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Interaction of drug based binuclear mixed-ligand complexes with DNA

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ABSTRACT

Here in we tried to increase an antibacterial activity of ciprofloxacin drug due to formation of mixed-ligand complexes. Synthesized compounds were found to be more potent compare to drugs, ligands and metal salt against selective gram$^{(+ve)}$ and gram$^{(-ve)}$ organisms. Interaction of the complexes with nucleic acid (DNA) were investigated using spectroscopic technique, viscosity measurement and gel electrophoresis and it was found that the complexes bind to DNA via intercalative mode.

1. Introduction

Quinolones are an important group of antibiotics and several quinolones are in common clinical use. The first quinolone antibiotic, nalidixic acid, was synthesized in 1962 by Lesher et al.[1] Ciprofloxacin is a typical second-generation fluoroquinolone, has been in clinical use for more than a decade and sold for $US 1.5 billion in 1996.[2] The drug has been the center of great interest and success and more than 15,000 articles have been published about ciprofloxacin. In connection with the outbreak of suspected anthrax biological warfare, many experts consider ciprofloxacin the drug of choice for treating victims. Binding of flouroquinolone to DNA is relatively weak, thus it is unlikely that their binding to DNA triggers the formation of gyrase–DNA complex.[3–6] Likewise, binding of fluoroquinolone to gyrase is weak, even though the presence of mutated gyrase alleles in resistant bacteria implicates gyrase in the interactions.[7–11] Numerous studies have shown that drug binding to DNA[12–15] and gyrase is enhanced in the presence of metal ions and metal ions are essential for antibacterial efficiency of drug–DNA interaction. Understanding the interactions between fluoroquinolone and DNA may help to elucidate the mechanism of action of this important class of antibacterial agents, and may ultimately lead to the design of better, more potent antibacterial agents with fewer side effects.

To this aim, we have investigated the DNA-binding assay of Co(II) mixed-ligand complexes with ciprofloxacin and some neutral bidentate ligands(NN) using a combination of spectroscopic (UV-spectroscopy), hydrodynamic techniques (viscometric) and gel electrophoresis technique. Antibacterial activity (MIC) has been assayed against five different microorganism namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Serratia merscences*.

2. Result and discussion

The structural characterizations of ligands have been done using IR spectra, ^{1}H and ^{13}C NMR spectra, and elemental analyses. The complexes under investigation have been characterized using IR spectra, magnetic measurements, TG analysis, FAB-mass spectra and electronic spectra. All the complexes were insoluble in ether, hexane, chloroform, water and methanol but were completely soluble in dimethyl sulphoxide.

2.1. ^{1}H NMR and ^{13}C NMR spectra of Schiff bases

The ^{1}H and ^{13}C NMR spectra of the ligands were carried out in $CDCl_3$/MeOD and are reported along with the possible assignment as below.

2.1.1. *N,N′*-Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^1∼bmpdme)

^{1}H NMR (ppm): 6.67–6.86 (8H, m, Ar-H), 2.07 (6H, s, Al-H), 3.73 (6H, s, OCH_3); ^{13}C NMR (ppm): 114.2–120.6 (Ar-C), 158.6 (Ar, C-O), 168.9 (C=N), 144.1 (C–N), 15.4 (Al–C), 55.4 (OCH_3).

2.1.2. *N,N′*-Bis-(4-methoxy-benzylidene)-ethane-1,2-diamine (A^2∼bmbed)

^{1}H NMR (ppm): 6.65–7.91 (8H, m, Ar-H), 3.8 (4H, t, Al-H), 8.3 (2H, s, CH=N), 3.4 (6H, s, OCH_3); ^{13}C NMR (ppm): 113.9–134.4 (Ar-C), 157.3 (C–O), 165.5 (C=N), 130.4 (C–N), 55.3 (OCH_3).

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2.1.3. *N,N′*-Bis-(1-phenylethylidene)-benzene-1,2-diamine (A^3∼bpebd)

^{1}H NMR (ppm): 6.5–7.5 (14H, m, Ar-H), 1.74 (6H, s, Al–H); ^{13}C NMR (ppm): 121.9–130.4 (Ar-C), 18.2 (Al–C), 170.4 (C=N), 148.4 (C–N).

The ^{1}H NMR spectra of ligands exhibiting peaks at about 6.5–7.91 ppm were assigned to the aromatic protons. The singlet peak which appeared at 8.3 ppm was assigned to the azomethine proton (–CH=N–). In the ^{13}C NMR spectra, the peak observed at about 113.9–134.4 was assigned to aromatic carbons. Peaks observed at about 130.4–148.4, 165.5–170.4 ppm were assigned to C–N and C=N carbons, respectively.

