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Dicoumarol complexes of Cu(II), Fe(II) and Fe(III): preparation, characterization, *in-vitro* antibacterial and DNA binding activity

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3-3′-Benzylidenebis[4-hydroxycoumarin] or 4-nitro,3-3′-benzylidenebis[4-hydroxycoumarin] or 4-methoxy,3-3′-benzylidenebis[4-hydroxycoumarin] and their complexes with Cu(II), Fe(II) and Fe(III) were synthesized and characterized using ^{1}H-NMR, ^{13}C-NMR, IR spectra, electronic spectra, magnetic measurements and elemental analyses. The ligands, metal salts, complexes, control and standard drug were tested for their *in-vitro* antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi*, and *Serratia marcescens*. The metal complexes exhibit good activity against bacterial strains compared with parental compounds and moderate compared with the standard drug (ciprofloxacin). *In-vitro* DNA-binding activity was carried out using agarose gel electrophoresis. The synthesized compounds show effective DNA-binding activity. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: antibacterial activity; DNA-binding activity; gel-electrophoresis; IR- and NMR spectra

INTRODUCTION

Coumarins are the best known lactones and well-known members of the benzopyrone class of flavonoid, the naturally occurring moiety from many plants and essential oils, and is useful for treatment of burns, brucellosis and rheumatic disorders.[1–3] Several coumarin derivatives have been reported for anti-HIV, anti-tumor and anti-platelet aggregation effects as well as acting as DNA gyrase, lipoxygenase and cyclooxygenase inhibitors.[4–7] The pharmacological activity of f-block bis-coumarin complexes have been studied and accurate density functional theory was also performed by Kostova *et al.*[8,9] Recently, a number of coumarin derivatives and their metal complexes have been synthesized and tested for their antihemorrahagic, antifungal, insecticidal, antibacterial, anticancer, antiallergic, anticoagulant, antibacterial, antifungal and pharmacological properties.[10–17] In current work we have synthesized nine novel complexes having moderate antibacterial and good DNA-binding activity.[18]

EXPERIMENTAL

Physical measurements

Carbon, hydrogen and oxygen elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The ^{1}H NMR and ^{13}C NMR was recorded on a Bruker Avance (400 MHz) using d^6-DMSO solvent. The reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO disks) 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}. The thermal study was done by recording thermogravimetricanalysis (TGA) with a 5000/2960 (T. A. Instruments, USA). 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).[19] 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.[20]

MATERIALS AND METHODS

All the chemicals used were of analytical grade. Phenol, malonic acid, zinc chloride, benzaldehyde, 4-methoxybenzal-

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dehyde, 4-nitrobenzaldehyde, cupric nitrate, ferrous sulfate and ferric nitrate were purchased from E. Merck Ltd (India). The organic solvents were purified by standard methods.[21] Agarose and ethidium bromide were purchased from Sigma Chemical Co., India. Bromophenol blue, xylene cyanol FF, tris(hydroxymethyl)methylamine, sucrose, acetic acid and EDTA were purchased from Qualigens Fine Chemicals, India. Agar-agar and luria broth ware purchased from Hi-media Laboratories Pvt. Ltd, India.

Preparation of ligands

4-Hydroxycoumarin

The 4-hydroxycoumarin was synthesized by the reported procedure.[22]

3-3′-Benzylidenebis[4-hydroxycoumarin] = A^1

The 4-hydroxycoumarin (0.1296 g, 0.04 mol) was dissolved in 20 ml of ethanol and heated in water bath for 4–5 h, to obtain a clear solution. Ethanolic solution of (20 ml) benzaldehyde (0.0424g, 0.02mol) was added to hot solution and refluxed in presence of 50 µl H_2SO_4 for 18 h. The fine crystals obtained were separated out and were recrystallized from ethanol. Experimental and physical parameters of ligands A^1–A^3 are summarized in Table 1.

