Polyhedron 29 (2010) 1918-1924

Contents lists available at ScienceDirect

Polyhedron



journal homepage: www.elsevier.com/locate/poly

Synthesis, characterization and biological studies of some homodinuclear complexes of zinc with second-generation quinolone drug and neutral bidentate ligands

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ARTICLE INFO

Article history: Received 23 August 2009 Accepted 4 March 2010 Available online 15 March 2010

Keywords: Ciprofloxacin DNA-binding Cleavage Minimum inhibitory concentration

ABSTRACT

The novel homodinuclear zinc(II) complexes with the quinolone antibacterial drugs ciprofloxacine and neutral bidentate ligands have been synthesized and characterized by elemental analysis, TG analyses and various spectroscopic techniques. The metal ion exhibits octahedral geometry with two water molecule in the inner sphere cavity environment. The interaction of complexes with DNA was determined using absorption titration, viscosity measurements and electrophoresis technique. The intrinsic binding constants (K_b) of complexes were determined, which were ranging from 1.0×10^4 to 3.5×10^4 per mole. Suggesting that complexes bind more strongly to DNA. Effect on viscosity has also been checked to authenticate the binding of metal complexes with DNA. An antimicrobial activity of all the ligands and metal complexes has been examined by minimum inhibitory concentration method (MIC).

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1. Introduction

Quinolones are a large and constantly expanding group of synthetic antibacterial agents [1,2], and ciprofloxacin (CPFL) is one of the most popular members of this family (Fig. 1). It is a synthetic, broad-spectrum antibacterial drug that is used to treat pneumonia, bronchitis and some types of gonorrhea, diarrhea caused by bacteria, typhoid fever, prostate, sinus and urinary tract infections [3,4]. It is active against DNA gyrase enzyme. DNA gyrase introduces negative supercoils in DNA [5] by wrapping the DNA around the enzyme. The enzyme then catalyzes the breakage of a segment of the wrapped DNA, the passage of a segment of the same DNA through the break and finally the relegation of the break [6]. In this way, a knot of DNA is resolved and is exposed for replication.

The design of drug based metal complexes is of particular interest in pharmacological research. Metal combinations with pharmaceutical agents are known to improve drug activity and to decrease their toxicity. Quinolone antibiotics are chelating agents for a variety of metal ions. The coordinated metal ions play an important role in maintaining proper structure and/or function. The interaction of quinolone with metal ions and the biologically active complexes formed as a result of this interaction are especially important [7,8]. Although reports indicate that the coordination of quinolones to metal ions appears to be important for the activity of quinolone antibiotics [9–11], it has detrimental effect on their absorption [12-16].

The role of zinc in various biological systems is important, since it is crucial for numerous cell processes and is a major regulatory ion in the metabolism of cells [17,18]. In the literature, different zinc complexes with biological activity have been reported, but only zinc complexes with drugs are used for the treatment of Alzheimers disease [19], while others show antibacterial [20], anticonvulsant [21], antidiabetic [22], anti-inflammatory [23] and antimicrobial activity [24].

Here, we present the interaction of Zn²⁺ with a second-generation quinolone, ciprofloxacin, and bidentate ligands in an attempt to examine the mode of coordination and biological properties of the prepared complexes. More specifically, the complexes have been synthesized and characterized using diverse spectroscopic techniques (IR and mass spectroscopy). The biological activity of the complexes has been evaluated by determining the minimum inhibitory concentration (MIC) against five microorganisms. To this aim, we have also investigated the DNA binding assay of the Zn(II) complexes with ciprofloxacin and some neutral bidentate ligands using a combination of spectroscopic (UV-Vis), hydrodynamic (viscometric) and gel electrophoresis techniques.

2. Experimental

2.1. Materials and methods

Ciprofloxacin hydrochloride (CPFL·HCl) was purchased from Bayer AG (Wuppertal, Germany). Ethylenediamine (A⁶-en), 2,3butanedione, p-anisaldehyde, p-anisidine, 1,8-diaminonaphthaline



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^{0277-5387/\$ -} see front matter © 2010 Elsevier Ltd. All rights reserved. doi:10.1016/j.poly.2010.03.007



Fig. 1. 4-(3-Carboxy-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)piperazin hydrochloride [CPFL·HCL].