2.2. Characterization of the mixed-ligand complexes

2.2.1. Infrared spectral resolution

In Table 1 the most characteristic absorptions of IR spectra of the complexes are listed. The ν(C=O) stretching vibration band appears at 1708 cm^{-1} for ciprofloxacin, where as for complexes it appear at 1615–1630 cm^{-1}; this shift towards lower energy suggests that coordination occurs through the carbonyl oxygen of pyridine ring.[16] The sharp band in ciprofloxacin at 3520 cm^{-1} is due to hydrogen bonding;[17] which is attributed to ionic resonance structure and peak observed because of free hydroxyl stretching vibration. This band completely vanished in the spectra of the metal complexes indicating deprotonation of the carboxylic proton. The data were further supported by ν(M–O) band which appeared at 504–512 cm^{-1}.[18] The strong absorption band obtained at 1624 cm^{-1} and 1340 cm^{-1} in ciprofloxacin were assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in the metal complexes these bands were observed at 1607 and 1383 cm^{-1}, respectively. The frequency separation ($\Delta\nu$) in the investigated complexes is greater than 200 cm^{-1}, suggesting a unidentate bonding nature for the carboxyl group.[16,19–23] The ν(C=N) peak for the ligands A^1–A^6 was observed at 1600–1629 cm^{-1} which on complexation were shifted to 1569–1602 cm^{-1}, which indicates the N–N coordination of the ligands.[24–27] This data was further supported by ν(M–N) which appeared at 535–542 cm^{-1}.[28]

2.2.2. Reflectance spectra

The characteristic bands of the electronic spectra of the complexes are given in Table 2. The Co(II) complexes exhibited well-resolved bands at 16,500–18,790 cm^{-1} and a strong high-energy band at 20,500–22,525 cm^{-1} (Table 2) were assigned to $^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)(\nu_1)$, $^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)(\nu_2)$, $^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)(\nu_3)$, transitions for a high-spin octahedral geometry.[29,30]

2.2.3. Magnetic behavior

The observed magnetic moments of cobalt(II) complexes are given in Table 2. The theory of magnetic susceptibility of cobalt(II) complex was given originally by Schlapp and Penney and the best summary of results on the magnetic behavior of cobalt compound is that of Figgis and Nyholm.[31,32] The observed values of magnetic moment for cobalt(II) complexes are generally diagnostic of the coordination geometry about the metal ion. The low-spin square-planar cobalt(II) complexes have magnetic susceptibility value 2.9 BM, arising from one unpaired electron along with contribution of apparently large orbital.[32] Both tetrahedral and high-spin octahedral cobalt(II) complexes possess three unpaired electrons but may be distinguished by the magnitude of the deviation of $\mu_{eff.}$ from the spin-only value. The magnetic moment of tetrahedral cobalt(II) complexes with an orbitally nongenerate ground term is increased above the spin-only value via contribution from higher orbitally degenerate terms and occurs in the range 4.2–4.7 BM.[33] Octahedral cobalt(II) complexes however maintain a large contribution due to $^4T_{1g}$ ground term and exhibit $\mu_{eff.}$ in the range 4.8–5.6 BM.[34] The magnetic measurements on the complexes reported herein 4.8–5.2 BM show that all are paramagnetic and have three unpaired electrons indicating a high-spin octahedral configuration.

2.2.4. Thermal response of synthesized complex

The thermal behavior of the complexes was determined using a 5000/2960 SDTA, TA instrument (USA) differential thermal analysis apparatus operating at a heating rate of 10 °C per minute in the range of 20–800 °C in N_2. The TG curves of Co(II) complexes showed in the following decomposition steps. It was observed that all the complexes showed a loss in weight corresponding to three water molecules in the range 50–130 °C, indicating that these water molecules were water of crystallization. In the second step weight loss during130–180 °C corresponding to two coordinated water molecules. For Co(II) complexes a loss in weight was seen corresponding to a piperazine (pip) molecule in the temperature range 180–250 °C, followed by liberation of Cip.(L) in the temperature range 250–500 °C. Finally, decomposition of A^n occurred in the temperature range 520–800 °C, and the remaining weight was consistent with metal oxide.[35] Probable structure of the complexes from the above analytical facts is given below in Figure 1.

Table 1
Infrared spectral data of the Co(II) complexes

Complexes	ν(C=O) cm^{-1} pyridone	$\nu(COO)_{asy}$ cm^{-1}	$\nu(COO)_{sym}$ cm^{-1}	$\Delta\nu$ cm^{-1}	ν(C–Cl) cm^{-1}	ν(C=N) cm^{-1} azomethine	ν(M–N) cm^{-1}	ν(M–O) cm^{-1}
I	1617	1607	1383	224	1145	1569	535	510
II	1623	1608	1388	220	1146	1602	538	509
III	1620	1587	1375	212	1130	–	540	504
IV	1615	1605	1384	221	1106	–	540	512
V	1627	1588	1383	205	1150	–	542	508
VI	1630	1600	1377	223	1101	–	536	510

Table 2
Electronic spectral data of Co(II) complexes

Complexes	ν_2	ν_3	B′	10Dq	β	%β	μ_{eff} BM
I	16,525	21,920	461.50	11403.40	0.4752	52.471	5.0
II	17,430	20,525	579.07	10760.86	0.5963	40.362	4.9
III	16,615	22,365	452.13	11622.30	0.4656	53.436	5.2
IV	16,577	21,920	465.46	11405.92	0.4793	52.063	4.8
V	17,125	20,620	553.12	10795.94	0.5696	43.035	4.9
VI	15,920	20,500	466.97	10686.53	0.4809	51.907	4.8

2.2.5. FAB-MS

The mass spectrum of the complex(I) $[Co_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2]\cdot 3H_2O$ (Fig. 2) shows a triplet peak at 1394(M), 1396(M+2) and 1398(M+4). The most abundance peak at 1394 may represent the molecular ion peak in absence of water of crystallization and presence of four H^+ ion that is, $[Co_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2] + 4H^+$. There also exists several triplet at with peak value 1679(M), 1681(M+2) and 1683(M+4) which corre-

