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Short communication

Antimicrobial active macrocyclic complexes of Cr(III), Mn(III) and Fe(III) with their spectroscopic approach

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ABSTRACT

A novel series of macrocyclic complexes of the type $[M(C_{48}H_{32}N_4)X]X_2$; where M = Cr(III), Fe(III) and Mn(III); X = Cl^-, NO_3^-, CH_3COO^- have been synthesized by template condensation of 1,8-diaminonaphthalene and benzil in the presence of trivalent metal salts in methanolic medium. The complexes have been characterized with the help of elemental analyses, conductance measurements, magnetic measurements, electronic, NMR, infrared, far infrared and mass spectral studies. On the basis of these studies, a five coordinate square pyramidal geometry for all of these complexes has been proposed. These metal complexes were also tested for their *in vitro* antimicrobial activities to assess their inhibiting potential.

1. Introduction

The design and study of well-arranged metal-containing macrocycles is an interesting field of chemistry [1]. Several numbers of synthetic and natural macrocyclic compounds have been investigated [2]. The chemistry of macrocyclic complexes has attracted the interest of both inorganic and bioinorganic chemists in recent years [3]. The field of macrocyclic chemistry of metals is developing very rapidly because of its importance in the area of coordination chemistry [4]. Macrocyclic compounds and their derivatives are interesting ligand system because they are good hosts for metal anions, neutral molecules and organic cation guests [5]. The metal-ion and host–guest chemistry of macrocyclic compounds are very useful in fundamental studies e.g. in phase transfer catalysis and biological studies [6]. The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades [7]. *In situ* one pot template condensation reactions lie at the heart of the macrocyclic chemistry [8]. Therefore template reactions have been widely used for synthesis of macrocyclic complexes [9], where generally the transition metal ions are used as templating agent [10]. The metal ions direct the reaction preferentially towards cyclic rather than oligomeric or polymeric product [11]. There is continued interest in synthesizing macrocyclic complexes because of their potential applications in fundamental and applied sciences [12,13]. Synthetic macrocyclic complexes mimic some naturally occurring macrocycles because of their resemblance with many natural macrocycles like metalloproteins, porphyrins and cobalamine [14–16]. So biologically active macrocyclic complexes are used in the identification of diseased and normal tissues [17]. Transition metal macrocyclic complexes have received a great attention because of their biological activities, including antiviral, anticarcinogenic [16], antifertile [18], antibacterial and antifungal [19]. Macrocyclic metal complexes of lanthanides e.g. Gd^{3+} are used as MRI contrast agents [20]. Macrocyclic metal chelating agents (DOTA) are useful for detecting tumour lesions [21]. Prompted by these, in the present paper, synthesis and characterization of Cr(III), Fe(III) and Mn(III) macrocyclic complexes derived from 1,8-diaminonaphthalene and benzil have been discussed. Besides the characterization of complexes by physicochemical technique like IR, NMR, elemental analyses, magnetic susceptibility and conductance measurements, the biological activities of the synthesized complexes have been examined against some antimicrobial strains *viz.* *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121)

Abbreviations: B.M., Bohr magneton; CFU, colony forming unit; DMF-*N*, *N*-dimethylformamide; DMSO, dimethylsulphoxide; IR, infrared; MIC, minimum inhibitory concentration; MRI, magnetic resonance imaging; MTCC, microbial type culture collection; MHA, Mueller Hinton agar; MHB, Mueller Hinton broth; MYEA, malt yeast extract agar; MBC, minimum bactericidal concentration; NMR, nuclear magnetic resonance; SDA, Sabouraud dextrose agar; DOTA, *tetra*-azacyclododecanetetra-acetic acid.

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(Gram-positive bacteria), *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) (Gram-negative bacteria) and *Aspergillus niger* (MTCC 282), *Aspergillus fumigatus* (MTCC 870) (molds), *Candida albicans* (MTCC 227), and *Saccharomyces cerevisiae* (MTCC 170) (yeasts) for evaluation of antibacterial and antifungal activities of the synthetic chemical compounds. The results obtained were compared with standard antibiotic: Ciprofloxacin and the standard antifungal drug: Fluconazole.

