Academic Sciences

International Journal of Chemistry Research

ISSN- 0976-5689 Vol 3, Issue 1, 2012

Research Article

SYNTHESIS, SPECTRAL AND ANTIBACTERIAL STUDIES OF 18- MEMBERED TETRAAZAMACROCYCLIC COMPLEXES DERIVED FROM CARBOHYDRAZIDE AND DIACETONYL

M. KAMBOJ[a#*], D.P. SINGH[a] AND K. JAIN[b]

[a]Department of Chemistry, National Institute of Technology, Kurukshetra 136119, India, [b]Department of Chemistry, M.L.N. College, Yamunanagar 135001, India, [#]Department of Chemistry, Amity School of Engineering and Technology, Amity campus, Lucknow 226010, India. Email: dpsinghchem@yahoo.co.in, monikakamboj@yahoo.com

Received: 19 Aug 2011, Revised and Accepted: 16 Nov 2011

ABSTRACT

A novel series of the macrocyclic complexes of type $[M(C_{14}H_{24}N_8O_2)X]X_2$, where M = Fe(III) or Cr(III) and X = Cl^{-1}, NO_3^{-1} or CH_3COO^{-1} has been synthesized by [2+2] condensation of carbohydrazide and acetonylacetone (diacetonyl) in the presence of trivalent metal salt in methanolic medium. The complexes were characterized with the help of various physicochemical techniques such as elemental analyses, conductance measurements, electronic and infrared spectral studies. The low value of molar conductance indicates them to be as 1:2 electrolytes. On the basis of various studies, a five coordinate square pyramidal geometry for these complexes has been proposed. All the synthesized macrocyclic complexes were also tested for their *in vitro* antibacterial activity against some pathogenic bacterial strains. The MIC values shown by the complexes against these bacterial strains were compared with those of the standard antibiotics *Ciprofloxacin.* Some of the complexes showed good antibacterial activities

Keywords: Antibacterial activity; Carbohydrazide; Macrocyclic complexes; Template condensation.

INTRODUCTION

Macrocyclic chemistry is the most substantial innovation within the coordination chemistry in recent years[1]. The study of macrocyclic ligand and their complexes allows us to probe many of more suitable aspects of reactivity of coordination compounds, which would not be possible in less stable complexes with open noncyclic ligands. Interest in macrocyclic complexes lies in the preparation of model compounds, which might mimic the biological species, involved in electron transfer and dioxygen activation processes[2–4]. More recently, the high stability of macrocyclic complexes has been utilized in construction of models for metalloproteinase and in a wide range of technological applications. Macrocyclic nickel complexes find use in DNA recognition and oxidation[5], while macrocyclic copper complexes find use in DNA binding and cleavage[6]. Template reactions have been widely used for the syntheses of macrocyclic complexes, where the transition metal ion is used as a templating agent[7]. Macrocyclic metal complexes have been useful because of their close relationship with natural products such as vitamin B_{12} and chlorophyll[8]. Some macrocyclic complexes have been reported to show antibacterial, antifungal, and anti-inflammatory activities[9–11]. Macrocyclic metal chelating agents (DOTA) are useful to detect tumor lesions[12]. Based on the above-mentioned studies, in the present paper, macrocyclic complexes of Fe(III) and Cr(III) derived by the template condensation reaction of carbohydrazide and acetonylacetone has been reported. The complexes have been characterized with the help of various physicochemical techniques like molar conductance, elemental analyses, magnetic susceptibilities, IR, electronic and mass spectra. These macrocyclic complexes were also screened for their *in vitro* antibacterial activity.

