Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 75 (10) 1369–1380 (2010)
JSCS–4059
UDC 546.732'742'562'472'482.004.12:
615.281/.282:66.097.8–914.7
Original scientific paper

Synthesis, characterization and antibacterial and antifungal studies of some tetraazamacrocyclic complexes

DHARAM PAL SINGH[1]*, VANDNA MALIK[1], RAMESH KUMAR[1], KRISHAN KUMAR[1] and SAURABH SUDHA DHIMAN[2]

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

(Received 29 January, revised 28 April 2010)

Abstract: A new series of complexes was synthesized by template condensation of malonyl dihydrazide and glyoxal in methanolic medium in the presence of divalent cobalt, nickel, copper, zinc and cadmium salts, whereby complexes of the type: $[M(C_5H_6N_4O_2)X_2]$ where M = Co(II), Ni(II), Cu(II), Zn(II) and Cd(II), and X = Cl^-, NO_3^- and OAc^-, were formed. The complexes were characterized with the aid of elemental analyses, conductance measurements, magnetic susceptibility measurements, and electronic, NMR and infrared spectral studies. Based on these studies, a six coordinate octahedral geometry is proposed for these complexes. The complexes were tested for their *in vitro* antibacterial and antifungal activities. The minimum inhibitory concentration shown by complexes was compared with that of standard drugs.

Keywords: antibacterial; antifungal; macrocyclic complexes; minimum inhibitory concentration.

INTRODUCTION

During the past few decades, a great deal of interest has been devoted to macrocyclic complexes containing oxygen and nitrogen atoms. Macrocyclic complexes are of great interest due to their resemblance to naturally occurring macrocycles and analytical, industrial and medical applications.[1–6] Macrocyclic metal complexes of lanthanides, *e.g.* Gd(III), are used as MRI contrast agents.[7] Macrocyclic metal chelating agents (DOTA) are useful for detecting tumour lesions.[8] The chemistry of macrocyclic complexes is also important due to their use as dyes and pigments,[9] as well as NMR shift reagents.[10] Additionally, some macrocyclic complexes have been found to exhibit potential antibacterial activities.[11] Prompted by these applications, in the present paper, the syntheses of macrocyc-

* Corresponding author. E-mail: dpsinghchem@yahoo.co.in
doi: 10.2298/JSC100129110S

lic complexes of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) obtained by template condensation reaction of malonyl dihydrazide and glyoxal are reported. The complexes were characterized with the aid of IR, NMR and electronic spectral studies, and magnetic susceptibilities, elemental analysis and molar conductance measurements. These complexes were also tested for their *in vitro* antibacterial and antifungal activities.

EXPERIMENTAL

Isolation of the complexes

The complexes were synthesized by the template method, *i.e.*, by condensation of malonyl dihydrazide and glyoxal in the presence of a divalent metal salt. To a hot stirred methanolic solution (≈50 mL) of malonyl dihydrazide (10 mmol) was added a divalent cobalt, nickel, copper, zinc or cadmium salt (Cl^-, NO_3^-, CH_3COO^-) (10 mmol) dissolved in the minimum quantity of methanol (≈20 mL). The resulting solution was boiled under reflux for 0.5 h. Subsequently, glyoxal (10 mmol) was added to the refluxing mixture and refluxing was continued for 8–10 h. The mixture was concentrated to half its volume and kept in a desiccator overnight. The complexes were then filtered, washed with methanol, acetone and ether and dried *in vacuo*; yield ≈50–60 %. The complexes were soluble in DMF and DMSO, but insoluble in other common organic solvents and water. They were found to be thermally stable up to ≈225–260 °C, after which they decomposed.

The template condensation of malonyl dihydrazide and glyoxal in the presence of divalent cobalt, nickel, copper, zinc and cadmium salts may be represented by the following scheme:

$$C_3H_8N_4O_2 + C_2H_2O_2 + MX_2 \xrightarrow[8\text{-}10\ h]{MeOH} [M(C_5H_6N_4O_2)X_2] + 2H_2O$$

where M = Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) and X = Cl^-, NO_3^- or CH_3COO^-.

Analytical and physical measurements

The microanalyses for C, H, and N were realized at SAIF, CDRI, Lucknow. The metal contents were determined by standard EDTA methods. The electronic spectra (DMF) were recorded on a Cary 14 spectrophotometer. The magnetic susceptibility measurements were performed at SAIF, IIT Roorkee. The IR spectra were recorded on an FT-IR spectrophotometer (Perkin Elmer) in the range 4000–200 cm^{-1} using the Nujol Mull method at SAIF, Punjab University, Chandigarh, India. The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz). The conductivity was measured on a digital conductivity meter (HPG System, G-3001).

