Tetraaza Macrocyclic Complexes: Synthesis, Characterization and their Antimicrobial Activities

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ABSTRACT
Template Condensation of triethylene tetraamine, 2,3-butanedione and metal salt in a 1:1:1 molar ratio results in the formation of a new series of tetraaza macrocyclic complexes: M[2,3-dimethyl-1,4,7,10-tetraaza cyclododeca-1,3-diene] where M = Ni(II), Co(II), Cu(II), Cr(III) and Fe(III). The Complexes were characterized by IR, ^{1}HNMR, UV-Vis spectral studies and magnetic susceptibility measurements. The metal complexes were also tested for their *in vitro* antimicrobial activities against the growth of some fungal and bacterial species in order to assess their inhibiting potential.

KEYWORDS : Tetraaza macrocycle, Antimicrobial activities, Template synthesis, Transition metal

INTRODUCTION
Over couple of years of extensive research work in many laboratories worldwide, macrocyclic chemistry is a well established and highly recognized branch of science. The synthetic, kinetic and structural aspects [1-3] of polyaza macrocyclic complexes have received considerable attention and a variety of such systems have been synthesized. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial, catalytic and medical applications [4-8]. Synthetic macrocyclic complexes emulate some naturally occurring macrocycles because of their resemblance to many natural macrocycles, such as metalloproteins, porphyrins and cobalamine.[9-10]. Transition metal macrocyclic complexes have received great attention due to their biological activities, including antiviral [11], anticarcinogenic [12], antifertile [13] antibacterial and antifungal [14-16].

Prompted by these facts, in the present paper we report the synthesis, characterization and antimicrobial activities of Ni(II), Co(II), Cu(II), Cr(III) and Fe(III) complexes of [Me_2(12)diene N_4] (Figure 1). The complexes were characterized with the help of various physico-chemical techniques, such as elemental analyses, IR, NMR and electronic spectral studies and magnetic susceptibility. These macrocyclic complexes were also screened for their in vitro antibacterial and antifungal activity.

EXPERIMENTAL
All the chemicals and solvents used in this study were of analytical grade.

Synthesis of complexes derived from [Me_2(12)dieneN_4]

Preparation of Ni(II),Co(II),Cu(II), Cr(III) and Fe(III) complexes(I-V) of [2,3-dimethyl-1,4,7,10-tetraaza cyclododeca-1,3-diene)

All the reported macrocyclic complexes were prepared by the template method. Solution of triethylenetetraamine (1.50 ml; 0.01 mol) and 2,3-butanedione (0.88 ml; 0.01 mol) in minimum quantity (20 ml) of dry and cold methanol were mixed and to this a methanolic solution of nickel(II) chloride (1.30g;0.01 mol) was added with constant stirring. The mixture was refluxed for 6h and than 1 ml concentrated hydrochloric acid was added. The mixture was further refluxed for 1h. The volume was than reduced to half on a steam bath and set aside for 30 minutes. The light brown coloured product formed was filtered off through a sintered crucible, washed with methanol and dried in *vacuo*.

A similar procedure was adopted for the preparation of cobalt (II), copper (II), chromium (III) and iron (III) complexes. Cobalt (II) nitrate hexahydrate (2.91g; 0.01 mol), copper (II) nitrate trihydrate (2.42g; 0.01mol), chromium (III) chloride hexahydrate (2.66g; 0.01 mol) and ferric (III) nitrate nonahydrate (4.04g; 0.01 mol) were used to give dark brown, light brown, greenish brown and reddish brown coloured complexes, respectively. In the case of metal nitrates, nitric acid in place of hydrochloric acid was used. The macrocyclic complexes were soluble in water, DMF and DMSO.

In vitro antibacterial and antifungal assay

Primary screening

The antimicrobial activities of the newly synthesized compounds were evaluated by the Serial dilution method against seven pathogenic and non-pathogenic bacterial strains i.e. Bacillus brevis MTCC 1952, Escherichia coli MTCC 1695, Klebsiella pneumonia MTCC 2405, Pseudomonas aeruginosa MTCC 2295, Staphylococcus aureus MTCC87, Staphylococcus epidermidis MTCC 435 and Salmonella typhimurium MTCC 98 and three fungal strains i.e. Aspergillus niger-ORS-4, Aspergillus flavus and Candida tropicalis. The bacterial cultures were maintained on the media prescribed by MTCC, IMT, Chandigarh by sub culturing them on a fresh slant after every 4-5 weeks and incubating them at the appropriate temperature for appropriate time and fungal strains were maintained on potato dextrose agar (2% dextrose, 2.5% agar in potato extract) slants, stored at 4^0C and renewed every month. Stock solutions (10 mg/ml) for all the macrocyclic complexes were prepared in DMSO to determine the Minimal Inhibitory Concentration (MIC). DMSO was used as control for all the test compounds. Antimicrobial activities of the synthetic compounds were studied according to the method as described [17].