Synthesis of metal complexes

*$[Cu(A^1)_2(H_2O)_2]$ (**I**)*

The 3-3′-benzylidenebis[4-hydroxycoumarin] (0.329 g, 0.04 mol) was dissolved in water(20 ml) by gradually adding aqueous solution of sodium hydroxide (0.1 mol l^{-1}), followed by addition of aqueous solution(20 ml) of $Cu(NO_3)_2 \cdot 3H_2O$ (0.096 g, 0.02 mol), stirred at 30 °C for 5 h and kept for overnight at room temperature. A fine amorphous powder was obtained by filtration and dried in air. Experimental and physical parameters of complexes **I–IX** are summarized in Table 1.

Minimum inhibitory concentration value

The minimal inhibitory concentration (MIC) was ascertained using the method of progressive double dilution in liquid media by variation of compound concentration.[23] All the compounds were found to be effective with different MIC values. The MIC results are expressed as µg ml^{-1} in Table 5.

In-vitro antibacterial activity study

The antibacterial activity of the control, standard drug (ciprofloxacin), ligands, metal salts and its complexes were analyzed against various bacterial cultures of *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Serratia marcescens* using the Agar-Plate technique.[24,25]

Gel electrophoresis

The super coiled pBR322 binding study was carried out in TAE [tris(hydroxymethyl)methylamine, acetic acid and EDTA] buffer pH 8.0. Experiments were designed as DNA alone (control), DNA with ligands and DNA with complex. DNA binding activity experiments were carried out by mixing pBR322 (50μM) in TE (40 mM Tris acetate and 1 mM EDTA) buffer (pH 8.0), and ligand or complex (50μM). The reaction mixture was incubated at room temperature for 1 h, then it was adjusted with 6× loading buffer (40% sucrose, 0.02% bromophenol blue and 0.02% xylene cyanol FF) and loaded onto 0.8% agarose gel. Electrophoresis was carried out on constant voltage (100 V) in a submarine electrophoresis unit (Genei, Bangalore, India). The gel was stained with ethidium bromide. The same experimental conditions were maintained in control assays performed in the existence of imprudent substance 2-mercaptoethanol. The gels were viewed on 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

Chemistry

The dicoumarol ligands A^1–A^3 were synthesized using the reported reaction (Scheme 1).[8,22,26] This reaction involved the removal of the water molecule by refluxing the aldehyde with 4-hydroxycoumarin in the presence of a catalytic amount of sulfuric acid (50 µl). Upon cooling, the products precipitated and were recrystallized from ethanol. The ligands were characterized using elemental analyses, m.p., TLC, IR, ^1H and ^{13}C NMR spectroscopy. The complexes were prepared using a modifying reported process.[8,27] The complexes (Scheme 2) were prepared in aqueous solution by dissolving coumarin by addition of dilute NaOH and followed by addition of metal salts for complex formation. The pH plays a vital role in precipitating the complex in aqueous solution. The complexes were insoluble in water, ether, DMF, ethanol, methanol, hexane, chloroform and dichloromethane with the exception of DMSO. All the complexes were analyzed using magnetic measurements, TGA, IR, ^1H NMR, ^{13}C NMR and electronic spectroscopy. The elemental analysis data are in good agreement with the proposed 1 : 2, metal–ligand formulation and theoretical expectation.

IR spectra

The IR spectra of ligands and complexes are shown in Table 2. The IR spectra of the dicoumarol derivatives show weak bands at ~3099, ~3058, ~1345 and ~1328 cm^{-1} corresponding to ν (O–H) and ν (C–OH) respectively; on complexation these peaks vanished, indicating deprotonation of phenolic proton.[28,29] The peaks at ~3250, ~1285 ~ 875 and ~750 cm^{-1} are attributed to OH stretching, banding, rocking and waging vibrations, respectively, due to the presence of water molecule[30]. These data are further supported by the formation of a metal–oxygen bond, and the peak appears at ~515 cm^{-1}.[31] The ν (C=O) of lacton rings observed at ~1665 and ~1650 cm^{-1} in the free ligand was shifted to a lower frequency (~30 cm^{-1})