In-vitro *antibacterial activity*

Primary screening. The antibacterial activities of the newly synthesized complexes were evaluated by the Agar Well Diffusion Assay Technique against two Gram-positive bacteria, *i.e.*, *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508) and two Gram-negative bacteria, *i.e.*, *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC 121). The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on 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 activity of the synthesized complexes, a suspension of each test microorganism was prepared. The 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^{-1}. A volume of 20 ml of agar media was poured into each Petri plate and the plates were swabbed with broth cultures of the respective micro-organisms and kept for 15 min for adsorption to occur. Using a punch, ≈8 mm diameter wells were bored 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 the control for all the test complexes. After holding the plates at room temperature for 2 h to allow diffusion of the compounds into the agar, the plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone. The entire tests were performed in triplicate and the mean of the diameter of inhibition was calculated. The antimicrobial activities of the complexes were compared against standard drugs.

*Minimum Inhibitory Concentration (*MIC*).* Nutrient broth was adjusted to pH 7.0 for the determination of the *MIC* of synthesized complexes.[12] The *MIC* is the lowest concentration of an antimicrobial agent that prevents the development of visible growth of microorganism after overnight incubation. The inoculum of the test microorganisms were prepared using 16 h-old cultures adjusted by reference to the 0.5 McFarland standards (10^8 cells ml^{-1}).[13] These cultures were further diluted up to 10-fold with nutrient broth to obtain an inoculum size of 1.2×10^7 CFU ml^{-1}. A positive control (containing inoculum but no complex) and a negative control (containing complex but no inoculum) were also prepared. A stock solution of 4 mg ml^{-1} of each compound was prepared in DMSO and further appropriately diluted to obtain final concentrations ranging from 250 to 0.03 µg ml^{-1}.[14] The requisite quantity of antifungal drug (cyclohexamide) was added to the broth to obtain its desirable final concentration of 100 µg ml^{-1}. Separate flasks were taken for each test dilution. To each flask was added 100 µl of inoculum. Then the 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 cultures were spotted in a predefined pattern by aseptically transferring 5 µl of each bacterial culture onto the surface of solidified agar-agar plates and incubated at 37 °C for 24 h for determining the *MIC* value.

In-vitro *antifungal activity*

Potato dextrose medium (PDA) was prepared in a flask and sterilized. To check the growth of bacterial culture in the medium, the requisite quantity of the standard antibiotic (ampicillin) was added to obtain the desired final concentration of 100 µg ml^{-1} of the medium. Test samples were prepared in different concentrations (10, 50 and 100 µg/ml*)* in dimethyl sulphoxide and 200 µl of each sample was spread on the PDA media contained in sterilized Petri plates. Mycelial discs taken from the standard cultures of fungi (*Aspergillus flavus* and *A. niger*) were grown on the PDA medium for 5–7 days. These cultures were used for the aseptic inoculation in the sterilized Petri dish. Standard cultures inoculated at 28±1 °C were also used as the control. The efficacy of each sample was determined by measuring the 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. The data are expressed as percent inhibition over the control obtained from the size of colonies. The percent inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100(C–T)/C$$

where C is the diameter of fungus colony in the control plate after incubation for 96 h and T is the diameter of the fungus colony in the tested plate after the same incubation period.

RESULTS AND DISCUSSION

The analytical data show the formula for macrocyclic complexes as: $[M(C_5H_6N_4O_2)X_2]$; where M = Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) and X = = Cl^-, NO_3^- or CH_3COO^-. The tests for the anions were positive only after decomposition of the complexes, indicating their presence inside the coordination sphere. All macrocyclic complexes were dark-coloured solids, which were soluble in DMF and DMSO. The measurements of molar conductance (conductance ≈10–20 S cm^2 mol^{-1}) in DMSO showed that these chelates are non-electrolytes.[15] All complexes gave satisfactory elemental analyses results, as shown in Table I.

