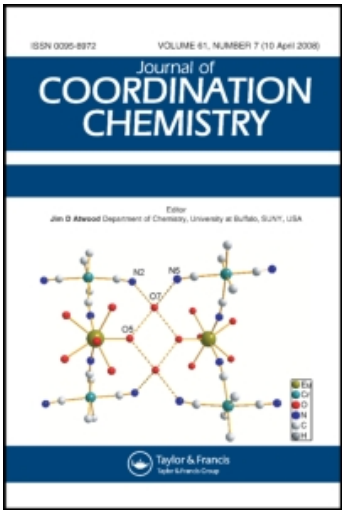

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Synthesis, characterization, *in-vitro* biocidal and nuclease activity of some coordination compounds

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Complexes of Cu(II), Fe(II) and Fe(III) have been synthesized with 2-nitro-3,3′-benzylidene *bis*[4-hydroxycoumarin]/4-chloro-3,3′-benzylidene *bis*[4-hydroxycoumarin]/4-hydroxy-3,3′-benzylidene *bis*[4-hydroxycoumarin]. They have been characterized using ^{1}H-NMR, ^{13}C-NMR, IR spectra, electronic spectra, magnetic measurements, elemental analyses and screened for their *in-vitro* biocidal activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, and *Serratia marcescens* bacterial strains and for their *in-vitro* antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Lasiodiplodia theobromae*. The metal complexes exhibit good activity against *bacterial strains* compared to parent compounds, but no significant antifungal activity against *fungal strains*. *In-vitro* nuclease activity has been carried out using agarose gel electrophoresis. The synthesized compounds show effective nuclease activity.

Keywords: Coordination compounds; Dicoumarols; *In-vitro* nuclease activity; Biocidal activity; Thermal and spectral studies

1. Introduction

Coumarins are members of the benzopyrone class flavonoid and both naturally occurring and synthetic derivatives of coumarin are known for their antibacterial, antifungal, insecticidal, anticancer, antiallergic, antihaemorrahagic and anticoagulant activity [1–8], and for treatment of burns, brucellosis, and rheumatic disorders [9, 10]. Several coumarin derivatives were reported for anti HIV activity, which may due to inhibition of DNA gyrase [11, 12], and hydroxy coumarin derivatives were reported as promising inhibitors of HIV integrase and HIV protease [13, 14]. Moreover, 3,3′-methylene *bis*[4-hydroxy coumarin] (dicoumarol) is the main part in naturally occurring anticoagulants derived from coumarin, metabolized from sweet clover (*Melilotus alba* and *Melilotus officinalis*) [15–17]. Dicoumarols and their metal complexes induce oxidative stress and cyatotoxicity toward cancerous pancreatic

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cells, as well as apoptosis, time and dose dependent [18–21]. In continuation of our earlier work [22], we investigate coordination of 2-nitro-3,3′-benzylidene *bis*[4-hydroxycoumarin], 4-chloro-3,3′-benzylidene *bis*[4-hydroxycoumarin] and 4-hydroxy-3,3′-benzylidene *bis*[4-hydroxycoumarin] in complexation with Cu(II), Fe(II) and Fe(III). The obtained metal complexes were characterized by ^{1}H–NMR, ^{13}C–NMR, IR spectroscopy, electronic spectroscopy, magnetic measurements and elemental analyses. The ligands, metal salts and complexes were screened for their *in-vitro* biocidal activity.

2. Experimental

2.1. *Materials and methods*

All the chemicals were of analytical grade. Phenol, malonic acid, zinc chloride, 2-nitrobenzaldehyde, 4-cholorobenzaldehyde, 4-hydroxybenzaldehyde, cupric nitrate, ferrous sulphate, phosphorus oxychloride and ferric nitrate were purchased from E. Merck Ltd., India. Luria broth and agar-agar were purchased from Hi-media Laboratories Pvt. Ltd., India. 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. The organic solvents were purified by standard methods [23].

2.2. *Physical measurements*

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance (400 MHz). Infrared spectra were recorded on an FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The reflectance spectra of the complexes were recorded in the range 1700–350 nm (as MgO discs) on a Beckman DK-2A spectrophotometer. Elemental analyses (C, H and N) were performed using a model 240 Perkin–Elmer elemental analyzer. The metal contents of the complexes were analyzed by EDTA titration [24] after decomposing with a mixture of $HClO_4$, H_2SO_4, and HNO_3 in ratio of 1 : 1.5 : 2.5. The water and halogen content were determined by Karl Fisher method and Stepnow's method, respectively. The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20°C), using Citizen Balance. Diamagnetic corrections were made using Pascal's constants [25].

2.3. *Preparation of ligands*

4-*Hydroxycoumarin*: the 4-hydroxycoumarin was synthesized as reported [26].