 $(A^4$ -*dan*), acetophenone, glycerol and zinc(II) acetate 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 the Sisco Research Lab., India. Bromophenol blue, *o*-phenylenediamine (A^5 -*opd*), acetic acid and EDTA were purchased from Sd Fine Chemicals, India. Sperm herring DNA was purchased from Sigma Chemical Co., India. All the chemicals and solvents used were of analytical grade.

2.2. Instrumentation

C, H and N elemental analyses were performed with a model Perkin Elmer 240 elemental analyzer. The metal contents of the complexes were analyzed by EDTA titration [25] after decomposing the organic matter with a mixture of HClO₄, H₂SO₄ and HNO₃ (1:1.5:2.5). IR spectra (400–4000 cm⁻¹) were recorded on a FT-IR Shimadzu spectrophotometer with the samples prepared as KBr pellets. Thermogravimetric analyses were obtained with a model 5000/2960 SDTA, TA instrument (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 FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The electronic spectra were recorded on a UV-160A UV–Vis spectrophotometer, Shimadzu (Japan)

2.3. Synthesis of the Schiff bases

The neutral bidentate ligands $A^1(bmpdme)$, $A^2(bmbed)$ and $A^3(bpebd)$ were synthesized and characterized as per the methodology previously reported by us [26] (Supplementary data 1: Structure of the ligands used).

Table 1

Experimental and physical parameters of the complexes.

2.4. Synthesis of the coordination compounds

 $[Zn_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2]\cdot 3H_2O$ (**I**). A methanolic solution (50 mL) of $Zn(OAc)_2\cdot H_2O$ (2.01 g, 10 mmol), was added to warm methanolic solution (50 mL) of *bmpdme* (2.96 g, 10 mmol), followed by addition of a previously prepared solution of CPFL·HCl (3.67 g, 10 mmol) in water (20 mL); the pH of the solution was adjusted to 6.0–7.0 using dilute NaOH solution. During the reaction, the piperazine ring of ciprofloxacin hydrochloride was substituted by a chloride ion in the presence of NaOH [27]. The resulting solution was refluxed for 8–10 h on a steam bath, and then was kept overnight at room temperature. A fine amorphous product was obtained which was washed with ether and dried in a vacuum desiccator.

In a similar way, complexes **II–VI** were prepared with the use of the corresponding ligands (Supplementary data 2: Proposed structures of the synthesized complexes). The physical parameters of all the complexes are shown in Table 1 and the proposed reaction is shown in Scheme 1.

2.5. Antibacterial activity - minimum inhibitory concentration

The antibacterial activity of the compounds (ligand and complexes) was studied against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia merscences*. All bacterial species were incubated and activated at 37 °C for 24 h by inoculating them to 2% Luria Broth (w/v) in double distilled water. The compounds were dissolved in DMSO and then diluted using cautiously adjusted Luria broth. The MIC was determined using twofold serial concentrations in liquid media containing 12 000–0.1 μ M of the compound being tested. Test cultures were incubated at 37 °C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of microorganisms were represented as the MIC in μ M. In each case triplicate tests were performed and an average was taken as the final reading [28].

2.6. DNA binding and cleavage experiments

2.6.1. UV–Vis spectroscopy

The concentration of DNA was measured by using its standard extinction coefficient at 260 nm ($6600 \text{ M}^{-1} \text{ cm}^{-1}$) [29]. Absorbances at 260 nm (A_{260}) and 280 nm (A_{280}) were measured to check the purity of the DNA. The ratio of A_{260} to A_{280} was found to be 1.8, indicating that the DNA was satisfactorily free from protein. Phosphate buffer (1 mM, pH 7.2) was used for the absorption titration experiment. The absorption titration was carried out by varying the DNA concentration ($0-20 \ \mu\text{M}$) and maintaining a constant concentration of the complex (4 μ M). Absorption spectra were recorded after each successive addition

