Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 795–803

Biologically active macrocyclic complexes derived from diaminonaphthalene and glyoxal: Template synthesis and spectroscopic approach

D. P. SINGH[1], KRISHAN KUMAR[1], SAURABH SUDHA DHIMAN[2], & JITENDER SHARMA[2]

[1]*Department of Chemistry, National Institute of Technology, Kurukshetra 136 119, Haryana, India, and* [2]*Department of Biotechnology, Kurukshetra University, Kurukshetra 136 119, Haryana, India*

(*Received 15 March 2008; accepted 3 July 2008*)

Abstract

A novel series of complexes of the type $[M(C_{24}H_{16}N_4)X_2]$; where M = Co(II), Ni(II), Cu(II), Zn(II) and Cd(II); X = Cl^{-1}, NO_3^{-1}, CH_3COO^{-1} has been synthesized by template condensation of 1,8-diaminonaphthalene and glyoxal in the presence of divalent metal salts in methanolic medium. The complexes have been characterized with the help of elemental analyses, conductance measurements, molecular weight determination, magnetic measurements, electronic, NMR, infrared and far infrared spectral studies. The low value of molar conductance indicates them to be non-electrolytes. On the basis of various studies a distorted octahedral geometry may be proposed for all of these complexes. These metal complexes were also tested for their *in vitro* antibacterial and antifungal activities to assess their inhibiting potential.

Keywords: *Biological activity, diaminonaphthalene, macrocyclic complexes, metal coordinate, spectroscopic studies*

Abbreviations: *B.M., Bohr Magneton; CFU, Colony Forming Unit; DMF, N,N dimethylformamide; DMSO, Dimethylsulphoxide; IR, Infra red; MIC, Minimum Inhibitory Concentration; MRI, Magnetic Resonance Imaging; MTCC, Microbial Type Culture Collection; NMR, Nuclear Magnetic Resonance; PDA, Potato Dextrose Agar*

Introduction

Macrocyclic compounds and their derivatives are interesting ligand-system because they are good hosts for metal anions, neutral molecules and organic cation guests [1]. 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 [2]. The family of complexes with aza-macrocyclic ligands has been remained a focus of scientific attention for many decades [3]. *In-situ* one pot template condensation reaction lie at the heart of the macrocyclic chemistry [4]. Therefore, template reactions have been widely used for synthesis of macrocyclic complexes where generally the transition metal ion is used as templating agent [5]. There is continued interest in synthesizing macrocyclic complexes because of their potential application in fundamental and applied sciences [6,7]. Macrocyclic complexes act as a model, mimicking naturally occurring 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 containg proteins have been identified [11]. Macrocyclic metal complexes of lanthanides e.g. Gd^{3+} are used as MRI contrast agents [12]. Macrocyclic metal chelating agent (DOTA) is useful for detecting tumour lesions [13]. The chemistry of macrocyclic complexes is also important due to their use as dyes and pigments [14] as well as NMR shift reagents [15].

Correspondence: D. P. Singh, Department of Chemistry, National Institute of Technology, Kurukshetra 136 119, Haryana, India. Tel: 91 1744 233512. Fax: 91 1744 238050. E-mail: dpsinghchem@yahoo.co.in

ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802397179

Some macrocyclic complexes have received special attention because of their mixed soft- hard donor character and versatile coordination behaviour [16] and of their pharmacological properties i.e. toxicity against bacterial and fungal growth [17]. Prompted by these, in the present paper, synthesis and characterization of cobalt(II), nickel(II), copper(II), zinc(II), and cadmium(II) macrocyclic complexes derived from 1,8-diaminonaphthalene and glyoxal 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 bacterial strains *viz*. *Bacillus subtilis* (MTCC 8509), *Bacillus stearothermophilus* (MTCC 8508), *Escherichia coli* (MTCC 51), *Pseudomonas putida* and some fungal strains *viz*. *Aspergillus flavus* and *Aspergillus niger*. The results obtained were compared with standard antibiotics: Chloroamphenicol, Streptomycin and the antifungal drug Cyclohexamide.

Experimental

All the chemicals used were of AnalaR grade. 1,8-diaminonaphthalene and glyoxal procured from Acros, metal salts were purchased from s.d.-fine, Merck, Ranbaxy and were used as received.

Chemistry

Isolation of complexes. All the complexes were synthesized by template method i.e. by condensation of 1,8-diaminonaphthalene and glyoxal in the presence of the respective divalent metal salts. To a hot stirring methanolic solution ($\sim 50\,cm^3$) of 1,8-diaminonaphthalene (10 mmol) was added divalent cobalt, nickel, copper, zinc and cadmium salt (5 mmol) dissolved in the minimum quantity of MeOH ($\sim 20\,cm^3$). The resulting solution was refluxed for 0.5 h. After that, glyoxal (10 mmol) was added in the refluxing mixture and refluxing was continued for 8-10 h. The mixture was concentrated to half of its 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 obtained ~50-65%. 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 ~280°C and then decomposed.

The template synthesis of complexes may be represented by the following scheme:

$$2\,C_{10}H_{10}N_2 + 2\,C_2H_2O_2 + MX_2 \xrightarrow[(8-10\,h)]{\text{Methanol}} [M(C_{24}H_{16}N_4)X_2]$$

Where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II)

$$X = Cl^{-1}, NO_3^{-1}, CH_3COO^{-1}$$

Analytical and physical measurements. The microanalysis of C, H, and N were carried out at SAIF, Punjab University, Chandigarh. The magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee. The metal contents in the complexes were determined by literature method [18]. The IR spectra were recorded on a FT-IR spectrophotometer (Perkin Elmer) in the range 4000-200 cm^{-1} using KBr pellets. The ^1H NMR spectra (at room temperature) (in DMSO d_6) were recorded on a Bruker AVANCE II 400 NMR spectrometer (400 MHz) at SAIF, Punjab University, Chandigarh. Electronic spectra (in DMSO) were recorded on Cary 14 spectrophotometer at room temperature. The FAB mass spectra (at room temperature) were recorded on TOF MS ES + mass spectrometer at SAIF, Punjab University, Chandigarh. The conductivity was measured on digital conductivity meter (HPG system, G-3001). Melting points were determined by using capillaries in electrical melting point apparatus. The molecular weights were determined by Rast camphor method [19].