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Table 1. Experimental and physical parameter of the compounds

No.	Reactants		Product/empirical formula/MW	Elemental analyses, % found/required				m.p. °C	Yield (g/%)
				C	H	O	M		
1	4-Hydroxycoumarin (0.1296 g, 0.04 mol)	Benzaldehyde (0.0424 g, 0.02 mol)	3-3′-Benzylidenebis[4-hydroxycoumarin] = A^1/$C_{25}H_{16}O_6$/(412.39)	72.70/72.81	3.94/3.91	23.27/23.28	—	227	0.11/65
2	4-Hydroxycoumarin (0.1296 g, 0.04 mol)	*p*-Anisaldehyde (0.0544 g, 0.02 mol)	4-Methoxy, 3-3′-benzylidenebis[4-hydroxycoumarin] = A^2/$C_{26}H_{18}O_7$/(442.42)	70.59/70.58	4.12/4.10	25.23/25.31	—	190	0.09/55
3	4-Hydroxycoumarin (0.1296 g, 0.04 mol)	*p*-Nitro benzaldehyde (0.0604 g, 0.02 mol)	4-Nitro,3-3′-benzylidenebis[4-hydroxycoumarin] = $A^3C_{25}H_{15}NO_8$/(457.40)	65.67/65.65	3.14/3.31	27.78/27.98	—	275	0.12/64
4	A^1 (0.329 g, 0.04 mol)	$Cu(NO_3)_2{\cdot}3H_2O$ (0.096 g, 0.02 mol)	$[Cu(A^1)_2(H_2O)_2]$ = I/$C_{50}H_{34}CuO_{14}$/(922.34)	65.10/65.11	3.65/3.72	24.30/24.29	6.87/6.89	250	0.27/64
5	A^2 (0.353 g, 0.02 mol)	$Cu(NO_3)_2{\cdot}3H_2O$ (0.096 g, 0.02 mol)	$[Cu(A^2)_2(H_2O)_2]$ = II/$C_{52}H_{38}CuO_{16}$/(982.39)	63.11/63.57	3.92/3.90	25.98/26.06	6.47/6.47	>360	0.30/68
6	A^3 (0.365 g, 0.02 mol)	$Cu(NO_3)_2{\cdot}3H_2O$ (0.096 g, 0.02 mol)	$[Cu(A^3)_2(H_2O)_2]$ = III $C_{50}H_{32}CuN_2O_{18}$/(1012.34)	59.18/59.32	3.07/3.19	28.26/28.45	6.15/6.28	295	0.32/70
7	A^1 (0.329 g, 0.04 mol)	$FeSO_4{\cdot}7H_2O$ (0.11 g, 0.02 mol)	$[Fe(A^1)_2(H_2O)_2]$ = IV/$C_{50}H_{34}FeO_{14}$/(914.64)	65.49/65.66	3.68/3.75	24.61/24.49	5.94/6.11	>360	0.26/59
8	A^2 (0.353 g, 0.02 mol)	$FeSO_4{\cdot}7H_2O$ (0.11 g, 0.02 mol)	$[Fe(A^2)_2(H_2O)_2]$ = V/$C_{52}H_{38}FeO_{16}$/(974.69)	64.24/64.08	4.03/3.93	26.30/26.26	5.78/5.73	295	0.29/63
9	A^3 (0.365 g, 0.02 mol)	$FeSO_4{\cdot}7H_2O$ (0.11 g, 0.02 mol)	$[Fe(A^3)_2(H_2O)_2]$ = VI/$C_{50}H_{32}FeN_2O_{18}$/(1004.64)	59.70/59.78	3.21/3.21	28.75/28.67	5.60/5.56	>360	0.31/65
10	A^1 (0.329 g, 0.04 mol)	$Fe(NO_3)_3{\cdot}9H_2O$ (0.161 g, 0.02 mol)	$[Fe(A^1)_2(H_2O)(OH)]$ = VII/$C_{50}H_{33}FeO_{14}$/(913.63)	65.65/65.73	3.54/3.64	24.49/24.52	6.08/6.11	280	0.32/65
11	A^2 (0.353 g, 0.02 mol)	$Fe(NO_3)_3{\cdot}9H_2O$ (0.161 g, 0.02 mol)	$[Fe(A^2)_2(H_2O)(OH)]$ = VIII/$C_{52}H_{37}FeO_{16}$/(973.69)	64.05/64.14	3.80/3.83	26.31/26.29	5.78/5.74	>360	0.30/58
12	A^3 (0.365 g, 0.02 mol)	$Fe(NO_3)_3{\cdot}9H_2O$ (0.161 g, 0.02 mol)	$[Fe(A^3)_2(H_2O)(OH)]$ = IX/$C_{50}H_{31}FeN_2O_{18}$/(1003.63)	59.90/59.84	3.07/3.11	28.57/28.69	5.66/5.56	>360	0.31/60

