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Iron(III) complexes: Preparation, characterization, antibacterial activity and DNA-binding**

PRAMOD B. PANSURIYA & M. N. PATEL

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

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Abstract
Iron(III) have been combined to well known quinolones (ciprofloxacin) and some Schiff bases with the help of coordination approach. Characterization of these compounds have been done using elemental analysis, magnetic measurements, thermogravimetric analysis, IR, UV-VIS, ^{1}H NMR and ^{13}C NMR spectral investigation. Analytical studies suggest that the iron(III)-quinolone complexes assume a six-coordinated dimeric distorted octahedral geometry. All the compounds show a good antibacterial activity against broad range of bacteria like *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Serratia marcescens*, whereas no significant inhibition towards growth of fungal strains like *Aspergillus Niger, Aspergillus flavus* and *Lasiodiplodia theobromae*. Analyses of all these compounds show effective sperm herring DNA inhibition.

Keywords: *Iron(III) complexes, antibacterial activity, absorption titration, DNA-binding, gel electrophoresis*

Introduction

Iron is the most abundant element found in the surrounding environment and plays crucial role for the survival of terrestrial organisms. Iron also participates in biochemical processes like ribonucleic reduction, energy production, photo synthesis, nitrogen reduction, oxygen transport, and oxygenation [3]. Ligands are designed and functionalized in order to provide tissue specificity for the declivity of metal toxicity and improvement of pharmacological properties. Several iron(III) complexes have been known as contrast agent for magnetic resonance imaging(MRI)[4]. Some Cu and Fe complexes have been known for catalytic, antitumor, hypoxic selective cyatotoxins, and antimicrobial activity [5–9]. Iron and its complexes both are known for its DNA damaging property [10–12]. In continuation of our earlier work we have synthesized eight quinolone blended iron(III) compounds with eight different neutral bidentate ligands, all the compounds have been screened for antibacterial activity, and their DNA binding property [1,2].

Experimental

Materials

All the chemicals used were of analytical grade. Ferric nitrate, m-chloro aniline, cyclohexanone, *o*-phenylenediamine, benzaldehyde, ethylenediamine, *p*-anisaldehyde, benzil, were purchased from the E. Merck (India) Limited, Mumbai. Ciprofloxacin hydrochloride was purchased form Bayer AG (Wuppertal, Germany). Thiophene-*o*-carboxaldehyde and 2,2′-bipyridylamine, 2-aminopyridine, and 1,8-diaminonaphthalene were purchased from Eastgate, White Lund, Morecambe, 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.

Corresponding author: M. N. Patel, Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120 Gujarat India. Tel: 91 2692 226857. Fax: 91 2692 236475. E-mail: jeenenpatel@yahoo.co.in

**For previous papers in series see [1] and [2].

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Chemistry

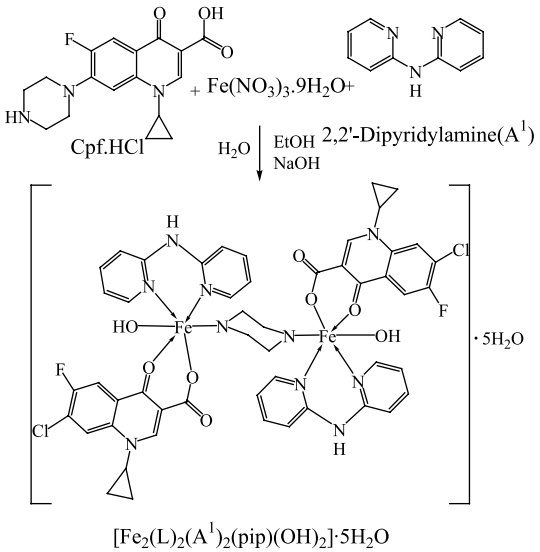
Synthesis of schiff bases. The A^2–A^8 Schiff bases were synthesized according to reported processes [1].

Synthesis of Fe(III)-complexes. A general preparative method for Fe(III) complexes is shown in Scheme I.

$[Fe_2(Cip)_2(bipym)_2(pip)(OH)_2]\cdot 5H_2O$ (I). A methanolic solution of $Fe(NO_3)_2\cdot 9H_2O$ (4.04 g, 10 mmol) was added to bipym (1.71 g, 10 mmol) in ethanol (100 mL). Followed by addition of formerly primed solution of Cpf·HCl (3.67 g, 10 mmol) in water; the pH was adjusted to 5.0 ~ 6.0 pH with dilute HNO_3 or NaOH solution. The resulting reddish brown solution was refluxed for 7 h, and then heated on steam bath to concentrate for 4–5 h. The reaction mixture was kept for overnight at room temperature. A fine colored product was obtained. Obtained product was washed with ether and dried over vacuum desiccator. Yield: 69%, m.p.: 345°C, Found %: C, 49.18, H, 4.38, N, 11.41; Fe, 9.07. $C_{50}H_{54}Cl_2F_2Fe_2N_{10}O_{13}$ (1223.62) requires %: C, 49.08, H, 4.45, N, 11.45; Fe, 9.13%.

