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ORIGINAL ARTICLE

Template synthesis, spectroscopic, antibacterial, and antifungal studies of trivalent transition metal ion macrocyclic complexes

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Abstract

A novel series of complexes of the type $[M(C_{36}H_{22}N_6)X]X_2$, where M = Cr(III), Mn(III), Fe(III); X = Cl^-, NO_3^-, CH_3COO^-; and ($C_{36}H_{22}N_6$) corresponds to the tetradentate macrocyclic ligand, have been synthesized by condensation of 1,8-diaminonaphthalene and isatin in the presence of trivalent metal salts in methanolic medium. The complexes have been characterized by elemental analysis, conductance and magnetic measurements, and UV/Vis, IR, and mass spectroscopy. On the basis of these studies, a five coordinate square pyramidal geometry for all of these complexes is proposed. All synthesized macrocyclic complexes have been tested for *in vitro* antimicrobial activities against some pathogenic bacterial strains, viz. *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative), and two fungal strains, viz. *Aspergillus niger, Aspergillus flavus*. The MICs shown by the complexes against these microbial strains have been compared with MICs shown by standard antibiotic ciprofloxacin and the antifungal drug amphotericin-B.

Keywords: *Antimicrobial activity; isatin; macrocyclic complexes; mass spectra*

Abbreviations: B.M., Bohr magneton; CFU, colony forming unit; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; IR, infrared; MIC, minimum inhibitory concentration; MRI, magnetic resonance imaging; MTCC, Microbial Type Culture Collection; MHA, Mueller Hinton agar; MHB, Mueller Hinton broth; MBC, minimum bactericidal concentration; SDA, Sabouraud dextrose agar; DOTA, tetra-azacyclododecanetetra-acetic acid; NBA, nitrobenzyl alcohol; NCCLS, National Committee for Clinical Laboratory Standards.

Introduction

The design and study of well-arranged metal-containing macrocycles is an interesting field of chemistry[1]. Some synthetic macrocyclic complexes (e.g. Cu-complex) have been investigated for accelerating the photodegradation of hazardous pollutants[2]. The *in situ* one-pot template condensation reaction lies at the heart of macrocyclic chemistry[3,4]. Therefore, template reactions have been widely used for the synthesis of macrocyclic complexes where generally the transition metal ions are used as the templating agent[5]. There is continued interest in synthesizing macrocyclic complexes because of their potential application in fundamental and applied sciences[6,7]. Synthetic macrocyclic complexes mimic some naturally occurring macrocycles because of their resemblance to many natural macrocycles, such as metalloproteins and metalloenzymes[8]. Macrocyclic nickel complexes find use in DNA recognition and oxidation[9]. Macrocyclic copper complexes find use in DNA binding and cleavage[10] and copper containing proteins have been identified[11]. Macrocyclic metal complexes of lanthanides, e.g. Gd^{3+}, are used as MRI contrast agents[12]. A macrocyclic metal chelating agent (DOTA) is useful for detecting tumor lesions[13]. The chemistry of macrocyclic complexes is also important, due to their use as dyes and pigments[14] as well as nuclear magnetic resosnance (NMR) shift reagents[15]. Some macrocyclic complexes have received special attention because of their mixed soft–hard donor character and versatile coordination behavior[16] and also their pharmacological properties, i.e. toxicity against bacterial and fungal growth[17]. Prompted by these facts, in the present article, the synthesis and characterization of chromium(III), manganese(III), and iron(III) macrocyclic complexes derived from 1,8-diaminonaphthalene and isatin are discussed. The

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complexes were characterized using various physicochemical techniques such as electronic, IR, and mass spectroscopy, elemental analysis, and magnetic susceptibility and conductivity measurements. All synthesized macrocyclic complexes were also tested for their *in vitro* antimicrobial activities against some bacterial strains, viz. *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) (Gram-positive), *Escherichia coli* (MTCC 1652), and *Pseudomonas aeruginosa* (MTCC 741) (Gram-negative) and two fungal strains, viz. *Aspergillus niger* (MTCC 282) and *Aspergillus flavus* (MTCC 871). The MICs shown by the complexes against these microbial strains have been compared with MICs shown by standard antibiotic ciprofloxacin and antifungal drug amphotericin-B.