2. Chemistry

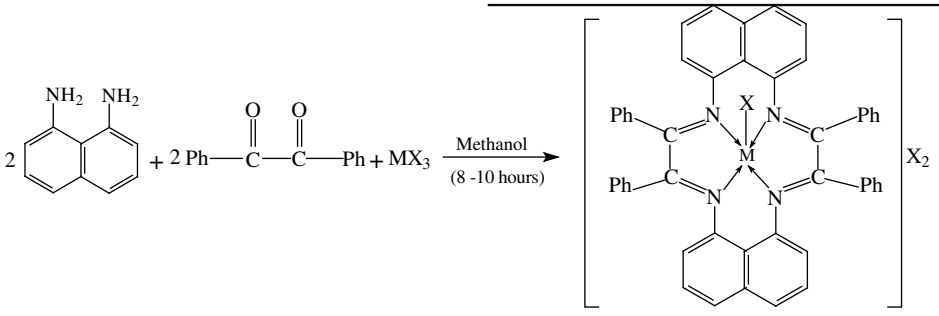
2.1. Reagents

All the chemicals used were of Analytical Reagent grade. 1,8-Diaminonaphthalene and benzil procured from Acros and s.d.-fine, respectively, metal salts were purchased from s.d.-fine, Merck, Ranbaxy and were used as received.

2.2. Isolation of complexes

All the complexes were synthesized by template method i.e. by condensation of 1,8-diaminonaphthalene and benzil in the presence of the respective trivalent metal salts. To a hot stirring methanolic solution (~50 cm^3) of 1,8-diaminonaphthalene (10 mmol, 1.58 g) was added trivalent chromium, manganese or iron salt (5 mmol) dissolved in the minimum quantity of MeOH (~20 cm^3). The resulting solution was refluxed for 0.5 h. After that, benzil (10 mmol, 2.10 g) was added in the refluxing mixture and refluxing was continued for 8–10 h. The mixture was concentrated to half of its original volume and kept in a desiccator overnight. On overnight cooling dark coloured precipitates formed which was filtered, washed with methanol, acetone, diethylether and dried *in vacuo*. Yield was obtained in ~45–55%. The complexes are soluble in DMF and DMSO, but are insoluble in common organic solvents and H_2O. They were found thermally stable up to ~260–285 °C and then decomposed.

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



Where M = Cr(III), Mn(III), Fe(III)
X = Cl-, NO_3-, CH_3COO-

3. Pharmacology

3.1. Test microorganisms

Eight microbial strains (four bacterial and four fungi) were selected on the basis of their clinical importance in causing diseases in humans. *S. aureus* (MTCC 96), *B. subtilis* (MTCC 121) (Gram-positive bacteria), *E. coli* (MTCC 1652) and *P. aeruginosa* (MTCC 741) (Gram-negative bacteria) and *A. niger* (MTCC 282), *A. fumigatus* (MTCC 870) (molds), *C. albicans* (MTCC 227), and *S. cerevisiae* (MTCC 170) (yeasts) were screened for evaluation of antibacterial and antifungal activities of the synthesized chemical compounds.

3.2. Medium

Three solid media namely Mueller Hinton Agar (MHA) (for bacteria), Malt Yeast Extract Agar (MYEA) (for yeasts) and Sabouraud Dextrose Agar (SDA) (for molds) were used for the biological assay.

3.3. In vitro *antimicrobial activity (for bacteria and yeasts)*

3.3.1. Primary screening

The antimicrobial activities (bacterial and yeasts) of the newly synthesized compounds were evaluated by agar well diffusion method [22]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml [23]. 20 ml of agar media was poured into each Petri plate and plates were swabbed with 100 μl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μl volume with concentration of 1.0 mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antimicrobial activity of all the synthesized compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). The medium with dimethylsulphoxide (DMSO) as solvent was used as a negative control whereas media with Ciprofloxacin (standard antibiotic) and Fluconazole (standard antifungal drug) were used as positive control. The experiments were performed in triplicates.