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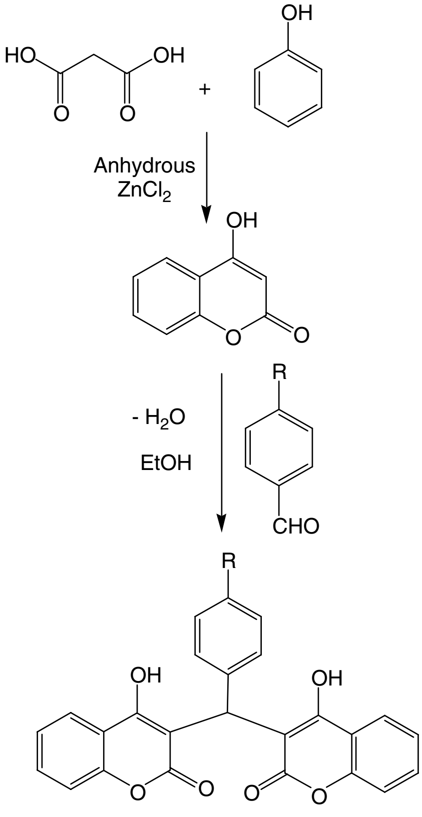
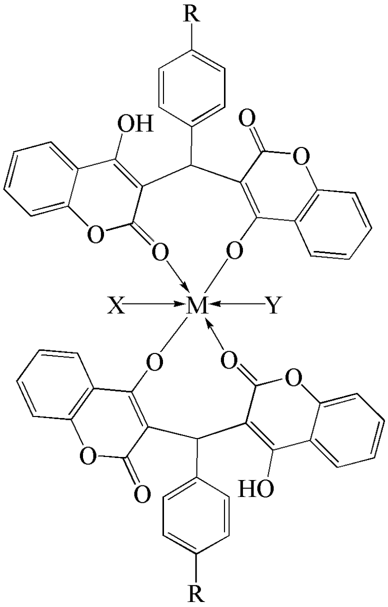


A^1 R=H, A^2 R=OCH_3, A^3 R=NO_2

Scheme 1. General reaction scheme for synthesis of dicoumarols.



Compounds	Metal	R	X	Y
I	Cu(II)	H	H_2O	H_2O
II	Cu(II)	OCH_3	H_2O	H_2O
III	Cu(II)	NO_2	H_2O	H_2O
IV	Fe(II)	H	H_2O	H_2O
V	Fe(II)	OCH_3	H_2O	H_2O
VI	Fe(II)	NO_2	H_2O	H_2O
VII	Fe(III)	H	H_2O	OH
VIII	Fe(III)	OCH_3	H_2O	OH
IX	Fe(III)	NO_2	H_2O	OH

Scheme 2. Proposed structure of complex..