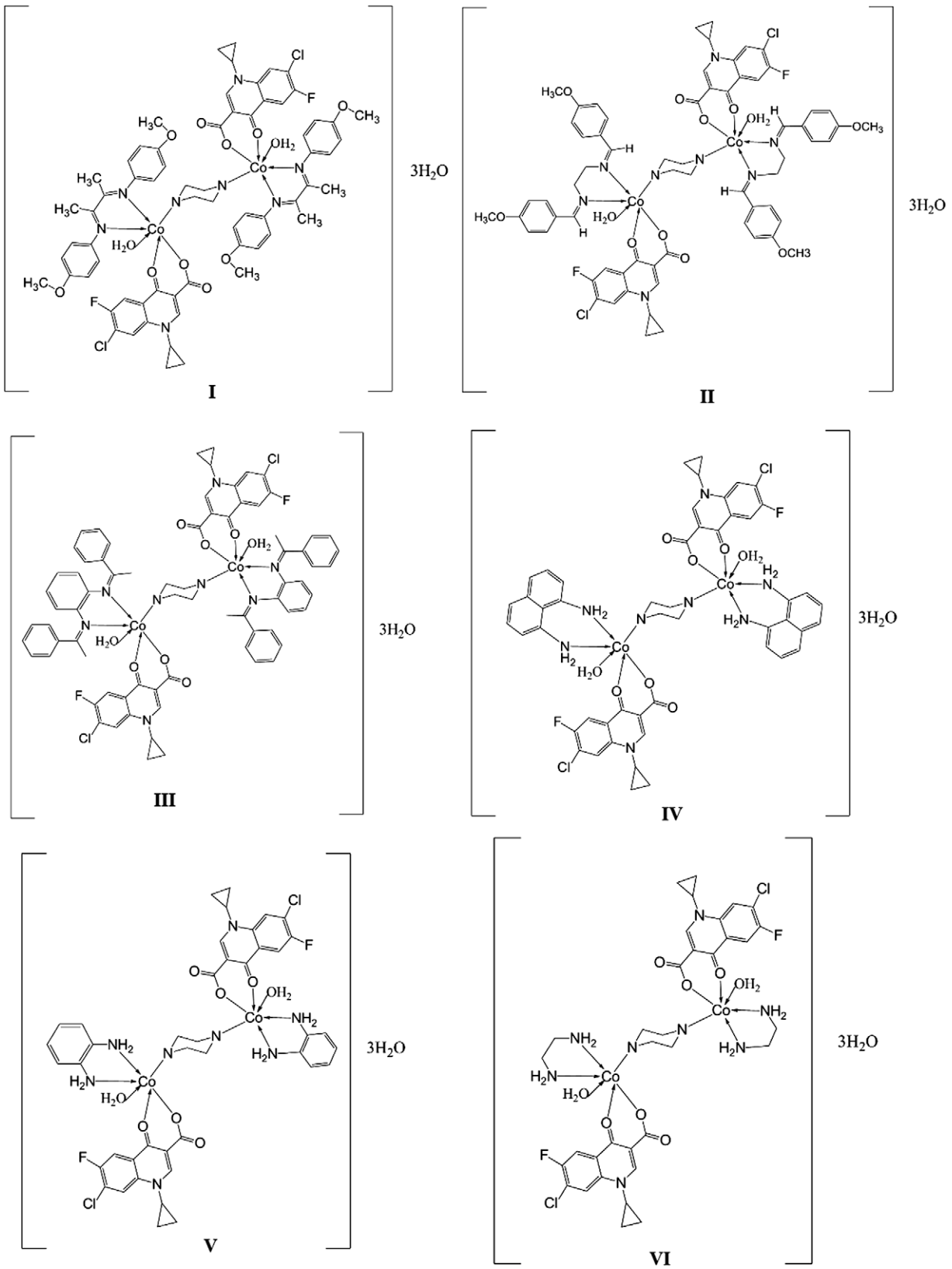


Figure 1. Probable structures of the complexes.

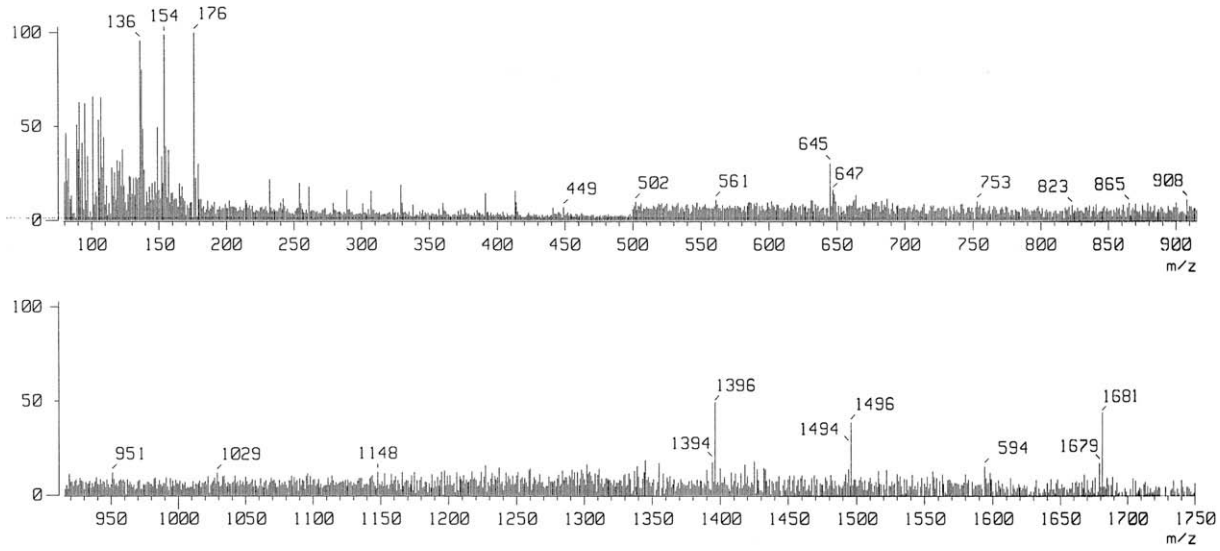


Figure 2. Mass spectrum of complex (I).