3.4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of synthesized compounds

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. The MIC of the chemically synthesized compound was tested against bacterial and yeast strains through a macrodilution tube method [23]. In this method, the test concentrations of chemically synthesized compounds were made from 128 to 0.25 μg/ml in the sterile tubes No. 1–11. Mueller Hinton Broth (MHB) medium was prepared and 100 μl sterile MHB were poured in each sterile tube

followed by addition of 200 μl compound in tube 1. Twofold serial dilutions were carried out from tube 1 to the tube 11 and excess broth (100 μl) was discarded from the last tube No. 11. 0.5 McFarland standard was made, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cells/ml. To each tube, 100 μl of standard inoculum was added. Ciprofloxacin (antibacterial drug) and Fluconazole (antifungal drug) were used as controls. All the tubes were incubated for 24 h at 37 °C.

A minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial compound that will prevent the growth of an organism after subculture on to antibiotic free media [23]. Minimum bactericidal concentrations (MBCs) were determined by spreading the 100 μl compound from one below MIC and MIC itself. All the tubes were incubated for 24 h at 37 °C. The growth was observed on each plate.

3.4.1. In vitro *antimicrobial activity (for molds)*

All the newly synthesized compounds were evaluated for their antimicrobial activity (molds) by poison food technique [24]. All the test molds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7–8 days. One week old culture of the mold was used as inoculum for evaluating antifungal activity of chemical compounds. The molten SDA (45 °C) was poisoned by the addition of 100 μl volume having concentration of 1.0 mg/ml of each compound reconstituted in the DMSO and poured into the sterile Petri plates. The prepared SDA plates containing the test compound were inoculated with fungal plugs (8 mm diameter) obtained from the actively growing margins of the fungal plates. Plates were incubated at 25 °C for 7 days. The medium with DMSO as solvent was used as a negative control whereas media with Fluconazole (standard antifungal drug) were used as positive control. The experiments were performed in triplicates. Diameter of fungal colony was measured and expressed as percentage inhibition and determined by the formula given below:

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

where dc = average fungal colony diameter in control sets; dt = average fungal colony diameter in treatment sets.

4. Result and discussion

The analytical data show the formula for macrocyclic complexes as: $[M(C_{48}H_{32}N_4)X]X_2$ where M = Cr(III), Fe(III), Mn(III) and X = Cl^-, NO_3^- and CH_3COO^-. The measurements of molar conductance in DMSO show that these chelates are 1:2 electrolytes [25] (conductance 160–189 ohm/cm^2/mol). The tests for anions are positive before decomposing and after decomposing the chelates with concentrated HNO_3 showing their presence outside as well as inside of coordination sphere. Several attempts failed to obtain a single crystal suitable for X-ray crystallography. However, the analytical, spectroscopic and magnetic data enable us to predict the possible structure of the synthesized complexes. All macrocyclic complexes are dark coloured solids and are soluble in DMF or DMSO. All complexes give satisfactory elemental analyses results as shown in Table 1.

4.1. IR spectra

It was noted that a pair of bands are present in the spectrum of 1,8-diaminonaphthalene at 3.350 and 3.390 cm^{-1} corresponding to $\nu(NH_2)$ group which are absent in the infrared spectra of all the complexes. Further, no strong absorption band was observed near 1.690 cm^{-1} indicating the absence of >C═O group of benzil moiety. The disappearance of these bands and appearance of a new strong absorption band near 1.585–1.629 cm^{-1} confirm the condensation of carbonyl group of benzil and amino group of diaminonaphthalene and formation of macrocyclic Schiff's base [26] as these bands may be assigned due to ν(C═N) stretching vibrations [27,28]. The value of ν(C═N) stretching vibration is found lower (1.585–1.629 cm^{-1}) than the expected value (1.650–1.690 cm^{-1}). This lower value of ν(C═N) stretching may be explained on the basis of a drift of lone pair density of azomethine nitrogen towards the metal atom [29,30] indicating that coordination takes place through nitrogen of (C═N) groups. The bands present in the range 3.010–3.050 cm^{-1} may be assigned due to ν(C–H) stretching vibrations of benzil and naphthalene ring [31]. The various absorption bands in the region 1.450–1.588 cm^{-1} may be assigned due to ν(C═C) aromatic stretching vibrations of the benzil and naphthalene ring [32,33]. The bands in the region 740–785 cm^{-1} may be assigned to ν(C-H) out of plane bending of aromatic rings [34,35]. The presence of the absorption bands at 1.408–1.440, 1.290–1.320 and 1.010–1.030 cm^{-1}, in the IR spectra of the Cr(III) and Fe(III) nitrato complexes suggests that the nitrate group is coordinated to the central metal ion in a unidentate fashion [36]. The IR spectra of the Chromium, Manganese and Iron acetate complexes, show an absorption band in the region 1.650–1.680 cm^{-1} which is assigned to $\nu(COO^-)$ asymmetric stretching of acetate ion and another in the region 1.258–1.290 cm^{-1} and which can be assigned to $\nu(COO^-)$ symmetric stretching vibration of acetate ion. A difference between $(\nu_{as} - \nu_s)$ is around 390–370 cm^{-1} which is greater than 144 cm^{-1} indicates the unidentate coordination of the acetate ion with the central metal ion [37]. The far infrared spectra show bands in the region 420–450 cm^{-1} corresponding to ν(M-N) vibrations [38–40]. The presence of bands in all complexes in the region 420–450 cm^{-1} originates from the (M-N) azomethine vibrational modes and identifies coordination of azomethine nitrogen [41]. The bands present in the range 300–320 cm^{-1} may be assigned due to ν(M-Cl) vibration [38–40]. The bands present in the region 220–250 cm^{-1} in all nitrato complexes are assigned to ν(M-O) stretching vibration [38,39].