TABLE I. Analytical data of the divalent Co, Ni, Cu, Zn and Cd complexes derived from malonyl dihydrazide and glyoxal

No.	Complex	Found (Calcd.), %				Colour	MW g mol^{-1}
		M	C	H	N		
1	$[Co(C_5H_6N_4O_2)Cl_2]$	20.56 (20.77)	21.09 (21.12)	2.02 (2.11)	19.63 (19.71)	Light brown	284
2	$[Co((C_5H_6N_4O_2)(NO_3)_2]$	17.42 (17.50)	17.71 (17.80)	1.65 (1.78)	24.76 (24.92)	Light brown	337
3	$[Co(C_5H_6N_4O_2)(OAc)_2]$	17.65 (17.82)	36.51 (36.62)	3.52 (3.62)	16.87 (16.91)	Shiny black	331
4	$[Ni(C_5H_6N_4O_2)Cl_2]$	20.43 (20.49)	21.17 (21.20)	2.06 (2.12)	19.53 (19.78)	Brown	283
5	$[Ni(C_5H_6N_4O_2)(NO_3)_2]$	17.23 (17.26)	17.88 (17.85)	1.76 (1.78)	24.89 (25.00)	Dark brown	336
6	$[Ni(C_5H_6N_4O_2)(OAc)_2]$	17.51 (17.56)	32.73 (32.72)	3.60 (3.63)	16.91 (16.96)	Shiny black	330
7	$[Cu(C_5H_6N_4O_2)Cl_2]$	21.89 (21.87)	20.81 (20.83)	2.03 (2.08)	19.46 (19.44)	Brown	288
8	$[Cu(C_5H_6N_4O_2)(NO_3)_2]$	18.41 (18.47)	17.51 (17.59)	1.72 (1.75)	24.69 (24.63)	Dark brown	341
9	$[Cu(C_5H_6N_4O_2)(OAc)_2]$	18.85 (18.80)	32.21 (32.23)	3.60 (3.58)	16.75 (16.71)	Greyish black	335
10	$[Zn(C_5H_6N_4O_2)(OAc)_2]$	19.26 (19.28)	32.02 (32.04)	3.55 (3.56)	16.64 (16.61)	Yellowish orange	337
11	$[Cd(C_5H_6N_4O_2)(OAc)_2]$	28.45 (29.16)	30.09 (28.12)	3.23 (3.12)	13.98 (14.58)	Reddish brown	384

IR Spectra

In the IR spectrum of malonyl dihydrazide, a pair of bands corresponding to ν(NH_2) was present at ≈3210 and ≈3270 cm^{-1}, but absent in the IR spectra of all the complexes.[16] However, a single broad medium band at 3360–3450 cm^{-1} was observed in the spectra of all the complexes, which may be assigned to ν(NH) stretching vibrations.[17,18] A strong peak at ≈1660 cm^{-1} in the IR spectrum of

malonyl dihydrazide is assigned to the >C=O group of the CONH moiety. This peak is shifted to lower frequencies (≈1620–1640 cm^{-1}) in the spectra of all the complexes,[19,20] suggesting the coordination of the oxygen of the carbonyl group with the metal. Furthermore, no strong absorption band was observed near 1700 cm^{-1} in the IR spectra of the complexes but was observed in the spectrum of glyoxal. This indicates the absence of >C=O groups of the glyoxal moiety in the complexes. These facts confirm the condensation of carbonyl groups of glyoxal and the amino groups of malonyl dihydrazide.[21,22] The IR spectra of the complexes showed a new strong absorption band in the region ≈1595–1610 cm^{-1}, which may be attributed to the ν(C=N) group.[23,24] These results provide strong evidence for the formation of the macrocyclic frame.[25] The lower value of ν(C=N) indicates coordination of the nitrogen of azomethine to the metal.[26] The bands present at ≈1300–1000 cm^{-1} are assigned to ν(C–N) vibration. The bands presents at ≈3040 cm^{-1} may be assigned to ν(C–H) vibrations of the glyoxal moiety. The IR spectra of the nitrate complexes display three (N–O) stretching bands at ≈1410–1455 cm^{-1} (ν_5), ≈1305–1315cm^{-1} (ν_1) and ≈1015–1030 cm^{-1} (ν_2). The separation of the two highest frequency bands ($\nu_5 - \nu_1$) suggest that both the nitrate groups are coordinated in a unidentate manner.[27] The acetate complexes showed two bands at ≈1630–1640 cm^{-1} (ν_1) and ≈1380–1390 cm^{-1} (ν_2). These indicate that the acetate group is coordinated in a unidentate manner.[28]