Experimental

Materials

All the chemicals and solvents used in this study were of AnalaR grade. 1,8-Diaminonaphthalene and isatin were procured from Acros, and metal salts were purchased from S.D.-Fine, Merck, and Ranbaxy, and were used as received.

Isolation of complexes

All the complexes were synthesized by template method, i.e. by condensation of 1,8-diaminonaphthalene and isatin in the presence of the respective trivalent metal salts. To a hot stirred methanolic solution (~50 mL) of 1,8-diaminonaphthalene (10 mmol) was added trivalent chromium, manganese, or iron salt (5 mmol) dissolved in the minimum quantity of MeOH (~20 mL). The resulting solution was refluxed for 0.5 h. Afterward, isatin (10 mmol) was added to the refluxing mixture and refluxing was continued for 8–10 h. The mixture was concentrated to half of its volume, cooled at room temperature, and kept in a desiccator overnight. After that, dark colored precipitates formed which were filtered, washed with methanol, acetone, and diethylether, and dried *in vacuo*. Yield was obtained of ~60–75%. The complexes were soluble in DMF and DMSO. They were found to be thermally stable up to ~260–285°C, after which decomposition occurred.

The template condensation of 1,8-diaminonaphthalene and isatin in the presence of trivalent metal salts, in the molar ratio 2:2:1, is shown by the following scheme:

$$2C_8H_5O_2N + 2C_{10}H_{10}N_2 + MX_3 \xrightarrow[(8-10\,h)]{MeOH} [M(C_{36}H_{22}N_6)X]X_2$$

where M = Cr(III), Mn(III), Fe(III); X = Cl^-, NO_3^-, CH_3COO^- for Cr(III), Fe(III); X = CH_3COO^- for Mn(III).

Analytical and physical measurements

The microanalysis of C, H, and N was carried out by elemental analyzer (PerkinElmer 2400) at SAIF, Punjab University, Chandigarh. Magnetic susceptibility measurements were carried out at IIC, IIT Roorkee, on a vibrating sample magnetometer (Model PAR 155). The metal contents in the complexes were determined by the literature method[18]. IR spectra were recorded on a Fourier transform (FT-IR) spectrophotometer (PerkinElmer) in the range 4000–200 cm^{-1} using Nujol mull. Electronic spectra (in DMSO) were recorded on a Cary 14 spectrophotometer at room temperature. Fast atom bombardment (FAB) mass spectra (at room temperature) were recorded on a time-of-flight mass spectrometry electrospray (TOF MS ES+) machine. The conductivity was measured on a digital conductivity meter (HPG system, G-3001). Melting points were determined by using capillaries in an electrical melting point apparatus.

Biological activity

Test microorganisms

Four bacteria, *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) (Gram-positive), *Escherichia coli* (MTCC 1652), and *Pseudomonas aeruginosa* (MTCC 741) (Gram-negative), and two fungi, *Aspergillus niger* (MTCC 282) and *Aspergillus flavus* (MTCC 871), were used in the present study.

In vitro antibacterial activity

The antibacterial activities of all the synthesized macrocyclic complexes were evaluated by the agar well diffusion method[19]. All the cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFU/mL[20]. Twenty milliliters of MHA medium was poured into each Petri plate and the agar plates were swabbed with a 100 µL inoculum of each test bacterium and kept for 15 min for adsorption. Using a sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume at concentration of 4.0 mg/mL of each complex reconstituted in DMSO. All the plates were incubated at 37°C for 24 h. The antibacterial activity of each complex was evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicate and the results are given as mean ± standard deviation.