4.2. NMR spectra

The ^{1}H NMR spectrum of the zinc(II) complex shows multiplets in the region 6.65–7.23 ppm corresponding to aromatic ring protons of naphthalene moiety (12H) [42]. The multiplets in the

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

Serial no.	Complexes	Found (calcd.) %				Colour	Mol. wt.
		M	C	H	N		
(1)	$[Cr(C_{48}H_{32}N_4)Cl]Cl_2$	6.28(6.32)	70.02(70.07)	3.84(3.89)	6.78(6.81)	Light green	822
(2)	$[Cr(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	5.73(5.76)	63.84(63.86)	3.55(3.55)	10.85(10.86)	Dark brown	902
(3)	$[Cr(C_{48}H_{32}N_4)(OAc)](OAc)_2$	5.80(5.82)	72.55(72.56)	4.57(4.59)	6.26(6.27)	Green	893
(4)	$[Mn(C_{48}H_{32}N_4)(OAc)](OAc)_2$	6.08(6.13)	72.28(72.32)	4.55(4.56)	6.25(6.25)	Dark grey	896
(5)	$[Fe(C_{48}H_{32}N_4)Cl]Cl_2$	6.75(6.76)	69.70(69.73)	3.81(3.87)	6.77(6.78)	Dark brown	826
(6)	$[Fe(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	6.11(6.16)	63.51(63.57)	3.49(3.53)	10.76(10.82)	Light brown	906
(7)	$[Fe(C_{48}H_{32}N_4)(OAc)](OAc)_2$	6.17(6.23)	72.16(72.24)	4.53(4.57)	6.19(6.24)	Reddish brown	897

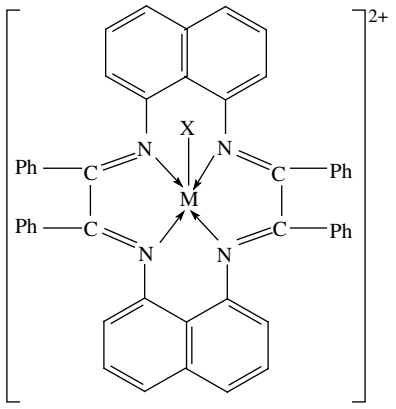


Fig. 1. Proposed structure of the complexes. Where M = Cr(III), Mn(III), Fe(III); X = Cl^-, NO_3^-, CH_3COO^-.

region 7.40–7.65 ppm may be assigned due to the aromatic ring protons of benzil (20H) [43].

4.3. Mass spectra

The FAB mass spectra of Cr(III), Mn(III) and Fe(III) macrocyclic complexes have been recorded. All the spectra exhibit parent peaks due to molecular ions (M^+). The proposed molecular formula of theses complexes was confirmed by comparing their molecular formula weights with *m*/*z* values. The molecular ion (M^+) peaks obtained for various complexes are as follows: (1) *m*/*z* = 822, (2) *m*/*z* = 902, (3) *m*/*z* = 893 (Chromium(III)complexes) (4) *m*/*z* = 896 (Manganese(III) complex) (5) *m*/*z* = 826, (6) *m*/*z* = 906, (7) *m*/*z* = 897 (Iron(III) complexes) This data is in good agreement with the proposed molecular formula for these complexes i.e. $[M(C_{48}H_{32}N_4)X]X_2$. Where M = Cr(III), Mn(III) and Fe(III), and X = Cl^-, NO_3^-, CH_3COO^-. This confirms the formation of the macrocyclic frame. In addition to the peaks due to the molecular ions, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the complexes. The peak intensity gives an idea of the stability of the fragments.

