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DNA-binding, antibacterial and spectral investigations of drug–Fe(II) complexes

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The antibiotic agent ciprofloxacin is well known for its drug design and coordinating ability towards metal ions. Iron(II) complexes of ciprofloxacin with various neutral bidentate ligands have been prepared. The structure of complexes has been investigated using spectral, physicochemical and elemental analyses. Antibacterial activity has been carried out using agar plate technique against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Serratia marcescens*. The results show a significant increase in antibacterial activity compared with parental ligands, metal salt and standard drugs (ofloxacin, levofloxacin). The DNA binding and cleavage efficacy were determined using absorption titration and gel electrophoresis techniques, respectively. The DNA binding and cleavage efficacy were increased in complexes compared with parental ligands and metal salt. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: iron–ciprofloxacin complexes; antibacterial activity; DNA-binding; absorption titration

INTRODUCTION

Fluoroquinolones are known for their wide-ranging applications in medicinal and life sciences.[1] Quinolones are known to have antimicrobial and complexation properties.[2–5] The mechanism of fluoroquinolone action involves intercalation of purine/pyrimidine of nucleic acids and inhibition of DNA gyrase, which is important for DNA replication.[6] The mechanism also involves the formation of metal complex as an intermediate.[7,8] It has also been proposed that the transport of ligands into cells can be facilitated by the formation of metal complex.[9] Several metal complexes are well known for their antibacterial, antifungal and biomimetic activities.[10–15] In continuation of earlier work,[16,17] it was planned to prepare the iron(II) complexes of ciprofloxacin with various neutral bidentate ligands and to determine their antibacterial activity, DNA binding and cleavage efficacy.

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade. Aniline, anthranilic acid, acetophenone, acetic anhydride, 2,3-butanedione, *p*-anisidine, benzil, benzaldehyde, benzoyl chloride, hydrazine hydrate and ferrous sulfate were purchased from the E. Merck (India) Limited, Mumbai. Ciprofloxacin hydrochloride was purchased form Bayer AG (Wuppertal, Germany). 1,8-Diaminonaphthalene was purchased from Lancaster, England. Luria broth and agar-agar ware purchased from SRL, India. Sperm herring DNA, sucrose, bromophenol blue, xylene cyanol FF, agarose, acetic acid and EDTA were purchased from Sigma Chemical Co., India. The organic solvents were purified by recommended methods.[18]

Preparation of ligands

The neutral bidentate ligands were synthesized according to reported methods.[17,19,20] Structures of ligands A^1–A^8 are shown in Scheme 1.

N,N′-Dicyclohexylidene-naphthalene-1,8-diamine (A^1 = dcnd)

An ethanolic solution (100 ml) of cyclohaxanone (1.96 g, 20 mmol) was added to ethanolic solution (100 ml) of 1,8-diamino naphthalene (1.58 g, 10 mmol). The mixture was stirred continuously for 4 h to obtain a fine yellow crystalline product. The crystalline product obtained was washed with *n*-hexane. The product was recrystallized in ethanol and dried in air. Yield: 68%; m.p. 135 °C; found (%): C, 83.00, H, 8.09, N, 8.74. $C_{22}H_{26}N_2$ (318.45) requires (%): C, 82.97, H, 8.23, N, 8.80. IR: 1600 (C═N), 1570 (C═C); ^{1}H NMR: 6.50–7.28 (6H,

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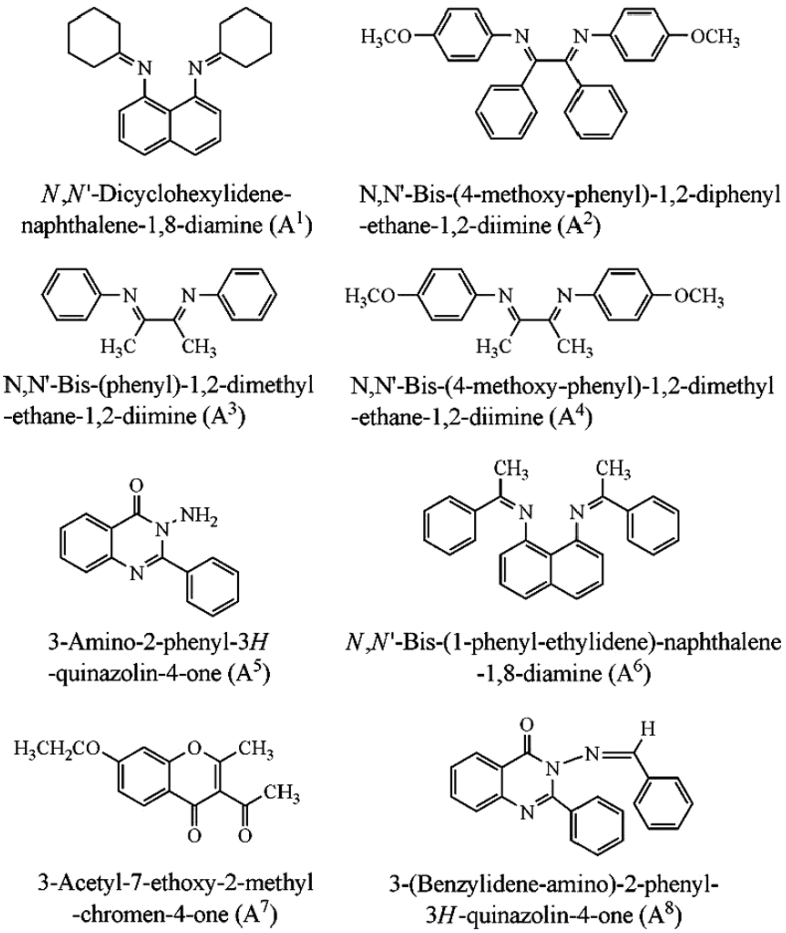