Biological assay

Test microorganisms. Two Gram-positive bacteria *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508), two Gram-negative bacteria *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC 121) and two fungal strains *Aspergillus flavus*, *Aspergillus niger* were used for biological assay.

In-vitro *antibacterial activity*

Primary screening. The antibacterial activities of the newly synthesized complexes were evaluated by Agar Well Diffusion Assay technique [20] against two Gram- positive bacteria: *B. subtilis* (MTCC 8509) and *B. stearothermophilus* (MTCC 8508) and two Gram-negative bacteria: *E. coli* (MTCC 51) and *P. putida* (MTCC 121). The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on the fresh slants after every 4-6 weeks and incubating them at the appropriate temperature for 24 h. All stock cultures were stored at 4°C. For the evaluation of antimicrobial activities of the synthetic compounds, suspension of each test microorganism was prepared. Turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile distilled water. The size of final inoculum was adjusted to 5×10^7 cfu/mL. A volume of 20 mL of agar media was poured into each petri plate and plates were swabbed with broth cultures of the respective

micro-organisms and kept for 15 min for adsorption to take place. Wells of ≈ 8 mm diameter were punched in the seeded agar plates and a 100 μL volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. To allow diffusion of the compounds in to the agar, the plates were hold at room temperature for 2 h. After that the palates were incubated at 37°C for 24 h. Antibacterial activities were determined by measuring the inhibition zone diameter. The entire tests were made in triplicates and mean of the diameter of inhibition was calculated.

Determination of minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation [21]. Nutrient broth was adjusted to pH 7.0 used for the determination of MIC [22]. The inoculum of the test microorganisms were prepared by using 16 h old cultures adjusted by reference to the 0.5 McFarland standards (10^8cells/mL) [23]. These cultures were further diluted up to 10 fold with nutrient broth to get the inoculum size of 1.2×10^7 cfu/mL. A positive control (containing inoculum but no compound) and a negative control (containing compound but no inoculum) were also prepared. A stock solution of 4 mg/mL of each compound was prepared in DMSO and further appropriately diluted to get final concentration ranging from 250 to 0.03 μg/mL [21]. Separate flasks were taken for each test dilution. To each flask was added the 100 μL of inoculum. Then appropriately diluted test sample was added to each flask having broth and microbial inoculum. The contents of the flask were mixed and incubated for 24 to 48 h at 37°C. The test bacterial culture were spotted in a predefined pattern by aseptically transferring 5 μL of each bacterial culture on the surface of solidified agar plates and incubated at 37°C for 24 h for determining the MIC values.

In-vitro *antifungal activity*. Antifungal activities of the synthesized compounds were carried out against two fungal strains i.e. *A. flavus* and *A. niger* by agar plate technique [20]. Further the antifungal (Percentage Inhibition) activities of these compounds were compared with standard drug cyclohexamide [24]. Potato Dextrose Agar (PDA) medium was prepared in flask and sterilized. To check the growth of bacterial culture in the medium, requisite quantity of the standard antibiotic (Ampicilline) was added, so as to get their desirable final concentration of 100 μg/mL of the medium. Test samples were prepared in different concentrations (10 μg, 50 μg, 100 μg per mL) in DMSO and 200 μL of each sample was spread on PDA media containing sterilized petri plates. Mycelial discs taken from the standard cultures *(A. flavus and A. niger)* of fungi were grown on PDA medium for 5-7 days. These cultures were used for the purpose of inoculation in sterilized petri dish, aseptically. Standard cultures inoculated at 28 ± 1°C, were also used as the control. The efficiency of each sample was determined by measuring radial mycelial growth. The radial growth of the colony was measured in two directions at right angle to each other, and the average of two replicates was recorded in each case. Data were expressed as percent inhibition over control from the size of colonies. The percent inhibition was given in the Table III was calculated using the formula:

$$\%\,\text{inhibition} = (C - T) \times 100/C$$

C = diameter of fungus colony in the control plate after 96 h incubation;
T = diameter of the fungus colony in tested plate after same incubation period.

Result and discussion

Chemistry

The analytical data show the formula for macrocyclic complexes as: $[M(C_{24}H_{16}N_4)X_2]$; where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and X = Cl^{-1} NO_3^{-1} and CH_3COO^{-1}. The tests for anions are positive only after decomposing the complexes with conc. HNO_3, indicating their presence inside the coordination sphere. The low molar conductance values (10-17 $ohm^{-1}\,cm^2\,mol^{-1}$) of complexes determined in DMSO indicates their non-electrolyte nature [25]. All complexes give satisfactory elemental analyses results as shown in Table I.

IR spectra. It was noted that a pair of bands are present in the spectrum of 1,8-diaminonaphthalene at 3,350 & 3,390 cm^{-1} corresponding to $\nu(NH_2)$ group are absent in the infrared spectra of all the complexes. Further, no strong absorption band was observed near 1,715 cm^{-1} indicating the absence of > C═O group of glyoxal moiety. The disappearance of these bands and appearance of a new strong absorption band near 1,590-1,629 cm^{-1} confirms the condensation of carbonyl group of glyoxal 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 lower value of ν(C═N) 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 glyoxal and naphthalene moiety [31]. The various absorption bands in the region 1,400-1,588 cm^{-1} may be assigned due to ν(C═C) aromatic stretching vibrations of the naphthalene ring [32,33]. The bands in the region 740-785 cm^{-1} may be assigned to ν(C—H) out of plane

Table I. Analytical data of divalent cobalt, nickel, copper, zinc and cadmium complexes derived from glyoxal and, 8-diaminonaphthalene.