The far IR spectra show bands in the region ≈420–450 cm^{-1}. corresponding to ν(M–N) vibrations in all the complexes.[29–31] The presence of a band in all the complexes in the ≈420–450 cm^{-1} region originate from (M–N) azomethine vibration modes and support the coordination of azomethine nitrogen with the metal.[32] The bands present at ≈310–315 cm^{-1} in the chloride complexes are due to ν(M–Cl).[29,31] and bands present at ≈210–240 cm^{-1} in all the nitrate complexes to ν(M–O).[29]

NMR Spectra

The ^{1}H-NMR spectrum of the zinc complex showed a broad singlet at 8.6 ppm due to protons of the –CONH moiety.[17,33] The singlet at 2.34 ppm may be due to $-CH_2$ protons.[19] The singlet in the region of 7.8 ppm may be assigned to protons of the glyoxal moiety.[34]

Magnetic measurements and electronic spectra

Cobalt complexes. The magnetic moments of the cobalt complexes were measured at room temperature and lie in the range 4.85–4.90 μ_B, which corresponds to 3 unpaired electrons. The solution spectra of the cobalt(II) complexes exhibited absorption in the region *ca*. 8100–9160 (ν_1), 12500–15700 (ν_2) and 18600–20500 cm^{-1} (ν_3). The spectra resemble those of complexes reported to be octahedral.[35] Thus, the various bands can be assigned to: $^4T_{1g} \rightarrow {}^4T_{2g}(F)$ (ν_1);

$^4T_{1g} \rightarrow {}^4A_{2g}(F)$ (ν_2) and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ (ν_3) transitions, respectively. It appears that the symmetry of these complexes is not idealized O_h but 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.[36] Various ligand field parameters, *Dq*, *B*, β and β% were calculated for the complexes and are listed in Table II (malonyl dihydrazide and glyoxal). *B* for a free cobalt(II) ion is 971 cm^{-1}. The values of β lie in the range 0.606–0.629. These values indicate the presence of covalent character in the metal–ligand "σ"' bond. The value of the ν_2/ν_1 ratio lies between 1.76–1.79, which identify the complexes as possessing a distorted octahedral structure.[37]

TABLE II. Ligand field parameters of the divalent cobalt and nickel complexes derived from malonyl dihydrazide and glyoxal

No.	Complexes	*Dq* cm^{-1}	*B* cm^{-1}	β	β%	ν_2/ν_1	μ_{eff} μ_B
1	$[Co(C_5H_6N_4O_2)Cl_2]$	1016	588	0.605	39.1	1.78	4.90
2	$[Co(C_5H_6N_4O_2)(NO_3)_2]$	1066	590	0.607	39.3	1.77	4.89
3	$[Co(C_5H_6N_4O_2)(OAc)_2]$	1071	591	0.608	39.2	1.78	4.93
4	$[Ni(C_5H_6N_4O_2)Cl_2]$	1185	540	0.519	48.1	1.41	2.88
5	$[Ni(C_5H_6N_4O_2)(NO_3)_2]$	1190	538	0.517	48.5	1.41	2.87
6	$[Ni(C_5H_6N_4O_2)(OAc)_2]$	1195	536	0.515	48.3	1.40	2.89

Nickel complexes. The magnetic moment of the nickel complexes at room temperature lie in the range 2.91–2.95 μ_B showing an octahedral environment around the Ni(II) ion in all complexes. The solution spectra of the Ni(II) complexes exhibited a well-discernable band with a shoulder on the low energy side. The other two bands generally observed in the region at *ca.* 16570–17240 cm^{-1} (ν_2), and 26860–28000 cm^{-1} (ν_3) are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g} \rightarrow$ $\rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively. The first two bands result from the splitting of one band, ν_1, are in the range ≈9700–10000 and 11800–12440 cm^{-1}, which can be assigned to $^3B_{1g} \rightarrow {}^3E_g$ and $^3B_{1g} \rightarrow {}^3B_{2g}$ transitions, respectively, assuming the effective symmetry to be D_{4h} (component of $^3T_{2g}$ in O_h symmetry).[36] The intense higher energy band at *ca.* 34000 cm^{-1} may be due to a π–π* transition of the (C=N) group. The various bands do not follow any regular pattern and seem to be anion independent. The spectra are consistent with the distorted octahedral nature of these complexes. Various ligand field parameters, *Dq*, *B*, β and β% were calculated for the complexes and are listed in Table II (malonyl dihydrazide and glyoxal). *B* for a free nickel(II) ion is 1040 cm^{-1}. The values of β lie in the range 0.509–0.519. These values indicate the presence of covalent character in the metal–ligand "σ" bond. The value of the ν_2/ν_1 ratio lies between 1.37–1.41 and shows that the complexes possess a distorted octahedral structure.[37]