2.3.1. 3,3′-((2-Nitrophenyl)methylene)*bis*(4-hydroxycoumarin) = A^{1}. Was prepared by condensing 4-hydroxycoumarin (0.04 mol; 0.129 g) dissolved in 20 mL of ethanol (warmed just to get a clear solution) and an ethanolic solution of 2-nitrobenzaldehyde (20 mL; 0.02 mol; 0.060 g) was added and kept under reflux for 18 h. Fine crystals of 3,3′-((2-nitrophenyl)methylene)*bis*(4-hydroxycoumarin) separated. Yield: 50%,

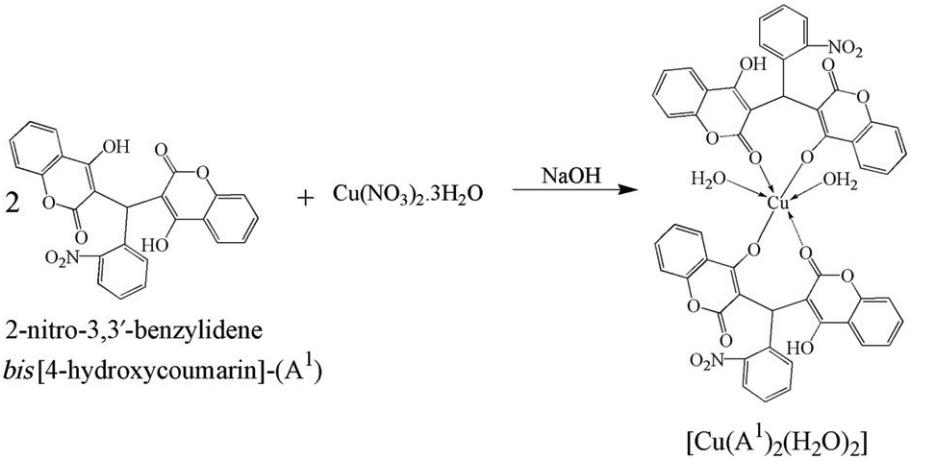
m.p.: 196°C, Found (%): C, 65.57; H, 3.28; N, 3.00. $C_{25}H_{15}NO_8$ (457.39) calculated (%): C, 65.65; H, 3.31; N, 3.06.

2.3.2. 3,3′-((4-Chlorophenyl)methylene)*bis*(4-hydroxycoumarin) = A^2. 4-Hydroxycoumarin (0.04 mol; 0.129 g) was dissolved in 20 mL of ethanol (warmed just to get a clear solution) and an ethanolic solution of 4-chlorobenzaldehyde (20 mL; 0.02 mol; 0.056 g) was added and refluxed for 18 h. Fine crystals of 3,3′-((4-chlorophenyl)methylene)*bis*(4-hydroxycoumarin) separated. Yield: 40%, m.p.: 188°C, Found (%): C, 67.19; H, 3.39; Cl, 7.90. $C_{25}H_{15}ClO_6$ (446.84) calculated (%): C, 67.20; H, 3.38; Cl, 7.93.

2.3.3. 3,3′-((4-Hydroxyphenyl)methylene)*bis*(4-hydroxycoumarin) = A^3. The 4-hydroxycoumarin (0.04 mol; 0.129 g) was dissolved in 20 mL of ethanol (warmed just to get a clear solution). An ethanolic solution of 4-hydroxybenzaldehyde (20 mL; 0.048 g, 0.02 mol) was added and refluxed for 18 h. Fine crystals of 3,3′-((4-hydroxyphenyl)-methylene)*bis*(4-hydroxycoumarin) separated. Yield: 45%, m.p.: 205°C, Found (%): C, 70.11; H, 3.84. $C_{25}H_{16}O_7$ (428.39) calculated (%): C, 70.09; H, 3.76.

2.4. *Synthesis of coordination compounds*

2.4.1. $[Cu(A^1)_2(H_2O)_2]$ (1). The 3,3′-((2-nitrophenyl)methylene)*bis*(4-hydroxycoumarin) A^1 (0.04 mol; 0.366 g) was dissolved in water by gradual addition of aqueous sodium hydroxide (20 mL; 0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (0.02 mol; 0.096 g), stirred at 30°C for 5 h and kept overnight at room temperature. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Proposed reaction is shown in scheme 1. Yield: 60%, m.p.: 245°C, Found (%): C, 59.36; N, 2.77; H, 3.08; Cu, 6.19. $C_{50}H_{32}CuN_2O_{18}$ (1012.34) calculated (%): C, 59.32; N, 2.77; H, 3.19; Cu, 6.28.