Sr. No.	Complexes	Found (Calcd.) %				Colour	Mol. Wt.
		M	C	H	N		
(1)	$[Co(C_{24}H_{16}N_4)Cl_2]$	11.95(12.02)	58.50(58.77)	3.09(3.26)	11.41(11.42)	Black	(480)490
(2)	$[Co(C_{24}H_{16}N_4)(NO_3)_2]$	10.50(10.76)	52.90(53.03)	2.90(2.94)	14.99(15.46)	Black	(534)543
(3)	$[Co(C_{24}H_{16}N_4)(OAc)_2]$	10.85(10.98)	61.87(62.56)	3.87(4.09)	10.29(10.42)	Dark grey	(520)537
(4)	$[Ni(C_{24}H_{16}N_4)Cl_2]$	11.90(12.00)	58.64(58.89)	3.18(3.27)	11.40(11.45)	Black	(470)489
(5)	$[Ni(C_{24}H_{16}N_4)(NO_3)_2]$	10.70(10.82)	53.10(53.13)	2.95(2.95)	15.28(15.49)	Black	(537)542
(6)	$[Ni(C_{24}H_{16}N_4)(OAc)_2]$	10.80(10.94)	62.33(62.68)	3.93(4.10)	10.45(10.44)	Black	(528)536
(7)	$[Cu(C_{24}H_{16}N_4)Cl_2]$	10.73(10.86)	58.30(58.29)	3.16(3.23)	11.20(11.33)	Dark grey	(488)494
(8)	$[Cu(C_{24}H_{16}N_4)(NO_3)_2]$	11.56(11.62)	52.47(52.65)	3.06(2.92)	15.03(15.36)	Dark grey	(529)547
(9)	$[Cu(C_{24}H_{16}N_4)(OAc)_2]$	11.66(11.74)	61.98(62.10)	4.01(4.06)	10.27(10.35)	Black	(530)541
(10)	$[Zn(C_{24}H_{16}N_4)(OAc)_2]$	11.96(12.04)	61.49(61.87)	3.89(4.05)	9.90(10.31)	Brown	(526)543
(11)	$[Cd(C_{24}H_{16}N_4)(OAc)_2]$	18.98(19.05)	56.26(56.95)	3.65(3.73)	8.93(9.49)	Grey	(565)590

bending of aromatic ring [34,35]. The bands present in the region 1,000-1,300 cm^{-1} in all complexes are assigned due to ν(C—N) stretching frequency [31].

Far IR spectra. The far infrared spectra show bands in the region 420-450 cm^{-1} corresponding to ν(M—N) vibrations [36–38]. The presence of bands in all complexes in the region 420-450 cm^{-1} originate from (M—N) azomethine vibrational modes and identify coordination of azomethine nitrogen [39]. The bands present in the range 300-320 cm^{-1} may be assigned due to ν(M—Cl) vibration [36–38]. The bands present in the region 220-250 cm^{-1} in all nitrato complexes are assignable to ν(M—O) stretching vibration [36,37].

NMR spectra. The ^{1}H NMR spectrum of the zinc(II) complex shows peaks in the region 6.62-7.32 p.p.m. corresponding to aromatic ring protons [peak B—8H

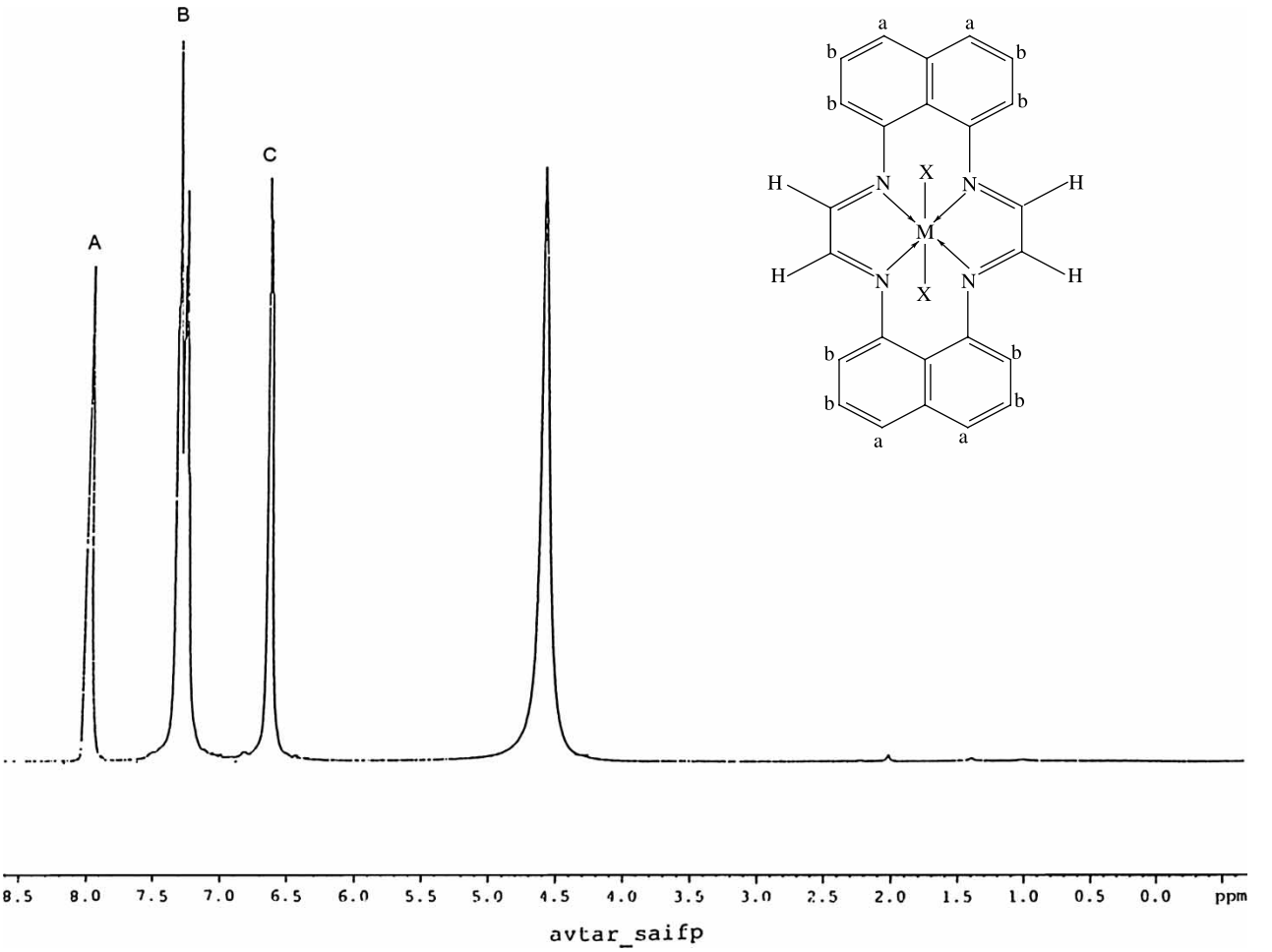

Figure 1. ^{1}H NMR spectrum of $[Zn(C_{24}H_{16}N_4)(OAc)_2]$ complex.