Determination of minimum inhibitory concentration (MIC) of chemically synthesized complexes

The minimum inhibitory concentration is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The MICs of the various complexes against the bacterial strains were determined through a macrodilution tube method as recommended by the NCCLS[21]. In this method, various test concentrations of the synthesized complexes were made, from 128 to 0.25 µg/mL, in sterile tubes, nos. 1 to 10. A volume of 100 µL of sterile MHB medium was poured into each sterile tube followed by the addition of 200 µL test compound in tube 1. Two-fold serial dilutions were carried out from tube 1 to tube 10, and excess broth (100 µL) was discarded from the last tube, no. 10. To each tube, 100 µL of standard inoculum (1.5×10^8 CFU/mL) was added. Ciprofloxacin was used as control. Turbidity was observed

after incubating the inoculated tubes at 37°C for 24 h. The minimum bactericidal concentration is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture onto antibiotic-free medium. The MBC was determined by spreading 100 µL of the complex from the test concentration that fell below the MIC to the MIC itself. All the plates were incubated for 24 h at 37°C. The growth was observed on each plate.

In vitro antifungal activity

The antifungal activities of the all the synthesized macrocyclic complexes were evaluated by the poisoned food technique[22]. Molds were grown on SDA medium at 25°C for 7 days and used as inocula. Fifteen milliliters of molten SDA medium (45°C) was poisoned by the addition of a 100 µL volume of each compound having a concentration of 4.0 mg/mL, reconstituted in DMSO, poured into a sterile Petri plate, and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8 mm diameter), obtained from the actively growing colony, and incubated at 25°C for 7 days. DMSO was used as the negative control whereas amphotericin-B was used as the positive control. The experiments were performed in triplicate. The diameter of the fungal colony was measured and expressed as percent mycelial inhibition determined by applying the formula given by Al-Burtamani *et al.*[22]:

$$\text{Inhibition of mycelial growth \%} = (dc - dt)/dc \times 100$$

where dc is the average diameter of the fungal colony in negative control plates, and dt is the average diameter of the fungal colony in experimental plates.

Results and discussion

Chemistry

The analytical data show the suggested formula for macrocyclic complexes as $[M(C_{36}H_{22}N_6)X]X_2$, where M = Cr(III), Mn(III), Fe(III); X = Cl^-, NO_3^-, CH_3COO^-; and ($C_{36}H_{22}N_6$) corresponds to the tetradentate macrocyclic ligand. Measurements of molar conductance in DMSO showed that these chelates are 1:2 electrolytes[23] (conductance 160–180 $ohm^{-1}\,cm^2\,mol^{-1}$). Various attempts such as crystallization using mixtures of solvents and low temperature crystallization were unsuccessful in obtaining a single crystal suitable for X-ray crystallography. However, the analytical, spectroscopic, and magnetic data enabled us to predict the possible structure of the synthesized complexes. All complexes gave satisfactory elemental analysis results as shown in Table 1.

IR spectra

It was noted that a pair of medium intensity bands were present in the spectrum of 1,8-diaminonaphthalene at 3350 and 3390 cm^{-1}, corresponding to the $\nu(NH_2)$ group, which were absent in the IR spectra of all the complexes. Further, no strong absorption band was observed near 1734 cm^{-1}, indicating the absence of the >C=O group of the isatin moiety. The disappearance of these bands and appearance of a new strong absorption band near 1590–1629 cm^{-1} confirmed condensation of the carbonyl group of isatin and amino group of diaminonaphthalene and formation of the macrocyclic Schiff base[24], as these bands may be assigned due to ν(C=N) stretching vibrations[25]. The value of the ν(C=N) stretching vibration was found to be lower (1590–1629 cm^{-1}) than the expected value (1670–1695 cm^{-1}). This lower value of ν(C=N) stretching may be explained on the basis of the drift of lone-pair density of the azomethine nitrogen toward the metal atom[26], indicating that coordination takes place through the nitrogen of the (C=N) group. The various absorption bands in the region of 1450–1600 cm^{-1} may be assigned due to ν(C=C) aromatic stretching vibrations of the naphthalene and isatin ring moieties[26]. The bands in the region 740–785 cm^{-1} may be assigned to ν(C-H) out of plane bending of aromatic rings[27,28]. The presence of absorption bands at 1410–1445, 1295–1320, and 1015–1030 cm^{-1} in the IR spectra of all the nitrato complexes suggests that the nitrate groups are coordinated to the central metal ion in a unidentate fashion[29]. The IR spectra of all the acetate complexes showed an absorption band in the region 1650–1680 cm^{-1}, assigned to $\nu(COO^-)_{as}$ asymmetric stretching vibrations of the acetate ion, and another in the region 1258–1290 cm^{-1} that can be assigned to $\nu(COO^-)_s$ symmetric stretching vibration of the acetate ion. The difference $\nu_{as} - \nu_s$, which is around 390–370 cm^{-1}, greater than 144 cm^{-1}, indicates the unidentate coordination of the acetate group with the central metal ion[30].