due to complex formation, and further supported by the shifting of ν (C–C), ν (C–O) and ν (C–O–C) stretching frequencies to higher frequencies.[32–34] Two bands at ~1620 and ~1550 cm^{-1} were assigned to stretching vibration of conjugate double bonding in the free ligand.[35] A broad band at ~3395 cm^{-1} observed in the complex was due to the ν (OH) characteristic peak of a coordinated water molecule.[36]

Table 2. IR data of ligands and complexes

Compounds	ν (C–OH) cm^{-1}	ν (H_2O) cm^{-1}	ν (C=O) cm^{-1}	ν (C=C) cm^{-1}	ν (C–C), ν (C–O), ν (C–O–C) cm^{-1}	ν (M–O) cm^{-1}
A^1	1345s, 1330m	3068m, 3032m	1662s, 1653s	1616s 1566s	1180m, 1157m, 1093m, 1053m, 798, 758	—
A^2	1349s, 1332m	3120m, 3070m	1668s, 1645s	1606s, 1550s	1184m, 1165m, 1094s, 1050m, 810, 756	—
A^3	1342s, 1328m	3111m, 3074m	1669s, 1649s	1618s, 1555s	1188m, 1162m, 1093s, 1036m, 811, 769	—
I	—	3396br	1658sh, 1602s	1527s	1205w, 1186m, 1155w, 1100s, 756	520
II	—	3396br	1666sh, 1606s	1510s	1215w, 1180m, 1163w, 1091s, 763	513
III	—	3398br	1649sh, 1600s	1524s	1218w, 1186m, 1160w, 1107s, 759	518
IV	—	3390br	1654sh, 1610s	1527s	1220w, 1185m, 1148w, 1100s, 750	512
V	—	3392br	1660sh, 1608s	1529s	1208w, 1180m, 1155w, 1106s, 754	505
VI	—	3396br	1658sh, 1612s	1532s	1198w, 1190m, 1157w, 1098s, 768	510
VII	—	3392br	1668sh, 1608s	1535s	1203w, 1184m, 1160w, 1107s, 756	515
VIII	—	3379br	1663sh, 1611s	1620s	1210w, 1186m, 1161w, 1100s, 766	510
IX	—	3395br	1656sh, 1609s	1619s	1220w, 1184m, 1153w, 1106s, 765	513

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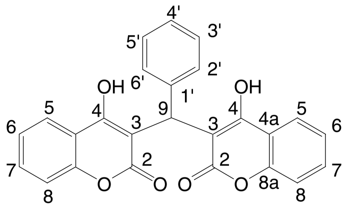
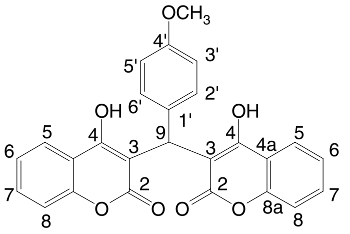
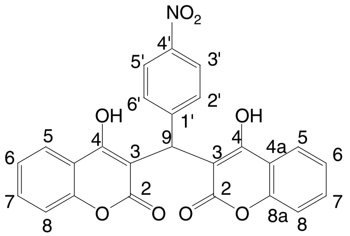
Table 3. ^{13}C NMR data of ligands and complexes