(b), peak C—4H (a)] Figure 1 [40]. The singlet at 7.98 p.p.m. (peak A) may be assigned due to the azomethine (**H**C═N) protons (4H) [41]

Mass spectra. The mass spectra of $[Co(C_{24}H_{16}N_4)(NO_3)_2]$ complex have been recorded and shown in Figure 2. The complex shows the peak at m/z = 544 corresponding to the molecular ion peak (M^+). This confirms the formation of the macrocyclic frame with molecular formula $[M(C_{24}H_{16}N_4)X_2]$. Another peaks at m/z = 353, 381, 413, 507 etc. may be corresponding to various fragments. The peak intensity gives an idea of the stability of the fragment.

Magnetic measurements and electronic spectra

Cobalt complexes. The magnetic moment of the cobalt complexes was measured at room temperature and lay in the range 4.90-4.99 B.M. which corresponds to three unpaired electrons. The electronic spectra of the cobalt(II) complexes recorded in DMSO exhibits three absorption peaks in the region 8,150-9,280 cm^{-1} (ν_1), 13,500-15,550 cm^{-1} (ν_2) and 18,500-20,500 cm^{-1} (ν_3) respectively. The spectra resemble to those complexes reported to be octahedral [42]. Thus, assuming the effective symmetry to be D_{4h}, the various bands may be assigned to the transitions: $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), (ν_1) 8,150-9,280 cm^{-1}; $^4T_{1g} \rightarrow {}^4A_{2g}$ (F), (ν_2) 13,500-15,550 cm^{-1}; $^4T_{1g} \rightarrow {}^4T_{1g}$ (P), (ν_3) 18,500-20,500 cm^{-1}; respectively. It appears that the symmetry of these complexes is not idealized O_h, but is D_{4h}. The assignment of the first spin-allowed band seems plausible since the first band appears approximately at half the energy of the visible band [43].

Nickel complexes. The magnetic moment of the nickel complexes at room temperature lay in the range 2.94-3.10 B.M. These values are in tune with a high spin configuration and show the presence of an octahedral environment around the Ni(II) ion in all complexes. The spectra of Ni(II) complexes recorded in DMSO solution exhibit a well discerned band with a shoulder on the low energy side. The other two bands generally observed in the region 16,500-17,200 cm^{-1} (ν_2), and 27,800-28,300 cm^{-1} (ν_3), are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2), and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) (ν_3), respectively. The first two bands result from the splitting of one band, ν_1 and are in the range ~ 9,650-10,200 and 11,850-12,350 cm^{-1}, which can be assigned to $^3B_{1g} \rightarrow {}^3E_g$ and $^3B_{1g} \rightarrow {}^3B_{2g}$, assuming the effective symmetry to be D_{4h} (component of $^3T_{2g}$ in O_h symmetry) [43]. The intense higher energy bands at 34,540 cm^{-1} may