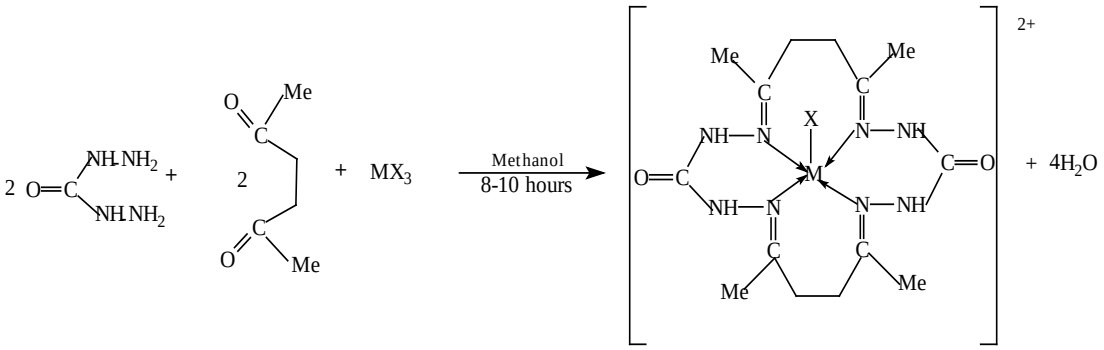
MATERIALS AND METHODS

Isolation of complexes

All the chemicals used were of analytical-reagent grade. All the employed solvents were of high purity. Moisture was excluded from the glass apparatus using $CaCl_2$ tubes.

All the complexes were obtained by template synthesis. To a stirring methanolic solution (~50 ml) of carbohydrazide (10 mmol) was added trivalent chromium or iron salt, respectively (5 mmol) dissolved in minimum quantity of methanol (~20 ml). The resulting solution was refluxed for 0.5 h. After that acetonylacetone (10 mmol) dissolved in ~20-30 ml methanol was added to the refluxing mixture and refluxing continued for 8-10 h. The mixture was concentrated to half of its volume and kept in desiccator overnight. The complexes were then filtered, washed with methanol, acetone and diethyl ether, dried in vacuo. The purity of complexes was checked by TLC.Yield ~55-65%.

The Template synthesis of the complexes of carbohydrazide and acetonylacetone in the presence of trivalent metal salts, in the molar ratio 2:2:1 is shown by the following scheme:



Where M = Cr(III) or Fe(III)

X = Cl^-, NO_3^-, CH_3COO^-

Analytical and physical measurements

The microanalyses of C, H, and N were carried out at Sophisticated Analytical Instrument Facility (SAIF), CDRI, Lucknow. The magnetic susceptibility measurements were carried at SAIF, IIT Roorkee on Vibrating Sample Magnetometer (ModelPAR155). The IR spectra were recorded on FT-IR spectrophotometer in the range 4000-200 cm^{-1} using Nujol Mull. The metal contents in the complexes were determined by literature method[13]. Electronic spectra (DMF) were recorded on Cary 14 spectrophotometer. The conductivity was measured on digital conductivity meter (HPG System, G-3001). Melting points were determined by using capillaries in electrical melting point apparatus.

Antibacterial activity

Four bacteria, *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) (Gram-positive), *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) (Gram-negative) were used in the present study. The antibacterial activity of synthesized macrocyclic complexes has been evaluated by the agar well diffusion method[14]. All the cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml[15]. A 20 ml of Mueller Hinton agar (MHB) medium was poured into each Petri plate and the agar plates were swabbed with 100 µl inocula of each test bacterium 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 4.0 mg/ml of each metal complex reconstituted in the DMSO. All the plates were incubated at 37°C for 24 h. Antibacterial activity of each synthesized complex was evaluated by measuring the zone of growth inhibition against the test microorganisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas *Ciprofloxacin* was used as a positive control. This procedure was performed in three replicate plates for each microorganism.

Determination of Minimum Inhibitory Concentration (MIC) of synthesized complexes

The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. MIC of the various complexes against various bacterial strains was tested through a macro dilution tube method[15]. In this method, various test concentrations of the synthesized metal complexes were made from 128 to 0.25 µg/ml in sterile tubes No. 1-10.

A 100 µl sterile Mueller Hinton Broth (MHB) medium was poured in each sterile tube followed by addition of 200 µl test complex in tube 1. Two fold serial dilutions were carried out from the tube 1 - 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.