Complexes empirical formula	Elemental analysis % found (required)			m.p. (°C)	% Yield	Formula weight (g/mol)	
	С	Н	Ν	М			
$C_{66}H_{74}Cl_2F_2N_8O_{15}Zn_2$ (I) [Zn ₂ (Cip) ₂ (bmpdme) ₂ (pip)(H ₂ O) ₂]-3H ₂ O	54.39 (54.33)	5.14 (5.11)	7.64 (7.68)	8.95 (8.97)	312	68.4	1459.06
$C_{66}H_{74}Cl_2F_2N_8O_{15}Zn_2$ (II) [$Zn_2(Cip)_2(bmbed)_2(pip)(H_2O)_2$].3H ₂ O	54.35 (54.33)	5.10 (5.11)	7.65 (7.68)	8.93 (8.97)	330	69.0	1459.32
$C_{74}H_{74}Cl_2F_2N_8O_{11}Zn_2$ (III)	59.58 (59.60)	4.98 (5.00)	7.49 (7.51)	8.75 (8.77)	280	66.5	1479.09
$[2I_{2}(CIp)_{2}(Dpebd)_{2}(pIp)(H_{2}O)_{2}]\cdot 3H_{2}O$ $C_{50}H_{54}Cl_{2}F_{2}N_{8}O_{11}Zn_{2}$ (IV) $[Zn_{2}(Cip)_{2}(dan)_{2}(pip)(H_{2}O)_{2}]\cdot 3H_{2}O)$	50.77 (50.78)	4.58 (4.60)	9.45 (9.47)	11.04 (11.06)	>360	67.8	1182.73
$C_{42}H_{50}Cl_2F_2N_8O_{11}Zn_2(\mathbf{V})$ $[Zn_2(Cip)_2(opd)_2(pip)(H_2O)_2].3H_2O_2$	46.59 (46.60)	4.65 (4.66)	10.34 (10.35)	12.05 (12.08)	356	68.2	1082.62
$\begin{array}{l} (2n_2(Cip)_2(0pa)_2(pp)_1(n_2o)_2) & 5n_2o\\ C_{34}H_{50}Cl_2F_2N_8O_{11}Zn_2 \ (\textbf{VI}) \\ [Zn_2(Cip)_2(en)_2(pip)(H_2O)_2]\cdot 3H_2O \end{array}$	41.35 (41.39)	5.10 (5.11)	11.35 (11.36)	13.24 (13.26)	300	65.7	986.53
$ \begin{split} & [2n_2(cqp)_2(bripdant_2)_2(p)(P_12O_2)^{-3}P_2O_2\\ & C_{66}H_7_4Cl_2F_2N_8O_{15}\ Zn_2\ (II)\\ & [Zn_2(cip)_2(bribed)_2(pip)(H_2O)_2]^{-3}H_2O\\ & C_{74}H_7_4Cl_2F_2N_8O_{11}Zn_2\ (III)\\ & [Zn_2(Cip)_2(bpebd)_2(pip)(H_2O)_2]^{-3}H_2O\\ & C_{50}H_54Cl_2F_2N_8O_{11}Zn_2\ (IV)\\ & [Zn_2(Cip)_2(dan)_2(pip)(H_2O)_2]^{-3}H_2O)\\ & C_{42}H_{50}Cl_2F_2N_8O_{11}Zn_2\ (V)\\ & [Zn_2(cip)_2(opd)_2(pip)(H_2O)_2]^{-3}H_2O\\ & C_{34}H_{50}Cl_2F_2N_8O_{11}Zn_2\ (V)\\ & [Zn_2(Cip)_2(en)_2(pip)(H_2O)_2]^{-3}H_2O \end{split} $	54.35 (54.33) 59.58 (59.60) 50.77 (50.78) 46.59 (46.60) 41.35 (41.39)	5.10 (5.11) 4.98 (5.00) 4.58 (4.60) 4.65 (4.66) 5.10 (5.11)	7.65 (7.68) 7.49 (7.51) 9.45 (9.47) 10.34 (10.35) 11.35 (11.36)	8.93 (8.97) 8.75 (8.77) 11.04 (11.06) 12.05 (12.08) 13.24 (13.26)	330 280 >360 356 300	69.0 66.5 67.8 68.2 65.7	1459.32 1479.09 1182.73 1082.62 986.53



[Zn₂(Cip)₂(bmpdme)₂(pip)(H₂O)₂]·3H₂O

Scheme 1. Proposed reaction scheme for synthesis of complex I.

of DNA followed by allowing it to attain equilibrium (approximately 10 min). For complexes I-VI, the observed data were further utilized to obtain the intrinsic binding constant, K_b using the following equation [30]:

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

where [DNA] is the concentration of DNA in terms of nucleotide phosphate, [NP] is the apparent absorption coefficient $\varepsilon_{\rm fr}$, $\varepsilon_{\rm a}$ and $\varepsilon_{\rm b}$ 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 yintercept.