Scheme 1. Structures of ligands A^1–A^8.

m, Ar–H), 1.51–2.37 (20H, m, Al–H); ^{13}C NMR: 105.9–134.7 (Ar–C), 173.0 (C═N), 140.0 (C–N), 22.3 (Al–C), 25.3 (Al–C), 36.9 (Al– C).

N,N′-*Bis-(4-methoxy-phenyl)-1,2-diphenyl-ethane-1,2-diimine (A^2 = bmpded)*

An ethanolic solution (100 ml) of benzil (2.10 g, 10 mmol) and *p*-anisidine (2.46 g, 20 mmol) was refluxed over a water bath for 24 h, concentrated up to one-third its volume and kept overnight over a sulfuric acid desiccator. The product obtained was filtered, recrystallized in ethanol and washed with 1 : 1 absolute ether : hexane. Yield: 64%; m.p.: 120 °C; found (%): C, 79.86, H, 5.78, N, 6.70. $C_{28}H_{24}N_2O_2$ (420.50) requires (%): C, 79.98, H, 5.75, N, 6.66. IR: 1601 (C═N), 1574 (C═C); ^{1}H NMR: 6.65–7.91 (18H, m, Ar–H), 3.72 (6H, s, OCH_3); ^{13}C NMR: 113.9–134.4 (Ar–C), 157.3 (C–O), 165.4 (C═N), 135.4 (C–N), 55.3 (OCH_3).

N,N′-*Bis-(phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^3 = bpdmed)*

An ethanolic solution (100 ml) of aniline (1.86 g, 20 mmol) was added drop-wise to ethanolic solution (100 ml) of 2,3-butanedione (0.86 g, 10 mmol) and refluxed over a water bath for 8 h. The resulting mixture was filtered. The crystalline yellow product obtained was recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 58%; m.p.: 114 °C; found (%): C, 81.49, H, 6.71, N, 11.69. $C_{16}H_{16}N_2$ (236.31) requires (%): C, 81.32, H, 6.82, N, 11.85. IR: 1613 (C═N), 1572 (C═C); ^{1}H NMR: 6.81–7.42 (10H, m, Ar–H), 2.19 (6H, s, Al–H); ^{13}C NMR: 118.8–129.0 (Ar–C), 168.3 (C═N), 150.9 (C–N), 15.4 (Al– C).

N,N′-*Bis-(4-methoxy-phenyl)-1,2-dimethyl-ethane-1,2-diimine (A^4 = 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 over a water bath for 8 h. The resulting mixture was filtered and the crystalline yellow product obtained was recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 56%; m.p.: 170 °C; found (%): C, 72.83, H, 6.64, N, 9.48. $C_{18}H_{20}N_2O_2$ (296.36) requires (%): C, 72.95, H, 6.80, N, 9.45. IR: 1610 (C═N), 1570 (C═C); ^{1}H NMR: 6.67–6.86 (8H, m, Ar–H), 2.07 (6H, s, Al–H), 3.73 (6H, s, OCH_3); ^{13}C NMR: 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).

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3-Amino-2-phenyl-3H-quinazolin-4-one (A^5 = apq)

The solution of anthranilic acid (1.37 g, 0.1 mol) was prepared in pyridine (100 ml) and followed by addition of benzoyl chloride (2.814 g, 0.2 mol). The resulting mixture was stirred for 0.5 h., and finally treated with 5% $NaHCO_3$ (15 ml). The separated solid was crystallized in ethanol. Yield: 80%; m.p.: 120 °C. The obtained 2-phenyl-3,1-benzoxazin-4-one (0.557 g, 0.05 mol) in ethanol (50 ml) and hydrazine hydrate (0.125 g, 0.05 mol) in ethanol (50 ml) were mixed and refluxed for 3 h. The obtained product was crystallized in ethanol. Yield: 85%; m.p.: 196 °C; found (%): C, 70.67, H, 4.62, N, 17.59. $C_{14}H_{11}N_3O$ (237.26) requires (%): C, 70.87, H, 4.67, N, 17.71. IR: 1680 (C═O), 1590 (C═N), 1545 (C═C); ^{1}H NMR: 7.28–8.19 (9H, m, Ar–H); ^{13}C NMR: 127.02–133.02(Ar–C), 164.7.6(C═O), 165.3 (C═N, ring), 145.5 (C– N).