RESULTS AND DISCUSSION

The complexes were soluble in DMF and DMSO, but were insoluble in common organic solvents and water. They were thermally stable up to ~270°C and then decomposed. The analytical data show the formula for macrocyclic complexes as: $[M(C_{14}H_{24}N_8O_2)X]X_2$ where M = Cr(III) or Fe(III) and X = Cl^{-1}, NO_3^{-1}, CH_3COO^{-1}.

The measurements of molar conductance in DMSO show that these chelates are 1:2 electrolytes (conductance 155-178 ohm^{-1} cm^2 mol^{-1})[16]. The tests for anions are positive before and after decomposing the chelates showing their presence outside as well as inside of coordination sphere. The synthesized metal complexes have been characterized with the aid of various physicochemical techniques such as infrared, electronic, magnetic susceptibility measurements, molecular weight determination. All complexes give satisfactory elemental analyses results as shown in Table -1.

Table 1: Analytical data of trivalent chromium and iron complexes derived from carbohydrazide and acetonylacetone. Found (Calcd.) %

Sr. No.	Complexes	M	C	H	N	Colour	Mol. Wt.
1	$[Cr(C_{14}H_{24}N_8O_2)Cl]Cl_2$	10.3(10.52)	33.8(34.0)	4.7(4.85)	22.10(22.67)	Dark brown	494
2	$[Cr(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	8.95(9.05)	29.1(29.26)	4.01(4.18)	26.4(26.82)	Brown	574
3	$[Cr(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	8.9(9.20)	42.2(42.47)	5.6(5.84)	19.63(19.82)	Brown	565
4	$[Fe(C_{14}H_{24}N_8O_2)Cl]Cl_2$	11(11.24)	33.6(33.73)	4.6(4.82)	22.2(22.49)	Brown	498
5	$[Fe(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	9.15(9.68)	28.89(29.06)	4.10(4.15)	26.1(26.64)	Brown	578
6	$[Fe(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	9.6(9.84)	42.0(42.18)	5.5(5.79)	19.52(19.68)	Brown	569

IR Spectra

The presence of single medium intensity bands in the region ~3240-3300 cm^{-1} in the complexes may be assigned due to N-H stretching vibrations[17]. It was noted that a pair of bands corresponding to $\nu(NH_2)$ stretching frequency appeared at ~3240-3300 cm^{-1} in the IR spectrum of carbohydrazide but are absent in the IR spectra of all the metal complexes. Further, no strong absorption band was observed near 1700 cm^{-1} indicating the absence of >C=O of acetonylacetone and thus, confirming the condensation of carbonyl group of acetonylacetone and amino group of carbohydrazide[18]. These results provide strong evidence for the formation of macrocyclic frame[19]. A strong absorption band in the region ~1590-1620 cm^{-1} may be assigned to (C=N) stretching vibrations[20]. The lower values of ν(C=N) may be explained on the basis of drift of lone pair density of azomethine nitrogen towards metal atom[21]. The band present in the range 1690-1710 cm^{-1} may be assigned due to the >C=O group of the CONH moiety in all the metal complexes[22]. The presence of the absorption bands in the region 1410-1430, 1280-1320 and 1010-1045 cm^{-1}, in the IR spectra of all the nitrate complexes suggest that both the nitrate groups are coordinated to the central metal ion in a unidentate fashion[23]. The IR spectra of the acetate complexes show an absorption bands in the region 1650-1685 cm^{-1} that is assigned to $\nu(COO^-)_{as}$ asymmetric stretching vibrations of acetate ion and another in the region 1258-1295 cm^{-1} that can be assigned to $\nu(COO^-)_s$ 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[23]. The far infrared spectra show bands in the region ~425-455 cm^{-1} corresponding to ν(M-N) vibrations[24]. The presence of these bands gives an idea about the coordination of azomethine nitrogen[25]. The bands present at 300-315 cm^{-1} may be assigned due to ν(M-Cl) vibrations[24]. The bands present at 230-250 cm^{-1} in all nitrate and acetate complexes are assigned due to ν(M-O) stretching vibrations[24].