2.6.2. Viscosity measurements

Viscosity measurements were carried out using an Ubbelohde 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. The flow time was measured with a digital stopwatch with a precision of ±0.1 s. Each sample was measured thrice and an average flow time was calculated. Data are presented as $(\eta/\eta_0)^{1/3}$ versus concentration ratio [Zn]/[DNA] [31], 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 the buffer alone (t_0), $\eta = t - t_0$.

2.6.3. Gel electrophoresis study

pUC19 DNA was prepared by transformation of pUC19 into safe competent cells (*E. coli* strain), and amplification of a clone as outlined by Sambrook and Russell [32]. After concentrating by ethanol precipitation, the DNA was stored in TE buffer (pH 8.0) at -20 °C. The relative amount of the supercoiled (SC) form was checked by gel electrophoresis on an agarose bed. Electrophoresis was carried out in a Submarine Mini-gel Electrophoresis Unit, also the degree of DNA cleavage activity was expressed in terms of the percentage of cleavage of the SC-DNA according to the following equation [33]:

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

2.7. Aliquots of complex

DMSO containing the metal complexes (50 μ M) was taken in a clean eppendroff tube and TE buffer (pH 8.0), pUC19 (0.12 μ g ml⁻¹) was added. The contents were incubated for 1 h at 37 °C, loaded on 1% agarose gel after mixing of the loading buffer (0.03% Bromophenol blue + 0.03% xylene cyanol + 60% glycerol sterilized, distilled), 60 mM EDTA was added and electrophoresis was performed at 100 V for 2 h in TAE buffer using 1.0% agarose gel containing 0.5 μ g ml⁻¹ ethidium bromide until the bromophenol blue reached to 3/4 of the gel. A control experiment was done in the presence of the reactive substances Zn(II) and ciprofloxacin.

The plasmid band was visualized by viewing the gel under a transilluminator and photographed. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked circular (NC) DNA from its supercoiled (SC) form by quantitatively estimating the intensities of the bands, and the extent of cleavage was determined by using volume quantization AlphaDigiDoc[™] RT. Version V.4.1.0 PC-Image software.

3. Results and discussion

All the complexes were insoluble in ether, hexane, chloroform, water and methanol, partially soluble in dimethyl formamide, and completely soluble in dimethylsulfoxide. Schiff bases A^1-A^3 were prepared by condensation of the amine and aldehyde/ketone in ethanol. The complexes under investigation were characterized using IR spectra, FAB-mass spectra, TG analysis and electronic spectra.

3.1. IR spectra

The v(C=0) stretching vibration band appears at 1708 cm⁻¹ for ciprofloxacin, whereas for complexes it appeared at 1619-1630 cm⁻¹; this shift towards lower energy suggests that the coordination occurs through the carbonyl oxygen of the pyridine ring [34]. A sharp band in ciprofloxacin at 3520 cm^{-1} [35] is due to hydrogen bonding; which is attributed to the ionic resonance structure and the peak is observed because of a free hydroxyl stretching vibration. This band completely vanished in the spectra of metal complexes indicating deprotonation of the carboxylic proton. The data were further supported by a v(M-O) [36] band which appeared at 502-514 cm⁻¹. The strong absorption band obtained at 1624 and 1340 cm⁻¹ in ciprofloxacin were assigned to $v(COO)_{as}$ and $v(COO)_{s}$ respectively, while in the metal complexes these bands were observed at 1591 and 1386 cm⁻¹, respectively. The frequency of separation (Δv) for the carboxyl group in the investigated complexes is greater than 200 cm⁻¹ suggesting a unidentate bonding nature [34,37–41]. The v(C=N)peak for the Schiff bases A^1-A^3 was observed at 1610-1640 cm⁻¹ which on complexation was shifted to 1564– 1580 cm⁻¹, which indicates the N–N coordinating behavior [42– 45]. This data was further supported by v(M–N) [46] which appeared at 537–548 cm⁻¹. Infrared spectral data of the complexes are shown in Table 2.

3.2. Electronic spectral measurements

In the electronic spectra of the zinc(II) complexes there are $M \rightarrow L$ charge-transfer bands at ~30 800 cm⁻¹, which were assigned to the octahedral structure. Zn(II) being d^{10} system, with no unpaired electron, always exhibits a diamagnetic nature [47,48].

Table 2	
Infrared spectral data of the Zn(II) complexed	es.