sponds to complex bound to matrix (289) that is, $[Co_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2] + 289$; 1494(M), 1496(M+2) and 1498(M+4) which corresponds to fragment $[Co_2(Cip)_2(bmpdme)_2(pip)] + 136$, these pattern indicates the presence of two Cl atom in this fragments.[17] There exist a doublet at 645(M) and 647(M+2) which corresponds to fragment $[Co(bmpdme)] + 289 + 4H^+$ consist of single Cl atom.[17] The other fragment was observed at 951(M) related to $[Co_2(bmpdme)_2(pip)] + 136 + 4H^+$. Thus the *m/z* of all the fragments of complex with the relative intensity confirms the stoichiometry of the complex as per Figure 1.

2.3. In vitro biological studies of mixed-ligand complexes

2.3.1. Effect of complexes on the microorganism

The complexes exhibit activities against three Gram$^{(-ve)}$ that is, *E. coli*, *S. merscences* and *P. aeruginosa* and two Gram$^{(+ve)}$ that is, *S. aureus, B. subtilis* microorganisms. The results concerning in vitro antimicrobial activity (MIC) of the metal salt, ligands, complexes and standard drug are represented in Table 3. The ligands (A^1–A^6) exhibit a little antimicrobial activity. The antimicrobial activity of the complexes against all the five microorganisms is much higher than metal salt, while in competition with the ciprofloxacin. The results of our study indicate that the in case of *S. aureus* the complexes **II**, **III**, **V** and **VI** (MIC = 0.65, 0.68, 0.28 and 1.03 μM) are more potent than all the standard drugs and complexes **I**, **IV** (MIC = 2.07, 1.73 μM) are less potent than ciprofloxacin. In case of *B. subtilis* except **III** and **IV** (MIC = 1.69 and 1.71 μM) all the complexes are much potent than the standard drugs. For *S. merscences* all the complexes are active compare to gatifloxacin and norfloxacin, but less active than ciprofloxacin. For *P. aeruginosa* complex **I** (MIC = 0.69 μM) is more potent than the gatifloxacin drugs. In case of E coli complexes **I**, **II**, **III** and **V** (MIC = 2.42, 2.27, 2.37 and 1.40 μM) are more potent than gatifloxacin norfloxacin and pefloxacin. It was observed that all the complexes were more potent bactericides than the ligands. It was found that the complex V having planer aromatic ancillary ligand

Table 3
Antimicrobial activity data (μM)

Compounds	Gram positive		Gram negative		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$Co(NO_3)_2{\cdot}6H_2O$	515.00	858.00	1374.00	1030.00	1030.00
Ciprofloxacin	1.63	1.09	1.63	1.36	1.36
Gatifloxacin	5.06	4.00	2.93	1.01	2.93
Norfloxacin	2.50	2.51	4.07	3.76	2.82
Enrofloxacin	1.95	3.90	1.67	1.39	1.39
Pefloxacin	2.10	2.40	5.10	5.70	2.70
Levofloxacin	1.66	2.21	1.67	1.66	0.97
Sparfloxacin	1.27	2.04	1.53	1.53	1.27
Ofloxacin	1.94	1.38	1.66	2.21	1.38
A^1	1855.00	1518.00	2024.00	2193.00	2361.00
A^2	1687.00	1518.00	1687.00	1855.00	2193.00
A^3	1760.00	1600.00	1760.00	1920.00	2080.00
A^4	3160.00	3476.00	4108.00	3792.00	4108.00
A^5	5086.00	5548.00	6010.00	6010.00	6473.00
A^6	8319.00	9151.00	9983.00	10815.00	10815.00
I	2.07	0.28	1.73	0.69	2.42
II	0.65	0.19	2.27	1.30	2.27
III	0.68	1.69	2.71	2.37	2.37
IV	1.71	1.71	3.42	3.42	2.99
V	0.28	0.37	2.80	0.93	1.40
VI	1.03	0.41	4.11	3.08	4.11

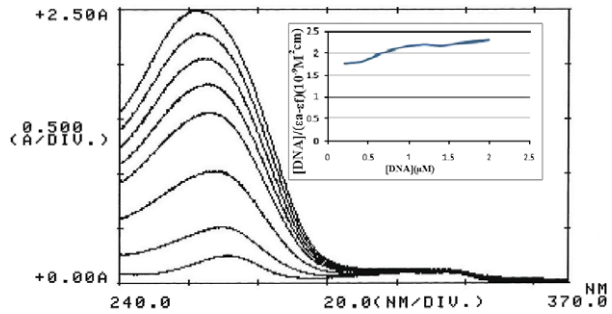


Figure 3. Absorption spectra of the complex I.