$[Fe_2(Cip)_2(bap)_2(pip)(OH)_2]\cdot 5H_2O$ (II). Prepared from bap (1.82 g, 10 mmol) was used instead of bipym (1.71 g, 10 mmol). Yield: 65%, m.p.: 130°C, Found %: C, 51.97, H, 4.58, N, 8.91; Fe, 9.06. $C_{54}H_{56}Cl_2F_2Fe_2N_8O_{13}$ (1245.66) requires %: C, 52.07, H, 4.53, N, 9.00; Fe, 8.97.

$[Fe_2(Cip)_2(tca)_2(pip)(OH)_2]\cdot 5H_2O$ (III). Prepared from tca (1.87 g, 10 mmol). Yield: 62%, m.p.: 355°C, Found %: C, 49.85, H, 4.23, N, 6.70; Fe, 8.95. $C_{52}H_{54}Cl_2F_2Fe_2N_6O_{13}S_2$ (1255.74) requires %: C, 49.74, H, 4.33, N, 6.69; Fe, 8.89.



Scheme 1. Preparation of Fe(III) complexes.

$[Fe_2(Cip)_2(bendan)_2(pip)(OH)_2]\cdot 5H_2O$ (IV). Prepared from bendan (3.34 g, 10 mmol). Yield: 59%, m.p.: >360°C, Found %: C, 60.50, H, 4.56, N, 7.19; Fe, 7.20. $C_{78}H_{72}Cl_2F_2Fe_2N_8O_{13}$ (1550.05) requires %: C, 60.44, H, 4.68, N, 7.23; Fe, 7.21.

$[Fe_2(Cip)_2(benen)_2(pip)(OH)_2]\cdot 5H_2O$ (V). Prepared from benen (2.36 g, 10 mmol). Yield: 58%, m.p.: 210°C, Found %: C, 54.93, H, 5.10, N, 8.25; Fe, 8.30. $C_{62}H_{68}Cl_2F_2Fe_2N_8O_{13}$ (1353.84) requires %: C, 55.00, H, 5.06, N, 8.28; Fe, 8.25.

$[Fe_2(Cip)_2(bmbbd)_2(pip)(OH)_2]\cdot 5H_2O$ (VI). Prepared from bmbbd (3.44 g, 10 mmol). Yield: 48%, m.p.: 235°C, Found %: C, 56.65, H, 4.90, N, 7.20; Fe, 7.04. $C_{74}H_{76}Cl_2F_2Fe_2N_8O_{17}$ (1570.03) requires %: C, 56.61, H, 4.88, N, 7.14; Fe, 7.11.

$[Fe_2(Cip)_2(bcpded)_2(pip)(OH)_2]\cdot 5H_2O$ (VII). Prepared from bcpded (4.29 g, 10 mmol). Yield: 45%, m.p.: 350°C, Found %: C, 56.60, H, 4.17, N, 6.37; Fe, 6.48. $C_{82}H_{72}Cl_6F_2Fe_2N_8O_{13}$ (1739.89) requires %: C, 56.61, H, 4.17, N, 6.44; Fe, 6.42.

$[Fe_2(Cip)_2(dcbd)_2(pip)(OH)_2]\cdot 5H_2O$ (VIII). Prepared from dcbd (2.68 g, 10 mmol). Yield: 46%, m.p.: >360°C, Found %: C, 55.91, H, 6.00, N, 7.84; Fe, 7.90. $C_{66}H_{84}Cl_2F_2Fe_2N_8O_{13}$ (1418.01) requires %: C, 55.90, H, 5.97, N, 7.90; Fe, 7.88.

Other methods. The organic solvents were purified by recommended methods [13]. The metal contents of the complexes were analyzed by EDTA titration [14] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5). 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). The diamagnetic correction was made using Pascal's constant [15].

Spectral investigation. The ^1H NMR and ^{13}C NMR was recorded on Bruker Avance (400 MHz). The diffuse reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO discs) on a Beckman DK-2A spectrophotometer. Infrared spectra were recorded on an FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. Carbon, hydrogen, nitrogen, and/or sulfur elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. Thermogravimetric analyses study were obtained with a model 5000/2960 SDTA, TA instrument (USA).

Preparation of stock solution. A stock solution of 2.5 ppm was prepared by dissolving 0.25 mg of each complex in 5% DMSO solution.

