects the drug against enzymatic degradations because of the inertness of certain metal–ligand linkages. The metal complex can have better hydrophobicity/hydrophilicity properties than the free ligand and, through this; it can improve the transport processes in the tissues. In addition, the metal complex can release the active drug(s) in a specific organ, and its activity can be reinforced by the combination of effects from the ligands and from the metal residue. The application of these principles has already resulted in the design of successful metal-based drugs [19,20].

A large number of mixed ligand copper(II) complexes have been shown to exhibit superoxide dismutase activity [21,22]. This activity depends on the Cu(II)/Cu(I) redox process, which is related to flexibility of the geometric transformation around the metal centers [23]. Complexation with copper enhances the biological activity of a wide variety of organic ligands [24,25].

In continuation of our previous work [26], herein we synthesized mixed ligand Cu(II) complexes with drug and neutral bidentate ligand. The *in vitro* antimicrobial activity of complexes was evaluated against two Gram(+ve) and three Gram(−ve) microorganisms. The synthesized complexes were checked for their DNA interactions using UV spectroscopy, viscosity measurement and gel electrophoresis. The SOD mimic behavior of the complexes was checked under non-enzymatic condition. The cytotoxic effects of the synthesized complexes were also checked using brine shrimp lethality bioassay.

Experiments

Material and reagents

All solvents, chemicals and reagents used were of analytical reagent grade and were used as such; double distilled water was used throughout the experiments. Ciprofloxacin (CFLH) was purchased from Bayer AG (Wuppertal, Germany). Cupric chloride was purchased from E. Merck (India) Ltd., Mumbai. 4-Fluoro benzaldehyde, 4-chloro benzaldehyde, 4-bromo benzaldehyde, 4-methyl benzaldehyde, 3-bromo benzaldehyde, 3-chlorobenzaldehyde and 2-acetyl thiophene were purchased Spectrochem Pvt. Ltd., Mumbai (India). Luria Broth, agarose, ethidium bromide, TAE (Tris-Acetyl-EDTA), bromophenol blue and xylene cyanol FF were purchased from Himedia (India). Nicotinamide adenine dinucleotide reduced (NADH), Nitroblue tetrazolium (NBT) and phenazin methosulphate (PMS) were purchased from Loba Chemie Pvt. Ltd. Herring Sperm DNA was purchased from Sigma Chemical Co. (India).

Physical measurements

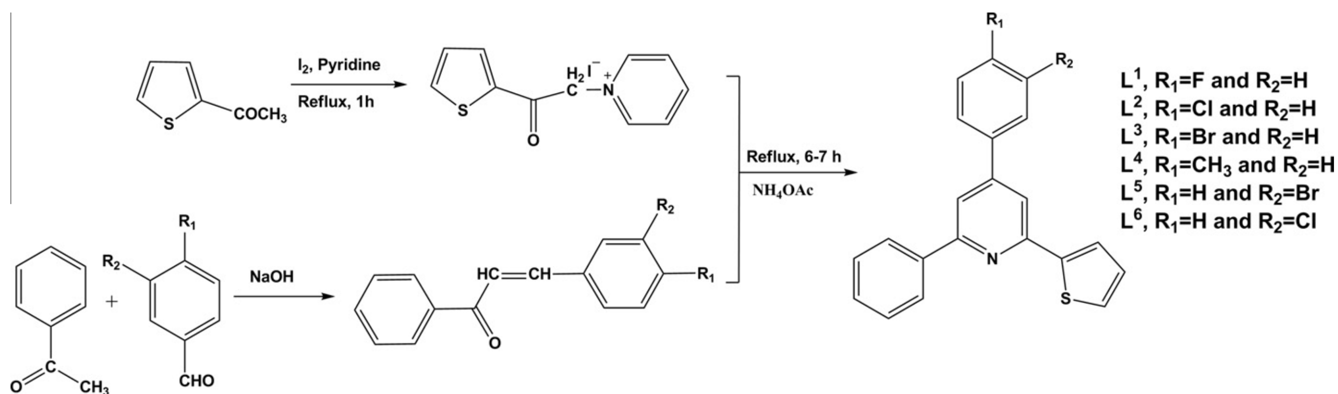
Elemental analyses (C, H and N) of the synthesized complexes were performed with a model 240 Perkin Elmer elemental analyzer, Massachusetts (USA). Metallic content of the complex was determined after decomposing it under effect of acid mixture and titrating against EDTA solution volumetrically. Room temperature magnetic measurement for the complexes was made using Gouy magnetic balance. The Gouy tube was calibrated using mercury(II) tetrathiocyanatocobaltate(II) as the calibrant (χ_g = 16.44 × 10^{-6} cgs units at 20 °C), Mumbai (India). The electronic spectra were recorded on a UV-160A UV–visible spectrophotometer, Shimadzu, Kyoto (Japan). Infrared spectra were recorded on a FT-IR ABB Bomen MB-3000 spectrophotometer (Canada) as KBr pellets in the range 4000–400 cm^{-1}. MIC study was carried out by means of laminar air flow cabinet, Toshiba, Delhi (India). The LC–MS spectra were recorded using Thermo mass spectrophotometer (USA). Photo quantization of the gel after electrophoresis was done using AlphaDigiDoc™ RT. Version V.4.0.0 PC-Image software, California (USA).