Copper complexes. The magnetic moment of the copper complexes lie in the range 1.77–1.82 μ_B. The electronic spectra of the copper complexes exhibit bands in the region *ca*. 17780–19000 cm^{-1} with a shoulder on the low energy side at ≈14600–16000 cm^{-1}, which show that these complexes have a distorted octahedral geometry.[35,36] 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 $xy \rightarrow x^2 - y^2$ ($^2B_{1g} \rightarrow {}^2E_g$) and $xz, yz \rightarrow x^2 - y^2$ ($^2B_{1g} \rightarrow \rightarrow {}^2A_{2g}$) transitions.[38] The band separation of the spectra of the complexes is of the order 2500 cm^{-1}, which is consistent with the proposed geometry of the complexes.[35] Therefore, it may be concluded that all the complexes formed by the macrocycles with Cu(II) metals are distorted octahedral.

Biological results and discussion

In this study, all the chemically synthesized complexes were evaluated against Gram-positive and Gram-negative bacteria. The *MIC* values of the synthetic complexes were determined by the method given by Andrews[39]. Standard antibiotics, namely streptomycin and chloramphenicol, were used for comparison with the antibacterial activities exhibited by these complexes. All the complexes of the tested series possessed some antibacterial activity against Gram-positive bacteria as well as Gram-negative bacteria (Table III). In the whole series, complexes **1** and **5** were found to be most effective against all the tested bacterial strains, showing zone of growth inhibition in the range from 46.2–49.2 mm

TABLE III. *In-vitro* antibacterial activity of the complexes determined by the agar well diffusion method for a concentration of 100 μg ml^{-1} (A – *Bacillus subtilis* (MTCC 8509), B – *Bacillus stearothermophilus* (MTCC 8508), C – *Escherichia coli* (MTCC 51), D – *Pseudomonas putida* (MTCC 121))

No.	Complex	Diameter of zone of growth inhibition[a], mm			
		A	B	C	D
1	$[Co(C_5H_6N_4O_2)Cl_2]$	48.2	46.2	49.2	47.3
2	$[Co((C_5H_6N_4O_2)(NO_3)_2]$	13.3	16.2	29.6	28.1
3	$[Co(C_5H_6N_4O_2)(OAc)_2]$	11.3	15.6	9.9	11.5
4	$[Ni(C_5H_6N_4O_2)Cl_2]$	10.3	19.2	16.5	21.4
5	$[Ni(C_5H_6N_4O_2)(NO_3)_2]$	36.3	37.1	38.2	36.2
6	$[Ni(C_5H_6N_4O_2)(OAc)_2]$	10.4	12.3	21.5	19.2
7	$[Cu(C_5H_6N_4O_2)Cl_2]$	13.7	24.8	26.4	23.2
8	$[Cu(C_5H_6N_4O_2)(NO_3)_2]$	15.4	13.5	14.8	21.4
9	$[Cu(C_5H_6N_4O_2)(OAc)_2]$	21.2	27.3	23.4	21.7
10	$[Zn(C_5H_6N_4O_2)(OAc)_2]$	10.3	19.2	12.4	13.9
11	$[Cd(C_5H_6N_4O_2)(OAc)_2]$	28.8	21.4	21.9	23.3
	Chloramphenicol[b]	64.2	77.2	65.4	71.2
	Streptomycin[b]	63.2	77.2	79.4	82.2

[a]Mean of three replicates; [b]standard drugs

and 36.2–38.2 mm, respectively. Complexes **9** and **11** exhibited good activity against all the tested bacterial strains with a zone of inhibition in the range from 21.2–
–27.3 mm and 21.4–28.8 mm, respectively. Complex **2** showed the highest zone of inhibition (29.6 and 28.1 mm) against *E. coli* and *P. putida*, respectively. Complex **7** showed the highest zone of inhibition 23.2–26.4 mm against *B. subtilis*, *E. coli* and *P. putida* (Table III).