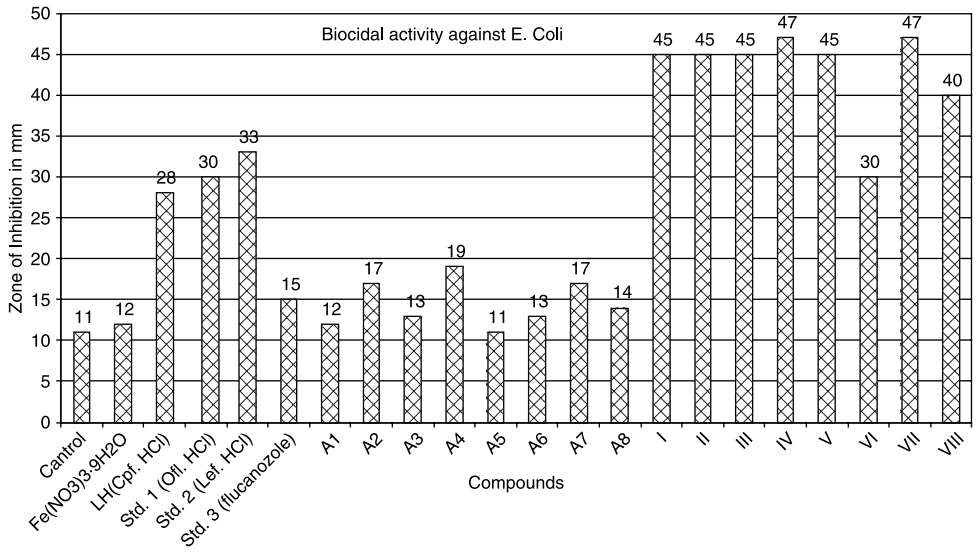
Biological studies

Determination of MIC value. The biocidal test was screened by minimal inhibitory concentration (MIC). MIC was determined with the help of progressive double dilution method [16] in liquid media containing 1ppm to 50ppm of the compound being tested. All the compounds were more effective with MIC value at 2.5ppm $\approx$2.5μg/mL. The biocidal activity of the ofloxacin, levofloxacin, flucanozole, ligands, $Fe(NO_3)_2{\cdot}9H_2O$, and its 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 [17].

Preparation of agar plates. The media was made up by dissolving bacteriological agar (20 g.) and luria broth (20 g.) in 1 L distilled water. The mixture was autoclave for 15 min at 120°C and then dispended into sterilized petri dishes, allowed to solidify, and then used for inoculation.

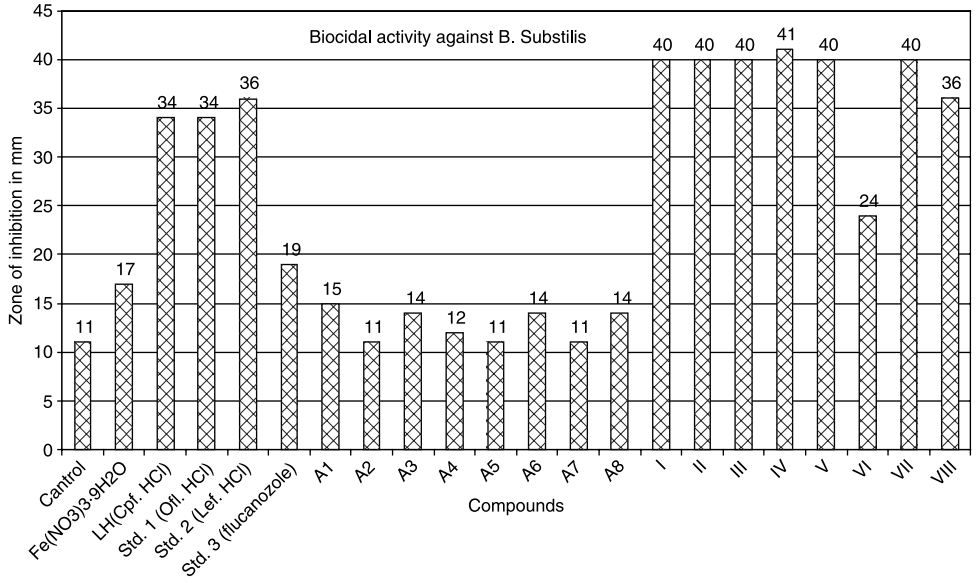
Procedure of inoculation. The target microorganism cultures were prepared separately in 15 mL of liquid LB medium for activation. Inoculation was done with the help of micropipette with sterilized tips; 100 μL of activated strain is placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterile, bent glass rod. Then two well having diameter 10 mm is done with the help of sterilized borer in each plate.

A



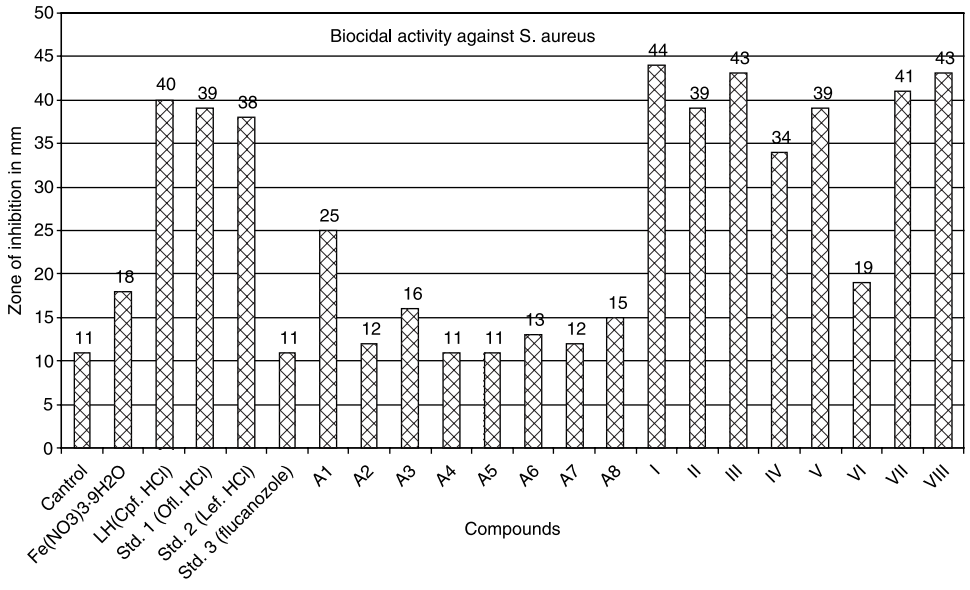
Inhibition order of activity: IV~VII > I~II~III~V > VIII > VI