N,N′-Bis-(1-phenyl-ethylidene)-naphthalene-1,8-diamine (A^6 = bpend)

An ethanolic solution of (100 ml) 1,8-diaminonaphthalene (10 mmol, 1.58 g) was added to an ethanolic solution (100 ml) of acetophenone (20 mmol, 2.34 g) and refluxed over a water bath for 8 h, then kept overnight in a refrigerator. The resulting mixture was filtered. The crystalline yellow product obtained was further recrystallized in ethanol, washed with *n*-hexane and dried in air. Yield: 56%; m.p.: 242 °C; found (%): C, 86.20, H, 6.31, N, 7.69. $C_{26}H_{22}N_2$ (362.46) requires (%): C, 86.15, H, 6.12, N, 7.73. IR: 1630 (C═N), 1575 (C═C); ^{1}H NMR: 7.24–7.67 (16H, m, Ar–H), 2.50 (6H, s, Al–H); ^{13}C NMR: 119.39–136.45 (Ar–C), 172.4 (C═N), 148.9 (C–N), 16.8 (Al– C).

3-Acetyl-7-ethoxy-2-methyl-chromen-4-one (A^7 = aemc)

The β-resacetophenone (10 g, 0.065 mol) was heated with fused sodium acetate (10 g) in acetic anhydride (20 ml) for 3 h at 150–160 °C under anhydrous conditions. The reaction mixture was poured over crushed ice, stirred and left overnight. The separated product was filtered and washed with water. The obtained 3-acetyl-7-ethoxy-2-methyl-chromen-4-one was crystallized in ethanol. Yield: 56%; m.p.: 127 °C; found (%): C, 68.34, H, 5.80, O, 26.09. $C_{14}H_{14}O_4$ (246.26) requires (%): C, 68.28, H, 5.73, O, 25.99. IR: 1680 (C═O), 1660 (C═O), 1550 (C═C); ^{1}H NMR: 7.17–8.24 (3H, m, Ar–H), 1.64 (3H. t, Al–H), 2.38 (3H. s, Al–H), 2.35 (2H. m, Al–H), 2.65 (2H. m, OCH_2); ^{13}C NMR: 110.7–127.4 (Ar–C), 154.7 (C–O), 155.8 (C–O), 168.5 (C–O), 175.2 (C═O), 19.79 (Al–C), 21.18 (Al–C), 32.25 (Al–C), 63.25 (OCH_2), 200.4 (C═ O).

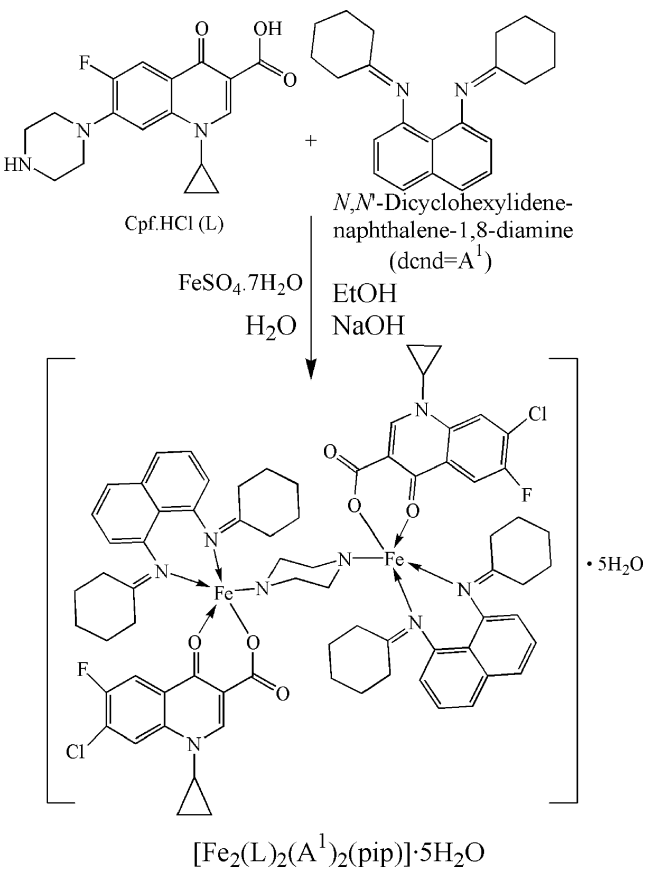
3-(Benzylidene-amino)-2-phenyl-3H-quinazolin-4-one (A^8 = bapq)

A methanolic solution of (100 ml) benzaldehyde (0.106 g, 0.01mol) was added to a methanolic solution (100 ml) of 3-amino-2-phenyl-3H-quinazolin-4-one (0.237 g, 0.01 mol). The mixture was refluxed over a water bath for 3 h, excess solvent was then removed under reduced pressure and it was kept overnight at room temperature. The resulting mixture was filtered and the obtained product was recrystallized in ethanol, and dried in air. Yield: 60%; m.p.: 180 °C; found (%): C, 77.60, H, 4.72, N, 13.03. $C_{21}H_{15}N_3O$ (325.36) requires (%): C, 77.52, H, 4.65, N, 12.91. IR: 1680 (C═O), 1618 (C═N), 1584 (C═N), 1564 (C═C); ^{1}H NMR: 7.24–8.20 (14H, m, Ar–H), 8.06 (1H, s, CH═N); ^{13}C NMR: 120.8–134.5 (Ar–C), 166.1 (C═O), 163.6 (C═N, ring), 138.8 (C–N), 164.1 (CH═ N).