General synthesis of ligands

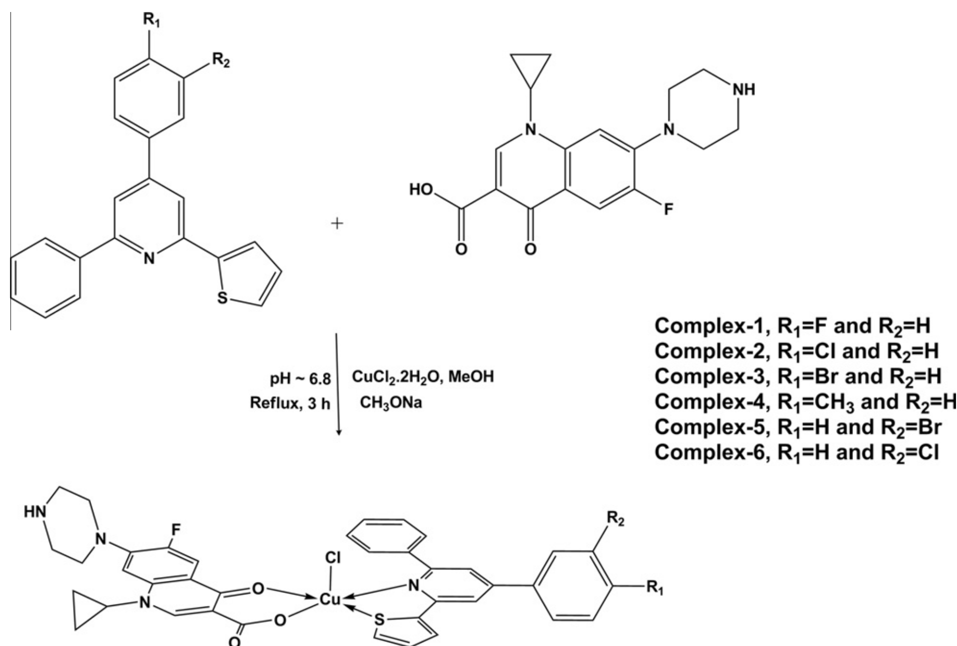
Ligands were prepared by modified Kronke pyridine synthesis [27] by using halo pyridinium salt of 2-acetyl thiophene, ammonium acetate and different chalcone in methanol. The mixture was reflux for 6–7 h on sand bath. The product was obtained by keeping the solution in ice bath. The product was purified by crystallization in *n*-hexane. Ligands were characterized by ^{1}H and ^{13}C NMR spectroscopy (Supplementary material 1: Physicochemical parameters of ligands L^1–L^6). The proposed structure of ligands is shown in Scheme 1.

General synthesis of complex

Methanolic solution of $CuCl_2{\cdot}2H_2O$ (1 mmol) was added to a methanolic solution of neutral bidentate ligand (L^n) (1 mmol), followed by addition of previously prepared methanolic solution of ciprofloxacin in presence of CH_3ONa (1 mmol). The pH of reaction mixture was adjusted at ~6.8 using dilute solution of CH_3ONa. The resulting solution was refluxed for 3 h on a water bath, followed by concentrating it to half of its volume. A fine amorphous product



Scheme 1. General synthesis of ligands and proposed structure.



Scheme 2. Proposed structure and general synthesis of complexes.

obtained was washed with chloroform and dried in vacuum desiccators. The proposed structure of complexes is shown in Scheme 2.

Biological evaluation of synthesized complexes

In vitro antimicrobial activity

In the present study, serial tube dilution technique was employed [28]. The tests were performed in triplicate and activities of the compounds were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Stapylococcus aureus*, *Bacillus subtilis* and *Serratia marcescens*. The former three are Gram(−ve) while later two are Gram(+ve) organism. Selection of *bacteria* is of choice in preliminary screening test organism for several reasons. They are systemic pathogens and seem to develop antibiotic resistance more readily than any other bacteria and laboratory animals can be readily infected with it. The inhibition of growth of this organism produced by various concentrations of the test compounds was compared under identical conditions with inhibition of growth of the same organism in presence of several fluoroquinolone drugs, metal salt, and ligands.