Magnetic measurements and electronic spectra

Chromium (III) complexes: Magnetic moment of chromium (III) complexes was found in the range of 4.18-4.35 B.M., at room temperature which was close to the predicted values for three unpaired electrons in the metal ion[25]. The electronic spectra of chromium complexes recorded in DMSO show bands at ~ 9020-9310 cm^{-1}, 13150-13320 cm^{-1}, 17550-18350 cm^{-1}, 27390-27750 cm^{-1} and 34820 cm^{-1}. These spectral bands were consistent with that of five coordinated square-pyramidal chromium(III) complexes, whose structure has been confirmed with the help of X-ray measurements[26]. Thus, on the basis of analytical data, spectral studies and electrolytic nature of these complexes, a five coordinated square-pyramidal geometry may be assigned for these complexes[27]. Thus, assuming the symmetry C_{4V} for these complexes, the various spectral bands may be assigned as: $^4B_1 \rightarrow {}^4E^a$, $^4B_1 \rightarrow {}^4B_2$, $^4B_1 \rightarrow {}^4A_2$ and $^4B_1 \rightarrow {}^4E^b$ [28,29].

Iron (III) complexes: The magnetic moment of iron(III) complexes was found in the range of 5.75-5.94 B.M., corresponding to the five

unpaired electrons and was close to the predicted high spin values for these metal ions[26]. The electronic spectra of iron complexes recorded in DMSO show various bands 9820-9950, 15520-15640, 27540-27710 cm^{-1} and these spectral bands were consistent with the range of spectral bands reported for five coordinate square-pyramidal iron(III) complexes[28,30]. 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 was difficult due to interactions of the metal-ligand л-bond systems lifting the degeneracy of the d_{xz} and d_{yz} pair

Mass Spectra

The mass spectra of chromium(III) and iron(III) macrocyclic complexes derived from acetonylacetone and carbohydrazide exhibit parent peaks due to molecular ions $[M]^+$ and $[M+2]^+$. The molecular ion $[M]^+$ peaks obtained for various complexes derived from acetonylacetone and carbohydrazide were as follows: (1) m/z = 492.6 (due to ^{35}Cl) & 494.6 (due to ^{37}Cl) [Mol.Wt.-494], (2) m/z = 573.4 [Mol.Wt.-574], (3) m/z = 563.5 [Mol. Wt.-565], (4) m/z = 497.6 (due to ^{35}Cl) & 499.6 (due to ^{37}Cl) [Mol.Wt.- 498], (5) m/z = 577.3 [Mol Wt.-578] and (6) m/z = 568.4 [Mol. Wt.- 569]. The proposed molecular formulas of these complexes were confirmed by comparing their molecular formula weights with m/z values. This confirms the formation of the macrocyclic frame.

Biological Results and Discussion

In this study, all the synthesized macrocyclic complexes were evaluated against Gram-positive and Gram-negative bacteria. Minimum Inhibitory concentration **(**MIC) of these synthesized macrocyclic complexes were determined by the method given by Andrews[15]. All the synthesized macrocyclic complexes of this series were screened for their antibacterial activities. All the tested complexes possessed variable antibacterial activities against both Gram-positive (*S. aureus, B. subtilis*) and Gram-negative (*E. coli, P. aeruginosa)* bacteria. Standard antibiotic namely *Ciprofloxacin* was used to compare the antibacterial activities shown by the synthesized complexes (Table-2, Table-3).

Table 2: *In vitro* antibacterial activity of synthesized complexes through agar well diffusion method

S. No.	Complexes	Diameter of growth of inhibition zone (mm)[x]			
		a	b	c	d
(1)	$[Cr(C_{14}H_{24}N_8O_2)Cl]Cl_2$	15.6	18.3	-	-
(2)	$[Cr(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	14	15.3	-	-
(3)	$[Cr(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	17.3	15.6	-	-
(4)	$[Fe(C_{14}H_{24}N_8O_2)Cl]Cl_2$	16.3	17	-	-
(5)	$[Fe(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	15.6	18.6	-	-
(6)	$[Fe(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	21.6	18.3	15.6	-
(7)	*Ciprofloxacin*	27.6	26.3	25.0	25.3