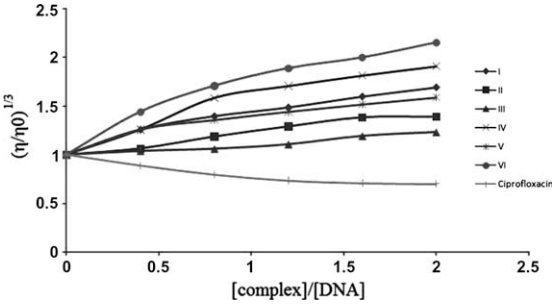


Figure 4. Viscosity data of the complexes with DNA.

was more potent bacteriostatic compare to the one with aliphatic ancillary ligand (complex **VI**). It was clearly observed that with increase in aromatic nature of ancillary ligands (complexes **III** and **IV**) potency of complex was not much affected, but the presence of methoxy group along with increase in aromatic character shows mark increase in potency against bacteria (complex **II**) which is further supported from K_b values from absorption titration and gel electrophoresis data. The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal center. This may support the argument that some type of biomolecular binding to the metal ions or intercalation or electrostatic interactions causing the inhibition of biological synthesis and preventing the organisms from reproducing. The strong antimicrobial activities of these complexes against tested organisms suggest further investigation on these complexes.

2.3.2. Interaction of the mixed-ligand complexes with DNA

2.3.2.1. Absorption spectroscopy. The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques.[36] Complex binding with DNA through intercalation usually results in hyperchromism and hypochromism, because intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The electronic absorption spectra of complexes mainly consist of two resolved bands. The low energy absorption band centered at 455–472 nm is assigned to metal-to-ligand charge transfer (MLCT) transition and the other band centered at 263–286 nm is attributed to intraligand (IL) $\pi-\pi^*$ transition by comparison with the spectrum of other polypyridyl Ru(II) complexes.[37] The absorption spectra of the complex (**I**) in the absence and presence of increasing amounts of DNA are illustrated in Figure 3. Change in absorbance at peak maximum shows moderate hypsochromism shift (~5 nm). For each complex, increasing concentration of DNA has been monitored for an evaluation of the intrinsic binding constant, which observed in the range of 1.0×10^4–4.0×10^4 M^{-1}. (Insert Fig. 3 for the plot for the calculation of intrinsic binding constant). The binding constants are given in Table 4. 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 titled complexes can intercalate into the double helix structure of DNA.

Table 4
The binding constants (K_b) of Co(II) complexes with DNA in phosphate buffer pH 7.2

Complexes	K_b (M^{-1})
I	1.0×10^4
II	4.0×10^4
III	1.5×10^4
IV	2.0×10^4
V	2.0×10^4
VI	2.5×10^4

2.3.2.2. Viscosity measurement. Furthermore, the interactions between the complex and DNA were investigated by viscosity measurements. Optical photophysical probes provided necessary, but not sufficient, clues to support a binding model. Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) of DNA are regarded as the least ambiguous and the most critical tests of binding mode in solution in absence of crystallographic structural data.[38,39] A classical intercalation model usually resulted in lengthening the DNA helix, as base pairs were separated to accommodate the binding ligand leading to the increase of DNA viscosity, where as non-classical intercalation mode usually results in static bend or kink in the helix and decreases the viscosity of DNA. As seen in Figure 4, the viscosity of DNA increased on increasing the ratio of Co(II) complexes to DNA. This result further suggested an intercalative binding mode of the complex with DNA and also parallel to the above spectroscopic results, such as hypochromism and hypsochromism of complexes in the presence of DNA. But the viscosity of DNA decreases on increasing the ratio of ciprofloxacin to DNA. This result suggests that ciprofloxacin bind DNA via partial, non-classical intercalation.

2.3.2.3. Oxidative and non-oxidative nucleic acid cleavage. There has been considerable interest in DNA cleavage reactions that are activated by transition metal complex.[40,41] The delivery of metal ion to the helix, in locally generating oxygen or hydroxide radicals, yields an efficient DNA cleavage reaction. Figure 5 illustrates the gel electrophoretic separations showing the cleavage of plasmid pBR322 DNA induced by the complexes under aerobic conditions and in presence of H_2O_2, respectively.[42] When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (SC). If one strand is cleaved, the supercoiled will relax to produce a slower-moving open circular form (OC). If both strands are cleaved, a nicked form (NC) will be generated that migrates in between. This clearly shows that the relative binding efficacy of the complexes to DNA is much higher than the binding efficacy of metal salt itself or ciprofloxacin (Table 5). The different DNA cleavage efficiency of the complexes was due to the different binding affinity of the complexes to DNA, which has been observed in other cases. One of

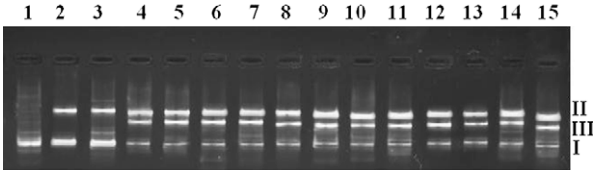


Figure 5. Gel electrophoresis data with pBR322: Lane 1: DNA (control); Lane 2: DNA + metal salt; Lane 3: DNA + cip; Lane 4: DNA + **I**; Lane 5: DNA + **II**; Lane 6: DNA + **III**; Lane 7: DNA + **IV**; Lane 8: DNA + **V**; Lane 9: DNA + **VI**; Lane 10: DNA + H_2O_2 + **I**; Lane 11: DNA + H_2O_2 + **II**; Lane 12: DNA + H_2O_2 + **III**; Lane 13: DNA + H_2O_2 + **IV**; Lane 14: DNA + H_2O_2 + **V**; Lane 15: DNA + H_2O_2 + **VI**.