A standard volume (10 mL) of Luria Broth that would support the growth of the test organism was added to several labeled sterile stopper identical assay tubes. Solution of each test compounds in a series of dilution was prepared. Dilution for metal salts, ligand and standard drugs were also prepared and a control tube containing no test compound was also included. All these operations were carefully performed under aseptic conditions. Assay tubes were incubated at 37 ± 1 °C for 24 h. The resultant faint turbidity was measured. The minimum inhibitory concentration (MIC) of a test compound is the lowest concentration showing no visible turbidity.

In addition to MIC, the bactericidal action of all compounds was evaluated against two Gram(+ve) and three Gram(-ve) bacteria in terms of colony forming unit (CFU). For CFU, inoculum of 10^6 viable bacteria/mL was prepared by diluting an overnight culture grown in Luria Broth. Bacteria were exposed to various concentrations 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 30–300 colonies.

DNA binding study by absorption titration

Binding of DNA via intercalation mode usually results in hypochromism and bathochromism [29–33], because intercalation mode involves a strong stacking interaction between an aromatic chromophore and the DNA base pair [34]. With a selection of an appropriate absorbance peak by performing spectrophotometric wavelength scans of each Cu(II) complexes. After addition of equivalent amount of DNA to reference cell, kept for 10 min incubation at room temperature followed by absorption measurement. This was specifically done to enable direct comparison between the assays that was required to interpret the results obtained. The intrinsic binding constant, K_b was determined by making it a subject in following equation [35]

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

where, [DNA] is the concentration of DNA in base pairs, εa the apparent extinction coefficient is obtained by calculating $A_{obs.}$/[complex], ε_f corresponds to the extinction coefficient of the complex in its free form and εb refers to the extinction coefficient of the complex in the fully bound form. When each set of data, fitted to the above equation, gave a straight line with a slope of 1/(εb − εf) and *y*-intercept of 1/*K*b(εb − εf). The K_b value was determined from the ratio of the slope to intercept.

Hydrodynamic volume measurement

Hydrodynamic volume change was measured using Ubbelohde viscometer immersed in a thermostatic bath maintained at 37 ± 0.1 °C. Flow times were measured with a digital stopwatch, each sample was measured three times, and an average flow time was calculated. Mixing of test compound and Hearing Sperm DNA were done by bubbling air to it. Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta = (t - t_0)$ [36].

DNA cleavage study

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out in TAE buffer (0.04 M Tris–Acetate, pH 8, 0.001 M EDTA). Fifteen microliters of reaction mixture containing plasmid DNA (150 μg/mL) in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and 200 μM complex were allowed to proceed for 24 h at 37 °C. All the reactions were quenched by addition of 5 μL loading buffer (40% sucrose, 0.25% bromophenol blue, 0.25% xylene cyanole FF, 200 mM EDTA). The aliquots were loaded directly onto 1% agarose gel and electrophoresed at 50 V in 1× TAE buffer. Gel was stained with 0.5 μg/mL ethidium bromide and was photographed on a UV trans-illuminator. The percentage of each form of DNA was quantified based on illumination using AlphaDigiDoc™ RT. The degree of DNA cleavage activity was expressed in terms of the percentage of cleavage of the SC-DNA according to the following equation [37]

$$\text{Percent of DNA cleavage} = (\text{Percent of SC-DNA})_{\text{control}} - (\text{Percent of SC-DNA})_{\text{sample}} / (\text{Percent of SC-DNA})_{\text{control}} \times 100$$

Enzymatic behavior

NBT/NADH/PMS system was used to study SOD-like behavior of the complexes. The superoxide radial produced by 79 μM NADH, 30 μM PMS in phosphate buffer (pH = 7.8) was responsible for 75 μM NBT in system, and 0.25–3.0 μM tested compound are responsible for retardation in the reduction rate of NBT. Retardation in the reduction rate of NBT was determined spectrophotometrically by monitoring the concentration of blue formazan form which absorbs at 560 nm. All measurements were carried out at room temperature. The percent inhibition (η) of NBT reduction was calculated using following equation [38],

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

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

Cytotoxic activity – Brine shrimp lethality bioassay

Brine shrimp (*Artemia cysts*) lethality bioassay technique was applied for the determination of general toxic property of complexes. The *in vitro* lethality test has been carried out using brine shrimp eggs i.e. *Artemia cysts*. Brine shrimp eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After 2 days nauplii were collected by a pipette from the lighter side. A stock solution of the test complex was prepared in DMSO. From this stock solution, solutions were transferred to the vials to make final concentration 2, 4, 6, 8, 10, 12 μM, etc. (three for each dilutions were used for each test sample and LC_{50} is the mean of three values) and three vial was kept as control having of DMSO only. After 2 days, when the of nauplii were ready, 1 mL of seawater and 10 of nauplii were added to each vial and the volume was adjusted with seawater to 2.5 mL per vial [39]. After 24 h each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. Data were analyzed by simple logit method to determine the LC_{50} values, in which log of concentration of samples were plotted against percent of mortality of nauplii [40].