Preparation of complexes

*$[Fe_2(L)_2(A^1)_2(pip)]\cdot 5H_2O$ (**I**)*

A methanolic solution (100 ml) of $FeSO_4 \cdot 7H_2O$ (2.78 g, 10 mmol) was added to methanolic solution (100 ml) of dcnd(A^1) (3.18 g, 10 mmol), followed by addition of a previously prepared solution (100 ml) of Cpf·HCl (3.67 g, 10 mmol) in water; the pH was adjusted to 4.5–6.0 pH with dilute NaOH solution. The resulting red solution was refluxed for 5 h., and then heated over a steam bath to evaporate up to half of the volume. The reaction mixture was kept overnight at room temperature. A fine colored crystalline product was obtained. The obtained product was washed with ether and dried over a vacuum desiccator. The reaction scheme is shown in Scheme 2.



Scheme 2. Synthesis of $[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$.

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Compounds **II–VIII** were prepared according to same method and their physicochemical parameters are summarized in Table 1.

Structural investigation

Thermogravimetric analyses and differential scanning calorimetric studies were carried out with a model 5000/2960 SDTA, TA instrument (USA). Infrared spectra were recorded on an FT-IR instrument. The ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Avance (400 MHz). Carbon, hydrogen and nitrogen elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The diffuse reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO disks) on a Beckman DK-2A spectrophotometer. The magnetic moments were measured using Gouy's method with 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.[21] The metal contents of the complexes were analyzed by EDTA titration[22] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1 : 1.5 : 2.5). Absorption titration was carried out using a Shimadzu UV–vis spectrophotometer. All the complexes were insoluble in water, methanol and dimethyl formamide, but were soluble in dimethyl sulfoxide.

Biocidal activity assay

A stock solution of 2.5 ppm was prepared by dissolving 0.25 mg of each complex in 5% DMSO solution. The biocidal test was screened by minimal inhibitory concentration (MIC). MIC was determined with the help of the progressive double-dilution method[23,24] in liquid media containing 1–50 ppm of the compound being tested. All the compounds were more effective with the MIC value at 2.5 ppm ≈2.5 μg/ml. The biocidal activities of the ofloxacin, levofloxacin, flucanozole, ligands, metal salts and their complexes were analyzed against various Gram-negative and Gram-positive bacterial cultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Serratia marcescens using* the Agar-plate technique.[16,25]

DNA-binding efficacy

Absorption titration

The DNA binding affinity study was performed on a Shimadzu UV–vis spectrophotometer. Absorption titration of compounds in DMSO, and the whole system in buffer (phosphate, pH 7.2), was done by keeping a fixed amount of iron compounds (where compound: **I** = 14.84, **II** = 16.88, **III** = 13.19, **IV** = 14.39, **V** = 13.21, **VI** = 15.72, **VII** = 13.35, **VIII** = 14.97 μg) and a variable amount of DNA, i.e. 0–6 μg, and the overall volume was maintained at 5 ml. Compound–DNA solutions were employed to record absorption spectra.

Gel analyses and quantification

The inspections of supercoiled pBR322 were carried out in TAE [tris(hydroxymethyl)methylamine, acetic acid and

Table 1. Physicochemical parameter of the complexes

Compounds/empirical formula	Elemental analyses, % found (required)				m.p. (°C)	Yield (%)	Molecular weight
	C	H	N	Fe(II)			
$[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O/C_{74}H_{86}Cl_2F_2Fe_2N_8O_{11}$ **(I)**	59.78/(59.89)	5.91/(5.84)	7.56/(7.55)	7.50/(7.53)	>350	50	1484.11
$[Fe_2(L)_2(A^2)_2(pip)] \cdot 5H_2O/C_{86}H_{82}Cl_2F_2Fe_2N_8O_{15}$ **(II)**	61.20/(61.18)	5.03/(4.90)	6.65/(6.64)	6.73/(6.62)	>350	59	1688.21
$[Fe_2(L)_2(A^3)_2(pip)] \cdot 5H_2O/C_{62}H_{66}Cl_2F_2Fe_2N_8O_{11}$ **(III)**	56.47/(56.42)	5.05/(5.04)	8.49/(8.49)	8.48/(8.46)	>350	67	1319.83
$[Fe_2(L)_2(A^4)_2(pip)] \cdot 5H_2O/C_{66}H_{74}Cl_2F_2Fe_2N_8O_{15}$ **(IV)**	55.42/(55.05)	5.13/(5.18)	7.80/(7.78)	7.69/(7.76)	200	58	1439.93
$[Fe_2(L)_2(A^5)_2(pip)] \cdot 5H_2O/C_{58}H_{56}Cl_2F_2Fe_2N_{10}O_{13}$ **(V)**	52.90/(52.71)	4.30/(4.27)	10.60/(10.60)	8.54/(8.45)	270	54	1321.72
$[Fe_2(L)_2(A^6)_2(pip)] \cdot 5H_2O/C_{82}H_{78}Cl_2F_2Fe_2N_8O_{11}$ **(VI)**	62.70/(62.65)	5.09/(5.00)	7.10/(7.13)	7.15/(7.10)	>350	50	1572.14
$[Fe_2(L)_2(A^7)_2(pip)] \cdot 5H_2O/C_{60}H_{66}Cl_2F_2Fe_2N_4O_{17}$ **(VII)**	54.03/(53.95)	4.88/(4.98)	4.18/(4.19)	8.45/(8.36)	>350	58	1335.78
$[Fe_2(L)_2(A^8)_2(pip)] \cdot 5H_2O/C_{72}H_{64}Cl_2F_2Fe_2N_{10}O_{13}$ **(VIII)**	57.85/(57.73)	4.30/(4.31)	9.46/(9.35)	7.45/(7.46)	>350	57	1497.93