3.3. TG analysis

TG curves of the Zn(II) complexes show 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 loss of water of crystallization. The second step weight loss, during 130–180 °C, corresponds to two coordinated water molecules. Loss in weight in the temperature range 180– 250 °C corresponds to a piperazine (pip) molecule followed by liberation of Cip 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 the metal oxide [49].

3.4. Mass spectrum

The FAB-mass spectrum of complex **I** (Fig. 2) shows the molecular ion peak at m/z 1406 due to [M+2H⁺] rid of three lattice water molecules. The peaks at m/z 1388 and 1370 are due to removal of coordinated water, one after another. The peaks occurring at m/z 641 and 726 correspond to the fragments [C₃₁H₂₈ClFN₃O₅Zn + H⁺] and [C₃₅H₃₆ClFN₅O₅Zn + H⁺], produced by cleavage of one of the Zn–N bonds with piperazine. The peak arising at m/z 296 corresponds to the ligand [C₁₈H₂₀N₂O₂], whereas the peak at m/z 344 corresponds to the fragment [C₁₃H₈ClFNO₃Zn] (Supplementary data 3: FAB-MS of Complexes **I–VI**).

3.5. Antimicrobial activity

The efficiencies of the ligands and the complexes have been tested against three Gram^(-ve), E. coli, S. merscences, P. aeruginosa, and two Gram^(+ve), S. aureus, B. subtilis, microorganisms. The results are presented in Table 3. From the experiment we found that all the metal complexes were more active than the metal salts and ligands. Complexes I, II and III have good activity against S. aureus compared to all standard drugs whereas complexes IV, V and VI were more active than gatifloxacin. In the case of *B. subtilis*, except for complex VI, all the complexes were more active than standard drugs. In the case of *S. merscences*, complex **V** had better activity than standard drugs whereas complexes I-IV were found to be more active than gatifloxacin, norfloxacin and pefloxacin. In the case of *P. aeruginosa*, complex I was the most potent among the six synthesized complexes, however the increasing order of their activity is I < III < II < V < VI < IV. In the case of E. coli the order of activity is III < II < I < V < IV < VI. The data shows that the metal complexes exhibit antimicrobial properties and it is important to note that these complexes exhibit enhanced activity in contrast to the free ligand. Enhanced antibacterial activity is because the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials, so liposolubility is an important factor controlling the antimicrobial activity [50].

It may be suggested that these complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more

Compounds	v(C=O) (cm ⁻¹) pyridone	$v(COO)_{as}$ (cm ⁻¹)	$v(COO)_s$ (cm ⁻¹)	Δv (cm ⁻¹)	v(C-Cl) (cm ⁻¹)	v(C==N) (cm ⁻¹) azomethine	v(M–N) (cm ⁻¹)	v(M-O) (cm ⁻¹)
I	1619	1591	1386	205	1130	1567	539	511
П	1625	1600	1380	220	1141	1564	537	508
Ш	1630	1610	1381	229	1125	1580	548	514
IV	1621	1587	1376	211	1128	-	540	509
v	1625	1598	1385	213	1126	-	538	505
VI	1622	1596	1372	224	1124	-	541	502



Fig. 2. FAB-mass spectrum of complex I.

Table 3 MIC data of the compounds (μM).

Compounds	Gram positive		Gram negative		
	S. aureus	B.	S.	Р.	E. coli
		subuus	merscences	aeruginosa	
Zn(OAc) ₂ ·H ₂ O	1373.4.00	1099.00	2198.00	1923.00	1923.00
Ciprofloxacin	1.63	1.09	1.63	1.36	1.36
Gatifloxacin	5.06	4.00	2.93	1.01	2.93
Norfloxacin	2.51	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	1.66	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	1856.00	1519.00	2025.00	2193.00	2362.00
A ²	1687.00	1518.00	1687.00	1856.00	2193.00
A ³	1761.00	1600.00	1761.00	1921.00	2080.00
A ⁴	3161.00	3477.00	4109.00	3793.00	4109.00
A ⁵	5086.00	5548.00	6011.00	6011.00	6473.00
A ⁶	8319.00	9151.00	9983.00	10815.00	10815.00
I	0.55	0.55	2.06	1.37	2.74
II	0.34	0.34	2.06	1.71	2.06
III	0.54	0.34	2.01	1.68	1.68
IV	2.54	0.85	2.96	2.54	2.96
V	3.23	0.92	0.92	1.85	2.77
VI	3.55	1.52	3.04	2.53	3.04