Result and discussion

Characterization of complexes

FT-IR, UV–visible spectroscopy, magnetic measurement and LC–MS techniques were used to evaluate the structure of the complexes. Physico-chemical parameters and microanalysis data (Supplementary material 2: Physico-chemical parameters and microanalysis data of complexes) are in good agreement with proposed structure.

Spectrophotometric titration

The amount of copper was determined by spectrophotometric titration technique [41,42]. The calculated results from the equivalent endpoint (Fig. 1, Supplementary material 3: Spectrophotometric titration data for the complexes) reveal the metallic content of the complex 1 (Supplementary material 4: Spectrophotometric titration curves for equivalent endpoint determination of the complexes).

IR spectroscopy

Major changes in IR spectra of ligands on complexation are comprised in Table 1 and some important points are as follows:

- Bands at 1780 and 1624 cm^{-1} in case of ciprofloxacin corresponds to $\nu(C{=}O)_{carb}$ and $\nu(C{=}O)_p$, respectively, which on complexation with metal ion shift to about 1580 and 1350 cm^{-1}, respectively [43].
- Unidentate nature for the carboxylato group of ciprofloxacin is proved by frequency separation of about 220 cm^{-1} ($\Delta\nu{=}COO_{as}{-}\nu COO_s$) [44].
- Deprotonation of the hydroxyl group of ciprofloxacin is confirmed by deduction of band at 3519 cm^{-1} from spectra due to hydrogen bonding [43].
- The band at 1624 cm^{-1} responsible for $\nu(C{=}O)_p$ in ciprofloxacin is observed between 1622 and 1632 cm^{-1} in case of complexes [12], these data are further supported by ν(M–O) appears at ~570–540 cm^{-1} [45], ν(M–N) appears at ~490–530 cm^{-1} and ν(M–S) appears at ~440–470 cm^{-1} [46].

Electronic and magnetic behavior

Copper(II) complexes, i.e. d^9 system with a simple ligand at low temperature exhibit an absorption band with a large width make them very difficult to interpret. Complexes of copper(II) with dif-

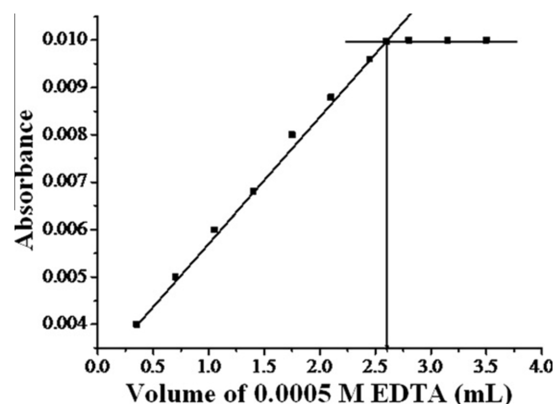


Fig. 1. The spectrophotometric titration curves for equivalent endpoint determination of the complex 1.

Table 1
Characteristic absorptions bands of IR spectra of the complexes and CPFH (cm^{-1}).

Compound	ν(C=O) pyridone	$\nu(COO)_{as}$	$\nu(COO)_s$	$\Delta\nu$	ν(M–O)	ν(M–N)	ν(M–S)
CPFH	1624	1708[a]	–	–	–	–	–
[Cu(CPF)L^1Cl] (1)	1625	1575	1352	223	565	510	455
[Cu(CPF)L^2Cl] (2)	1629	1574	1349	225	570	505	465
[Cu(CPF)L^3Cl] (3)	1627	1578	1358	220	540	525	440
[Cu(CPF)L^4Cl] (4)	1632	1577	1354	223	560	520	485
[Cu(CPF)L^5Cl] (5)	1622	1580	1355	228	575	490	470
[Cu(CPF)L^6Cl] (6)	1630	1576	1355	221	545	515	450

[a] As ν(COOH).