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EDTA] buffer pH 8.0. The pattern of inspection was DNA alone (control), DNA in the presence of ligands and DNA in the presence of Fe(II) complexes. Nuclease activity experiments were accomplished by mixing pBR322 (50 μM) in TE (40 mM Tris acetate and 1 mM EDTA) buffer (pH 8.0), and ligand or Fe(II) (50 μM). Reaction mixture was incubated at room temperature for 1 h. then it was amended with 6× loading buffer (40% sucrose, 0.02% bromophenol blue and 0.02% xylene cyanol FF) and loaded on 0.8% agarose gel. Electrophoresis was carried out at constant voltage (100 V) in the Submarine Electrophoresis Unit (Genei, Banglore, India). Gel was stained with ethidium bromide. The same experimental conditions were maintained in control assays. The gels were viewed on a UV transilluminator; images were captured with an attached camera and estimated using AlphaDigiDoc™ RT Version V.4.1.0 PC-Image software.

RESULTS AND DISCUSSION

The structural investigation of all the prepared ligands was done using elemental analyses, IR. ^{1}H and ^{13}C-NMR spectroscopy. The Fe(II) complexes were prepared by reaction of ferrous sulfate, Cpf.HCl with variable ligands A^1–A^8 in a 1:1:1 ratio. The Fe(II) coordinated to deprotonated carboxylate oxygen, pyridone oxygen and N–N/N–O/O–O of neutral bidentate ligands and nitrogen of piperazine ring to form a square pyramidal geometry. The thermal analysis suggests decomposition of crystalline water molecules and stepwise decomposition of complexes. The preparation of $[Cu_2(Cip)_2(bpy)_2(pip)].6H_2O$ and its crystal structure have been reported by Wu *et al*.[26] They proposed a possible reaction scheme for dimeric complex formation and liberation of piperazine ring from ciprofloxacin. In addition, a number of Fe(II) and Cu(II) compounds have been synthesized by Patel *et al*.[16,17]

All the complexes are insoluble in water, ethanol, methanol, dichloromethane, chloroform, acetonitrile, hexane and DMF, while they are soluble in DMSO, so it is difficult to grow a single crystal for X-ray diffraction analyses. The elemental analyses were in good agreement with the proposed 1:1:1 Fe(II):Cip:A^n formulation of dimeric complexes.

IR spectra

The IR spectral data of complexes are shown in Table 2. The ν(C=O) stretching vibration band appeared at 1708 cm^{-1} in the spectra of ciprofloxacin, while in complexes this band shifted towards lower energy at ~1624 cm^{-1}, suggesting that coordination occurs through the pyridone oxygen atom.[27] The absorption bands observed at 1624 and 1340 cm^{-1} in ciprofloxacin were assigned to $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in complexes these bands were observed at ~1598 and ~1382 cm^{-1}. The frequency separation ($\Delta\nu = \nu COO_{asy} - \nu COO_{sym}$) in the investigated complexes was greater than 200 cm^{-1}, suggesting a unidentate bonding nature for the carboxylato group.[28] The sharp band in ciprofloxacin[29] at 3520 cm^{-1} was due to hydrogen bonding, which contributed to ionic resonance structure and peak observed because of free hydroxyl stretching vibration. This band absolutely vanished in the spectra of complexes, indicating deprotonation of carboxylic proton. The ν(C=O) peak for A^5, A^7 and A^8 was observed at ~1680 cm^{-1} (cyclic) and ~1660 cm^{-1} (acetyl), which was shifted to 1574 cm^{-1} on formation of complexes.[30] These data were further supported by a ν(M–O)[25] band appearing at ~510 cm^{-1}. The band at ~1478 cm^{-1} was assigned to δ(C–H) bending of <N–CH_2–CH_2–N→.[30] The ν(C=N) band for A^1–A^4, A^6 and A^8 was observed at ~1612 cm^{-1}, which shifted in the range 1560–1601 cm^{-1} in complexes, indicating the bidentate N–N coordination of the ligand.[17,31] These data were further supported by a ν(M–N) band[32] appearing at ~540 cm^{-1}.