Table 5
Gel electrophoresis data of the compounds

Compounds	% SC	% NC	% OC
DNA control	100	–	–
DNA + $Co(NO_3)_2{\cdot}6H_2O$	58	–	42
DNA + Cip.	50	14	36
DNA + **I**	19	26	55
DNA + **II**	21	33	46
DNA + **III**	20	35	45
DNA + **IV**	21	35	44
DNA + **V**	26	30	44
DNA + **VI**	29	37	44
DNA + **I** + H_2O_2	17	25	58
DNA + **II** + H_2O_2	14	34	52
DNA + **III** + H_2O_2	19	36	45
DNA + **IV** + H_2O_2	18	28	54
DNA + **V** + H_2O_2	24	27	49
DNA + **VI** + H_2O_2	18	32	50

the most interesting electrophoretic results of the complexes takes place when experiment is done in presence of H_2O_2 in TAE buffer. The DNA + complex (10 μM) + H_2O_2 (100 μM) systems (Fig. 5, Lane 10–15) cleave the supercoiled DNA form (**I**) and convert into nicked form (**II**) and linear form (**III**) more than complex alone. Therefore, we concluded that the mixture of complex with H_2O_2 have been found to be efficient oxidant.

3. Conclusion

In all the complexes, the quinolone ligand bound to cobalt(II) via the pyridone oxygen and one carboxylate oxygen. Interaction of Co(II) with the deprotonated quinolone ligand and neutral bidentate, results in the formation of the neutral dinuclear complexes. For all the complexes, an octahedral environment around Co(II) has been suggested. The interaction of complexes **I**–**VI** with DNA revealed that all the complexes can bind to DNA by the intercalative mode. The best inhibition among the compounds studied in this work is provided by the compound **V** (MIC = 0.28 μM) against *S. aureus* while compounds **I** (MIC = 0.28 μM), **II** (MIC = 0.19 μM), **V** (MIC = 0.37 μM) and **VI** (MIC = 0.41 μM) against *B. subtilis*. In case of *P. aeruginosa* **I** and **V** (MIC = 0.69 and 0.93 μM) prove to be better active. Hence complexation of ligands with metal is responsible for the better MIC value than ligands itself. From the gel electrophoresis data it is crystal clear that there occurs a mark increase in DNA cleavage property of ciprofloxacin on complexation.

4. Experimental

4.1. Materials and methods

4.1.1. Drug and chemicals

All the chemicals used were of analytical grade. 1,8-Diaminonaphthalene (*dan*∼A^4) was purchased from Lancaster, England. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). Ethylenediamine (*en*∼A^6), 2,3-butanedione, *p*-anisaldehyde, *p*-anisidine, acetophenone, glycerol, and cobalt nitrate were purchased from E. Merck (India) Ltd, Mumbai. Xylene cyanol FF, ethidium bromide and luria broth were purchased from Himedia, India. Agarose was purchased from Sisco research Lab., India. Bromophenol blue, *o*-phenylenediamine (*opd*∼A^5), acetic acid and EDTA were purchased from Sd fine chemicals, India. Sperm herring DNA was purchased from Sigma Chemical Co., India. Organic solvents were purified by standard methods.[43]

4.1.2. Instrumentation

Infrared spectra were recorded on a FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. C, H and N elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO discs) on a Beckman DK-2A spectrophotometer. The metal contents of the complexes were analyzed by EDTA titration after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5).[44] MIC study was carried out by means of laminar air flow cabinet, Toshiba, Delhi, India, Thermogravimetric analyses was obtained with a model 5000/2960 SDTA, TA instrument (USA). The ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance (400 MHz). The electronic spectra were recorded on a UV-160A UV–vis. spectrophotometer, Shimadzu (Japan). The 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), Citizen Balance. The diamagnetic correction was made using Pascal's constant.[45] The FAB-mass spectra were recorded on a Jeol SX 102/Da-600 mass spectrometer/Data System using Argon/Xenon (6 kv, 10mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. *m*-Nitro benzyl alcohol (NBA) was used as the matrix. The matrix peaks appear at *m/z* 136, 137, 154, 289, 307.

4.2. Method

4.2.1. Synthesis of ligand

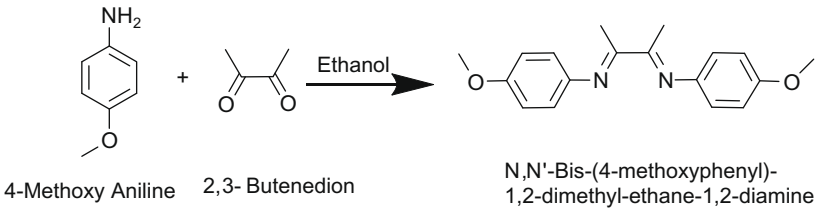
4.2.1.1. *N,N′*-Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^1∼bmpdme). An ethanolic solution (100 mL) of *p*-anisidine (2.46 g, 20 mmol) was added drop wise to ethanolic solution (100 mL) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed on water bath for 8 h. The resulting mixture was filtered. The obtained crystalline yellow product was recrystallized in ethanol, washed with *n*-hexane and dried in air (see Scheme 1).

4.2.1.2. *N,N′*-Bis-(4-methoxy-benzylidene)-ethane-1,2-diamine (A^2∼bmbed). An ethanolic solution of ethylenediamine (0.60 g, 10 mmol) and *p*-anisaldehyde (2.50 g, 20 mmol) were refluxed on water bath for 8 h. The resulting mixture was filtered. The obtained crystalline yellow product was recrystallized in ethanol, washed with n-hexane and dried in air.