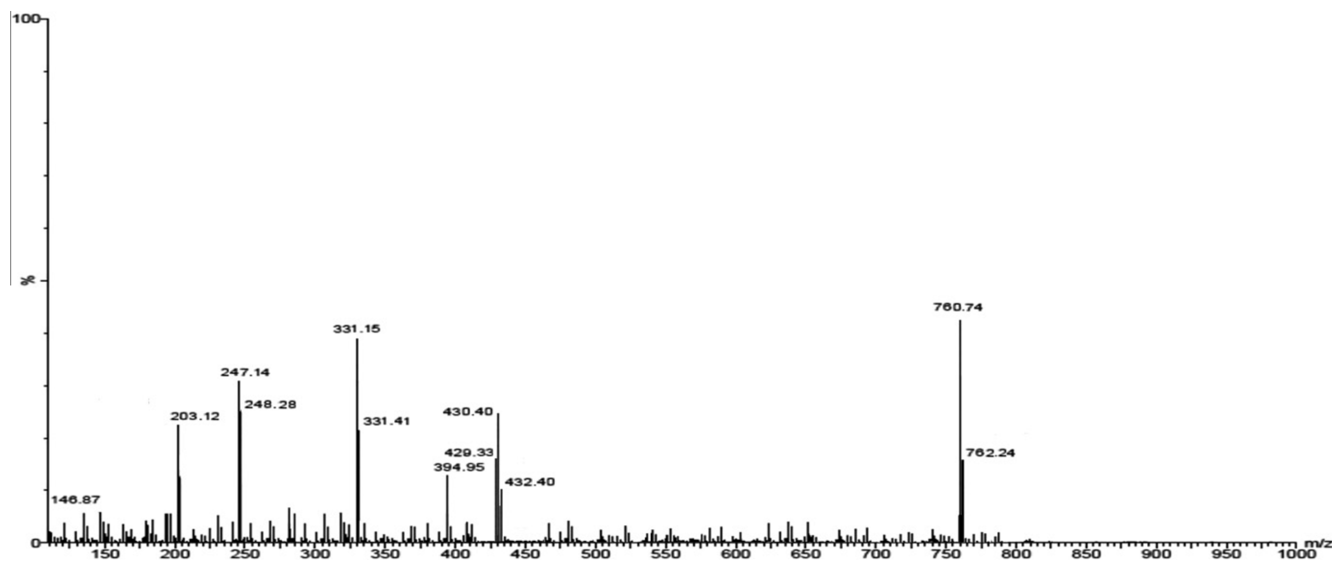


Fig. 2. LC–MS spectrum of complex [Cu(CPF)L^1Cl].

ferent coordination numbers resulting in different geometry. The copper(II) complexes exhibit a broad band at ~15,300 cm^{-1} [47,48] corresponding to a characteristic d–d transition in tetragonal field, suggesting distorted square pyramidal geometry for copper(II) complexes.

The magnetic moments measurement for any geometry in copper(II) complexes generally results in 1.8 BM, which is very close to spin-only value, i.e. 1.73 BM. The observed values in our case are very close to the spin-only values (Supplementary material 2: Physico-chemical parameters and microanalysis data of complexes) expected for $S = ½$ system (1.73 BM) which lead to a path of conclusion that metal center in synthesized complexes posses five coordination number with one unpaired electron responsible for $S = ½$ system [49,50].

LC–MS spectra analysis

LC–MS spectrum of complex [Cu(CPF)(L^1)Cl] is shown in Fig. 2. Mass spectrum of the complex show molecular ion [M^+] at 760.74 *m/z* and [M+2] at 762.24 *m/z*. Peaks at 430.40 and 432.40 *m/z* are due to ligand attached with copper and one chlorine atom. The peak at 429.33 and 431.33 *m/z* corresponds to ciprofloxacin attached to copper(II) including coordinated chlorine atom. The peak at 394.95 *m/z* is due to removal of chlorine atom from neutral bidentate ligand. The peak at 331.41 *m/z* is due to the loss of copper from neutral bidentate ligand. The peak at 331.15 *m/z* corresponds to ciprofloxacin moiety. The peak at 248.28 *m/z* is due to fragment of neutral bidentate ligand. Peak at 247.14 *m/z* corresponds to loss of piperidine moiety from ciprofloxacin. Peak at 203.21 *m/z* is due to loss of COOH group from fragment of ciprofloxacin (Supplementary material 5: Proposed mass fragmentation pattern of complex 1).

Biological evaluation of synthesized complexes

In vitro antimicrobial activity

The complexes were screened for *in vitro* antimicrobial activity against two Gram(+ve) i.e. *S. aureus*, *B. subtilis* and three Gram(−ve), i.e., *S. marcescens*, *P. aeruginosa* and *E. coli* microorganisms using serial tube dilution technique. The antimicrobial activity data are shown in Table 2, indicate that:

- Except complex 4, all the complexes are more active against *S. aureus*.
- In case of *B. subtilis* the complexes 1–3 are more active than the complexes 4–6.
- All the complexes are more active in case of *S. marcescens*.
- Complexes 1 and 2 are more active, 3 is comparatively active while complexes 4–6 are not active against *P. aeruginosa*.
- Complexes 1, 2, 3 and 6 are more active while 4 and 5 are not active against *E. coli*.