Reflectance spectra and magnetic properties

Reflectance spectral data and magnetic moments for Fe(II) are presented in Table 3. Figure 1 shows reflectance spectra of $[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$. According to our visual observation, Fe(II) complexes are intense greenish brown in color, but the origin of this color is uncertain. The five-coordinated Fe(II) complexes characterized by spectrophotometric technique have been rarely reported.[33] The reflectance spectra of diiron(II) complexes $[Fe_2(L)_2(A^n)_2(pip)] \cdot 5H_2O$ exhibited three bands at about ~36 300, ~17 800 and ~12 000 cm^{-1},[34,35] which were assigned to the transitions

Table 2. Infrared spectral data of complexes

Compounds	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(C=N) (cm^{-1}), ring	ν(M–N) (cm^{-1})	ν(M–O) (cm^{-1})
I	1620	1604	1378	226	1140	1572	—	535	510
II	1626	1596	1381	215	1145	1564	—	540	512
III	1625	1608	1382	226	1130	1572	—	536	508
IV	1612	1589	1384	205	1142	1562	—	540	510
V	1619	1591	1386	205	1135	—	1598	539	510
VI	1625	1598	1382	216	1141	1604	—	537	508
VII	1630	1612	1383	229	1145	—	—	550	507
VIII	1621	1587	1376	211	1128	1575	1602	540	509

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Table 3. Reflectance spectral data of Fe(II) complexes

Compounds	λ_{max} in DMSO			μ_{eff}
	$\pi-\pi^*$ transition	d–d transition	MLCT	BM
I	38 100	16 800	12 800	4.91
II	36 700	18 500	12 500	5.07
III	36 300	17 550	13 350	4.70
IV	35 300	15 150	11 100	5.12
V	38 000	19 200	11 100	4.82
VI	35 900	20 000	12 000	4.96
VII	35 500	16 900	11 800	4.85
VIII	35 300	18 500	11 600	4.76

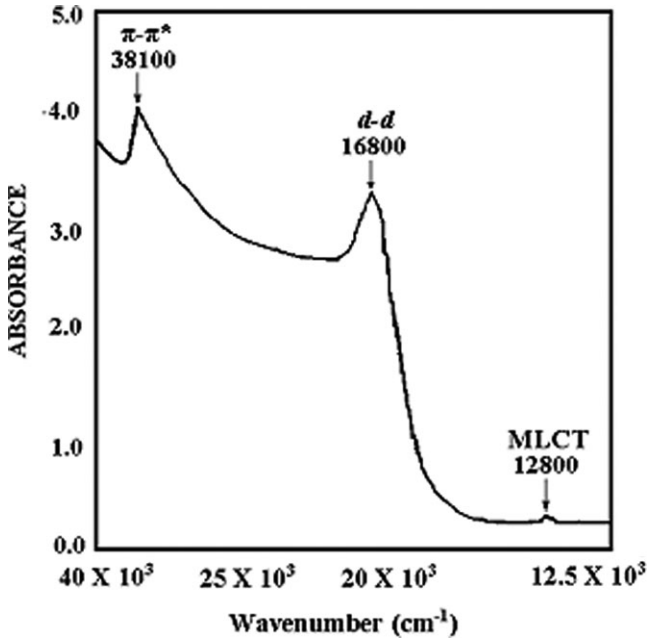

Figure 1. Reflectance spectra of $[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$.

$\pi \rightarrow \pi^*$, d–d, and MLCT, respectively. In the case of complexes $[Fe_2(L)_2(A^n)_2(pip)] \cdot 5H_2O$ the molecules exhibited effective magnetic moment in the range 4.70–5.07 BM that is typical for such penta-coordinated Fe(II) complexes, indicating the presence of four unpaired electrons and a quintet ground state ($S = 2$) and consistent with the presence of four unpaired electrons,[36,37] suggesting paramagnetic nature. The magnetic moment and reflectance spectra suggest that Fe(II) is in a distorted square pyramidal coordination environment.

Thermogravimetric analysis

The thermogravimetric analysis (TGA)for the complexes were carried out within a temperature range from 20–800 °C in a N_2 atmosphere to establish their compositional differences as well as to ascertain the nature of associated water molecules.[38] The determined temperature ranges and corresponding percentage mass losses accompanying the changes in the complexes on heating revealed the following findings. The TG curves of Fe(II) complexes showed four decomposition steps. It was observed that all the complexes showed a loss in weight corresponding to five water molecules in the range 50–130 °C, indicating that these water molecules were water of crystallization. For Fe(II) complexes a loss in weight was seen corresponding to a piperazine (pip) molecule in the temperature range 130–240 °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 iron oxide.

Antibacterial activity

Comparative analysis shows that the complexes exhibited higher antibacterial activity as compared with the free ligands, metal salts, control (DMSO) and standard drugs ofloxacin and levofloxacin; the data are summarized in Table 4.

The antibacterial activity order for Fe(II) complexes against each bacterial strain is given below in ascending sequence.

B. cereus:

Control $\approx$ Std 3 $\approx A^2 < A^1 \approx A^3 \approx A^6 < A^4 < A^5 \approx A^7 < A^8 \approx FeSO_4.7H_2O <$ **I** < Std 2 < **II** < Std 1 < LH $\approx$ **VI** $\approx$ **VII** < **III** $\approx$ **IV** < **V** < **VIII.**

S. aureus:

Control < Std 3 $\approx A^2 < A^1 \approx A^6 < A^3 < A^4 < A^7 < FeSO_4 \cdot 7H_2O \approx A^5 \approx A^8 <$ **V** < **VII** < **III** < Std 2 $\approx$ **IV** $\approx$ **VIII** < Std 1 $\approx$ **VI** < LH < **I** $\approx$ **II.**

E. coli:

Control $\approx A^2 \approx A^3 < A^4 < FeSO_4 \cdot 7H_2O \approx A^1 <$ Std 3 $< A^7 < A^5 < A^6 \approx A^8 <$ LH < Std 1 < Std 2 < **VII** < **III** $\approx$ **V** < **IV** $\approx$ **VIII** < **I** < **VI** < **II.**