4.4. Magnetic measurements and electronic spectra

4.4.1. Chromium complexes

Magnetic moments of chromium(III) complexes were found in the range of 4.15–4.52 B.M. at room temperature which is close to the predicted values for three unpaired electrons in the metal ion [44]. The electronic spectra of chromium(III) complexes show bands at ~9010–9320 cm^{-1}, 13 030–13 340 cm^{-1}, 17 460–18 320 cm^{-1}, 27 420–27 850 cm^{-1} and 34 810 cm^{-1}. The spectral bands are consistent with that of five coordinated Cr(III) complexes, whose structure has been confirmed with the help of X-ray measurements [45]. 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 these complexes [46,47], the various spectral bands may be assigned as: $^4B_1 \rightarrow {}^4E^a$, $^4B_1 \rightarrow {}^4B_2$, $^4B_1 \rightarrow {}^4A_2$ and $^4B_1 \rightarrow {}^4E^b$.

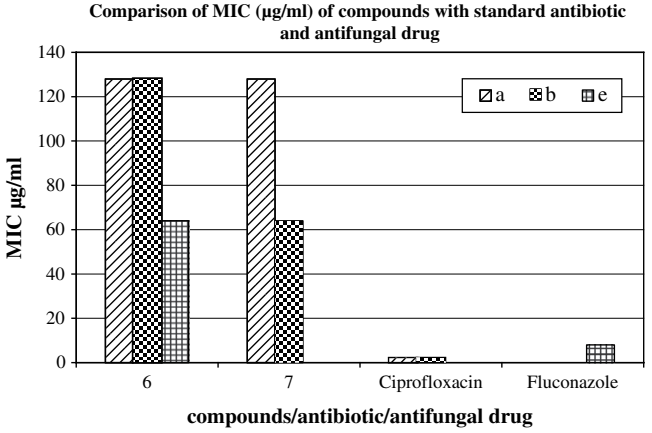


Fig. 2. Comparison of MIC (µg/ml) of compounds with standard antibiotics and antifungal drug: a – *Staphylococcus aureus* (MTCC 96); b – *Bacillus subtilis* (MTCC 121); e – *Saccharomyces cerevisiae* (MTCC 170); Ciprofloxacin – Standard antibiotic; Fluconazole – standard drug.

4.4.2. Manganese complexes

The magnetic moment of manganese(III) complex is 4.90 B.M. which indicates the high spin d^4 system [44]. The electronic spectra of manganese complex show three d–d bands which lay in the range 12 250–12 590, 16 050–18 920 and 35 420–35 750 cm^{-1}. The higher energy band at 35 440–35 750 cm^{-1} may be assigned due to charge transfer transitions. The spectra resemble to those reported for five coordinate square pyramidal manganese(III) complexes [46,47]. 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: $d_z^2 \rightarrow d_{x-y}^{22}$, $d_{xy} \rightarrow d_{x-y}^{22}$ and d_{xz}, $d_{yz} \rightarrow d_{x-y}^{22}$, respectively, in order of increasing energy. However, the complexes do not have idealized C_{4V} symmetry.

4.4.3. Iron complexes

The magnetic moment of iron(III) complexes lay in the range 5.81–5.91 B.M. corresponding to the five unpaired electrons and is close to the predicted high spin values for these metal ions [44]. The electronic spectra of iron(III) complexes show various bands in the

Table 2
In vitro antimicrobial activity of synthesized macrocyclic complexes through agar well diffusion method.