This increase in antimicrobial activity may be due to Overtone's concept [51], chelation theory [52,53] or the following factors [54] such as: (i) the chelate effect of the ligands; (ii) the nature of the NS-donor ligands; (iii) the total charge of the complex; (iv) the existence and the nature of the ion neutralizing the ionic complex; (v) the nuclearity of the metal center in the complex.

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 NS-donor ligand and the nature of the ligands. This is probably one of the main reasons for the diverse antibacterial activities shown by the com-

Table 2
Antimicrobial activities of CPFH, copper(II) salt and complexes in terms of minimum inhibitory concentration (MIC) (μM).

Compound	Gram positive		Gram negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$CuCl_2 \cdot 2H_2O$	2698.0	2815.0	2756.0	2404.0	3402.0
CPFH	1.52	1.04	1.52	1.32	1.36
[Cu(CPF)(L^1)Cl] (1)	0.75	0.30	0.45	0.95	0.80
[Cu(CPF)(L^2)Cl] (2)	0.80	0.40	0.75	1.15	0.90
[Cu(CPF)(L^3)Cl] (3)	0.92	0.45	0.80	1.30	1.12
[Cu(CPF)(L^1)Cl] (4)	1.55	1.40	1.35	1.73	1.52
[Cu(CPF)(L^5)Cl] (5)	1.30	1.22	1.28	1.65	1.40
[Cu(CPF)(L^6)Cl] (6)	1.23	1.10	0.95	1.60	1.25

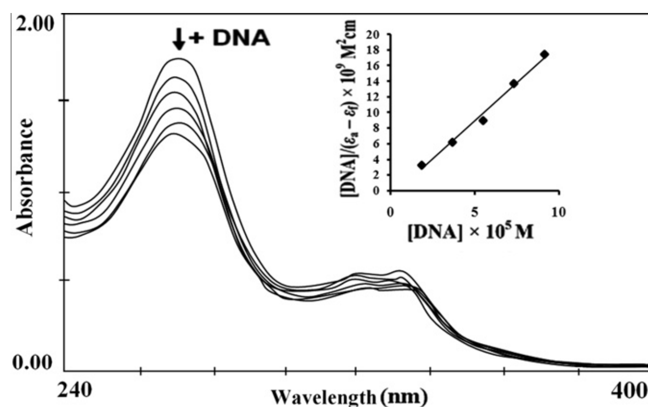


Fig. 3. Electronic absorption spectra of [Cu(CPF)L^1Cl] in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2) in the absence and presence of increasing amount of DNA. The [Cu] complex = 10 μM; [DNA] = 0–150 μM. The incubation period is 10 min. at room temperature, Inset: Plot of [DNA]/$(\varepsilon_a - \varepsilon_f)$ *versus* [DNA]. Arrow shows the absorbance change upon increasing DNA concentrations.

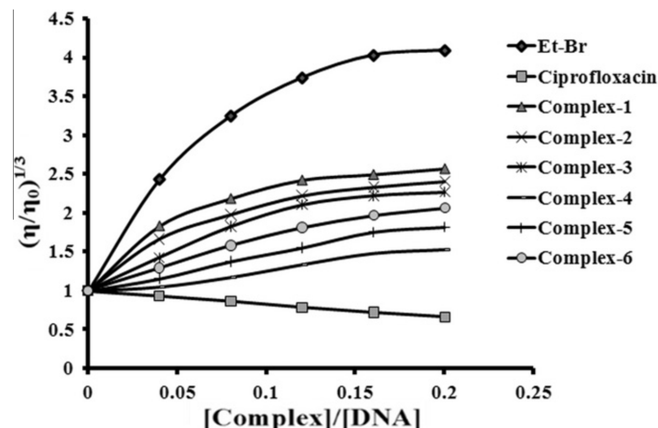


Fig. 4. Effect of increasing amount of EtBr, CPFH and complexes on the relative viscosity of Herring Sperm DNA at 37 ± 0.1 °C.

Table 3
Binding constant (K_b), IC_{50} and LC_{50} values of synthesized complexes.