A series of tubes containing culture media, prepared by dissolving appropriate amounts of the media components in 100 ml of double distilled water were used. The final pH was adjusted with 1N NaOH and 1N HCl. The broth was sterilized at 121^0C for 15 minutes. The media after sterilization was inoculated with 1% of the seeded culture and 5 ml of the inoculated culture media were dispensed into rimless 'pyrex' test tubes plugged with sterile non-absorbent cotton wool under aseptic condition. The various concentration of each of the variety of compounds were added. The tubes were incubated and examined with respective controls after required time intervals. For each compound the lowest concentration that is able to completely prevent the growth of microorganisms was determined and represented as minimal inhibitory concentration (MIC in mg/ml) of the compound. The compound having the lowest MIC values would have the highest antimicrobial activity against the pathogen.

RESULT AND DISCUSSION

All the complexes are stable at room temperature, insoluble in water but soluble in DMF and DMSO.

The analytical data (Table 1) of the complexes suggest the formula of the macrocyclic complexes as: $[M(C_{10}H_{20}N_4)]Cl_2$ (I), $[M'(C_{10}H_{20}N_4)(NO_3)_2]$(II, III), $[M''(C_{10}H_{20}N_4)Cl_2]^+.Cl^-$ (IV), $[M'''C_{10}H_{20}N_4)(NO_3)_2]^+.NO_3^-$ (V), where M = Ni(II), M′= Co(II), Cu(II), M″= Cr(III) and M‴= Fe(III), respectively. All complexes give satisfactory elemental analyses results, as shown in Table 1and fit well the following structures (Fig. I-V):

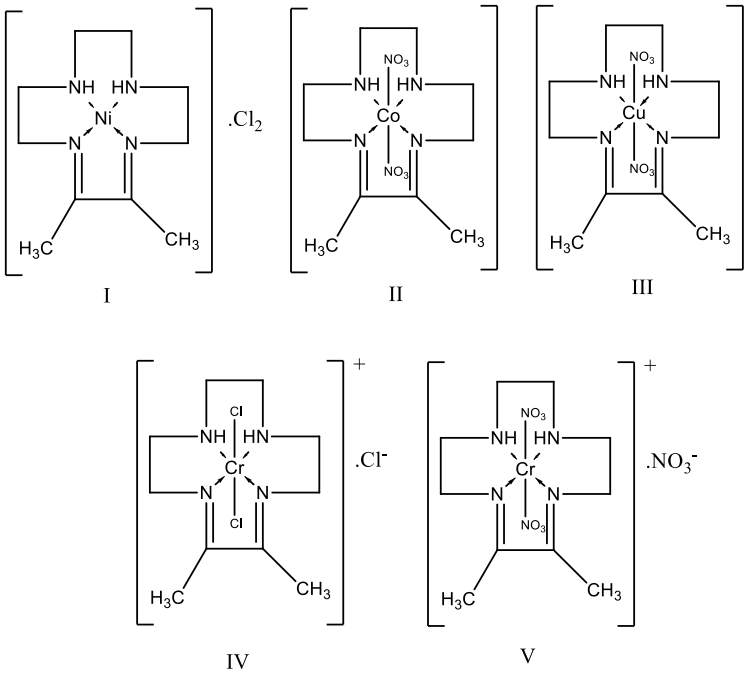

Fig.1 Proposed Structures of the synthesized complexes of [Me_2(12)dieneN_4]

Table 1: Analytical, physical and spectral data of the complexes (I-V) derived from [Me_2(12)dieneN_4]