Serial no.	Complexes	Diameter of growth of inhibition zone (mm)[x]					
		a	b	c	d	e	f
(1)	$[Cr(C_{48}H_{32}N_4)Cl]Cl_2$	14.3	–	–	–	15.6	–
(2)	$[Cr(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	–	–	–	–	–	–
(3)	$[Cr(C_{48}H_{32}N_4)(OAc)](OAc)_2$	–	–	–	–	12.3	–
(4)	$[Mn(C_{48}H_{32}N_4)(OAc)](OAc)_2$	–	11.6	–	–	–	–
(5)	$[Fe(C_{48}H_{32}N_4)Cl]Cl_2$	–	12.6	–	–	12.8	–
(6)	$[Fe(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	14.6	20.5	–	–	–	–
(7)	$[Fe(C_{48}H_{32}N_4)(OAc)](OAc)_2$	19.6	18.5	–	–	14.3	–
	Ciprofloxacin	26.3	25.6	25.0	23.3	–	–
	Fluconazole	–	–	–	–	14.0	15.3

(–) No activity; [x]Values, including diameter of the well (8 mm), are means of three replicates; a – *Staphylococcus aureus*; b – *Bacillus subtilis*; c – *Escherichia coli*; d – *Pseudomonas aeruginosa*; e – *Saccharomyces cerevisiae*; f – *Candida albicans*; Ciprofloxacin – Standard antibiotic; Fluconazole – standard drug.

Table 3
Minimum inhibitory concentrations (MIC) (in µg/ml) of compounds by using macrodilution method.

Serial no.	Complexes	a	b	e
(6)	$[Fe(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	128	128	64
(7)	$[Fe(C_{48}H_{32}N_4)(OAc)](OAc)_2$	128	64	–
	Ciprofloxacin	2	2	–
	Fluconazole	–	–	8

Table 4
Minimum bactericidal concentrations (MBC) (in μg/ml) of compounds by using macrodilution method.

Serial no.	Complexes	a	b	e
(6)	$[Fe(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	128	64	–
(7)	$[Fe(C_{48}H_{32}N_4)(OAc)](OAc)_2$	128	128	64
	Ciprofloxacin	2	2	–
	Fluconazole	–	–	8

a – *Staphylococcus aureus* (MTCC 96); b – *Bacillus subtilis* (MTCC 121); e – *Saccharomyces cerevisiae* (MTCC 170); Ciprofloxacin – Standard antibiotic; Fluconazole – standard drug.

range 9820–9970, 15 510–15 575, 27 600–27 730 cm^{-1} and are consistent with the range of spectral bands reported for five coordinate square pyramidal iron(III) complexes [47,48]. 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.

Based on various studies like elemental analyses, conductance measurements, magnetic susceptibilities, infrared, NMR and electronic and mass spectral studies the structure shown in Fig. 1 may be proposed for all of the complexes.

5. Biological results and discussion

In the present study, seven chemically synthesized compounds were evaluated against two Gram-positive, two Gram-negative bacteria and four fungi (two mold and two yeast strains). Minimum inhibitory concentration (MIC) of some of these synthesized compounds against some Gram-positive bacteria and yeast was determined by the method given by Andrews [23]. Standard antibiotics namely Ciprofloxacin and standard antifungal drug Fluconazole were used for comparison with antibacterial and antifungal activities shown by compounds (Table 2, Fig. 2). All the compounds of the tested series possessed good antibacterial activity against Gram-positive bacteria (*S. aureus*, *B. subtilis*) and antifungal activity against the yeast (*S. cerevisiae*) and molds (*A. niger* and *A. fumigatus*). However the compounds in this series were not effective against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and yeast (*C. albicans*) (Table 2). On the basis of maximum inhibitory activity shown against bacteria and yeast, compounds (6) and (7) were found to be most effective. In the whole series, MIC of chemical compound (6) was found to be 128 μg/ml for *S. aureus* and 64 μg/ml for *B. subtilis* whereas MIC of compound (7) was found to be 128 μg/ml for both *S. aureus*, *B. subtilis* (Table 3, Fig. 2). The MBC of compound (6) was found to be more than 128 μg/ml for *S. aureus* and 128 μg/ml for *B. subtilis*. MBC of compound (7) was found to be more than 128 μg/ml for both organisms (*S. aureus* and *B. subtilis*) (Table 4).

Table 5
In vitro antimicrobial activities of synthesized complexes through poisoned food method.