B



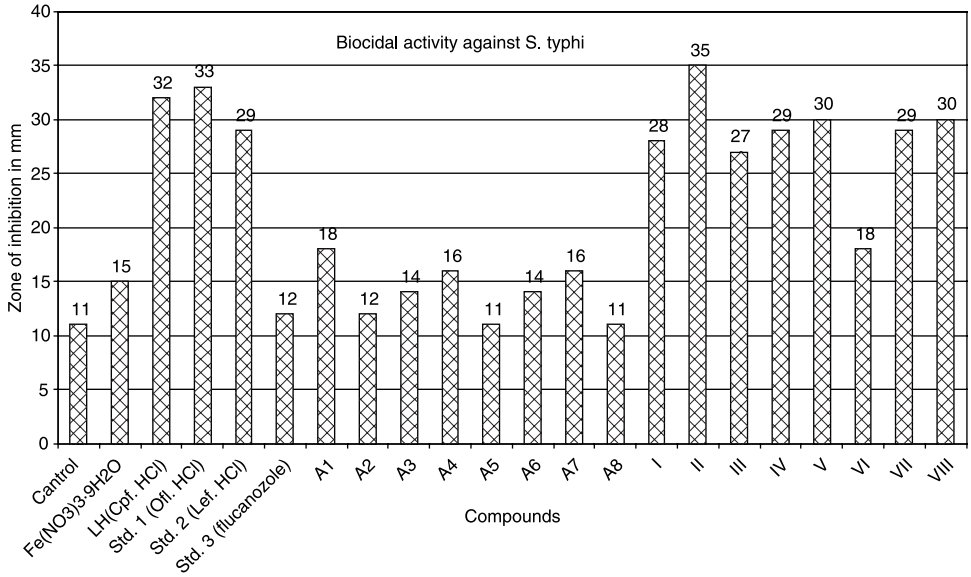
Inhibition order of activity: IV > I~II~III~V~VII > VIII > VI

C



Inhibition order of activity: I > VIII~III > VII > V~II > IV > VI

D



Inhibition order of activity: II > V~VIII > IV~VII > I > III > VI

Application of discs. Sterilized stock solutions (2.5 μg/mL) were used for the application in the well of previously inoculated agar plates. When the discs were applied, they were incubated at 37°C for 24 h. The control experiment was performed and then the zone of inhibition was measured (in mm) around the disc and the results are represented in Figure I. All experiments were performed in triplicate and ofloxacin, levofloxacin, and flucanozole were used as a standard drug. The growth was compared with solvent as the control and is expressed as zone of inhibition.

Absorption titration. DNA binding affinity study was performed on Shimadzu UV-VIS spectrophotometer. Absorption titration of compounds were done by keeping fixed amount of iron compounds (where compound: I = 12.23, II = 12.45, III = 12.55, IV = 15.50, V = 13.53, VI = 15.70, VII = 17.39, VIII = 14.18 μg), variable amount of DNA i.e.0 to 7 μg and maintaining total volume of 20 mL using phosphate buffer, pH 7.2. Compound-DNA solutions were employed to record absorption spectra.

Gel analyses and quantification. The inspection of super coiled pBR322 have been done in TAE [tris(hydroxymethyl)methylamine(T), acetic acid(A) and EDTA(E)] buffer pH 8.0. Pattern of inspection designed as DNA alone (control), DNA in presence of ligands and DNA in presence of Fe(III)-complexes.