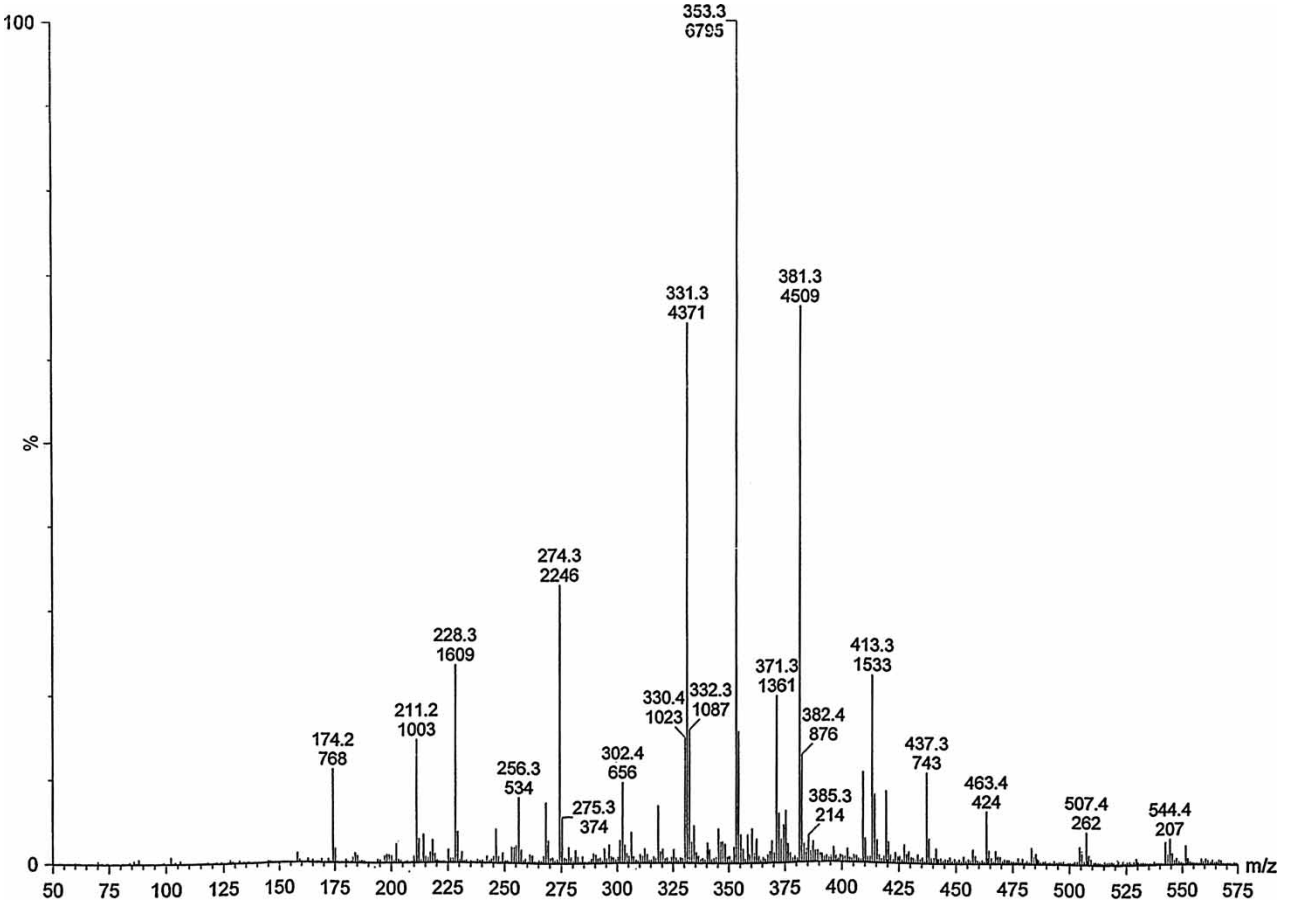

Figure 2. FAB mass spectra of $[Co(C_{24}H_{16}N_4)(NO_3)_2]$ complex.

be due to a π-π* transition of the (C=N) group. Various bands do not follow any regular pattern and seem to be anion independent. The spectra are consistent with distorted octahedral nature of these complexes.

Copper complexes. The magnetic moment of copper complexes lay in the range 1.76-1.80 B.M. The electronic spectra of the Cu(II) complexes exhibit bands in the region 17,750-19,500 cm^{-1}, with a shoulder on the low energy side at ~14,500-16,000 cm^{-1}, and show that these complexes are distorted octahedral [42,43]. Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence for these complexes may be: x^2-y^2 > z^2 > xy > xz > yz and the shoulder can be assigned to: $z^2 \rightarrow x^2$-y^2 ($^2B_{1g} \rightarrow {}^2B_{2g}$) and the broad band contains both the xy → x^2-y^2 ($^2B_{1g} \rightarrow {}^2E_g$) and xy, yz → x^2-y^2 ($^2B_{1g} \rightarrow {}^2A_{2g}$) transition [44]. The band separation of the spectra of the complexes is of the order 2,500 cm^{-1}, which is consistent with proposed geometry of the complexes [44]. Therefore it may be concluded that all the complexes formed by macrocycles with Cu(II) metals are distorted octahedral.

Proposed structure of complexes

Based on various studies like elemental analyses, conductance measurements, magnetic susceptibilities, infrared, NMR and electronic spectral studies the structure shown in Figure 3 may be proposed for all of the complexes.

Biological result and discussion

The newly synthesized compounds (**1-11**) were tested in the present investigation for their *in-vitro* antibacterial as well as antifungal activities. The antibacterial activities were studied against the two Gram-positive bacteria i.e. *B. subtilis* (MTCC 8509), *B. stearothermophilus* (MTCC 8508) and two Gram-negative bacteria i.e. *E. coli* (MTCC 51), *P. putida* (MTCC 121). The minimum inhibitory concentration (MIC) of all the complexes against Gram-positive and Gram-negative bacteria were determined by the method of NCCLS [21] and given in the Table II. In the whole series, compound (**7**) showed highest MIC 1 μg/mL against *P. putida* and *E. coli* (Table II). Compound (**6**) possessed MIC of 2 μg/mL against *B. subtilis*, *P. putida* and *E. coli*. Compound (**3**) showed MIC of 2 μg/mL against the bacteria *P. putida* and MIC of 4 μg/mL against *B. subtilis* and *E. coli.* Compound (**5**) and (**6**) showed MIC of 4 μg/mL against *B. subtilis* and *B. stearothermophilus,* respectively. Further the antibacterial activities of these complexes were compared with standard antibiotics *viz.* Chloramphenicol and Streptomycin (Figure 4). In general all the synthesized compounds showed the antibacterial activities, but they were found more potent inhibitors against Gram-negative bacteria as compared to the Gram-positive ones (Table II). Some compounds were found to be more potent than the standard antibiotics against some species of bacteria. Compounds (**3**), (**6**) and (**7**) were found to be more potent than the commercial antibiotics by showing MIC of (1-2 μg/mL) (Table II, Figure 4).

The antifungal activities of complexes were carried out against two fungal strains i.e. *A. flavus* and *A. niger* and then compared with standard drug Cyclohexamide (Table III, Figure 5). The antifungal activities (percentage inhibition) are given in Table III. In the whole series, compound (**4**) showed the highest percentage inhibition, whereas none of the tested compounds restrict the fungal growth excellently (Table III). However, the tested compounds (**1**), (**5**), (**7**), (**8**) and (**9**) showed a moderate capability to check the growth of these fungal species (Figure 5).

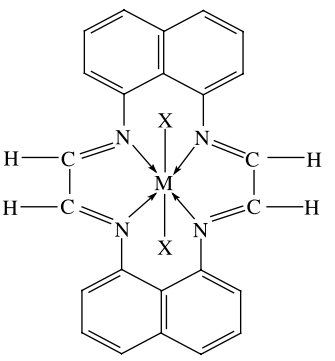

Figure 3. Structure of the complexes. Where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II); X = Cl^{-1}, NO_3^{-1}, CH_3COO^{-1}.

Table II. Minimum inhibitory concentration (MIC) of the complexes against test bacteria by using agar dilution assay.