B. subtilis:

Control $\approx A^2 \approx A^3 < A^4 \approx A^6 < A^1 < FeSO_4 \cdot 7H_2O \approx A^7 < A^5 <$ Std 3 $\approx A^8 <$ LH $\approx$ Std 1 < **VII** < Std 2 $\approx$ **III** < **V** < **IV** $\approx$ **VI** $\approx$ **VIII** < **I** < **II.**

S. typhi:

Control $\approx A^1 \approx A^4 <$ Std 3 $\approx A^3 < FeSO_4 \cdot 7H_2O \approx A^2 < A^7 < A^5 < A^6 < A^8 <$ **II** < Std 2 $\approx$ **I** < **VI** < LH < Std 1 $\approx$ **VII** < **III** < **IV** < **V** $\approx$ **VIII.**

S. marcescens:

Control $\approx A^3 <$ Std 3 $\approx A^2 \approx A^4 < A^1 \approx A^6 < A^5 \approx A^7 < FeSO_4 \cdot 7H_2O \approx A^8 <$ **VI** < **I** < Std 1 < Std 2 < LH $\approx$ **VII** < **II** $\approx$ **V** < **III** $\approx$ **VIII** < **IV.**

It was observed that all the iron complexes were more potent bactericides than the ligand. This enhancement in the activity can be explained on the basis of chelation theory and/or may be due to Overtone's concept.[39,40] Chelation reduces the polarity of the metal ion considerably, mainly because of the partial sharing of its positive charge with donor groups and possible π-electron delocalization on the whole chelate ring. The lipids and polysaccharides are some important constituents of cell walls and membranes, which are preferred for metal ion interaction. In addition to this, the

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Table 4. Biocidal activity data of ligands and complexes

Compounds	Zone of inhibition (mm)					
	E. coli	*B. substilis*	*S. aureus*	*S. typhi*	*B. cereus*	*S. marcesens*
Control	11	11	10	11	11	11
Fe $SO_4 \cdot 9H_2O$	13	16	19	14	18	19
LH(Cpf. HCl)	28	34	40	32	31	37
Std 1 (Ofl. HCl)	30	34	39	33	30	32
Std 2 (Lef. HCl)	33	36	38	29	28	34
Std 3 (Fluconozole)	11	11	15	19	12	12
A^1	13	15	12	11	13	14
A^2	11	11	11	14	11	12
A^3	11	11	14	12	13	11
A^4	12	12	15	11	14	12
A^5	17	18	19	16	16	17
A^6	18	12	12	17	13	14
A^7	16	16	18	15	16	17
A^8	18	19	19	18	18	19
I	42	40	44	29	25	31
II	44	43	44	27	29	38
III	37	36	37	35	33	41
IV	38	38	38	36	33	42
V	37	37	34	37	36	38
VI	43	38	39	30	31	27
VII	36	35	35	33	31	37
VIII	38	38	38	37	39	41

cell wall also contains many aminophosphates, and carbonyl and cysteinyl ligands, which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Chelation can considerably reduce the polarity of the metal ion, which in turn increases the lipophilic character of the chelate. Thus, interaction between metal ion and the lipid is favored. This may lead to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cell processes. If the geometry and charge distribution around the molecule are incompatible with the geometry and charge distribution around the pores of the bacterial cell wall, penetration through the wall by the toxic agent cannot take place and this will prevent the toxic reaction within the pores. In addition to these, some important factors that contribute to the activity are the nature of the metal ion, the nature of the ligand, coordinating sites, geometry of the complex, concentration, hydrophilicity, lipophilicity and the presence of co-ligands. Certainly, steric and pharmokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent. Apart from this, the mode of action of these complexes may also invoke the hydrogen bond though the $C_{55}N–N_{55}CH–$ group with the active centre and thus interfere with normal cell processes. The presence of lypophilic and polar substituents is expected to enhance antibacterial activity. Heterocyclic ligands with multifunctionality have a greater chance of interaction either with nucleoside bases (even after complexation with metal ion) or with biologically essential metal ions present in the biosystem, and can be promising candidates as bactericides since they always look to enact especially with some enzymatic functional groups, to achieve a higher coordination number. Thus, the antibacterial property of metal complexes cannot be ascribed to chelation alone but it is an intricate blend of all the above contributions.

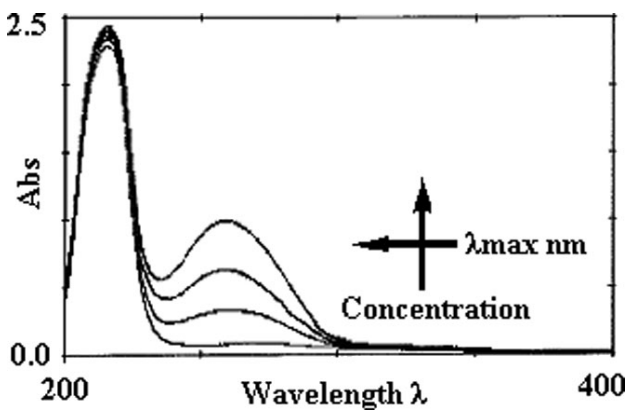

Figure 2. Absorption titration curve of $[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$.