Serial no.	Complexes	Mycelial growth inhibition (%)	
		g	h
(1)	$[Cr(C_{48}H_{32}N_4)Cl]Cl_2$	25.6	43
(2)	$[Cr(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	40.6	57.1
(3)	$[Cr(C_{48}H_{32}N_4)(OAc)](OAc)_2$	35.6	50
(4)	$[Mn(C_{48}H_{32}N_4)(OAc)](OAc)_2$	53	37.5
(5)	$[Fe(C_{48}H_{32}N_4)Cl]Cl_2$	51.2	61.2
(6)	$[Fe(C_{48}H_{32}N_4)(NO_3)](NO_3)_2$	54	62.1
(7)	$[Fe(C_{48}H_{32}N_4)(OAc)](OAc)_2$	–	52.5
	Fluconazole	75.3	80.2

g – *Aspergillus niger* (MTCC 282); h – *Aspergillus fumigatus* (MTCC 870); Fluconazole – standard drug.

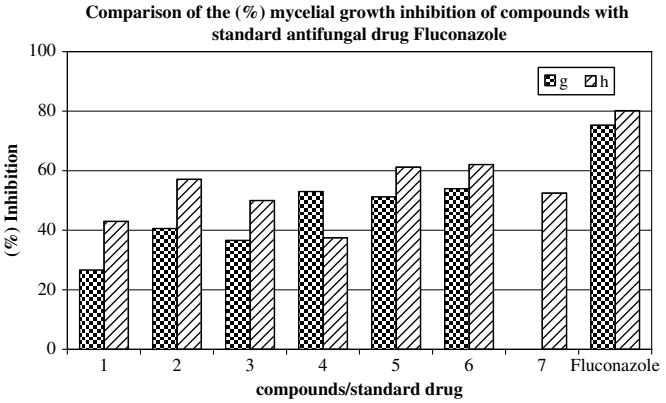


Fig. 3. Comparison of the (%) mycelial growth inhibition of compounds with standard antifungal drug Flucanozole: g – *Aspergillus niger* (MTCC 282); h – *Aspergillus fumigatus* (MTCC 870); Fluconazole – standard drug.

The antifungal activities of all the complexes were carried out against two fungal strains i.e. *A. niger* and *A. fumigatus* and then compared with standard antifungal drug Fluconazole (Table 5, Fig. 3). The antifungal activities (percentage inhibition) are given in Table 5. In the whole series, compound (6) shows the highest percentage inhibition against both fungal strains, whereas none of the tested compounds restrict the fungal growth excellently (Table 5). However, of all the tested compounds (4), (5) and (6) showed nearly 50% inhibition of mycelial growth against *A. niger* whereas compounds (2), (3), (5), (6) and (7) showed 50–60% inhibition of mycelial growth against *A. fumigatus* (Table 5, Fig. 3).

Keeping in view the rising problems of antimicrobial resistance, these chemical compounds may be used for formulating novel chemotherapeutic agents and further investigation will be necessary to identify the active principle.

6. Experimental protocols

The microanalysis of C, H, and N were estimated by elemental analyzer (Perkin Elmer 2400), at SAIF, Punjab University, Chandigarh.

The magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee, on Vibrating Sample Magnetometer (Model PAR 155).

The IR spectra were recorded on a FT-IR spectrophotometer (Perkin Elmer) in the range 4000–200 cm^{-1} using Nujol Mull.

The ^1H NMR spectra (at room temperature) (in DMSO-d_6) were recorded on a Bruker AVANCE II 400 NMR spectrometer (400 MHz) with reference to Me_4Si (0.0 ppm), at SAIF, Punjab University, Chandigarh.

Electronic spectra (in DMSO) were recorded on a Hitachi 330 spectrophotometer (850–200 nm) at room temperature.

The FAB mass spectra (at room temperature) were recorded on VG-70-S mass spectrometer at SAIF, Punjab University, Chandigarh.

The metal contents in the complexes were determined by literature method [49].

The conductivity was measured on digital conductivity meter (HPG system, G-3001).

Melting points were determined by using capillaries in electrical melting point apparatus.

7. Conclusions

It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its

positive charge with donor group within the whole chelate ring system [50,51]. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively thus increasing the activity of the complexes. Besides from this many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be possible reasons for remarkable antibacterial activities of these complexes [52]. It also has been observed that some moieties such as azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive biological activities that may be responsible for the increase in hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the microorganism and enhance biological utilization ratio and activity of complexes [53].

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