		MIC (μg/mL)*			
Sr. No.	Compounds	Bs	Bst	Pp	Ec
(1)	$[Co(C_{24}H_{16}N_4)Cl_2]$	>64	32	16	16
(2)	$[Co(C_{24}H_{16}N_4)(NO_3)_2]$	>64	>64	32	64
(3)	$[Co(C_{24}H_{16}N_4)(OAc)_2]$	04	08	02	04
(4)	$[Ni(C_{24}H_{16}N_4)Cl_2]$	16	32	>64	>64
(5)	$[Ni(C_{24}H_{16}N_4)(NO_3)_2]$	04	08	16	08
(6)	$[Ni(C_{24}H_{16}N_4)(OAc)_2]$	02	04	02	02
(7)	$[Cu(C_{24}H_{16}N_4)Cl_2]$	16	08	01	01
(8)	$[Cu(C_{24}H_{16}N_4)(NO_3)_2]$	>64	>64	64	32
(9)	$[Cu(C_{24}H_{16}N_4)(OAc)_2]$	32	16	08	08
(10)	$[Zn(C_{24}H_{16}N_4)(OAc)_2]$	>64	32	16	32
(11)	$[Cd(C_{24}H_{16}N_4)(OAc)_2]$	>64	>64	32	64
	Chloramphenicol	02	02	04	02
	Streptomycin	02	02	04	04

*Mean of three replicates. Bs—*Bacillus subtilis* (MTCC 8509); Bst—*Bacillus stearothermophilus* (MTCC 8508); Pp—*Pseudomonas putida* (MTCC 121); Ec—*Escherichia coli* (MTCC 51). Chloramphenicol, Streptomycin—standard antibiotics.

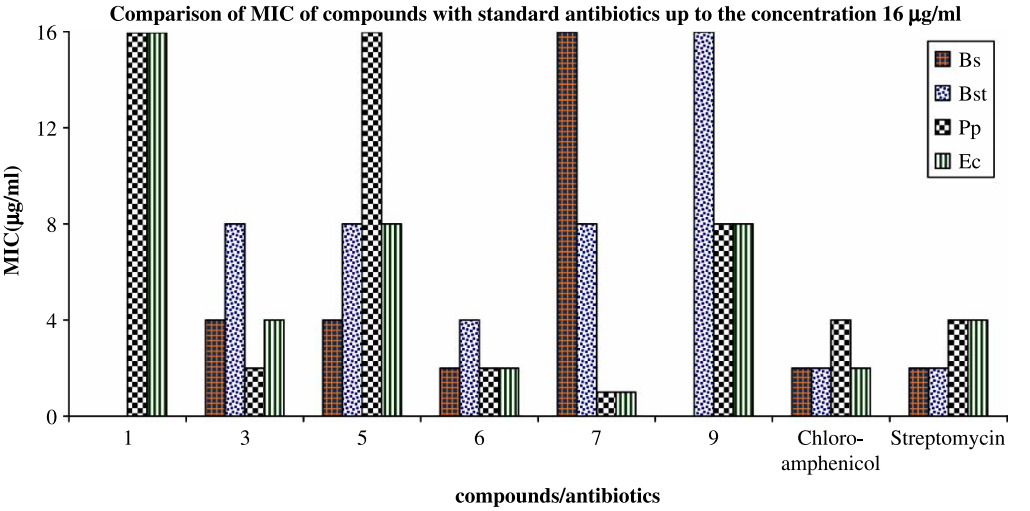

Figure 4. Comparison of MIC of compounds with standard antibiotics up to the concentration 16 µg/mL. Bs—*Bacillus subtilis* (MTCC 8509); Bst—*Bacillus stearothermophilus* (MTCC 8508); Pp—*Pseudomonas putida* (MTCC 121); Ec—*Escherichia coli* (MTCC 51); Chloramphenicol, Streptomycin—standard antibiotics.

Table III. Antifungal (percentage inhibition) activities of the complexes against the fungal strains. (for concentration—100 µg/mL).

Sr. No.	Compounds	Percentage inhibition AF	AN
(1)	$[Co(C_{24}H_{16}N_4)Cl_2]$	33.82	21.37
(2)	$[Co(C_{24}H_{16}N_4)(NO_3)_2]$	40.29	43.18
(3)	$[Co(C_{24}H_{16}N_4)(Oac)_2]$	33.69	29.07
(4)	$[Ni(C_{24}H_{16}N_4)\ Cl_2]$	42.68	49.31
(5)	$[Ni(C_{24}H_{16}N_4)(NO_3)_2]$	20.52	36.49
(6)	$[Ni(C_{24}H_{16}N_4)(OAc)_2]$	20.66	19.42
(7)	$[Cu(C_{24}H_{16}N_4)Cl_2]$	31.60	34.19
(8)	$[Cu(C_{24}H_{16}N_4)(NO_3)_2]$	39.41	38.90
(9)	$[Cu(C_{24}H_{16}N_4)(Oac)_2]$	33.81	32.20
(10)	$[Zn(C_{24}H_{16}N_4)(Oac)_2]$	25.77	24.62
(11)	$[Cd(C_{24}H_{16}N_4)(Oac)_2]$	15.33	18.71
	Cyclohexamide	87.34	89.91

AF—*Aspergillus flavus*; AN—*Aspergillus niger*; Cyclohexamide—standard drug.

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 [45,46]. 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 [47]. It also has been observed that some moieties such as azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive

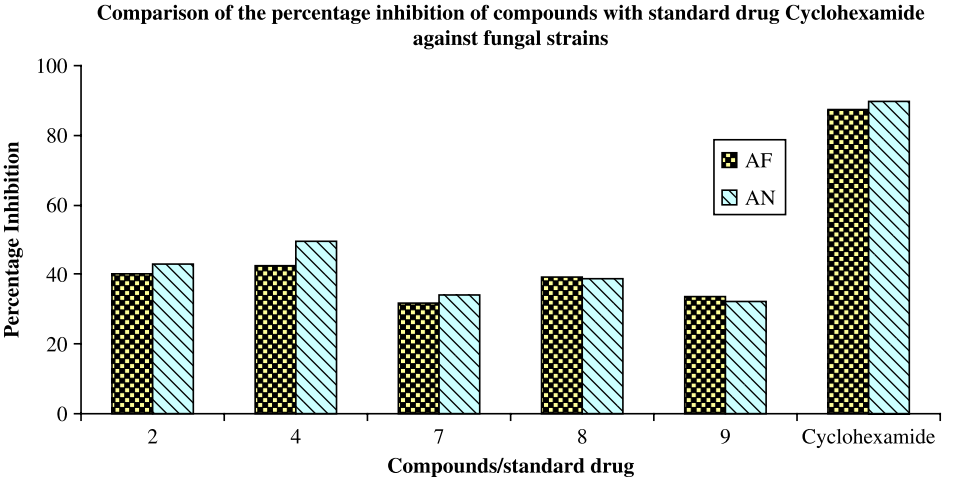

Figure 5. Comparison of the percentage inhibition of compounds with standard drug Cyclohexamide against fungal strains. AF—*Aspergillus flavus*; AN—*Aspergillus niger*; Cyclohexamide—standard drug.