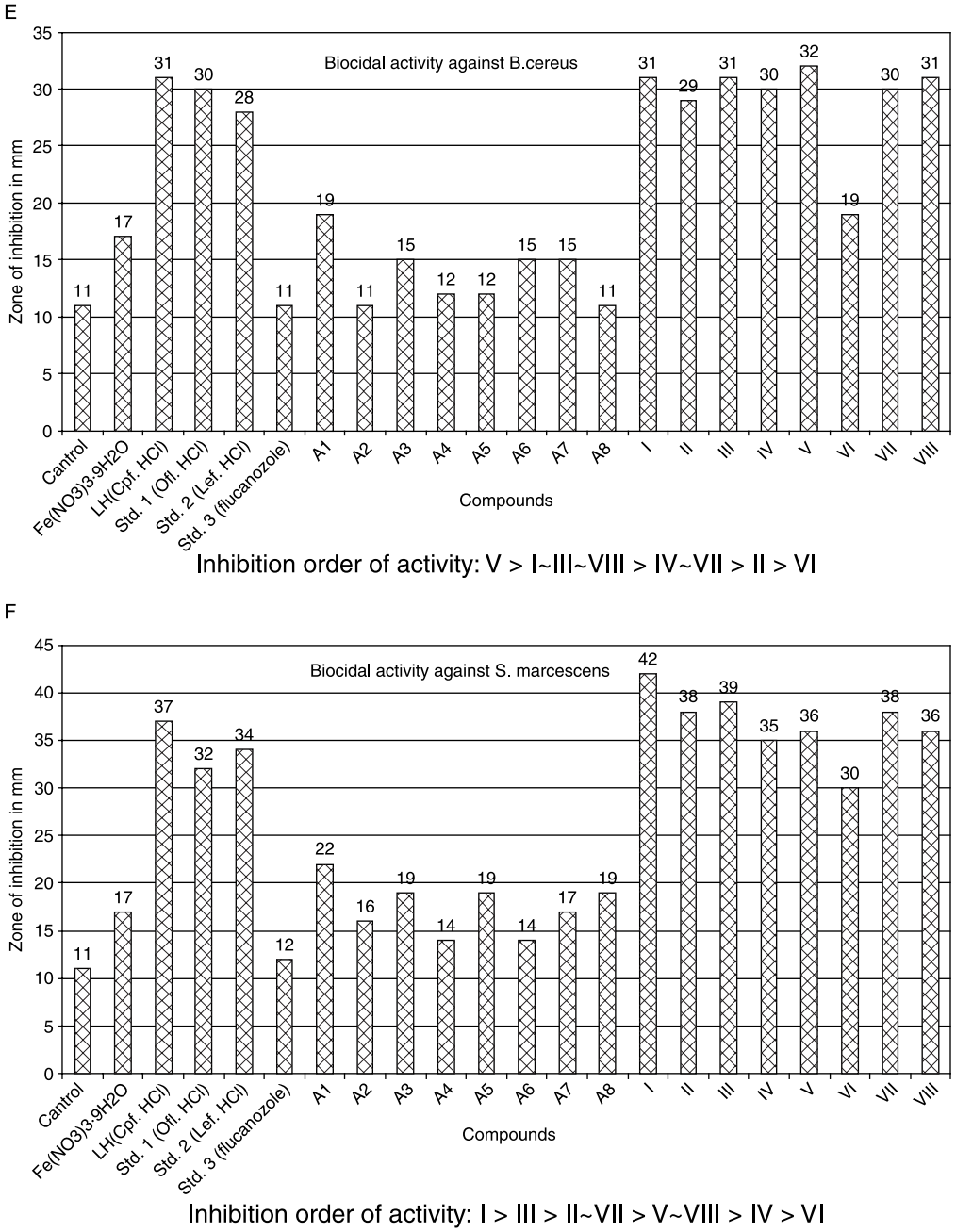


Figure 1. Biocidal activity of synthesized compounds.

Nuclease activity experiments have been accomplished by mixing pBR322 (50 μM) in TE [40 mM Trisacetate(T) and 1 mM EDTA(E)] buffer (pH 8.0), and ligand or Fe(III)-complexes (50 μM). Reaction mixture was incubated at room temperature for 1 h. then it was diluted 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 on constant voltage (100V) in 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 UV transilluminator, images captured with an attached camera and estimated using AlphaDigiDoc^{TM} RT. Version V.4.1.0 PC-Image software.

Results and discussion

Chemistry

The ligands A^2–A^8 were isolated by reacting aldehyde/ketone with amine in ethanol as reported in earlier work of our laboratory [11]. The synthesized ligands were analyzed using elemental analyses, IR, ^{1}H and ^{13}C NMR spectroscopy. The iron(III) complexes of ciprofloxacin were synthesized by reacting ferric nitrate and variable ligands A^1–A^8 in a 1:1:1 ratio.

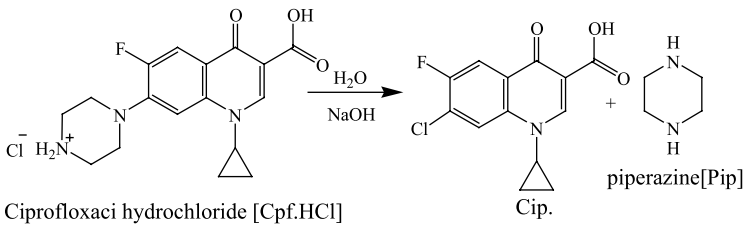
Iron complexes assume six coordinated octahedral geometry coordinating with N–N/N–S of neutral bidentate ligands, two oxygen of cip.(LH = 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3- carboxylic acid), nitrogen of piperazine ring and oxygen of hydroxyl group. The iron complexes are dimeric in nature. In continuation of earlier work on Cu(II) and Fe(II) complexes [1,2] we have synthesized eight dimeric Fe(III) complexes which follow the proposed probable reaction scheme 2.