Atom												
	A^1	**I**	**IV**	**VII**	A^2	**II**	**V**	**VIII**	A^3	**III**	**VI**	**IX**
						^{1}H-NMR						
H$_5$–H$_8$, m	7.13–7.33 8H	7.09–7.24 16H	7.01–7.37 16H	7.00–7.50 16H	7.34–7.93 8H	7.25–7.80 16H	7.21–7.79 16H	7.28–7.92 16H	7.58–8.18 8H	7.22–7.98 16H	7.29–7.81 16H	7.20–7.83 16H
H$_9$, s	6.34, 1H	6.25, 2H	6.26, 2H	6.17, 2H	6.34, 1H	6.20, 2H	6.18, 2H	6.02, 2H	6.14, 1H	6.08, 2H	6.09, 2H	6.11, 2H
H$_{3'}$—H$_{5'}$, d	—	—	—	—	7.08, 2H	6.97, 4H	7.02, 4H	7.12, 4H	7.32, 2H	6.71, 4H	6.70, 4H	6.65, 4H
H$_{2'}$—H$_{6'}$, d	—	—	—	—	6.83, 2H	6.73, 4H	6.94, 4H	7.04, 4H	7.44, 2H	7.19, 4H	7.23, 4H	7.13, 4H
H$_{2'}$—H$_{6'}$, m	7.56–7.89 5H	7.50–7.80 10H	7.48–7.84 10H	7.62–8.02 10H	—	—	—	—	—	—	—	—
						^{13}C-NMR						
C=O	168.35	169.94	169.80	169.45	165.38	168.35	168.65	167.98	168.64	169.52	169.38	168.54
C–O	152.76	152.95	153.08	153.11	152.56	165.01	165.24	165.37	159.48	152.84	153.61	154.36
C–O	165.30	167.56	167.54	166.97	164.92	157.30	157.28	157.16	165.76	166.61	165.59	164.80
C–N	—	—	—	—	—	—	—	—	147.45	146.56	145.18	144.91
C–OCH$_3$	—	—	—	—	157.94	152.90	153.13	152.95	—	—	—	—
Aromatic	104.42–132.18	106.08–142.83	106.18–142.75	105.91–142.97	105.04–132.61	104.32–135.89	104.17–135.71	104.50–136.59	105.82–132.84	105.02–138.73	104.11–139.65	103.25–138.47
Aliphatic	36.43	37.15	37.17	37.23	35.69, 55.42	35.78, 55.35	35.84, 55.39	36.03, 55.37	36.54	35.27	36.31	35.20

NMR spectra

The ^1H NMR spectra and ^{13}C NMR spectra of the ligands and complexes were studied in DMSO-d_6. The ^1H NMR and ^{13}C NMR spectral data are reported along with the possible assignment in Table 3. In the case of ^1H NMR spectra of the ligand, peaks observed at ~6.65–8.18 ppm were assigned to the aromatic protons. The singlet peak appearing at ~6.20 ppm was assigned to aliphatic proton. Hydroxyl protons were exhibited at about ~11.50 ppm in ligands. In ^{13}C NMR spectra peaks observed at 104.17–142.83 ppm were assigned to aromatic. Peaks observed at ~168, ~153 and ~165 ppm were assigned to C═O, C–O and C–OH carbons respectively. Aliphatic carbon was exhibited at ~36 ppm. The differences in chemical shifts were observed in the complexes and these changes were attributed to coordination of the ligands to metal. The chemical shift differences were observed for the adjacent carbons of the complex and they confirmed the expected coordination of the ligand through deprotonated hydroxyl and carbonyl oxygen atoms because of electron transfer from the hydroxyl and carbonyl oxygen atoms to the metal. The other carbon atoms were only slightly affected by the coordination of the metal.

Electronic spectra and magnetic properties

Magnetic moments and electronic spectral data are presented in Table 4. The reflectance spectra of Cu(II) complexes displayed two broad bands at ~10,000 and ~14,000 cm^{-1} corresponding to $d_{z^2} \rightarrow d_{x^2-y^2}$ and $d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$, respectively. The Cu(II) complexes exhibited a magnetic moment of 1.76–1.94 B.M. This value is close to the spin-allowed value expected for an $S = 1/2$ system (1.73 B.M.). The electronic spectral and magnetic measurement data suggest that copper(II) complexes may exhibit the distorted octahedral geometry.[37,38] The diffuse reflectance spectra of iron(II) complexes $[Fe(A^n)_2(H_2O)_2]$ exhibited a band at about ~10,100 cm^{-1} due to the $^5T_{2g} \rightarrow ^5E_g$ transition. The magnetic moment values of Fe(II) complexes were in the range 4.70–5.10 B.M. The diffuse reflectance spectra of iron(III) complexes $[Fe(A^n)_2(H_2O)(OH)]$ exhibited two bands at about ~20,000 cm^{-1}, assigned to the $^6A_{1g} \rightarrow ^4T_{2g}$ transitions, and at ~32,500 cm^{-1}, assigned to MLCT in the d^5-system of the Fe(III) atom. The Fe(III) complexes exhibited magnetic moment of 5.93–5.98 B.M. From the electronic spectra and magnetic measurement data of Fe(II) and Fe(III) complexes, an octahedral geometry around the central metal ion is suggested.[37,39,40]