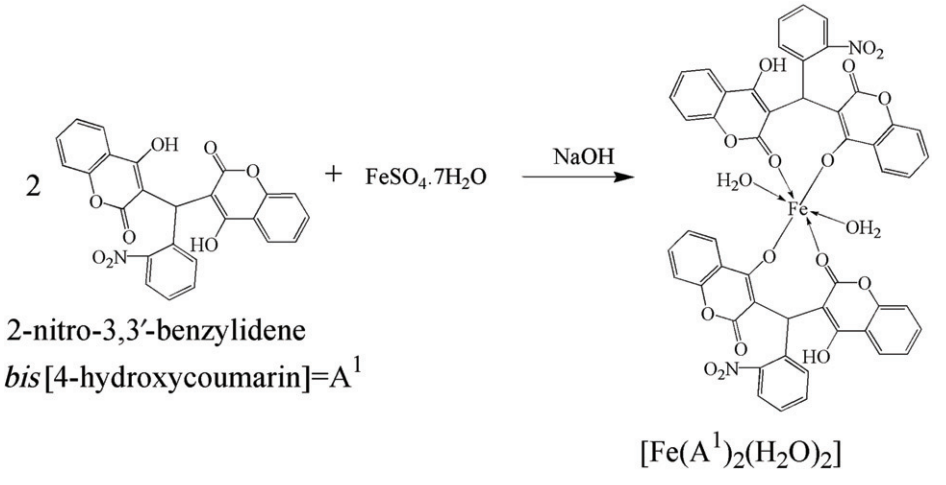
Scheme 1. Synthesis of **1**.

2.4.2. $[Cu(A^2)_2(H_2O)_2]$ (2). The 3,3′-((4-chlorophenyl)methylene)*bis*(4-hydroxycoumarin) A^2 (0.04 mol, 0.357 g) was dissolved in water (20 mL) by gradual addition of aqueous sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (0.02 mol; 0.096 g), stirred at 30°C for 5 h and kept overnight at room temperature. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 50%, m.p.: 240°C, Found (%): C, 60.67; H, 3.31; Cl, 7.10; Cu, 6.29. $C_{50}H_{32}Cl_2CuO_{14}$ (991.23) calculated (%): C, 60.58; H, 3.25; Cl, 7.15; Cu, 6.41.

2.4.3. $[Cu(A^3)_2(H_2O)_2]$ (3). The 3,3′-((4-hydroxyphenyl)methylene)*bis*(4-hydroxycoumarin) A^3 (0.343 g, 0.04 mol) was dissolved in water (20 mL) by gradual addition of aqueous sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (0.02 mol, 0.096 g), stirred at 30°C for 5 h and kept overnight at room temperature. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 40%, m.p.: 250°C, Found (%): C, 62.98; H, 3.51; Cu, 6.58. $C_{50}H_{34}CuO_{16}$ (954.34) calculated (%): C, 62.93; H, 3.59; Cu, 6.66.

2.4.4. $[Fe(A^1)_2(H_2O)_2]$ (4). The 3,3′-((2-nitrophenyl)methylene)*bis*(4-hydroxycoumarin) (0.04 mol; 0.366 g) was dissolved in water (20 mL) by gradually adding aqueous sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $FeSO_4 \cdot 7H_2O$ (0.02 mol; 0.11 g), stirring at 30°C for 7 h and storing overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Proposed reaction is shown in scheme 2. Yield: 58%, m.p.: > 360°C, Found (%): C, 59.87, H, 3.14; N, 2.82; Fe, 5.59. $C_{50}H_{32}FeN_2O_{18}$ (1004.64) calculated (%): C, 59.78; H, 3.21; N, 2.79; Fe, 5.56.

2.4.5. $[Fe(A^2)_2(H_2O)_2]$ (5). 3,3′-((4-Chlorophenyl)methylene)*bis*(4-hydroxycoumarin) A^2 (0.04 mol; 0.357 g) was dissolved in water (20 mL) by gradual addition of aqueous



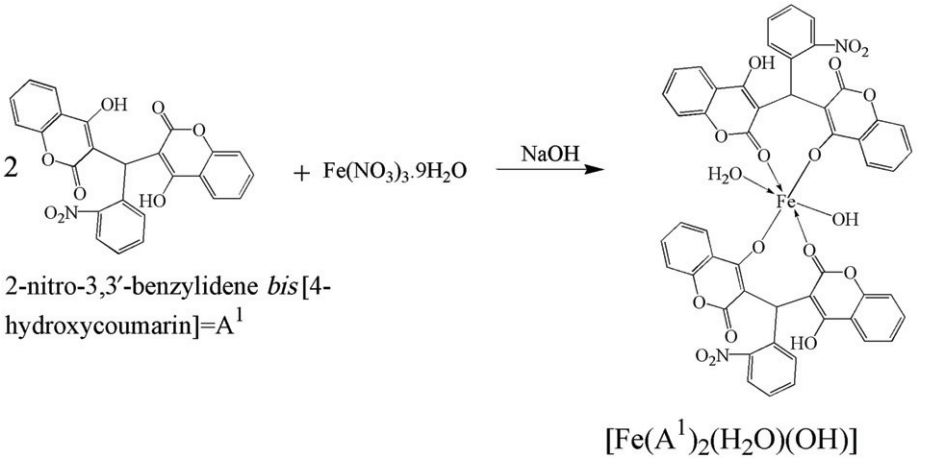
Scheme 2. Synthesis of **4**.

sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $FeSO_4 \cdot 7H_2O$ (0.02 mol; 0.11 g), stirring at 30°C for 7 h and storing overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 45%, m.p.: 210°C, Found (%): C, 59.97; H, 3.27; Cl, 7.20; Fe, 5.79. $C_{50}H_{32}Cl_2FeO_{14}$ (983.53) calculated (%): C, 61.06; H, 3.28; Cl, 7.21; Fe, 5.68.