proteins of the cell, which as a result, impairs normal cellular processes. The following five principal factors [51–54] should be considered for metal complexes showing antimicrobial activity, (i) the chelate effect, i.e. ligands that are bound to metal ions in a bidentate fashion, like quinolones and NN-donor ligands; (ii) the nature of the ligands; (iii) the total charge of the complex; generally the antimicrobial efficiency decreases in the order cationic > neutral > anionic complex; (iv) the nature of the ion neutralizing the ionic complex; (v) the nuclearity of the metal center in the complex; dinuclear centers are usually more active than mononuclear ones. Thus, factors (i), (ii) and (v) can be considered to increase in the antibacterial activity in this study.

3.6. DNA binding studies

3.6.1. Absorption titration

Increasing amounts of DNA were titrated with preset amounts $(4 \mu M)$ of the complexes. The electronic spectral traces are given

in Fig. 3. For the complexes, the absorption spectra clearly show that addition of DNA to the complexes yields hyperchromism and a blue shift corresponding to the ratio of [DNA]/[*M*]. Obviously, these spectral characteristics suggest that all the complexes interact with DNA, most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA. Addition of increasing amounts of DNA resulted in hypso-chromism of the peak maxima in the UV–Vis spectra of the binuclear zinc(II) complexes.

An electronic interaction between the complex chromophore and DNA bases meets one of the criteria for intercalative binding [55]. Using the absorption measurements for **I**, a linear plot of [DNA]/($\varepsilon_{\rm f} - \varepsilon_{\rm a}$) versus [DNA] (Fig. 3, inset) was obtained. Assuming all the complexes were bound with DNA, the experimental K_b was obtained by substituting the absorbance into Beer's law. The binding constants (K_b) are given in Table 4. The binding constant K_b for the Zn(II) complexes varies in the range $1.0-3.5 \times 10^4 \,\mathrm{M^{-1}}$, which is in good agreement with that determined experimentally. The DNA-binding constant of the title complexes are comparable to



Fig. 3. Absorption spectra of $[Zn_2(Cip)_2(bmpdme)_2(pip)(H_2O)_2]\cdot 3H_2O$ (4 μ M) in the absence and in the presence of increasing amounts of DNA, where *a* to *h* represents the increasing amount of DNA, inset: plot of $[DNA]/(\varepsilon_f - \varepsilon_a)$ vs. [DNA]. Arrow shows the absorption change upon increasing the DNA concentration.

Table 4	
The binding constants (K_b) of Zn(II) complexes with DNA in phosphate buffer pH	17.2.

• • • • • •	
Complexes	$K_b (\mathrm{M}^{-1})$
I II III	$\begin{array}{c} 1.0 \times 10^{4} \\ 3.5 \times 10^{4} \\ 1.0 \times 10^{4} \end{array}$
IV V VI	$\begin{array}{c} 1.0 \times 10^{4} \\ 2.0 \times 10^{4} \\ 2.0 \times 10^{4} \end{array}$

those of some DNA intercalative polypyridyl Ru(II) complexes, 1.0– 4.8 × 10⁴ M⁻¹ [56–58]. In the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], the binding constant K_b is given by the ratio of the slope to the intercept.

3.6.2. Viscosity measurements

The binding of intercalative drugs to DNA has also been characterized recently through the changes in viscosity of DNA. Fig. 4 summarizes the relative viscosity (η_0) of DNA in the absence and presence of the complexes. The viscosity measurements were carried out by varying the concentration of the complexes. The intercalation of drugs with DNA usually causes an increase in the viscosity of DNA [59]. The viscosity experiment displays that the relative viscosity (η_0) of DNA increased with an increasing concentration of the complexes, but the extent of increase was lesser than that for the known intercalator EB [60]. For all the complexes, as the amounts of complexes increased, the viscosity of DNA increased steadily, which is similar to that of the classical intercalative complex $[Ru(phen)_2(DPPZ)]^{2-}$. From Fig. 4 we can say that complexes I, II and VI intercalate more strongly than complexes III, IV and V. This indicates that I, II and VI bind to DNA more strongly than the **III**, **IV** and **V**. Based on the above experiments, the results clearly indicate the different ability of the ligands in the all complexes to stack and overlap with the base pairs.