4.2.1.3. *N,N′*-Bis-(1-phenylethylidene)-benzene-1,2-diamine (A^3∼bpebd). The preparation of *N,N′*-bis-(1-phenylethylidene)-benzene-1,2-diamine was carried out by refluxing an ethanolic solution of acetophenone (2.4 g, 20 mmol) and *o*-phenylenediamine (1.08 g, 10 mmol) on water bath for 8 h. Fine yellow crystalline product obtained on filtration was further crystallized in ethanol, washed with *n*-hexane and dried in air.

4.2.2. Synthesis and physical properties of the mixed-ligand complexes

4.2.2.1. $[Co_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2]{\cdot}3H_2O$ (I). A methanolic solution of $Co(NO_3)_2{\cdot}6H_2O$ (2.91 g, 10 mmol) was added to a methanolic solution of *bmpdme* (2.96 g, 10 mmol), followed by addition of a previously prepared solution of Cip·HCl (3.67 g, 10 mmol) in water; pH was adjusted to 6–7.5 pH using dilute NaOH solution. During reaction the piperazine ring of ciprofloxacin was substituted by chloride ion in the presence of NaOH.[46] The resulting red solution was refluxed for 8–10 h. on a steam bath, and then was kept overnight at room temperature. A fine reddish brown crystalline product was obtained which was washed with ether and dried in a vacuum desiccator. The physical parameters of the complexes are shown in Table 6 and probable reaction scheme is shown in Scheme 2. Yield 68.5%, mp >360 °C, Calcd for



Scheme 1. Reaction scheme of A^1∼bmpdme.

$C_{66}H_{74}C_{l2}Co_2F_2N_8O_{15}$: C, 54.82; H, 5.16; N, 7.75; Co, 8.15; M^+, 1446.28. Found: C, 54.80; H, 5.19; N, 7.72; Co, 8.17; M^+, 1446.11.

4.2.2.2. $[Co_2(Cip)_2(bmbed)_2(pip)(H_2O)_2]\cdot 3H_2O$ (II). It was prepared by using *bmbed* (2.96 g, 10 mmol). Yield 69.4%, mp 350 °C, Calcd for $C_{74}H_{74}C_{l2}Co_2F_2N_8O_{15}$: C, 57.63; H, 4.84; N, 7.27; Co, 7.64; M^+, 1542.54. Found: C, 57.60; H, 4.85; N, 7.24; Co, 7.61; M^+, 1542.19.

4.2.2.3. $[Co_2(Cip)_2(bpebd)_2(pip)(H_2O)_2]\cdot 3H_2O$ (III). It was prepared by using *dan* (3.12 g, 10 mmol). Yield 67.5%, mp 255 °C, Calcd for $C_{74}H_{74}C_{l2}Co_2F_2N_8O_{11}$: C, 60.13; H, 5.05; N, 7.58; Co, 7.97; M^+, 1478.19. Found: C, 60.14; H, 5.04; N, 7.56; Co, 7.95; M^+, 1478.20.

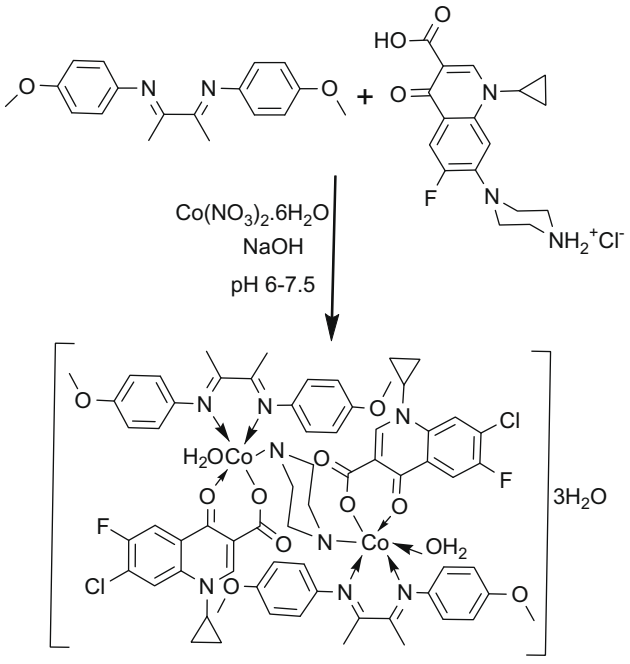
4.2.2.4. $[Co_2(Cip)_2(dan)_2(pip)(H_2O)_2]\cdot 3H_2O$ (IV). It was prepared by using *dan* (1.58 g, 10 mmol). Yield 68.0%, mp 350 °C, Calcd for $C_{50}H_{54}C_{l2}Co_2F_2N_8O_{11}$: C, 51.34; H, 4.65; N, 9.58; Co, 10.08; M^+, 1169.78. Found: C, 51.30; H, 4.66; N, 9.56; Co, 10.06; M^+, 1169.78.

4.2.2.5. $[Co_2(Cip)_2(opd)_2(pip)(H_2O)_2]\cdot 3H_2O$ (V). It was prepared by using *opd* (1.08 g, 10 mmol). Yield 68.0%, mp 360 °C (dec.), Calcd for $C_{42}H_{50}C_{l2}Co_2F_2N_8O_{11}$: C, 47.16; H, 4.71; N, 10.48; Co, 11.02; M^+, 1069.73. Found: C, 47.15; H, 4.72; N, 10.45; Co, 11.01; M^+, 1069.66.