2.4.6. $[Fe(A^3)_2(H_2O)_2]$ (6). The 3,3′-((4-hydroxyphenyl)methylene)*bis*(4-hydroxycoumarin) A^3 (0.04 mol, 0.343 g) was dissolved in water (20 mL) by gradual addition of aqueous sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $FeSO_4 \cdot 7H_2O$ (0.02 mol; 0.11 g), stirred at 30°C for 7 h and kept overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 42%, m.p.: 265°C, Found (%): C, 63.58; H, 3.51; Fe, 5.82. $C_{50}H_{34}FeO_{16}$ (946.64) calculated (%): C, 63.44; H, 3.62; Fe, 5.90.

2.4.7. $[Fe(A^1)_2(H_2O)(OH)]$ (7). The 3,3′-((2-nitrophenyl)methylene)*bis*(4-hydroxycoumarin) A^1 (0.04 mol; 0.366 g) was dissolved in water (20 mL) by gradual addition of aqueous sodium hydroxide (0.1 mol L^{-1}), followed by addition of 20 mL aqueous solution of $Fe(NO_3)_3 \cdot 9H_2O$ (0.02 mol; 0.161 g), stirring at 30°C for 8 h and storing overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Proposed reaction is shown in scheme 3. Yield: 65%, m.p.: 185°C, Found (%): C, 59.91; H, 3.22; N, 2.82; Fe, 5.54. $C_{50}H_{31}FeN_2O_{18}$ (1003.63) calculated (%): C, 59.84; H, 3.11; N, 2.79; Fe, 5.56.

2.4.8. $[Fe(A^2)_2(H_2O)(OH)]$ (8). The 3,3′-((2-nitrophenyl)methylene)*bis*(4-hydroxycoumarin) A^1 (0.04 mol; 0.357 g) was dissolved in water (20 mL) as before, followed by addition of 20 mL aqueous solution of $Fe(NO_3)_3 \cdot 9H_2O$ (0.02 mol; 0.161 g), stirred at 30°C for 8 h and kept overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 54%, m.p.: 240°C, Found (%): C, 61.08;



Scheme 3. Synthesis of **7**.

Table 1. Minimum inhibitory concentration ($\mu g\,mL^{-1}$) of compounds against bacteria.

Compounds	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhi*	*Serratia marcescens*
A^1	>540	>540	>540	>540	>540	>540
A^2	>532	>532	>532	>532	>532	>532
A^3	>512	>512	>512	>512	>512	>512
1	404	404	404	404	404	404
2	396	396	396	396	395	396
3	380	380	380	381	381	380
4	401	404	401	404	404	404
5	392	393	392	393	393	392
6	378	378	378	378	378	378
7	401	350	401	404	400	401
8	392	392	392	392	392	392
9	377	377	377	378	377	377

H, 3.23; Cl, 7.20; Fe, 5.70. $C_{50}H_{31}Cl_2FeO_{14}$ (982.52) calculated (%): C, 61.12; H, 3.18; Cl, 7.22; Fe, 5.68.

2.4.9. $[Fe(A^3)_2(H_2O)(OH)]$ (9). The 3,3′-((4-hydroxyphenyl)methylene)*bis*(4-hydroxycoumarin) A^3 (0.04 mol; 0.343 g) was dissolved in water (20 mL) as above, followed by addition of 20 mL aqueous solution of $Fe(NO_3)_3 \cdot 9H_2O$ (0.02 mol; 0.161 g), stirring at 30°C for 8 h and kept overnight. Fine amorphous product obtained by filtration was washed with *n*-hexane and dried in air. Yield: 48%, m.p.: 295°C, Found (%): C, 63.48; H, 3.56; Fe, 5.80. $C_{50}H_{33}FeO_{16}$ (945.63) calculated (%): C, 63.51; H, 3.52; Fe, 5.91.

2.5. *Minimum inhibitory concentration (MIC) value*

The minimal inhibitory concentration (MIC) was determined using the method of progressive double dilution in liquid media by varying concentration [27]. All the compounds were found to be effective with different MIC values. The MIC results are expressed as $\mu g\,mL^{-1}$ in table 1.