The synthesized dimeric compounds are characterized using IR spectra, electronic spectra, magnetic measurements and thermogravimetric analyses. The thermal decomposition suggests five water molecule of crystallization and stepwise decomposition of complexes. The elemental analyses were in good agreement with the proposed 1:1:1; Fe(III):Cip:A^n formulation of all complexes.

IR spectra. The IR spectral bands for stretching and bending vibrations are listed in Table I. The sharp band in ciprofloxacin at 3520 cm^{-1} [18] is due to hydrogen bonding; which is contributed to ionic resonance structure and peak observed because of free hydroxyl stretching vibration. This band completely absent in the spectra of metal complexes indicating deprotonation of carboxylic proton. The broad absorption band obtained at 1624 cm^{-1} and 1384 cm^{-1} in ciprofloxacin is observed at ~1579 cm^{-1} and ~1367 cm^{-1} for ν $(COO)_{asy}$ and ν $(COO)_{sym}$ in complexes respectively, in present case separation frequency $\Delta\nu > 200\ cm^{-1}$ ($\Delta\nu = \nu$ COOasy - ν COO_{sym}) suggesting the unidentate binding of carboxylato group [19–22]. The ν (C═O) stretching vibration band appears at 1708 cm^{-1} in the spectra of ciprofloxacin, in the complexes this band shifted towards lower energy at about 1630 cm^{-1}; suggesting that coordination occurs through the carbonyl oxygen atom [19]. In the investigated compound the ν(C═N) of 2, 2′-bipyridylamine appears at 1580 cm^{-1}. This band shifted to higher frequency at 1612 cm^{-1} [23] in complexes indicates the bidentate N–N coordination of the ligand. Similarly for benzylidene-2-amino pyridine two strong band at 1619 and 1593 cm^{-1} assigned to ν(C═N) stretching vibration of azomethine and pyridine ring respectively. On complexation these bands are shifted to 1664 cm^{-1} and 1619 cm^{-1} suggesting the bidentate N–N participation in coordination [24,25]. The ν (C═N) peak for synthesized Schiff bases A^3–A^8 observed at 1601–1629 cm^{-1}. On complexatation of ligand these bands are shifted to 1550–1610 cm^{-1} indicating the N–S or N–N bidentate coordination of ligand [26–30]. Absorption at about 3420 cm^{-1} for the ν_{OH} frequency indicates the coordinated hydroxo anion to iron, and is further supported by shoulder at about 340 nm in UV-vis spectra [31]. These data are further supported by ν (M–O) [32], and ν (M–N) [33] appear at 500 ~ 515 cm^{-1}, and 535 ~ 545 cm^{-1} respectively. In case of $[Fe_2(Cip)_2(tca)_2(pip)(OH)]\cdot 5H_2O$ new band observed at 420 cm^{-1} which can be assigned to ν (M–S) [27,34] mode.

Electronic spectra and magnetic measurements. Electronic spectral data and magnetic moments are summarized in Table II. The diffuse reflectance spectra of diiron complexes $[Fe_2(L)_2(A^n)_2(pip)(OH)_2]\cdot 5H_2O$ have been taken in solid state. The electronic spectra of the complexes exhibit three absorption bands at about ~19000, ~23000, and ~25500 cm^{-1}, which may be assigned to transitions $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{2g}$, $^6A_{1g} \rightarrow {}^4A_{1g}$, ^4E_g, respectively [35] and suggests octahedral geometry. The magnetic moment of all compounds obtained in between 5.71–6.09 B.M. is good agreement for six-coordinated dinuclear iron(III) system and consistent with the presence of a five-unpaired electrons [36].

Thermogravimetric analysis. The thermogravimetric analysis suggest that initially there is weight loss occurring in the 50–130°C temperature range for all Fe(III)-complexes is attributed to as a loss of the water of crystallization. In the second step weight loss during 140–180°C corresponding to two hydroxyl (OH) molecules. In case of third step weight loss during 180–220°C corresponding to piperazine (pip) molecule, followed by liberation of (L) in between



Scheme 2.

Table I. Infrared spectral data of complexes.

Compounds	ν (C=O) cm^{-1} Pyridone	ν $(COO)_{asy}$ cm^{-1}	ν $(COO)_{sym}$ cm^{-1}	$\Delta\nu$ cm^{-1}	ν (C–Cl) cm^{-1}	ν (C=N) cm^{-1} Azo methine	ν (C=N) cm^{-1} Ring	ν (M–N) cm^{-1}	ν (M–O) cm^{-1}	ν (M–S) cm^{-1}
L	1708	1624	1384	240	–	–	–	–	–	–
I	1630	1595	1381	202	1146	–	1612	535	507	–
II	1629	1612	1382	230	1145	1664	1619	545	508	–
III	1625	1580	1368	212	1139	1584	–	535	515	420
IV	1632	1574	1352	222	1134	1590	–	540	511	–
V	1628	1590	1381	209	1114	1610	–	540	505	–
VI	1608	1600	1390	210	1145	1570	–	535	510	–
VII	1629	1593	1382	211	1102	1595	–	540	500	–
VIII	1616	1589	1381	208	1141	1550	–	540	507	–

220–490°C. At last decomposition of A^n occurs in temperature range 510–710°C, and remaining weight is in good agreement with oxide of iron.