Based on the *MIC* values shown by these complexes against all the bacterial strains, complex **1** was found to be most effective by showing a *MIC* of 4 μg ml^{-1} for *E. coli*, which is equal to the *MIC* shown by the standard antibiotic chloramphenicol and streptomycin against the same bacterial strain. Complex **1** also exhibited *MIC* values in the range from 4 to 8 μg ml^{-1} for the other bacterial strains. In the whole series, the *MIC* value of complex **5** was found to be 16 μg ml^{-1} for *E. coli* and 32 μg ml^{-1} for *B. subtilis*, *B. stearothermophilus* and *P. putida*. Complex **11** showed an *MIC* of 64 μg ml^{-1} for *B. subtilis* (Table IV).

TABLE IV. Minimum inhibitory concentration (*MIC*) shown by the complexes against the test bacteria determined by agar dilution assay (A – *Bacillus subtilis* (MTCC 8509), B – *Bacillus stearothermophilus* (MTCC 8508), C – *Escherichia coli* (MTCC 51), D – *Pseudomonas putida* (MTCC 121))

No.	Complex	*MIC* / μg ml^{-1}			
		A	B	C	D
1	$[Co(C_5H_6N_4O_2)Cl_2]$	4	8	4	8
2	$[Co((C_5H_6N_4O_2)(NO_3)_2]$	>128	>128	64	64
3	$[Co(C_5H_6N_4O_2)(OAc)_2]$	>128	>128	>128	>128
4	$[Ni(C_5H_6N_4O_2)Cl_2]$	>250	>250	>250	>250
5	$[Ni(C_5H_6N_4O_2)(NO_3)_2]$	32	32	16	32
6	$[Ni(C_5H_6N_4O_2)(OAc)_2]$	>250	>250	128	>128
7	$[Cu(C_5H_6N_4O_2)Cl_2]$	>250	>128	>128	>128
8	$[Cu(C_5H_6N_4O_2)(NO_3)_2]$	>250	>250	>250	>128
9	$[Cu(C_5H_6N_4O_2)(OAc)_2]$	>128	128	>128	>128
10	$[Zn(C_5H_6N_4O_2)(OAc)_2]$	>250	>250	>250	>250
11	$[Cd(C_5H_6N_4O_2)(OAc)_2]$	64	>128	>128	>128
	Chloramphenicol[a]	2	2	4	2
	Streptomycin[a]	2	2	4	4

[a]Standard drugs

The antifungal activities of all the complexes were determined against two fungal strains, *i.e.*, *A. flavus* and *A. niger*, and then compared with the standard antifungal drug cyclohexamide (Table V). In the whole series, complex **1** showed the highest percentage inhibition (41–43 %) against both fungal strains but none of the tested complex restricted fungal growth excellently. However, of all the tested complexes, complex **5** showed nearly 30–31 % inhibition of mycelial growth against both fungal strains *i.e.*, *A. flavus* and *A. niger*, whereas complexes **10** and

11 showed nearly 15–24 % inhibition of mycelial growth against *A. flavus* and *A. niger* (Table V).

TABLE V. Antifungal activities of the complexes against the fungal strains for a concentration of 100 μg ml^{-1}

No.	Complex	Inhibition, %	
		Aspergillus flavus	*Aspergillus niger*
1	$[Co(C_5H_6N_4O_2)Cl_2]$	43.29	41.18
2	$[Co((C_5H_6N_4O_2)(NO_3)_2]$	10.69	11.07
3	$[Co(C_5H_6N_4O_2)(OAc)_2]$	11.68	15.31
4	$[Ni(C_5H_6N_4O_2)Cl_2]$	10.52	14.49
5	$[Ni(C_5H_6N_4O_2)(NO_3)_2]$	30.66	31.42
6	$[Ni(C_5H_6N_4O_2)(OAc)_2]$	12.33	10.66
7	$[Cu(C_5H_6N_4O_2)Cl_2]$	19.33	10.33
8	$[Cu(C_5H_6N_4O_2)(NO_3)_2]$	13.81	10.20
9	$[Cu(C_5H_6N_4O_2)(OAc)_2]$	16.77	12.62
10	$[Zn(C_5H_6N_4O_2)(OAc)_2]$	15.33	18.71
11	$[Cd(C_5H_6N_4O_2)(OAc)_2]$	23.77	16.66
	Cyclohexamide[a]	87.34	89.91

[a]Standard drug

CONCLUSIONS

Based on elemental analyses, conductivity and magnetic measurements, and electronic, IR and NMR spectral studies, the structure shown in Fig. 1 may be proposed for all the synthesized complexes.