2.5.1. Procedure for determination of MIC value. A solution of compound having 2000 μM concentration was sterilized by autoclaving followed by filtration. Two rows of 12 sterile tubes (7.5 × 1.3 cm) were capped and solution of the compound in 10 tubes with 5, 10, 15, 20, ... μL made to 20 mL with 2% culture medium sterilized by autoclaving. Under sterile conditions bacterial species were added and incubated for 18 h at 37°C. Very faint turbidity may be given by the inoculum itself; the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC is used as the control to check reagents and conditions.

2.6. In-vitro *biocidal activity*

The biocidal activity of the control, ciprofloxacin, ligands, metal salts and complexes was performed against various microbial cultures of *Staphylococcus aureus*, *Bacillus*

Table 2. *In-vitro* biocidal activity data against bacteria (zone of inhibition in mm).

Compounds	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhi*	*Serratia marcescens*
Control	11	10	11	11	10	11
$Cu(NO_3)_2 \cdot 3H_2O$	12	11	12	11	11	12
$FeSO_4 \cdot 7H_2O$	11	10	11	11	11	11
$Fe(NO_3)_3 \cdot 9H_2O$	11	12	11	12	11	12
Ciprofloxacin	29	35	28	31	30	35
A^1	13	12	14	14	13	14
A^2	12	16	16	14	13	14
A^3	11	13	15	11	12	14
1	15	14	16	16	15	17
2	13	17	18	15	14	15
3	12	14	17	14	13	16
4	15	14	15	15	16	15
5	14	16	17	15	13	15
6	13	15	16	13	14	15
7	16	15	17	16	15	17
8	13	17	17	15	14	15
9	14	14	19	13	15	19

subtilis, *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli* and *Serratia marcescens* and three fungi cultures, namely *Aspergillus niger*, *Aspergillus flavus* and *Lasiodiplodia theobromae*, using the Agar-Plate technique [28]. The biocidal activity data are summarized in table 2.

2.7. *Gel electrophoresis*

Inspection of super coiled pBR322 is carried out in TAE [*tris*(hydroxymethyl)methylamine, acetic acid and EDTA] buffer pH 8.0 of DNA alone (control), DNA in presence of ligands and DNA in presence of complexes. Nuclease activity experiments have been accomplished by mixing pBR322 (50 μM) in TE [40 mM tris acetate and 1 mM EDTA] buffer (pH 8.0), and ligand or complex (50 μM). Reaction mixture was incubated at room temperature for 1 h then 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 on constant voltage (100 V) in a submarine electrophoresis unit (Genie, Banglore, India). Gel was stained with ethidium bromide. The same experimental condition was maintained for control assays performed in the presence of 2-mercaptoethanol. The gels were viewed on UV transilluminator; images were captured with an attached camera and estimated using AlphaDigiDoc^{TM} RT, Version V.4.1.0 PC-Image software.

3. Results and discussion

The ligands (A^1–A^3) have been prepared by refluxing an ethanolic solution of 4-hydroxy coumarin with the corresponding aldehydes in mole ratio of 2:1, respectively. The structures of the synthesized ligands were established by

microanalytical data, NMR and IR spectra. All metal complexes (I-IX) were prepared by stoichiometric reaction of the corresponding ligand with the respective metal salt in a molar ratio M : L of 1 : 2 and have been characterized using IR spectra, electronic spectra, magnetic measurements, thermal analysis and elemental analyses. The elemental analyses are in good concurrence with theoretical expectation. All are air and moisture stable, colored, amorphous solids insoluble in ether, hexane, chloroform, water, methanol and dimethyl formamide and soluble in DMSO. Poor solubility of complexes in common organic solvents has inhibited crystal growth.

3.1. *^{1}H and ^{13}C NMR spectra*

The ^{1}H and ^{13}C NMR spectral data are reported with possible assignment in table 3. The ^{1}H and ^{13}C NMR spectra of the ligands and complexes were taken in DMSO-d_6. In ^{13}C NMR spectra, peaks observed at 91.57–149.94 ppm were assigned to aromatic carbons, peaks at 166.69, 159.45, 163.13 ppm assigned to C=O, C–O, and C–OH carbons, respectively, and aliphatic carbon at 36.72 ppm. For ^{1}H NMR spectra of the ligands, peaks observed in the range 7.11–8.16 ppm were assigned to aromatic protons, the singlet at 6.35 ppm to aliphatic proton and hydroxyl protons at 11.25 ppm. Metal coordinates with ligand by one oxygen of C=O and one deprotonated hydroxyl group, in good agreement with ^{1}H and ^{13}C–NMR spectra. The difference in chemical shift shows that the metal binds to ligand through deprotonated OH; there is also a peak at about 9.81 ppm corresponding to free OH group. The chemical shift differences observed for neighboring carbons of the complex confirmed coordination through deprotonated hydroxyl and carbonyl oxygen atoms. The other carbons were only slightly affected due to coordination.