The far infrared spectra showed bands in the region 420-450 cm^{-1}, corresponding to ν(M-N) vibrations[31–33]. The presence of bands in all complexes in the region 420–450 cm^{-1} originates from (M-N) azomethine vibrational modes and identifies coordination of the azomethine nitrogen[34]. The bands present in the range 300–320 cm^{-1} may be assigned to ν(M-Cl) vibration[31–33]. The bands present in the region 220–250 cm^{-1} in all nitrato complexes are related to the ν(M-O) stretching vibration[31,32].

Table 1. Analytical data of trivalent chromium, manganese, and iron complexes derived from 1,8-diaminonaphthalene and isatin.

		Found (calcd.) (%)					
Sr. no.	Complex	M	C	H	N	Color	Mol. wt.
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	7.40 (7.46)	62.02 (62.02)	3.15 (3.16)	12.05 (12.06)	Light green	696.49
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	6.62 (6.69)	55.61 (55.67)	2.80 (2.83)	16.24 (16.24)	Dark green	775.99
(3)	$[Cr(C_{36}H_{22}N_6)(OAc)](OAc)_2$	6.58 (6.68)	65.63 (65.71)	4.00 (4.04)	10.91 (10.95)	Black	766.99
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	7.16 (7.24)	65.37 (65.38)	4.02 (4.02)	10.83 (10.89)	Light grey	770.84
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	7.79 (7.85)	61.76 (61.76)	3.13 (3.14)	11.99 (12.00)	Dark brown	699.43
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	6.93 (7.05)	55.43 (55.46)	2.82 (2.82)	16.16 (16.17)	Dark brown	778.93
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	7.10 (7.13)	65.41 (65.46)	4.01 (4.03)	10.92 (10.91)	Reddish	769.93

Mass spectra

The FAB mass spectra of the Cr(III), Mn(III), Fe(III) macrocyclic complexes were recorded using an *m*-nitrobenzyl alcohol (NBA) matrix. All the spectra exhibited parent peaks due to molecular ions $[M]^+$ and $[M+2]^+$. The proposed molecular formula of these complexes was confirmed by comparing their molecular formula weights with *m/z* values. The molecular ion $[M]^+$ and $[M+2]^+$ peaks obtained for the various complexes are shown in Table 2. The data are in good agreement with the proposed molecular formula for these complexes, i.e. $[M(C_{36}H_{22}N_6)X]X_2$. This confirms formation of the macrocyclic frame. In addition to the molecular ion peaks, the spectra exhibited other peaks assignable to various fragments arising from thermal cleavage of the complexes (Table 2). The peak intensity gives an idea of the stability of the fragments.

Magnetic measurements and electronic spectra

Chromium complexes

The magnetic moment of chromium(III) complexes was found in the range of 3.90–4.30 B.M., at room temperature, which is close to the predicted values for three unpaired electrons in the metal ion[26]. The electronic spectra of the chromium complexes showed bands at ~9010–9320 cm^{-1}, 13,030–13,350 cm^{-1}, 17,460–18,320 cm^{-1}, 27,420–27,850 cm^{-1}, and 34,810 cm^{-1}. However, these spectral bands cannot be interpreted in terms of a four or six coordinated environment around the central metal atom. In turn, the spectra are consistent with that of five coordinated chromium(III) complexes, whose structure has been confirmed with the help of X-ray measurements[35]. On the basis of the analytical data, spectral studies, and electrolytic nature of these complexes, a five coordinated square pyramidal geometry may be assigned for these complexes. Thus, assuming the symmetry C_{4V} for the complexes[36–38], the various spectral bands may be assigned as: $^4B_1 \rightarrow {}^4E^a$, $^4B_1 \rightarrow {}^4B_2$, $^4B_1 \rightarrow {}^4A_2$, and $^4B_1 \rightarrow {}^4E^b$.