[x]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

On the basis of zone of inhibition produced against the test bacterium, complex (6) was found to be most effective against *S. aureus* showing the maximum zone of inhibition of 21.6mm. Complexes (1), (2), (3) (4) and (5) also showed the zone of inhibition ranges from 18.6 to 14.0 mm against some of the tested bacteria strains. Complex (6) showed activity against all the tested bacteria except *P. aeruginosa* and complexes (1) ,(2) (3),(4) and (5) showed activity only against Gram-positive (*S. aureus, B. subtilis*) bacteria. On the basis of MIC shown by these complexes against the selected bacterial strains, complex (6) was found to be most effective by showing the MIC of 32 µg/ml for *S. aureus* . (Table-3, Fig 1).

Table 3: Minimum inhibitory concentration (MIC) (in µg/ml) of the complexes by using macro dilution method

S. No.	Complexes	MIC (µg/mL)			
		a	b	c	d
(1)	$[Cr(C_{14}H_{24}N_8O_2)Cl]Cl_2$	256	64	-	-
(2)	$[Cr(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	>256	256	-	-
(3)	$[Cr(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	128	256	-	-
(4)	$[Fe(C_{14}H_{24}N_8O_2)Cl]Cl_2$	128	128	-	-
(5)	$[Fe(C_{14}H_{24}N_8O_2)(NO_3)](NO_3)_2$	256	64	-	-
(6)	$[Fe(C_{14}H_{24}N_8O_2)(OAc)](OAc)_2$	32	64	128	-
(7)	*Ciprofloxacin*	05	05	05	05

(-) 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

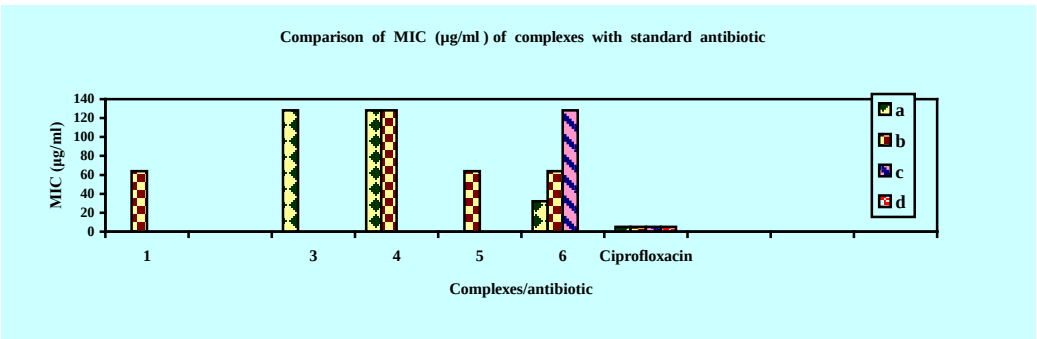


Fig. 2: Comparsion of MIC (µg/ml) of complexes with antibiotic

a- *Staphylococcus aureus* (MTCC 96)
b- *Bacillus subtilis* (MTCC 121)
c- *Escherichia coli* (MTCC 1652)
d- *Pseudomonas aeruginosa* (MTCC 741)
Ciprofloxacin-Standard antibiotic

In the whole series, MIC of the complexes (1), (5) and (6) was found to be 64 µg/ml for the bacterial strains *B. subtilis.* Whereas MIC of complexes (3) and (6) were found to be 128 µg/ml for the bacterial strains *S. aureus* and *E. coli,* respectively. The complex (4) also showed the MIC of 128 µg/ml for both *S. aureus* and *B. subtilis*.

CONCLUSION

Based on elemental analyses, conductivity, magnetic measurements, electronic, IR spectral and mass spectral studies, the structure as shown in Figure: 2 may be proposed for all of the synthesized complexes. 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 groups within the whole chelate ring system. 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.