3.6.3. Gel analysis and quantification of pUC19 DNA

There has been considerable interest in DNA cleavage reactions that are activated by transition metal complexes [61,62]. The delivery of the metal ion to the helix, locally generating oxygen or hydroxide radicals, yields an efficient DNA cleavage reaction. Figs. 5 and 6 illustrate electrophoretic separations presenting the cleavage of plasmid pUC19 DNA by the complexes under aerobic conditions in the presence and absence of H_2O_2 , respectively [63]. 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 form 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 the



Fig. 4. Change in viscosity of DNA on addition of the complexes.



Fig. 5. Gel electrophoresis data with pUC19. Lane 1: DNA (control); Lane 2: DNA + metal salt; Lane 3: DNA + CPFL-HCl; Lane 4: DNA + **I**; Lane 5: DNA + **II**; Lane 6: DNA + **II**; Lane 7: DNA + **IV**; Lane 8: DNA + **V**; Lane 9: DNA + **V**I.



Fig. 6. Gel electrophoresis data with pUC19. Lane 1: DNA (control); Lane 2: DNA + H_2O_2 ; Lane 3: DNA + H_2O_2 + metal salt; Lane 4: DNA + H_2O_2 + CPFL; Lane 5: DNA + H_2O_2 + **I**; Lane 6: DNA + H_2O_2 + **II**; Lane 7: DNA + H_2O_2 + **II**; Lane 8: DNA + H_2O_2 + **IV**; Lane 9: DNA + H_2O_2 + **V**; Lane 10: DNA + H_2O_2 + **V**I.

Table 5		
Gel electro	phoresis	data

Compounds	% SC	% NC	% OC	% Cleavage
DNA control	58	31	11	0
DNA + metal salt	49	39	12	15.52
DNA + CPFL·HCl	44	32	24	24.14
DNA + I	48	35	24	17.24
DNA + II	47	42	11	18.97
DNA + III	48	41	11	17.24
DNA + IV	47	39	15	18.97
DNA + V	48	41	11	17.24
DNA + VI	48	39	13	17.24
$DNA + H_2O_2$	35	24	41	39.66
DNA + H ₂ O ₂ + metal salt	34	26	40	41.38
DNA + H ₂ O ₂ + CPFL·HCl	31	30	39	46.55
$DNA + H_2O_2 + I$	42	47	11	27.59
$DNA + H_2O_2 + II$	44	48	18	24.14
$DNA + H_2O_2 + III$	42	46	12	27.59
$DNA + H_2O_2 + IV$	45	43	12	22.41
$DNA + H_2O_2 + V$	47	41	12	18.97
$DNA + H_2O_2 + VI$	46	40	14	20.69

metal salt itself (Table 5). The different DNA cleavage efficiencies of the complexes is due to the different binding affinity of the complexes to DNA, which has been observed in other cases. One of the most interesting electrophoretic results of the complexes takes place when the experiment was carried out in the presence of H_2O_2 in TAE buffer. The DNA + complex + H_2O_2 systems (Fig. 6) cleaved the supercoiled DNA form (I) and converted it into the nicked form (II) and linear form (III) more than just the complex alone. It can be concisely seen from the data that the mixture of complex with H_2O_2 proves to be an efficient cleaving agent via the electrostatic mode of interaction [64].

4. Conclusion

The antimicrobial activity shows that the complexes have been found to be more potent against $Gram^{(+ve)}$ than $Gram^{(-ve)}$ species. The DNA-binding constants of the title complexes varies in the

range $1.0-3.5 \times 10^4 \,\text{M}^{-1}$, which are comparable to the values of some DNA intercalative polypyridyl Ru(II) complexes, 1.0- $4.8 \times 10^4 \,\text{M}^{-1}$. All the complexes exhibit good binding and cleavage activity. Complexes I, II and VI intercalate more strongly than complexes III, IV and V. This indicates that complexes I, II and VI bind to DNA strongly than complexes III, IV and V. The mixture of the complexes with H₂O₂ have been found to be efficient oxidants via electrostatic interaction by generating more strand breaks in plasmid DNA compare to the complexes alone.

Acknowledgments

We wish to express our gratitude to the Head, Department of Chemistry, Vallabh Vidyanagar, Gujarat, India for providing necessary laboratory facilities. The authors are also thankful to the UGC for financial assistance of UGC grant 32-226/2006(SR).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.poly.2010.03.007.

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