Biology

Biocidal activity. Comparative analysis show higher biocidal activity of the Fe(III)-complexes than free ligands, metal salt and the control (DMSO). The Fe(III)-complexes exhibit higher activities except complex VI against *S. aureus, B. subtilis, B. cereus, E. coli, S. marcescens* and moderate activity against *S. typhi* as compared to the standard drugs ofloxacin, levofloxacin, and flucanozole. Comparative study of biocidal activity shown in Figure 2. Average potency of biocidal activity is determined and order of activity has been found out. The compound-VI has lowest activity while, compound-I has highest activity may be due to the compound-I have highest number of N atoms in its geometry. Order of average potency to inhibit the microorganism is I > II > III ~ VII > V > IV ~ VIII > VI. All the iron(III) complexes exhibit marked augment in biocidal activity may be due to the effect of the metal ion on the normal cell process [37]. A possible mode for increase in biocidal activity may be considered in light of Overtone's concept [38] and Tweedy's chelation theory [39].

DNA binding. Absorption titration is comprehensively used and renowned to decide the binding of the complexes with DNA helix. Complexes bound to DNA through interaction results in red shift (bathochromism) and blue shift (hypochromism) due to interaction between chromophores and the base pair of DNA helix. The level of hypochromism is generally consistent with the strength of intercalative interaction [40–42]. The data of the DNA binding with complexes are represented in Table III. The maxima at about 271 nm is observed in spectrum of Fe(III)-complexes in absence of DNA, which is decreases as the amount of DNA increases and observed at about 249 nm in presence of 7 μgm DNA. The absorption spectra of the complex $[Fe_2(Cip)_2(bipym)_2(pip)(OH)_2].5H_2O$ is shown in Figure 3. Similarly in case of variable ligands (A^1 to A^8), $Fe(NO_3)_2.9H_2O$, and ciprofloxacin maxima observed at about 260 nm in absence of DNA and in presence of 7 μgm DNA maxima observed about 245 nm. All data leads to suggest that in presence of 7 μgm of DNA (maxima at about 245 nm) whole complex dissociate, and free Fe(III), constant ligand(Cip), and variable ligands(A^n) bind with DNA. The effect of compounds in presence of DNA and in absence of DNA have been studied on the basis of difference in λ_{max} of compounds and presented in Figure 4. Order of effect is

Table II. Electronic spectral data of complexes.

Compounds	d–d transition in cm^{-1}			Charge Transfer	μ_{eff}. B.M.
	$^6A_{1g} \rightarrow {}^4T_{1g}$	$^6A_{1g} \rightarrow {}^4T_{2g}$	$^6A_{1g} \rightarrow {}^4A_{1g}, {}^4E_g$		
I	20490	22720	25800	36490	6.02
II	18040	23800	25775	36500	6.00
III	20160	22830	26000	36490	5.85
IV	18010	22730	25800	35700	6.09
V	19050	23800	25770	35580	6.08
VI	20790	23690	24690	35700	5.89
VII	19920	23800	25790	36360	5.94
VIII	20700	23310	25770	35460	5.71

VIII > III > IV > II > I > VI > VII > V, and suggesting that compound- VIII has highest binding property while; compound-V has lowest binding property.

Electrophoretic behavior of complexes-DNA systems. The effect of the binding of complexes on supercoiled (SC) pBR322 was determined by its ability to make it bulky by binding with reactive sites of pBR322 DNA and produce changes in its conformation from supercoiled(SC) to nicked open circular(OC) form. When pBR322 is subjected to electrophoresis, the fastest migration is observed for SC. If one strand is cleaved due to binding with reactive species, the SC form is converted in OC form. Figure 5 shows the electrophoretic process of all eight Fe(III)-complexes after incubation for 1 h. and the comparison of same experiments have been carried out with Fe(III) and ligands. Fe(III)-complexes exhibit nuclease higher activity than that of Fe(III).

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

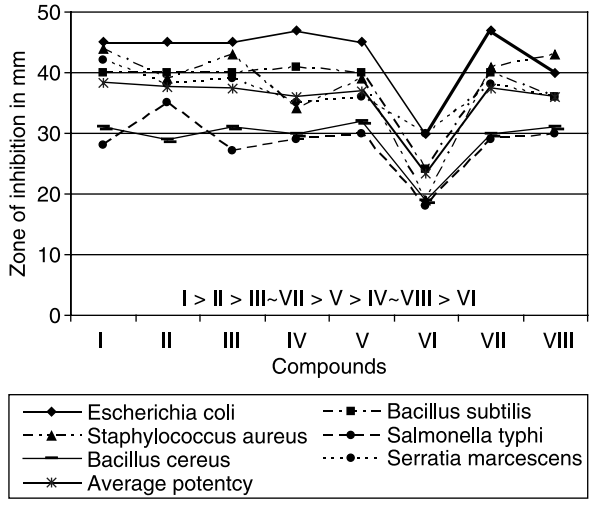


Figure 2. Comparative biocidal activity of compounds.