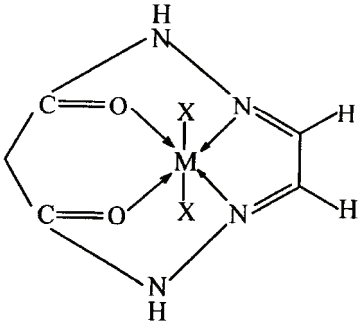

Fig. 1. Structure of the synthesized complexes (M = = Co(II), Ni(II), Cu(II), Zn(II) or Cd(II) and X = Cl^-, NO_3^- or CH_3COO^-).

However, none of the synthesized macrocyclic metal complexes showed good antibacterial and antifungal activities against all the bacterial and fungal strains, but some of the complexes, such as **1** and **5**, were found to be most effective against various bacterial and fungal strains. It is suggested that chelation/coordination reduces the polarity of the metal ion, mainly because of the partial sharing of its positive charge with a donor group within the whole chelate ring system.[39] 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. In addition to this, many other factors, such as solubility, dipole moment and conductivity, which are influenced by the metal ion may be the possible reasons for the antibacterial activities of these metal complexes.[40] It was also 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 the biological utilization ratio and activity of the complexes.[41]

Acknowledgements. DPS thanks the University Grants Commission, New Delhi, India, 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 the authorities of N.I.T., Kurukshetra, India, for providing the necessary research facilities.

Abbreviations

MIC – Minimum inhibitory concentration; MTCC – microbial type culture collection; CFU – colony forming unit; DMF – *N*,*N*-dimethylformamide; DMSO – dimethyl sulphoxide; PDA – potato dextrose medium.

ИЗВОД

СИНТЕЗА, КАРАКТЕРИСАЊЕ, АНТИБАКТЕРИЈСКА И АНТИФУНГАЛНА ИЗУЧАВАЊА НЕКИХ ТЕТРААЗАМАКРОЦИКЛИЧНИХ КОМПЛЕКСА

DHARAM PAL SINGH[1], VANDNA MALIK[1], RAMESH KUMAR[1], KRISHAN KUMAR[1] и SAURABH SUDHA DHIMAN[2]

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

Применом темплатне кондензационе методе полазећи од малонилдихидразида и глиоксала у метанолу као растварачу у присуству соли Co(II), Ni(II), Cu(II), Zn(II) и Cd(II) јона синтетизовани су нови комплекси $[M(C_5H_6N_4O_2)X_2]$-типа (M = неки од наведених јона метала; X = Cl^-, NO_3^- и OAc^-). За карактеризацију ових комплекса употребљени су елементарна микроанализа, магнетна и кондуктометријска мерења. Поред тога, за карактеризацију комплекса употребљена је електронска, NMR и инфра црвена спектроскопија. На бази ових изучавања за добивене комплексе претпостављена је октаедарска геометрија. Приказани су резултати *in vitro* испитивања антибактеријске и антифунгалне активности изолованих комплекса.

(Примљено 29. јануара, ревидирано 28. априла 2010)

REFERENCES

1. D. P. Singh, R. Kumar, V. Malik, P. Tyagi, *Transition Met. Chem.* **32** (2007) 1051
2. L. F. Lindoy, *The Chemistry of Macrocyclic Ligand Complexes*, Cambridge University Press, Cambridge, 1989