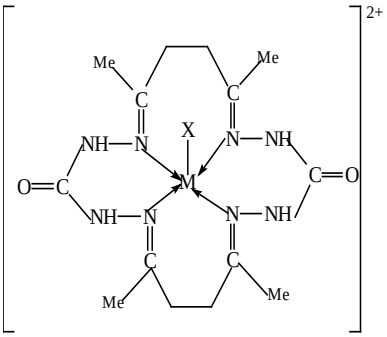


Fig. 2: Structure of complex

Abbreviations: B.M.-Bohr Magneton, CFU-Colony Forming Unit, DMF-N,N dimethylformamide, DMSO-Dimethylsulphoxide, IR-Infrared, MIC-Minimum Inhibitory Concentration, MTCC-Microbial Type Culture Collection, NCCLS-National Committee for Clinical Laboratory Standards, MHA-Mueller Hinton Agar, MHB-Mueller Hinton Broth

ACKNOWLEDGEMENT

Authors are thankful to the authorities of the NIT, Kurukshetra and Amity University, Uttar Pradesh for providing the necessary research facilities.

REFERENCE

1. Paryzek WR, Patroniak V, Lisowski J. Coord Chem Rev (2005) 249: 2156.
2. Chandra S, Gupta LK, Aggarwal S. Transition Met Chem (2007) 32: 240.
3. Constable EC. Coordination Chemistry of Macrocyclic Compounds. Oxford: Oxford University Press(1999).
4. Singh DP, Kumar R. J Serb Chem Soc (2007) 72: 1069.
5. Muller JG, Chen X, Dadiz AC. Pure Appl Chem (1993) 65: 545.
6. Liu J, Lu TB, Deng H. Transition Met Chem (2003) 28: 116.
7. Lindoy LF. The Chemistry of Macrocyclic Ligand Complexes. Cambridge: Cambridge University. Press(1989).
8. Niasari MS, Bazarganipour M, Ganjali MR, Norouzi P. Transition Met Chem (2007) 32: 9.
9. Keypour H, Khanmohammadi H, Wainwright KP, Taylor MR. Inorg Chim Acta (2007) 357: 1283.
10. Singh DP, Kumar R, Malik V, Tyagi P. J Enzym Inhib Chem (2007) 22: 177.
11. Singh RV, Chaudhary A. J Inorg Biochem (2004) 98: 1712.
12. Kosmos C, Snook D, Gooden CS. Cancer Res (1992) 52: 904.
13. Vogel AI. A Text Book of Qualitative Chemical analysis, 5th Ed, Longman, London (1989).
14. Singh DP, Kumar R, Singh J. Eur J Med Chem (2009) 44: 1731; J Enzym Inhib Med Chem (2009) 24: 883; Ahmad I, Beg AZ. J Ethnopharmacol (2001) 74: 113.
15. Andrews JM. J Antimicrob Chemother (2001) 48: 5.
16. Kumar R, Singh R. Turk J Chem (2006) 30: 77
17. Singh AK, Singh R, Saxena P. Transition Met Chem (2003) 28: 160.
18. Srinivasan S, Athappan P. Transition Met Chem (2001) 26: 588.
19. Singh DP, Kumar R, Tyagi P. Transition Met Chem (2006) 31: 970.
20. Mohamed AK, Islam KS, Hasan SS, Shakir M. Transition Met Chem (1999) 24: 198.
21. Chandra S, Sharma SD. Transition Met Chem (2002) 27: 732.
22. Souza P, Mendiola MA, Matesanz AI, Fernandez V. Transition Met Chem (1995) 20: 157.
23. K. Nakamoto. Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B, 5th Ed, Wiley, New York (1997).
24. Singh DP, Malik V, Kumar R, Kumar K, Singh J. Russ J Coord Chem (2009) 35: 740 .
25. Singh DP, Kumar R ,Malik V, P. Tyagi. Transition Met Chem (2007) 32:1051.
26. Figgis BN, Lewis J. The Magneto Chemistry of Chelates in Modern Coordination Chemistry, Interscience, New York (1960).
27. Wood JS. Prog Inorg Chem (1972) 16: 227.
28. Singh DP, Rana VB. Polyhedron (1995) 14: 2901.
29. Singh DP, Kumar R, Sharma C. Eur J Med Chem (2009) 44: 3299.
30. Lever ABP. Inorganic Electronic Spectroscopy, Amsterdam: Elsevier (1984).