Thermal study

The thermal decomposition curve for the complex was obtained at a heating rate of 10 °C min^{-1} in N_2 atmosphere over the temperature range 50–800 °C using TGA. The initial weight loss was in the range of 150–180 °C, which is equivalent to coordinated water molecules, followed by loss of ligand molecules in the temperature range 180–800 °C.[36]

Table 4. Electronic spectral and magnetic measurement data of complexes

Compounds	d–d transition in cm^{-1}	Assignment	$\mu_{eff.}$ B.M.
I	13,700	$d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$	1.89
	10,100	$d_{z^2} \rightarrow d_{x^2-y^2}$	
II	13,900	$d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$	1.76
	10,100	$d_{z^2} \rightarrow d_{x^2-y^2}$	
III	13,800	$d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$	1.94
	10,200	$d_{z^2} \rightarrow d_{x^2-y^2}$	
IV	10,050	$^5T_{2g} \rightarrow ^5E_g$	4.70
V	10,050	$^5T_{2g} \rightarrow ^5E_g$	5.06
VI	10,000	$^5T_{2g} \rightarrow ^5E_g$	5.10
VII	20,200	$^6A_{1g} \rightarrow ^4T_{2g}$	5.96
	32,900	MLCT	
VIII	20,000	$^6A_{1g} \rightarrow ^4T_{2g}$	5.98
	31,200	MLCT	
IX	18,900	$^6A_{1g} \rightarrow ^4T_{2g}$	5.93
	32,700	MLCT	

Table 5. Minimum inhibitory concentration (μg ml^{-1}) of compounds against bacteria

Compounds	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhi*	*Serratia marcescens*
A^1	>495	>495	>495	>495	>495	>495
A^2	>530	>530	>530	>530	>530	>530
A^3	>550	>550	>550	>550	>550	>550
I	368	370	375	368	365	360
II	392	393	395	392	390	390
III	404	405	400	405	400	405
IV	365	365	360	367	360	370
V	389	390	389	390	385	380
VI	401	402	400	402	401	400
VII	365	360	365	366	365	360
VIII	389	390	390	395	389	380
IX	401	401	400	405	402	400

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Table 6. *In vitro* antimicrobial activity data against bacteria (zone of inhibition in millimetres)

Compounds	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhi*	*Serratia marcescens*
Control	11	10	11	11	10	11
$Cu(NO_3)_2^{\bullet}3H_2O$	12	11	12	11	11	12
$FeSO_4^{\bullet}7H_2O$	11	10	11	11	11	11
$Fe(NO_3)_3^{\bullet}9H_2O$	11	12	11	12	11	12
Ciprofloxacin	29	35	28	31	30	35
A^1	14	14	14	13	13	13
A^2	15	15	15	16	14	16
A^3	12	14	12	12	14	18
I	14	18	16	14	15	17
II	16	15	16	17	15	17
III	13	14	15	14	15	19
IV	14	15	15	15	14	17
V	17	16	16	17	15	17
VI	14	16	14	13	15	20
VII	15	16	14	14	14	17
VIII	16	17	17	17	14	18
IX	15	15	14	13	14	18

Figure 1. pBR322 in presence of ligands and complexes. Lane 1: Control(pBR 322), Lane 2: Cu(II) + pBR322, Lane 3: Fe(II) + pBR322, Lane 4: Fe(III) + pBR322, Lane 5: A^1 + pBR322, Lane 6: **I** + pBR322, Lane 7: **IV** + pBR322, Lane 8: **VII** + pBR322, Lane 9: A^2 + pBR 322, Lane 10: **II** + pBR322, Lane 11: **V** + pBR322, Lane 12: **VIII** + pBR322, Lane 13: A^3 + pBR322, Lane 14: **III** + pBR322, Lane 15: **VI** + pBR322, Lane 16: **IX** + pBR322.