Table III. DNA binding with complexes data.

Compound	DNA μgm	Compound λ_{max} nm	A^n λ_{max} nm	L λ_{max} nm	Fe(III) λ_{max} nm
I	0	268.2	260.4	260.0	262.6
	3	264.0	259.6	259.2	261.2
	5	261.0	258.6	258.8	259.0
	7	246.2	245.0	247.4	247.8
II	0	270.9	260.6	260.0	262.6
	3	260.8	258.4	259.2	261.2
	5	259.4	258.0	258.8	259.0
	7	248.6	249.4	247.4	247.8
III	0	274.6	260.0	260.0	262.6
	3	260.6	258.2	259.2	261.2
	5	259.4	257.8	258.8	259.0
	7	249.4	246.6	247.4	247.8
IV	0	272.3	263.0	260.0	262.6
	3	263.0	259.2	259.2	261.2
	5	260.2	258.2	258.8	259.0
	7	249.4	244.2	247.4	247.8
V	0	270.6	265.2	260.0	262.6
	3	260.6	259.2	259.2	261.2
	5	260.0	258.2	258.8	259.0
	7	253.0	246.6	247.4	247.8
VI	0	268.5	261.7	260.0	262.6
	3	260.0	258.8	259.2	261.2
	5	258.2	258.0	258.8	259.0
	7	248.6	244.6	247.4	247.8
VII	0	268.4	259.0	260.0	262.6
	3	262.8	258.4	259.2	261.2
	5	260.4	258.0	258.8	259.0
	7	248.6	245.4	247.4	247.8
VIII	0	274.0	260.3	260.0	262.6
	3	260.4	259.4	259.2	261.2
	5	259.4	258.4	258.8	259.0
	7	248.6	247.4	247.4	247.8

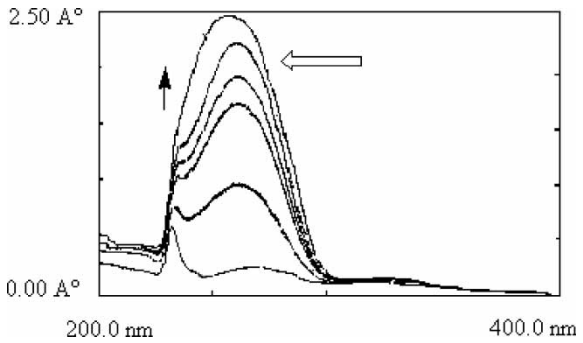


Figure 3. Absorption titration curve of complex $[Fe_2(L)_2(A^1)_2(pip)(OH)_2]\cdot 5H_2O$.

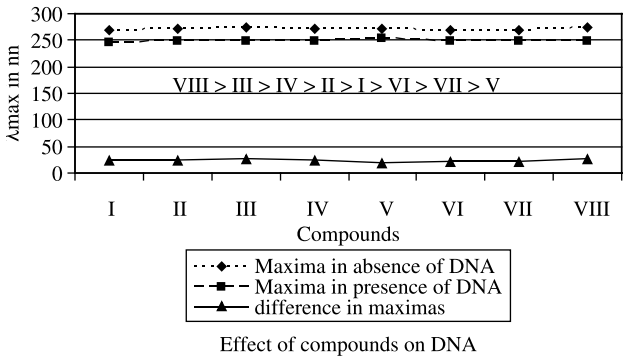


Figure 4. Effect of compounds on DNA.

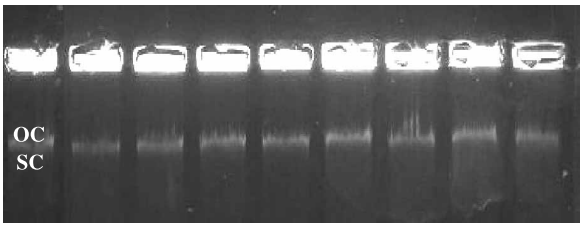


Figure 5. Effect of complexes on pBR322.

7: pBR322 + VI; lane 8: pBR322 + VII; lane 9: pBR322 + VIII

The more cleavage activity of complexes respect to the ligands is clearly evidenced from the figure and data (Table IV). It is observed that SC migrates faster than OC. SC smudge on the gel while OC stay behind in well. It may have two reasons, (I) OC become bulky having high molecular weight due to intercalation of compounds. (II) OC requires more time to run on gel than SC. From the experiment we can conclude that the conversion of SC to OC is higher in presence of complexes than that of in presence of free ligands and Fe(III).

Table IV. Cleavage of pBR322 DNA.

	DNA %			DNA %	
Compounds	SC	OC	Compounds	SC	OC
Fe(III)	66	34	Fe(III)	66	34
A^1	55	45	I	45	55
A^2	75	25	II	23	77
A^3	48	52	III	42	58
A^4	85	15	IV	22	78
A^5	47	53	V	31	69
A^6	84	16	VI	19	81
A^7	81	19	VII	23	77
A^8	87	13	VIII	18	82

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