4.2.2.6. $[Co_2(Cip)_2(en)_2(pip)(H_2O)_2]\cdot 3H_2O$ (VI). It was prepared by using *en* (0.60 g, 10 mmol). Yield 67.9%, mp 360 °C (dec.), Calcd for $C_{34}H_{50}C_{l2}Co_2F_2N_8O_{11}$: C, 41.94; H, 5.18; N, 11.51; Co, 12.11; M^+, 973.58. Found: C, 41.93; H, 5.15; N, 11.49; Co, 12.10; M^+, 973.58.

4.2.3. Function of complexes on the microorganism

All the bacteria were incubated and activated at 30 °C by inoculation into luria broth for 24 h. The compounds were dissolved in DMSO and then diluted using luria broth. Twofold serial concentrations of the compounds were employed to determine the Minimum Inhibitory Concentration (MIC) ranging from 11,000 μM to 0.1 μM. Test cultures were incubated at 37 °C (24 h). The lowest concentrations of antimicrobial agents that resulted in complete inhibition of growth were represented as MIC (μM). In each case triplicate tests were performed and the average was taken as the final value.[47]



Scheme 2. Synthesis of Complex I $[Co_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2]\cdot 3H_2O$.

4.2.4. Function of complexes on the DNA

Isolation of plasmid DNA from pure culture of *E. coli* was carried out by conventional

Method,[48] the basic principle employed for isolation is based on three steps:

1. Weakening of bacterial cell wall by the action of lysozyme.
2. Cells are lysed by EDTA and detergent at high pH.
3. Insoluble cells debris consisting of genomic DNA and protein is precipitated with high salt and centrifuged down leaving the plasmid in solution (supernant).

The basic principle employed is 'alkali-lysis' in which at the alkaline pH, both the genomic and plasmid DNA are denatured. On reduction of the pH the plasmid DNA molecule being small in size,

Table 6
Experimental and physical parameters of the complexes

Complexes empirical formula	Elemental analysis % found (required)				mp (°C)	% Yield	Formula weight (gm/mol)
	C	H	N	M			
$C_{66}H_{74}C_{l2}Co_2F_2N_8O_{15}$ (**I**)	54.80 (54.82)	5.19 (5.16)	7.72 (7.75)	8.17 (8.15)	>360	68.5	1446.11
$C_{74}H_{74}C_{l2}Co_2F_2N_8O_{15}$ (**II**)	57.60 (57.63)	4.85 (4.84)	7.24 (7.27)	7.61 (7.64)	350	69.4	1542.19
$C_{74}H_{74}C_{l2}Co_2F_2N_8O_{11}$ (**III**)	60.14 (60.13)	5.04 (5.05)	7.56 (7.58)	7.95 (7.97)	255	67.5	1478.20
$C_{50}H_{54}C_{l2}Co_2F_2N_8O_{11}$ (**IV**)	51.30 (51.34)	4.66 (4.65)	9.56 (9.58)	10.06 (10.08)	350	68.0	1169.78
$C_{42}H_{50}C_{l2}Co_2F_2N_8O_{11}$ (**V**)	47.15 (47.16)	4.72 (4.71)	10.45 (10.48)	11.01 (11.02)	360 dec.	68.0	1069.66
$C_{34}H_{50}C_{l2}Co_2F_2N_8O_{11}$ (**VI**)	41.93 (41.94)	5.15 (5.18)	11.49 (11.51)	12.10 (12.11)	360 dec.	67.9	973.58

quickly reanneals itself while the large genomic DNA is not. The denatured genomic DNA is then sediment while the plasmid DNA remains in solution. This is then precipitated.

The concentration of DNA was measured by using its standard extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}).[49] Absorption titration was carried out by varying the DNA concentration (2–20 μM) and maintaining constant concentration of the complex (4 μM). Absorption spectra were recorded after each successive addition of DNA followed by allowing it to attain equilibrium (approximately 10 min). The observed data were then utilized to obtain the intrinsic binding constant, K_b using Eq. (1).[50]

$$\frac{[DNA]}{(\epsilon_a - \epsilon_f)} = \frac{[DNA]}{(\epsilon_b - \epsilon_f)} + \frac{1}{K_b(\epsilon_b - \epsilon_f)} \quad (1)$$

where, [DNA] is the concentration of DNA in terms of nucleotide phosphate [NP], the apparent absorption coefficient ε_f, ε_a and ε_b correspond to the extinction coefficient of the free complex, the extinction coefficient for each addition of DNA to the complex and the extinction coefficient for the complex in the fully bound form, respectively and K_b is the ratio of the slope to the y intercept.

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 with an approximate average length of 200 base pairs were prepared by sonication in order to minimize complexities arising from DNA flexibility.[51] Flow time was measured with a digital stopwatch. Each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio [Co]/[DNA],[52] where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t >100 s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.12 μg) in TE buffer was treated with different Co(II) complexes(10 μM); and in the presence of hydrogen peroxide(100 μM) in the reaction solution. The samples were incubated for 30 min at 37 °C, then a loading buffer containing 10 mM TE (pH 7.5), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA was added and electrophoresis was performed at 100 V for 2 h in TAE buffer using 1.0% agarose gel containing 1.0 mg/mL ethidium bromide. Bands were visualized by UV light and photographed on a capturing system (AlphaDigiDocTM RT. Version V.4.1.0 PC-Image software).

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