Manganese complex

The magnetic moment of the manganese(III) complex was found to be 4.89 B.M., which indicates the high spin (d^4) system[26]. The electronic spectra of the manganese complex showed three d-d bands, which lay in the regions 12,350–12,590, 16,050–18,820, and 35,420–35,700 cm^{-1}. The higher energy band at 35,440–35,750 cm^{-1} may be assigned to charge transfer transitions. The spectra resemble those reported for five coordinate square pyramidal manganese complexes[36–38]. This idea is further supported by the presence of the broad ligand field band at 20,400 cm^{-1}, diagnostic of C_{4V} symmetry, and thus the various bands may be assigned as follows: $^5B_1 \rightarrow {}^5A_1$, $^5B_1 \rightarrow {}^5B_2$, and $^5B_1 \rightarrow {}^5E$, respectively. The band assignment in single electron transition may be made as: $d_{z^2} \rightarrow d_{x^2-y^2}$, $d_{xy} \rightarrow d_{x^2-y^2}$, and d_{xz}, $d_{yz} \rightarrow d_{x^2-y^2}$, respectively, in order of increasing energy.

Iron complexes

The magnetic moment of iron complexes lay in the range of 5.81–5.90 B.M., corresponding to the five unpaired electrons, which is close to the predicted high spin values for these metal ions[26]. The electronic spectra of the iron complexes showed various bands at 9820–9970, 15,520–15,575, and 27,550-27,730 cm^{-1}, and these bands do not suggest octahedral or tetrahedral geometry around the metal atom. The spectral bands are consistent with the range of spectral bands reported for five coordinate square pyramidal iron(III) complexes[37–39]. Assuming C_{4V} symmetry for these complexes, the various bands can be assigned as: $d_{xy} \rightarrow d_{xz}$, d_{yz} and $d_{xy} \rightarrow d_{z^2}$. Any attempt to make accurate assignment is difficult due to interactions of the metal–ligand π-bond systems lifting the degeneracy of the d_{xz} and d_{yz} pair.

Biological activity

All the synthesized macrocyclic complexes were screened for their antibacterial and antifungal activities. All the tested complexes possessed variable antibacterial and antifungal activities against both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria and against the fungal strains (*A. niger*, *A. flavus*). To compare the antibacterial and antifungal activities shown by the synthesized complexes, a standard antibiotic, namely ciprofloxacin, and standard antifungal drug, amphotericin-B, were used (Tables 3 and 6, Figures 1 and 2). On the basis of the MICs shown by these complexes against the bacteria, compounds (4) and (7) were found to be most effective

Table 2. FAB mass spectral data of the trivalent chromium, manganese, and iron complexes derived from 1,8-diaminonaphthalene and isatin.