Complex	K_b (M^{-1})	IC_{50} value (μM)	LC_{50} (μM)
[Cu(CPF)(L^1)Cl] (1)	2.45×10^5	0.55	8.07
[Cu(CPF)(L^2)Cl] (2)	2.04×10^5	0.75	11.18
[Cu(CPF)(L^3)Cl] (3)	1.41×10^5	0.89	13.01
[Cu(CPF)(L^4)Cl] (4)	1.02×10^5	1.25	25.67
[Cu(CPF)(L^5)Cl] (5)	1.29×10^5	1.16	21.64
[Cu(CPF)(L^6)Cl] (6)	1.37×10^5	0.95	15.89

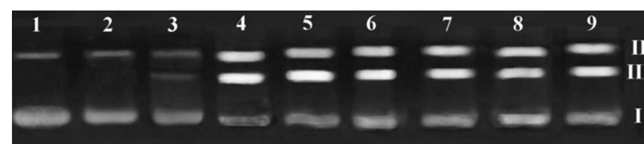


Fig. 5. Photogenic view of cleavage of pUC19 DNA (300 μg/mL) with series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μL, for 24 h at 37 °C. Lane 1, DNA control; Lane 2, $CuCl_2 \cdot 2H_2O$; Lane 3, ciprofloxacin; Lane 4, [Cu(CPF)L^1Cl]; Lane 5, [Cu(CPF)L^2Cl]; Lane 6, [Cu(CPF)L^3Cl]; Lane 7, [Cu(CPF)L^4Cl]; Lane 8, [Cu(CPF)L^5Cl]; Lane 9, [Cu(CPF)L^6Cl].

plexes. 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.

In addition, our study regarding bactericidal activity in terms of CFU/mL of above metal complexes against same microorganisms reveals that, decrease in number of colonies with increasing the concentration of complexes. The CFU/mL for different microorganism against complexes is shown in Supplementary material 6.

DNA binding study by absorption titration

Basic principle of absorption titration is change in spectral transition of coordination compounds on interaction with DNA. With increase in DNA to complex ratio, hypochromism and red shift was observed (Fig. 3). The extent of binding strength of complexes was quantitatively determined by measuring an intrinsic binding constants K_b (Table 3). This is lower than K_b value of classical intercalator ethidium bromide but higher than reported [Cu^{II}(phen)(bnp)H_2O] [55], [CuL^1(ClO_4)$_2$] [56], [Zn(erx)$_2$(H_2O)$_2$] [57], [Ni(sf)$_2$(bipyam)] [58] and [Ni(oxo)$_2$(H_2O)$_2$] complexes [59]. Thus, there is a possibility of intercalation of complexes. The highest binding constant of complex 1 is due to electron withdrawing group present on the ancillary ligand [60].

Hydrodynamic volume measurement

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, but not provide conclusive evidences for the exact mode of binding. Viscosity of DNA is increased in the case of classical intercalator due to increase in the length of DNA helix, as base pairs are separated to accommodate the intercalator.

In absence of crystallographic study, it is found that relative viscosity measurement study is the most critical tests for exploding interaction properties between complexes and DNA, in solution state [61]. Fig. 4 shows that the binding ability of classical intercalator ethidium bromide is more compare to all complexes. In our case increase in viscosity was observed, hence complexes bind to DNA via intercalation mode [62] and the magnitude of increase in relative viscosity is same as shown by Haq et al. [63], and out of all, complex 1 interact more strongly compare to other.

Table 4
Complex mediated DNA cleavage data by gel electrophoresis.

Lane No.	Compound	Form I (SC)	Form II (OC)	Form III (LC)	%Cleavage
1	DNA	81	19	–	–
2	$CuCl_2 \cdot 2H_2O$	76	24	–	6.17
3	Ciprofloxacin	63	23	14	22.22
4	$[Cu(CPF)(L^1)Cl]$ (1)	10	50	40	87.65
5	$[Cu(CPF)(L^2)Cl]$ (2)	20	50	30	75.31
6	$[Cu(CPF)(L^3)Cl]$ (3)	25	41	34	69.14
7	$[Cu(CPF)(L^1)Cl]$ (4)	26	40	34	61.72
8	$[Cu(CPF)(L^5)Cl]$ (5)	28	37	35	65.43
9	$[Cu(CPF)(L^6)Cl]$ (6)	31	33	36	67.90