The residual weight was in good agreement with metal oxides. These results are in good agreement with the proposed composition of the complexes.

Biology

In vitro *antibacterial activity*

The antibacterial activity data are summarized in Tables 5 and 6. Comparative analysis shows higher antibacterial activity of the complexes than free ligands, metal salt and control (DMSO). The complexes exhibit moderate activities as compared with the standard drug ciprofloxacin. This enhancement in antibacterial activity is rationalized on the basis of Overtone's concept, Tweedy's chelation theory and the partial sharing of the positive charge of metal ions with donor groups.[41–48]

DNA-binding activity

The outcome from binding of complexes with pBR322 was determined by its ability to create it bulky by binding with reactive sites of pBR322 DNA and to make changes in its conformation from supercoiled (SC) to nicked open circular (OC) form. When pBR322 was subjected to electrophoresis, the fastest migration was observed for SC. If one strand was cleaved due to binding with reactive species, the SC form was converted to the OC form. To rule out the observed binding in the DNA being a consequence of a direct interaction of complexes, additional experiments were carried out using ligands, metal salts, ligands with 2-mercaptoethanol and complexes with 2-mercaptoethanol. The results (Figs 1 and 2; Table 7) indicate that the ligands and complexes converted SC DNA to OC DNA, while the extent of conversion was less in the case of ligands and metal ions.

The data suggests that the complexes have good cleaving and binding efficacy compared with ligands and metal ions. In the presence of the reactive substance 2-mercaptoethanol with ligands or complexes, good efficacy of binding was observed (lanes 2–13, Fig. 2), i.e. SC DNA was completely converted into OC DNA, while no effect was observed in the case of pBR322 with 2-mercaptoethanol alone (lane 1, Fig. 2).

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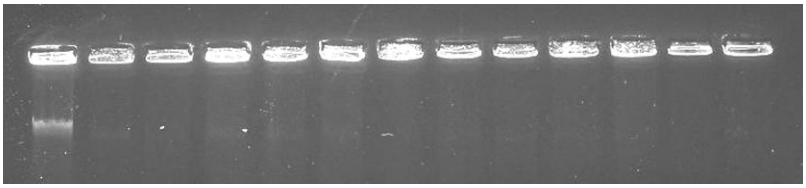

Figure 2. pBR322 in presence of ligands and complexes with 2-mercaptoethanol. Lane 1, control (pBR 322); lane 2, A^1 + pBR322; lane 3, I + pBR322; lane 4, **IV** + pBR322; lane 5, **VII** + pBR322; lane 6, A^2 + pBR 322; lane 7, **II** + pBR322; lane 8, **V** + pBR322; lane 9, **VIII** + pBR322; lane 10, A^3 + pBR322; lane 11, **III** + pBR322; lane 12, **VI** + pBR322; lane 13, **IX** + pBR322.

Table 7. DNA binding data of ligands and complexes

% DNA/ compounds	Control	Cu(II)	A^1	**I**	**II**	**III**
SC	100.0	72.15	9.48	8.06	6.41	9.43
OC	00.00	27.85	90.52	91.94	93.59	90.57
% DNA/ compounds	Control	Fe(II)	A^2	**IV**	**V**	**VI**
SC	100.0	73.57	10.64	7.82	7.73	9.45
OC	00.00	26.43	89.36	92.18	92.27	90.55
% DNA/ compounds	Control	Fe(III)	A^3	**VII**	**VIII**	**IX**
SC	100.0	65.85	10.50	7.40	6.94	9.16
OC	00.00	34.15	89.50	92.60	93.06	90.84

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