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 [48].

Acknowledgements

D.P. Singh thanks the University Grants Commission, New Delhi for financial support in the form of Major Research Project [MRP-F.No. 32-196/2006(SR)] and Krishan Kumar for the award of Project Fellowship under the above project. Thanks are also due to authorities of N.I.T., Kurukshetra for providing necessary research facilities.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Chandra S, Gautum A, Tyagi M. Synthesis and spectroscopic characterization of transition metal complexes of a 12-membered tetraaza [N_4] macrocyclic ligand and their biological activity. Transition Met Chem 2007;32:1079–1084.

[2] Bozic LT, Marotta E, Traldi P. Efficient solid-state microwave-promoted complexation of a mixed dioxa-diaza macrocycle of a sodium ethyl 4-benzeneazophosphonate complex. Polyhedron 2007;26:1663–1668.

[3] Lindoy LF. The chemistry of macrocyclic ligand complexes. Cambridge: Cambridge University Press; 1989.

[4] Curtis NF. Macrocyclic coordination compounds formed by condensation of metal-amine complexes with aliphatic carbonyl compounds. Coord Chem Rev 1968;3:3–47; Niasari MS, Bazarganipour M, Ganjali MR, Norouzi P. Bis (macrocyclic) dinickel(II) complexes containing phenylene bridges between 13-membered triaza dioxa macrocyclic ligands: *In-situ* one pot template synthesis, characterization and catalytic oxidation of cyclohexene. Transition Met Chem 2007;32:9–15.

[5] Niasari MS, Davar F. *In situ* one-pot template synthesis (IOPTS) and characterization of copper(II) complexes of 14-membered hexaaza macrocyclic ligand 3,10-dialkyl-dibenzo-1,3,5,8,10,12-hexaazacyclotetradecane. Inorg Chem Commun 2006;9:175–179.

[6] Kumar DS, Alexander V. Synthesis of lanthanide(III) complexes of chloro and bromo substituted 18-membered tetraaza macrocycles. Polyhedron 1999;18:1561–1568.

[7] Ilhan S, Temel H, Ziyadanogullari R, Sekerci M. Synthesis and spectral characterization of macrocyclic Schiff base by reaction of 2,6-diaminipyridine and 1,4-bis(2-carboxyaldehydephenoxy) butane and its Cu(II), Ni(II), Pb(II), Co(III), La(III) complexes. Transition Met Chem 2007;32:584–590.

[8] Shakir M, Khatoon S, Parveen S, Azim Y. Synthesis and spectral studies of a 12-membered tetraimine macrocyclic ligand and its complexes. Transition Met Chem 2007; 32:42–46.

[9] Muller JG, Chen X, Dadiz AC, Rokita SE, Burrows CJ. Macrocyclic nickel complexes in DNA recognition and oxidation. Pure Appl Chem 1993;65:545–550.

[10] Liu J, Lu TB, Deng H, Ji LN, Qu LH, Zhou H. Synthesis, DNA-binding and cleavage studies of macrocyclic Cu(II) complexes. Transition Met Chem 2003;28:116–121.

[11] Akilan P, Thirumavalavan M, Kandaswamy M. Synthesis of mono and binuclear copper(II) complexes using new macrocyclic tricompartmental unsymmetrical ligands: Magnetic, electrochemical and catalytic studies. Polyhedron 2003;22:3483–3492.

[12] Singh DP, Kumar R, Malik V, Tyagi P. Template synthesis, spectroscopic studies and biological activities of macrocyclic complexes derived from thiocarbohydrazide and glyoxal. J Enz Inhib Med Chem 2007;22:177–182.

[13] Kosmos C, Snook D, Gooden CS, Courtenay Luck NS, Mc Calla MJ, Meares CF, Epenetos AA. Development of humoral immune responses against a macrocyclic chelating agent (DOTA) in cancer patients receiving radioimmunoconjugates for imaging and therapy. Cancer Res 1992;52:904–911.

[14] Seto J, Tamura S, Asai N, Kishii N, Kijima Y, Matsuzawa N. Macrocyclic functional dyes: Applications to optical disk media, photochemical hole burning and non-linear optics. Pure Appl Chem 1996;68:1429–1434.

[15] Dong W, Yang R, Yan L. Rare earth complexes of a new Schiff base macrocyclic ligand derived from 2,2′-(ethylenedioxy) bis-benzaldehyde and 2,2′-(ethylenedioxy) bis-benzylamine. Indian J Chem 2001;40A:202–206.

[16] Chandra S, Gupta R, Gupta N, Bawa SS. Biologically relevant macrocyclic complexes of copper spectral, magnetic, thermal and antibacterial approach. Transition Met Chem 2006;31:147–151.

[17] Chandra S, Gupta LK, Agrawal S. Synthesis spectroscopic and biological approach in the characterization of novel [N_4] macrocyclic ligand and its transition metal complexes. Transition Met Chem 2007;32:558–563.

[18] Vogel AI. A text book of quantitative chemical analysis. 5th ed. London: Longman; 1989.

[19] Vogel AI. Practical organic chemistry including quantitative organic analysis. 6th ed. London: Longman; 2006.

[20] Collee JG, Miles RS, Watt B. Laboratory control of antimicrobial therapy. Practical medical microbiology. 14th ed. New York: Churchill Livingstone; 1996.

[21] NCCLS. Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically, approved standards. 5th ed. Villanova, PA: National Committee for Clinical Laboratory Standards; 2000.

[22] Greenwood D, Slack R, Peutherer J. Medical microbiology: A guide to microbial infections: Pathogenesis, immunity, laboratory diagnosis and control. 15th ed. Edinburgh: Churchill Livingstone; 1997.

[23] McFarland J. The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc 1907;49: 1176–1178.

[24] Koshy B, Shivaprakash MK, Kulkarni V. Effect of antibacterial and antifungal agents on mega sporocarp germination in Azolla filiculoides. J Ecobiol 2001;13:119–122.

[25] Geary WJ. The use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord Chem Rev 1971;7:81–122.