3. W. Ma, Y. Tian, S. Zhang, J. Wu, *Transition Met. Chem.*,**31** (2006) 97
4. *Current Trends and Future Perspectives*, K. Gloe, Ed., Springer, Amsterdam, 2005
5. *Coordination Chemistry of Macrocyclic Compounds*, E. C. Constable, Ed., Oxford University Press, Oxford, 1999
6. D. P. Singh, R. Kumar, V. Malik, P. Tyagi, *J. Enzym. Inhib. Med. Chem.* **22** (2007) 177
7. *Magnetic Resonance Imaging of the Body*, A. D. Watson, S. M. Rockladge, Eds., Raven Press, New York, 1992
8. C. Kosmas, D. Snook, C. S. Gooden, N. S. Courtenay-Luck, M. J. McCall, F. Claude, C. F. Meares, A. A. Epenetos, *Cancer Res.* **52** (1992) 904
9. J. Seto, S. Tamura, N. Asai, N. Kishii, Y. Kijima, N. Matsuzawa,, *Pure Appl. Chem.* **68** (1996) 1429
10. W. Dong, R Yang, L. Yan, *Indian J. Chem.* **40A** (2001) 202
11. D. P. Singh, R. Kumar, P. Tyagi, *Transition Met. Chem.* **31** (2006) 970
12. D. Greenwood, R. Slack, J. Peutherer, *Medical Microbiology: A Guide to Microbial Infections: Pathogenesis, Immunity, in Laboratory Diagnosis and Control*, 15th ed., ELST Publishers, Edinburgh, 1997
13. J. McFarland, *J. Am. Med. Assoc.* **14** (1907) 1176
14. NCCLS, *Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically, in Approved Standards*, 5th ed., M7-A5, National Committee for Clinical Laboratory Standards, Villanova, PA, 2000
15. S. Chandra, S. D. Sharma, *Transition Met. Chem.* **27** (2002) 732
16. R. N. Prasad, M. Mathur, A. Upadhyay, *J. Indian Chem. Soc.* **84** (2007) 1202
17. T. A. Khan, M. A. Rather, N. Jahan, S. P. Varkey, M. Shakir, *Transition Met. Chem.* **23** (1998) 283
18. A. K. Singh, A. Panwar, R. Singh, S. Beniwal, *Transition Met. Chem.* **28** (2003) 160
19. D. L. Pavia, G. M. Lampman, G. S. Kriz, *Introduction to Spectroscopy*, Harcourt College Publishers, New York, 2001
20. N. Nishat, Rahis-ud-din, M. M. Haq, K. S. Siddiqi, *Transition Met. Chem.* **28** (2003) 948
21. R. N. Prasad, M. Mathur, *J. Indian. Chem. Soc.* **83** (2006) 1208
22. Q. Zeng, J. Sun, S. Gou, K. Zhou, J. Fang, H. Chen, *Transition Met. Chem.* **23** (1998) 371
23. A. K. Singh, R. Singh, P. Saxena, *Transition Met. Chem.* **29** (2004) 867
24. L. K. Gupta, S. Chandra, *Transition Met. Chem.* **31** (2006) 368
25. A. K. Mohamed, K. S. Islam, S. S Hasan, M. Shakir, *Transition Met. Chem.* **24** (1999) 198
26. C. Lodeiro, R. Bastida, E. Bertolo, A. Macias, R. Rodriguez, *Transition Met. Chem.* **28** (2003) 388
27. a) S. Chandra, L. K. Gupta, *Spectrochim. Acta A* **60** (2004) 2767; b) M. Shakir, S. P. Varkey, P. S. Hameed, *Polyhedron* **12** (1993) 2775
28. K. Nakamoto, *Infrared & Raman Spectra of Inorganic & Coordination Compounds Part B*, 5th ed., Wiley Interscience, New York, 1997
29. M. Shakir, K. S. Islam, A. K. Mohamed, M. Shagufta, S. S. Hasan, *Transition Met. Chem.* **24** (1999) 577
30. M. Shakir, S. P. Varkey, *Polyhedron* **14** (1995) 1117
31. F. M. A. M. Aqra, *Transition Met. Chem.* **24** (1999) 337
32. S. Chandra, R. Kumar, *Transition Met. Chem.* **29** (2004) 269
33. Z. A. Siddiqi, S. M. Shadab, *Indian J. Chem.* **43A** (2004) 2274

34. D. M. Boghaei, S. Mohebi, *J. Mol. Catal. Chem.* **179** (2002) 41
35. V. B. Rana, D. P. Singh, P. Singh, M. P. Teotia, *Transition Met. Chem.* **6** (1981) 36
36. A. B. P. Lever, *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam, 1984
37. D. N. Sathyanarayana, *Electronic Absorption Spectroscopy and Related Techniques*, 1st ed., Universities Press, New Delhi, 2001
38. A. B. P. Lever, E. Mantovani, *Inorg. Chem.* **10** (1971) 817
39. J.M. Andrews, *J. Antimicrob. Chemother*. **48** (2001) 5
40. N. Raman, S. R. Johnson, A. Sakthivel, *J. Coord. Chem*. **62** (2009) 691
41. Z. H. Chohan, M. U. Hassan, K. M. Khan, C. T. Supuran, *J. Enzym. Inhib. Med. Chem*. **20** (2005) 183
42. K. Singh, D. P. Singh, M. S. Barwa, P. Tyagi, Y. Mirza, *J. Enzym. Inhib. Med. Chem.* **21** (2006) 749.