Sr. no.	Complex	Molecular ion peak $[M]^+$ and $[M+2]^+$ at *m/z*	Important peaks due to complex fragmentation
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	$[M]^+$ = 695.4 (^{35}Cl), $[M+2]^+$ = 697.4 (^{37}Cl)	$[Cr(C_{36}H_{21}N_6)Cl]^+$ = 624.5, $[Cr(C_{36}H_{21}N_6)]^+$ = 589.0, $[C_{36}H_{21}N_6]^+$ = 537.1, $[C_{36}H_{21}N_6\text{-}2H]^+$ = 535.1
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	$[M]^+$ = 774.9	$[Cr(C_{36}H_{21}N_6)(NO_3)]^+$ = 650.9, $[Cr(C_{36}H_{21}N_6)]^+$ = 588.9, $[C_{36}H_{21}N_6]^+$ = 537.0, $[C_{36}H_{21}N_6\text{-}2H]^+$ = 535.0
(3)	$[Cr(C_{18}H_{22}N_6)(OAc)](OAc)_2$	$[M]^+$ = 765.9	$[Cr(C_{36}H_{21}N_6)(OAc)]^+$ = 647.9, $[Cr(C_{36}H_{21}N_6)]^+$ = 588.9, $[C_{36}H_{21}N_6]^+$ = 536.9, $[C_{36}H_{21}N_6\text{-}2H]^+$ = 534.9
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	$[M]^+$ = 768.8	$[Mn(C_{36}H_{20}N_6)(OAc)]^+$ = 650.8, $[Mn(C_{36}H_{20}N_6)]^+$ = 591.8, $[C_{36}H_{20}N_6]^+$ = 535.9, $[C_{36}H_{20}N_6\text{-}2H]^+$ = 533.9
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	$[M]^+$ = 697.4 (^{35}Cl), $[M+2]^+$ = 699.4 (^{37}Cl)	$[Fe(C_{36}H_{20}N_6)Cl]^+$ = 626.5, $[Fe(C_{36}H_{20}N_6)]^+$ = 591.0, $[C_{36}H_{20}N_6]^+$ = 536.1, $[C_{36}H_{20}N_6\text{-}2H]^+$ = 534.1
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	$[M]^+$ = 777.9	$[Fe(C_{36}H_{21}N_6)(NO_3)]^+$ = 654.0, $[Fe(C_{36}H_{21}N_6)]^+$ = 592.0, $[C_{36}H_{21}N_6]^+$ = 537.1, $[C_{36}H_{21}N_6\text{-}2H]^+$ = 535.1
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	$[M]^+$ = 768.9	$[Fe(C_{36}H_{21}N_6)(OAc)]^+$ = 650.9, $[Fe(C_{36}H_{21}N_6)]^+$ = 591.9, $[C_{36}H_{21}N_6]^+$ = 536.9, $[C_{36}H_{21}N_6\text{-}2H]^+$ = 534.9

Table 3. *In vitro* antibacterial activity of synthesized macrocyclic complexes through agar well diffusion method.

Sr. no.	Complex	Diameter of growth of inhibition zone (mm)*			
		a	b	c	d
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	22.6 ± 0.58	21.6 ± 0.58	—	16.6 ± 0.58
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	20.3 ± 0.58	21.6 ± 0.58	16.3 ± 0.58	13.6 ± 0.58
(3)	$[Cr(C_{36}H_{22}N_6)(OAc)](OAc)_2$	21.6 ± 0.58	19.3 ± 0.58	16.6 ± 0.58	—
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	26.6 ± 0.58	24.3 ± 0.58	19.6 ± 0.58	19.6 ± 0.58
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	23.6 ± 0.58	24.6 ± 0.58	18.3 ± 0.58	17.6 ± 0.58
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	20.3 ± 0.58	21.5 ± 0.58	16.3 ± 0.58	13.6 ± 0.58
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	26.6 ± 0.58	24.3 ± 0.58	19.6 ± 0.58	19.6 ± 0.58
	Ciprofloxacin	26.0 ± 0.58	24.0 ± 0.58	25.0 ± 0.58	22.0 ± 0.58

Note. —, no activity; *values, including diameter of the well (8 mm), are means of three replicates. a, *Staphylococcus aureus* (MTCC 96); b, *Bacillus subtilis* (MTCC 121); c, *Escherichia coli* (MTCC 1652); d, *Pseudomonas aeruginosa* (MTCC 741). Ciprofloxacin, standard antibiotic.