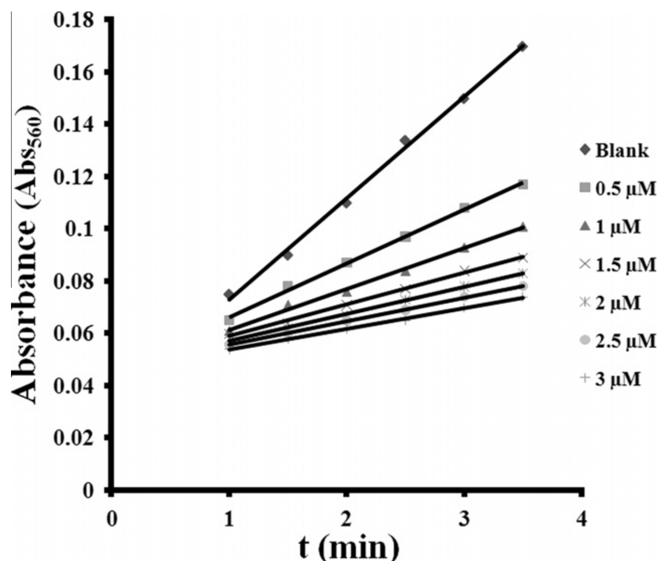


Fig. 6. Plot of absorbance (Abs_{560}) as a function of time (t) to determine% inhibition of formazan formation at various concentration of complex 1 (0.5–3 μm) as function of time.

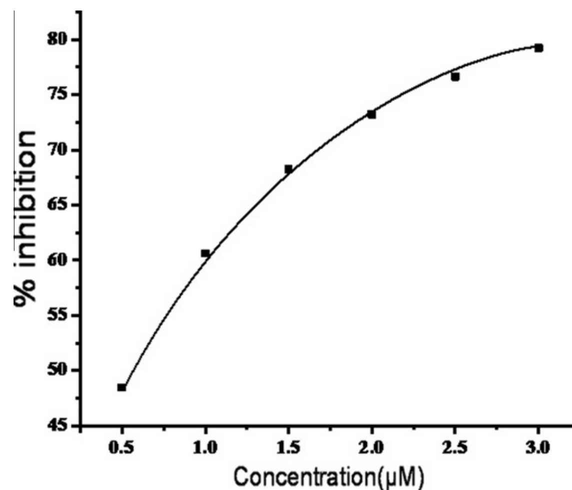


Fig. 7. The plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex 1.

DNA cleavage study

Transition metal complex mediated DNA cleavage is the center of interest [64,65]. When plasmid DNA was subjected to electrophoresis after interaction, upon illumination of gel (Fig. 5) the fastest migration was observed for super coiled (SC) Form I, where as the slowest moving was open circular (OC) Form II and the intermediate moving is the linear (LC) Form III, generated on cleavage of open circular. The data of plasmid cleavage are presented in Table 4. Here the complex 1 shows the maximum cleavage ability compared to all synthesized complexes. The different DNA-cleavage efficiency of the complexes, metal salt and drugs is due to the difference in binding affinity of the complexes to DNA and the structural dissimilarities of ligands.

Enzymatic behavior

NBT/NADH/PMS system was used to check SOD like activity of the synthesized complexes. The percentage inhibition of formazan formation at various concentrations of complexes as a function of time was measured by measuring the absorbance at 560 nm and plotted to have a straight line (Fig. 6). With increase in concentration of tested compounds decrease in slope (m) was observed. Percentage inhibition of the reduction of NBT plotted against concentration of the complex (Fig. 7). The SOD activity of the present synthesized complexes is given in Table 3. All the complexes show better radical scavenging ability than complexes reported by Chao et al. [66]. It is clear that the complexes exhibit greater scavenging activity toward superoxide radicals, which may be accredited to the redox potential of Cu(II) complex, geometry at metal center, the nature of coordinating ligand and acidic anion on apical position. Distorted geometry of complexes may favor the geometrical change, which is essential for the catalysis of copper in the Cu–Zn SOD that also changes distorted square pyramidal geometry [67,68].

Cytotoxic activity – Brine shrimp lethality bioassay

LC_{50} values of test complexes observed after 24 h are shown in Table 3. From the results it was found that the complexes showed considerable cytotoxicity in the brine shrimp (*Artemia cysts*) lethality bioassay, but complex 1 showed maximum activity than rest of the complexes. The increasing order of cytotoxic assay of complexes are as follow 1 > 2 > 3 > 6 > 5 > 4.

Conclusion

Here in this work, we have synthesized six Cu(II) metallointercalators with different neutral bidentate ligands and ciprofloxacin. The antibacterial activity of ciprofloxacin is changed upon coordination with metal ion. Hypochromism and bathochromism of band in absorption titration and increase in relative viscosity of DNA suggest that all complexes bind with DNA via classical intercalative mode. Complexation of drug with copper(II) ion enhances their DNA cleavage ability. Ligand which can facilitate the stabilization of bonding between metal center and oxygen radical anion favors enhancement in enzymatic behavior. These results suggest that the synthesized complexes can be kept forward for their in vivo nuclease, antibacterial and enzymatic behavior.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2012.05.037.

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