3.2. *IR spectra*

The modes of binding of the ligands to metal were also elucidated using IR spectra. Bands at 3122, 3070, 1696, 1640, 1606, 1554, 1489, 1344, 1335, 1180, 1163, 1091, 1033, 800, 763 cm^{-1} are found in IR spectra of **1**. Bands about 1606 and 1554 cm^{-1} are stretching vibrations of the conjugated olefinic system [29] and the band at 1489 cm^{-1} to the aromatic systems. A broad band at 3300–3400 cm^{-1} corresponds to ν(O–H) of coordinated water as well as phenolic OH [30, 31]. A comparison of the infrared spectra of the ligand and of the complex reveal that bands in free ligand at 3122, 3070 cm^{-1} and 1344, 1335 cm^{-1} associated with the stretching and deformation of the phenolic OH groups, still remain in spectra of the complexes, indicates the presence of phenolic proton. Bands at 1606, 1554, 1489, 800, 763 cm^{-1} can be attributed to stretching of nitro group of A^1, which still remain in **1**. The bands at 1696 and 1640 cm^{-1} were attributed to stretching vibrations of the carbonyl groups ν(C=O) of the lactone rings [32], shifted to 1658 and 1614 cm^{-1} on complexation, indicating participation of C=O in coordination. The C–C and C–O stretches and the C–O–C bands were shifted higher in the complex [33–35]. These data were supported by ν(M–O) band at 505–512 cm^{-1} [36]. Interpretation of IR spectra of remaining ligands and their respective complexes was carried out; IR data for all ligands and complexes are condensed in table 4.

Table 3. ^{1}H and ^{13}C NMR data of ligands and complexes.

Compounds	^{1}H NMR δ-ppm	^{13}C NMR δ-ppm
A^{1}	7.28–7.98 (Aromatic), 6.54 (Aliphatic), 10.92 (OH phenolic)	99.07–149.94 (Aromatic), 34.68 (Aliphatic), 168.29 (C=O), 157.68 (C–O),163.82 (C–OH)
A^{2}	7.27–8.16 (Aromatic), 5.88 (Aliphatic), 11.25 (OH phenolic)	91.57–135.53 (Aromatic), 37.60 (Aliphatic), 166.69 (C=O), 162.71 (C–O),163.13 (C–OH)
A^{3}	7.11–7.84 (Aromatic), 6.35 (Aliphatic), 11.30, 9.89 (OH phenolic)	98.81–135.50 (Aromatic), 36.72 (Aliphatic), 162.85 (C=O), 159.45 (C–O),162.11 (C–OH)
1	7.16–7.86 (Aromatic), 6.37 (Aliphatic)	98.00–144.13 (Aromatic), 32.71 (Aliphatic), 169.89, 155.45, 164.97 (C=O, C–O, C–O)
2	7.19–7.94 (Aromatic), 5.67 (Aliphatic)	90.15–133.38 (Aromatic), 41.83 (Aliphatic), 170.04, 159.97, 165.27 (C=O, C–O, C–O)
3	6.59–7.77 (Aromatic), 5.88 (Aliphatic)	94.45–134.43 (Aromatic), 39.45 (Aliphatic), 160.95, 155.59, 168.71 (C=O, C–O, C–O)
4	7.19–7.81 (Aromatic), 6.41 (Aliphatic)	97.59–143.39 (Aromatic), 31.74 (Aliphatic), 169.48, 154.72, 165.01 (C=O, C–O, C–O)
5	7.20–8.02 (Aromatic), 5.71 (Aliphatic)	89.96–130.84 (Aromatic), 39.92 (Aliphatic), 169.63, 160.08, 164.70 (C=O, C–O, C–O)
6	6.89–7.65 (Aromatic), 6.21 (Aliphatic)	94.18–134.24 (Aromatic), 39.87 (Aliphatic), 160.16, 159.45, 169.16 (C=O, C–O, C–O)
7	7.14–7.83 (Aromatic), 6.39 (Aliphatic)	96.98–144.05 (Aromatic), 32.61 (Aliphatic), 170.00, 154.03, 166.75 (C=O, C–O, C–O)
8	7.16–7.91 (Aromatic), 5.68 (Aliphatic)	90.09–131.00 (Aromatic), 40.68 (Aliphatic), 169.03, 161.15,163.88 (C=O, C–O, C–O)
9	7.01–7.62 (Aromatic), 6.16 (Aliphatic)	98.81–134.08 (Aromatic), 38.53 (Aliphatic), 160.74, 155.51,168.94 (C=O, C–O, C–O)

Table 4. IR data of ligands and complexes.