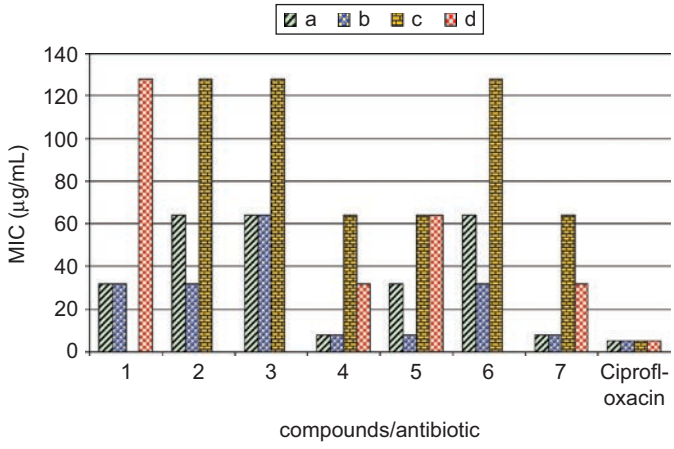

Figure 1. Comparison of MIC (µg/mL) of the synthesized macrocyclic complexes with standard antibiotic. a, *Staphylococcus aureus* (MTCC 96); b, *Bacillus subtilis* (MTCC 121); c, *Escherichia coli* (MTCC 1652); d, *Pseudomonas aeruginosa* (MTCC 741). Ciprofloxacin, standard antibiotic.

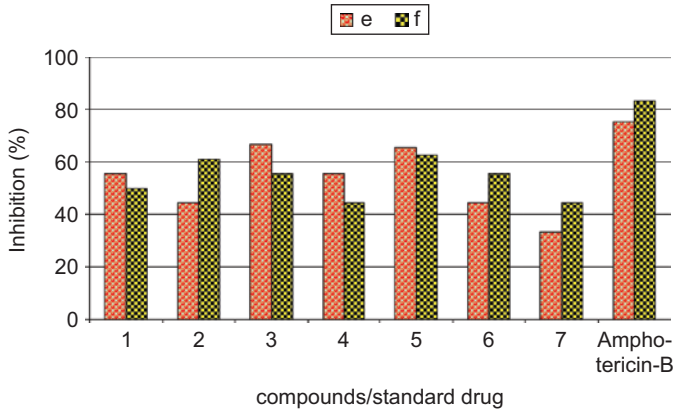

Figure 2. Comparison of the (%) mycelial growth inhibition of complexes with standard antifungal drug amphotericin-B. e, *Aspergillus niger* (MTCC 282); f, *Aspergillus flavus* (MTCC 871). Amphotericin-B, standard drug.

against both Gram-positive bacterial strains *S. aureus* and *B. subtilis*, showing an MIC of 8 µg/mL. In the whole series, the MIC of complexes (1) and (5) was found to be 32 µg/mL for *S. aureus*, whereas the MIC of complexes (1), (2), and (6) was 32 µg/mL for *B. subtilis* (Table 4, Figure 1). Complexes (4) and (7) showed an MIC of 32 µg/mL against *P. aeruginosa* and MIC of 64 µg/mL against *E. coli*. Similarly, the MBC of complexes (4) and (7) was found to be 16 µg/mL for *S. aureus* and *B. subtilis* and 64 µg/mL for *P. aeruginosa*, whereas the MBC of complexes (1), (2), and (6) was 64 µg/mL for *B. subtilis*. Similarly, complexes (1) and (5) showed an MBC of 64 µg/mL against *S. aureus* (Table 5).

The antifungal activities of all the complexes were determined against two fungal strains, i.e. *Aspergillus niger* and *Aspergillus flavus*, and then compared with the standard antifungal drug amphotericin-B (Table 6, Figure 2). The antifungal activities (percentage inhibition) are given in Table 6. In the whole series, complexes (3) and (5) showed the highest percentage inhibition, around 65%, against fungal strain *A. niger*, whereas (2) and (5) showed the highest percentage inhibition, around 61%, against *A. flavus*. None of the tested complexes restricted fungal growth excellently (Table 6). However, of all the tested compounds, (1) and (4) showed about 55% inhibition of mycelial growth against *A. niger*, whereas compounds (1), (3), and (6) showed 50–55% inhibition of mycelial growth against *A. flavus* (Table 6, Figure 2).

Conclusions

Based on various studies such as elemental analysis, conductance measurements, magnetic susceptibilities, and IR, electronic, and MS spectral studies, a five coordinate square pyramidal geometry for all of these complexes is proposed. The proposed structure is shown in Figure 3.