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Figure 3. Gel of pBR322 with compounds. Lane 1: pBR322 (control); lane 2: pBR322 + **I**; lane 3: pBR322 + **II**; lane 4: pBR322 + **III**; lane 5: pBR322 + **IV**; lane 6: pBR322 + **V**; lane 7: pBR322 + **VI**; lane 8: pBR322 + **VII**; lane 9: pBR322 + **VIII**.

Table 5. Absorption titration data of ligands and complexes with DNA

Sample no.	DNA (μg)	Fe(II) complexes, λ_{max} (nm)	A^n λ_{max} (nm)	L λ_{max} (nm)	Fe(II) λ_{max} (nm)
I	0	275.0	261.6	260.0	268.4
	2	268.0	260.0	259.7	266.3
	4	261.2	258.6	259.0	259.0
	6	258.0	257.0	258.4	257.8
II	0	272.0	264.1	260.0	268.4
	2	265.0	262.8	259.7	266.3
	4	259.2	259.0	259.0	259.0
	6	258.0	255.0	258.4	257.8
III	0	275.0	261.8	260.0	268.4
	2	273.0	260.8	259.7	266.3
	4	260.2	258.2	259.0	259.0
	6	259.0	256.4	258.4	257.8
IV	0	274.7	263.6	260.0	268.4
	2	270.0	261.4	259.7	266.3
	4	259.4	259.0	259.0	259.0
	6	258.4	257.1	258.4	257.8
V	0	276.0	262.2	260.0	268.4
	2	271.0	260.0	259.7	266.3
	4	259.0	258.4	259.0	259.0
	6	258.4	256.7	258.4	257.8
VI	0	272.8	262.6	260.0	268.4
	2	270.0	261.0	259.7	266.3
	4	260.0	260.1	259.0	259.0
	6	258.0	258.3	258.4	257.8
VII	0	270.0	261.8	260.0	268.4
	2	268.0	259.0	259.7	266.3
	4	259.4	257.6	259.0	259.0
	6	257.0	256.8	258.4	257.8
VIII	0	275.0	263.2	260.0	268.4
	2	269.0	261.5	259.7	266.3
	4	260.0	259.1	259.0	259.0
	6	258.2	257.0	258.4	257.8

DNA binding

Absorption titration

Absorption spectroscopy is broadly and well known to determine the binding of the complexes with DNA. Complexes bound to DNA binding results in bathochromism (red shift) and hypochromism (blue shift) due to interaction between chromophores and the base pair of DNA. The extent of hypochromism is commonly consistent with the strength of intercalative interaction.[41–44] Figure 2 shows the absorption titration curve of $[Fe_2(L)_2(A^1)_2(pip)] \cdot 5H_2O$ with sperm herring DNA.

The DNA binding data of the complexes are represented in Table 5. The maxima at about ~275 nm was observed in the spectrum of the complex without DNA, which decreased as the amount of DNA increased and was observed at about ~258 nm in the presence of 6 μg DNA. In the case of variable ligands (A^1–A^8), $FeSO_4 . 7H_2O$ and ciprofloxacin, the maxima were observed at about ~261 nm in the absence of DNA and ~258 nm in the presence of 6 μg of DNA. All the data lead to the suggestion that, in the presence of 6 μg DNA whole complex dissociate, and free Fe(II), constant ligand(Cip) and variable ligands (A^1–A^8) interact with DNA or complex binding with DNA (i.e. N of purines or pyrimidine ring) through coordinating atoms of the complex [i.e. Fe(II), N and O].

Table 6. Gel electrophoresis data of ligands and complexes with DNA

Compounds	DNA % SC	DNA % OC	Compounds	DNA % SC	DNA % OC
Control	100	00	Fe(**II**)	74	26
A^1	84	16	**I**	34	66
A^2	67	33	**II**	64	36
A^3	80	20	**III**	49	51
A^4	74	26	**IV**	45	55
A^5	75	25	**V**	44	56
A^6	70	30	**VI**	24	76
A^7	67	33	**VII**	18	82
A^8	85	15	**VIII**	34	66

Gel quantification of complexes-DNA systems

Quinolone metal complexes can bind with DNA by two unique binding sites; namely groove binding and intercalation. This behavior is of great significance with regard to the relevant biological role of quinolones antibacterial in living systems. The binding of complexes with supercoiled (SC) pBR322 was determined by its ability to make it bulky by changing in conformation of pBR322 DNA due to binding with reactive sites of DNA. When pBR322 is subjected to electrophoresis, the fastest migration is observed for SC DNA. If one strand is cleaved due to binding with reactive species, the SC form is converted in open nicked circular DNA (OC) form. Figure 3 and Table 6 show the electrophoretic process of complexes, Fe(II) ions, metal complexes and ligands. Complexes exhibit higher nuclease activity than that of Fe(II) ions and corresponding ligands.

It is observed that SC smear on the gel while OC remain in the well. This may be due to OC becoming bulky owing to their high molecular weight, due to intercalation of

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compounds, and/or OC requiring more time to run on the gel than SC. From the experiment we can conclude that the conversion of SC to OC is higher in the presence of complexes than that in the presence of free ligands and Fe(II).

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