Compounds	$\nu_{(O-H/H_2O)}\,cm^{-1}$	$\nu_{(C=O)}\,cm^{-1}$	$\nu_{(C=C)}\,cm^{-1}$	$\nu_{(C-O)}\,cm^{-1}$	δ(COH)	$\nu_{(M-O)}\,cm^{-1}$
A^1	3122m, 3070m	1696s, 1640s	1606s, 1554s	1180m, 1163m, 1091m, 1033m, 800, 763	1344m, 1335m	–
A^2	3120m, 3068m	1685s, 1660s	1624s, 1556s	1180m, 1161m, 1095s, 1033m, 781, 754	1345m, 1336m	–
A^3	3125m, 3066m	1690s, 1662s	1601s, 1564s	1182m, 1168m, 1097s, 1043m, 810, 761	1355m, 1386m	–
1	3396br	1658sh, 1614s	1523s	1213w, 1184m, 1166w, 1099s, 767	1358m, 1336w	510
2	3393br	1664sh, 1610s	1521s	1209w, 1182m, 1160w, 1104s, 764	1356m, 1335w	508
3	3390br	1665sh, 1612s	1526s	1198w, 1180m, 1162w, 1078m, 760	1355m, 1336m	505
4	3373br	1654sh, 1608s	1525s	1207w, 1186m, 1150w, 1105s, 761	1357m, 1335w	509
5	3393br	1658sh, 1614s	1522s	1213w, 1183m, 1165w, 1097s, 767	1358m, 1335w	510
6	3385br	1659sh, 1614s	1523s	1199w, 1184m, 1165w, 1080s, 758	1357m, 1337w	509
7	3396br	1668sh, 1612s	1525s	1209w, 1184m, 1149w, 1107s, 769	1361m, 1348m	505
8	3389br	1664sh, 1614s	1524s	1203w, 1182m, 1163w, 1099s, 759	1362m, 1347m	508
9	3379br	1655sh, 1613s	1521s	1202w, 1186m, 1161w, 1090m, 757	1361m, 1351m	511

Table 5. Electronic, spectral and magnetic measurement data of complexes.

Compounds	d–d transition in cm^{-1}	Assignment	μ_{eff} BM
1	13850	$d_{xy}, d_{yz} \rightarrow d_{x^2-y^2}$	1.73
	10050	$d_{z^2} \rightarrow d_{x^2-y^2}$	
2	15500	$d_{xy}, d_{yz} \rightarrow d_{x^2-y^2}$	1.91
	10300	$d_{z^2} \rightarrow d_{x^2-y^2}$	
3	16600	$d_{xy}, d_{yz} \rightarrow d_{x^2-y^2}$	1.78
	10050	$d_{z^2} \rightarrow d_{x^2-y^2}$	
4	10050	$^5E_g \rightarrow {}^5T_{2g}$	4.78
5	11100	$^5E_g \rightarrow {}^5T_{2g}$	4.78
6	9950	$^5E_g \rightarrow {}^5T_{2g}$	4.95
7	19600	$^6A_{1g} \rightarrow {}^4T_{2g}$	6.20
	32700	MLCT	
8	19400	$^6A_{1g} \rightarrow {}^4T_{2g}$	6.40
	32250	MLCT	
9	19200	$^6A_{1g} \rightarrow {}^4T_{2g}$	6.10
	32900	MLCT	

MLCT: metal to ligand charge transfer.

3.3. *Reflectance spectra and magnetic properties*

Magnetic moments and electronic spectral data are presented in table 5. The reflectance spectra of Cu(II) complexes display two broad bands at 10,000 cm^{-1} and 14,000 cm^{-1} corresponding to $d_{z^2} \rightarrow d_{x^2-y^2}$ and d_{xy}, $d_{yz} \rightarrow d_{x^2-y^2}$, respectively. The Cu(II) complexes have magnetic moments of 1.73–1.91 BM, very close to the spin-allowed value for $S = 1/2$ (1.73 BM) and may indicate distorted octahedral geometry around the Cu(II) [37]. The diffuse reflectance spectra of iron(II) complexes exhibit a band at 10000 cm^{-1} due to the $^5E_g \rightarrow {}^5T_{2g}$ transition. The magnetic moment values of Fe(II) complexes were in the range 4.75–4.95 BM, indicating an octahedral complex with high-spin [38].

The diffuse reflectance spectra of iron(III) complexes exhibit bands at about 19,000 cm^{-1} assigned to $^6A_{1g} \rightarrow {}^4T_{2g}$ transitions and at 32,500 cm^{-1} assigned to MLCT (metal to ligand charge transfer) in d^5 Fe(III) atom. The Fe(III) complexes exhibit magnetic moments of 6.20–6.40 BM, assigned to octahedral geometry around Fe(III) [37, 39]. Reflectance spectra of **1**, **4** and **7** are provided in Supplementary Material.