It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor group within the whole chelate ring system[40]. This process of chelation thus increases the lipophilic nature of the central metal atom, which, in turn, favors its permeation through the lipid layer of the membrane, thus causing the metal complex to cross the bacterial membrane more effectively, and hence increasing the activity of the complex. Besides this, many other factors such as solubility, dipole moment, and conductivity influenced by the metal ion may be possible reasons for the remarkable antibacterial activities of these complexes[41].

Table 4. Minimum inhibitory concentration (MIC) (in μg/mL) of synthesized macrocyclic complexes by using macrodilution method.

		MIC (μg/mL)			
Sr. no.	Complex	a	b	c	d
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	32	32	—	128
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	64	32	128	>128
(3)	$[Cr(C_{36}H_{22}N_6)(OAc)](OAc)_2$	64	64	128	—
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	8	8	64	32
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	32	8	64	64
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	64	32	128	>128
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	8	8	64	32
	Ciprofloxacin	05	05	05	05

Note. —, no activity. a, *Staphylococcus aureus* (MTCC 96); b, *Bacillus subtilis* (MTCC 121); c, *Escherichia coli* (MTCC 1652); d, *Pseudomonas aeruginosa* (MTCC 741). Ciprofloxacin, standard antibiotic.

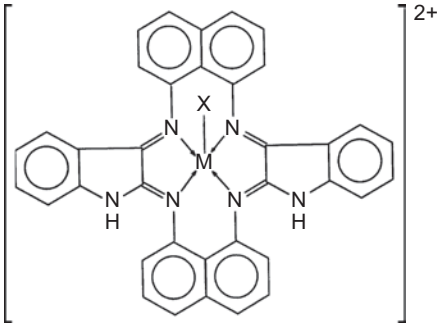

Figure 3. Proposed structure of the synthesized macrocyclic complexes, where M = Cr(III), Mn(III), Fe(III); X = Cl^-, NO_3^-, CH_3COO^- for Cr(III), Fe(III); X = CH_3COO^- for Mn(III).

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Declaration of interest

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Table 5. Minimum bactericidal concentration (MBC) (in μg/mL) of synthesized macrocyclic complexes by using macrodilution method.

		MBC (μg/mL)			
Sr. no.	Complex	a	b	c	d
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	64	64	—	>128
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	128	64	>128	>128
(3)	$[Cr(C_{36}H_{22}N_6)(OAc)](OAc)_2$	128	128	>128	—
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	16	16	128	64
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	64	16	128	128
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	128	64	>128	>128
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	16	16	128	64
	Ciprofloxacin	05	05	05	05

Note. —, no activity. a, *Staphylococcus aureus* (MTCC 96); b, *Bacillus subtilis* (MTCC 121); c, *Escherichia coli* (MTCC 1652); d, *Pseudomonas aeruginosa* (MTCC 741). Ciprofloxacin, standard antibiotic.

Table 6. *In vitro* antifungal activities of synthesized complexes through poisoned food method.

		Mycelial growth inhibition (%)	
Sr. no.	Complex	e	f
(1)	$[Cr(C_{36}H_{22}N_6)Cl]Cl_2$	55.5	50.0
(2)	$[Cr(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	44.4	61.1
(3)	$[Cr(C_{36}H_{22}N_6)(OAc)](OAc)_2$	66.6	55.5
(4)	$[Mn(C_{36}H_{22}N_6)(OAc)](OAc)_2$	55.5	44.4
(5)	$[Fe(C_{36}H_{22}N_6)Cl]Cl_2$	65.5	62.5
(6)	$[Fe(C_{36}H_{22}N_6)(NO_3)](NO_3)_2$	44.4	55.5
(7)	$[Fe(C_{36}H_{22}N_6)(OAc)](OAc)_2$	33.3	44.4
	Amphotericin-B	75.3	83.3

Note. e, *Aspergillus niger* (MTCC 282); f, *Aspergillus flavus (*MTCC 871). Amphotericin-B, standard drug.

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