[26] Singh DP, Kumar R, Tyagi P. Template synthesis, spectroscopic studies and biological screening of macrocyclic complexes derived from thiocarbohydrazide and benzyl. Transition Met Chem 2006;31:970–973; Athappan PR, Rajagopal G. Synthesis, spectroscopic and redox behavior of copper(II), nickel(II) and cobalt(II) complexes of some macrocyclic multidentates. Polyhedron 1996;15:527–534; Sakata K, Hashimoto M, Hamada T, Matsuno S. Synthesis and characterization for five 7,16-dibenzylated tetraaza [14] annulene nickel(II) complexes. Polyhedron 1996;15:967–972.

[27] Siddiqi ZA, Khan M, Khalid M, Kumar S. Spectral and electrochemical characterization of bimetallic complexes of a

novel 32-membered unsymmetrical [N_{12}] macrocycle. Transition Met Chem 2007;32:927–935.

[28] Singh DP, Shishodia N, Yadav BP, Rana VB. Synthesis and characterization of bivalent metal complexes of a tetradentate [N_6] macrocyclic ligand. Polyhedron 1997;16:2229–2232.

[29] Chandra S, Sharma SD. Chromium(III), manganese(II), cobalt(II), nickel(II), copper(II) and palladium(II) complexes of a 12-membered tetraaza [N_4] macrocyclic ligand. Transition Met Chem 2002;27:732–735.

[30] Lodeiro C, Basitida R, Bertolo E, Macias A, Rodriguez R. Synthesis and characterization of four novel N_xO_y- Schiff base macrocyclic ligands and their metal complexes. Transition Met Chem 2003;28:388–394.

[31] Singh DP, Kumar R, Malik V, Tyagi P. Synthesis and characterization of complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) with macrocycle 3,4,11,12-tetraoxo-1,2,5,6,9,10,13,14-octaazacyclohexadeca-6,8,14,16-tetraene and their biological screening. Transition Met Chem 2007;32:1051–1055.

[32] Prasad RN, Mathur M, Upadhayay A. Synthesis and spectroscopic studies of Cr(III), Fe(III) and Co(III) complexes of hexaazamacrocycles. J Indian Chem Soc 2007;84:1202–1204.

[33] Costamagna J, Ferraudi G, Villagran M, Wolcan E. Ligand luminescence and photoinduced charge separation in bis(naphthalene) substituted fourteen-membered tetraaza-macrocyclic complexes of Cu(II) and NI(II). J Chem Soc Dalton Trans 2000;2631–2637.

[34] Chandra S, Gupta LK. Designing and synthesis of macrocyclic Schiff base ligand: Study of interaction with Mn(II), Co(II), Ni(II) and Cu(II) and biological screening. J Indian Chem Soc 2005;82:454–458.

[35] Singh DP, Shishodia N, Yadav BP, Rana VB. Trivalent transition metal ion directed macrocycles. J Indian Chem Soc 2004;81:287–290.

[36] Shakir M, Nasman OSM, Varkey SP. Binuclear [N_6] 22-membered macrocyclic transition metal complexes: Synthesis and characterization. Polyhedron 1996;15:309–314.

[37] Shakir M, Islam KS, Mohamed AK, Shagufa M, Hasan SS. Macrocyclic complexes of transition metals with divalent polyaza units. Transition Met Chem 1999;24:577–580.

[38] Chandra S, Kumar R. Synthesis and spectral studies on mononuclear complexes of chromium(III) and manganese(II) with 12-membered tetradentate N_2O_2, N_2S_2 and N_4 donor macrocyclic ligand. Transition Met Chem 2004;29:269–275.

[39] Rana VB, Singh DP, Singh P, Teotia MP. Trivalent chromium, manganese, iron and cobalt chelates of a tetradentate [N_6] macrocyclic ligand. Transition Met Chem 1982;7:174–177.

[40] Jiang H, Sun H, Zhang S, Hua R, Xu Y, Jin S, Gong H, Li L. NMR investigations of inclusion complexes between β-cyclodextrin and naphthalene/anthraquinone derivatives. J Incl Phenom 2007;58:133–138.

[41] Boghaei DM, Mohebi S. Synthesis, characterization and study of vanadyl tetradentate Shiff base complexes as catalyst in aerobic selective oxidation of olefins. J Mol Catal A: Chem 2002;179:41–51.

[42] Rana VB, Singh DP, Singh P, Teotia MP. Divalent nickel, cobalt and copper complexes of a tetradentate [N_6] macrocyclic ligand. Transition Met Chem 1981;6:36; Divalent nickel, cobalt and copper complexes of tetradentate macrocycle, dibenzo (f, n) 2,4,10,12-tetramethyl-1,5,9,13-tetraazacyclohexadeca [16] 1,3,9,11-tetraene. Polyhedron 1982;1:377–381.

[43] Lever ABP. Inorganic electronic spectroscopy. Amsterdam: Elsevier; 1968.

[44] Lever ABP, Mantovani E. The far infrared and electronic spectra of some bis(ethylenediamine) and related complexes of copper(II) and the relevance of these data to tetragonal distortion and bond strengths. Inorg Chem 1971;10:817–826.

[45] Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT. Isatin-derived antibacterial and antifungal compounds and their transition metal complexes. J Enz Inhib Med Chem 2004;19:417–423.

[46] Chohan ZH, Supuran CT, Scozzafava A. Metal binding and antibacterial activity of ciprofloxacin complexes. J Enz Inhib Med Chem 2005;20:303–307.

[47] Chohan ZH, Scozzafava A, Supuran CT. Unsymmetrical 1,1′-disubstituted ferrocenes: Synthesis of Co(II), Cu(II), Ni(II) and Zn(II) chelates of ferrocenyl-1-thiadiazolo-1′-tetrazole,-1-thidiazolo-1′-triazole and -1-tetrazolo-1′-triazole with antimicrobial properties. J Enz Inhib Med Chem 2002;17:261–266.

[48] Chohan ZH, Hassan MU, Khan KM, Supuran CT. *In-vitro* antibacterial, antifungal and cytotoxic properties of sulfonamide-derived Schiff's base and their metal complexes. J Enz Inhib Med Chem 2005;20:183–188.