3.4. *Thermal study*

Thermogravimetric analyses were carried out with heating rate of 10°C min^{-1} for all complexes. The complexes were stable even at high temperature and degrade via two steps. Percentage of coordinated water molecules were calculated from TGA. Each inflection on the TG curves accompanied by their endo- or exothermic peak on the DTA curve correspond to certain phase transformations. Initial weight loss occurring in the 130–180°C temperature range for Cu(II) and Fe(III) complexes were attributed to loss of two water molecules [36], while for Fe(III) complexes it corresponds to loss of one water and one hydroxyl group, leading to formation of anhydrous chelate [40]. The inflection of the TG curve in temperature range 390–750°C indicates decomposition of the organic part of the chelates leaving metal oxide as residue [41]. The analytical data for TGA summarized in table 6 are in good agreement with the expected formulas. The thermal degradation of the complex can be depicted as shown in scheme 4.

Table 6. Thermoanalytical results (TGA) of metal complexes.

Metal complexes	TG range/°C	Mass loss/% obs (% cal)	Assignment
1	50–180	3.53 (3.55)	Loss of 2 coordinated water molecules
	180–630	90.18 (90.15)	Removal of the ligand and leaving CuO residue
2	50–180	3.69 (3.63)	Loss of 2 coordinated water molecules
	180–650	90.01 (89.94)	Removal of the ligand and leaving CuO residue
3	50–180	3.82 (3.77)	Loss of 2 coordinated water molecules
	180–650	89.55	Removal of the ligand and leaving CuO residue
4	50–180	3.58	Loss of 2 coordinated water molecules
	180–660	90.08	Removal of the ligand and leaving Fe_2O_3 residue
5	50–180	3.83	Loss of 2 coordinated water molecules
	180–630	89.38	Removal of the ligand and leaving Fe_2O_3 residue
6	50–180	3.80	Loss of 2 coordinated water molecules and OH^-
	180–630	89.47	Removal of the ligand and leaving Fe_2O_3 residue
7	50–180	3.48	Loss of 1 coordinated water molecule and OH^-
	180–650	90.07	Removal of the ligand and leaving Fe_2O_3 residue
8	50–180	3.56	Loss of 1 coordinated water molecule and OH^-
	180–650	89.85	Removal of the ligand and leaving Fe_2O_3 residue
9	50–180	3.70	Loss of 1 coordinated water molecules
	180–660	89.46	Removal of the ligand and leaving Fe_2O_3 residue

$$[Cu(A^1)_2(H_2O)_2] \xrightarrow{130-180^\circ C} [Cu(A^1)_2] \xrightarrow{180-800^\circ C} [CuO] + 2A^1$$

$$[Fe(A^1)_2(H_2O)_2] \xrightarrow{130-180^\circ C} [Fe(A^1)_2] \xrightarrow{180-800^\circ C} [Fe_2O_3] + 2A^1$$

$$[(FeA^1)_2(H_2O)(OH)] \xrightarrow{130-180^\circ C} [Fe(A^1)_2] \xrightarrow{180-800^\circ C} [Fe_2O_3] + 2A^1$$

Scheme 4. Thermal degradation of the complexes.

3.5. In-vitro *biocidal activity*

The increase in biocidal activity of the complexes may be due to the effect of metal coordination. This improvement in activity of A^1–A^3 was also rationalized on the basis of their structural activity relationship (SAR). Increase in biocidal activity may be considered in light of Overtone's concept [42, 43] and Tweed's chelation theory [44–46]. Thus, complexes block the synthesis of proteins which restrict further augmentation of the organisms [48, 49]. Comparative analysis shows higher biocidal activity of the complexes than free ligands, metal salt and the control (DMSO). The complexes exhibit moderate activities compared to that of standard drug ciprofloxacin; no significant antifungal activity has been observed.

3.6. *Nuclease activity*

The effect of the binding of complexes on pBR322 was determined by its ability to make it bulky by binding with reactive sites of pBR322 DNA producing changes in its conformation from super coiled (SC) to nicked open circular (OC) form. When pBR322

Table 7. DNA binding data of ligands and complexes.

% DNA/ Compounds	Control	A^1	A^2	A^3	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
SC	100	10.75	5.75	13.48	8.46	3.70	9.32	9.03	4.05	11.10	4.89	5.28	10.31
OC	–	89.25	94.25	86.52	91.54	96.30	90.68	90.97	95.95	88.90	95.11	94.72	89.68

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 to OC. To rule out that the observed binding in the DNA is consequence of a direct interaction of complexes, additional experiments were carried out using ligands, ligands with 2-mercaptoethanol and complexes with 2-mercaptoethanol. In free ligands with pBR322, the conversion of the SC form to the OC is less, while in presence of complexes with pBR322, noticeable conversion of SC to OC was observed. This indicates that the complexes have better cleaving and binding efficacy than ligands. All the data have been summarized in table 7. In presence of reactive substance *i.e.* 2-mercaptoethanol with ligands or complexes; good efficacy of binding is observed *i.e.* SC DNA converted into OC DNA. In the case of pBR322 with 2-mercaptoethanol no effect is observed. Data are given in Supplementary Material.

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