Complex/Colour	M.Pt. (^{0}C)	Yield%	μ_{eff} (B.M.)	Analysis%:Found (Calcd.)						UV-Vis Spectra λ_{max} (cm^{-1})
				C	H	N	O	Cl	M	
$[Ni(C_{10}H_{20}N_4)]Cl_2$ Light brown	317	60	0	36.3 (36.8)	6.3 (6.2)	17.0 (17.2)	-	21.2 (21.7)	17.9 (18.1)	15850, 19982, 32463
$[Co(C_{10}H_{20}N_4)(NO_3)_2]$ Dark brown	310	72	4.16	30.8 (31.7)	5.1 (5.3)	21.9 (22.1)	-	-	15.9 (15.5)	13000, 15910, 33320
$[Cu(C_{10}H_{20}N_4)(NO_3)_2]$ Light brown	165	58	1.77	30.6 (31.3)	4.9 (5.3)	22.0 (21.9)	25.0 (25.3)	-	16.2 (16.6)	15480, 26420, 36331
$[Cr(C_{10}H_{20}N_4)Cl_2]^+.Cl^-$ Greenish brown	180	64	3.76	33.6 (33.9)	5.3 (5.7)	15.2 (15.8)	25.4 (25.0)	29.4 (29.9)	15.0 (14.7)	15625, 37420
$[Fe(C_{10}H_{20}N_4)(NO_3)_2]^+.NO_3^-$ Reddish brown	341	70	5.86	33.9 (33.5)	5.8 (5.6)	15.1 (15.6)	-	29.2 (29.7)	15.9 (15.6)	12320, 15820, 37665

IR Spectra

The IR spectra of all the complexes (Table 2) show a single sharp band in theregion 3240-3260 cm^{-1} due to N-H stretching vibrations of secondary amines [18] moiety. The appearance of a strong absorption band in the region 1630-1650 cm^{-1} corresponds to C=N stretching frequency [19]. No band is observed around 1700-1800 cm^{-1} indicating the condensation of amine and the ketone. A band appearing at 420-460 cm^{-1} region can be ascribed [20] to ν(M-N) vibrations which further confirms the coordination of these groups with the metal ion.

Table 2: Infrared spectral data of the complexes (I-V) derived from [Me_2(12)dieneN_4]

Complex	C=N (cm-1)	NH (cm-1)	M-N (cm-1)
$[Ni(C_{10}H_{20}N_4)].Cl_2$	1634	3248	468
$[Co(C_{10}H_{20}N_4)(NO_3)_2]$	1650	3250	421
$[Cu(C_{10}H_{20}N_4)(NO_3)_2]$	1640	3240	420
$[Cr(C_{10}H_{20}N_4)Cl_2]^+ \cdot Cl^-$	1630	3260	455
$[Fe(C_{10}H_{20}N_4)(NO_3)_2]^+ .NO_3^-$	1650	3255	448

^{1}H NMR

The ^{1}H NMR spectrum of macrocyclic copper (II) complex in DMSO-d_6 shows a broad singlet at δ1.64-1.84 attributable to the imine methyl (CH_3-C=N, 6H) protons. Two multiplets in the region 3.12-3.38 and2.28-2.38 ppm may be due to the non equivalent methylene protons (C-CH_2-N=, 4H) and (C-CH_2-N, 8H) of the amine moiety. A multiplet in the region 8.02-8.06 ppm corresponds to the secondary amino protons (C-NH-C, 2H).

Magnetic measurements and electronic spectra

The observed value of the magnetic moment (Table 1) for nickel (II) macrocyclic complex [20] indicates a square planar geometry around the metal ion. Which is being further confirmed by the appearance of two bands in its electronic spectrum at 15850 cm^{-1} and 19982 cm^{-1}, reasonably be assigned to $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1A_{2g}$ transitions, respectively . The observed magnetic moment values for copper (II), cobalt (II), chromium (III) and iron (III) complexes suggest their octahedral geometry. The Electronic spectra of the complexes are dominated by an intense single band in the range 32463-37665 cm^{-1} due to charge transfer transition.

Antimicrobial Screening

In this study, all the chemically synthesized metal complexes were screened for antimicrobial activity against bacterial and fungal strains. The minimum inhibitory concentrations (MIC) values of these synthetic complexes were determined by Serial dilution method. All the metal complexes show significant antibacterial activity against some pathogens (Table 3). Complexes 3 and 5 exhibited good activities against all the tested bacterial strains except II and VII ranging from 0.045 to 0.099 mg/ml. Complex 3 showed the highest inhibition (0.045 and 0.046 mg/ml) against Bacillus brevis and Staphylococcus(Table 4). Based on the MIC values shown by these complexes against bacteria, copper and iron complexes were found to be the most effective. The antifungal activities of all the complexes were determined against three fungal strains, i.e., Aspergillus niger and Aspergillus flavus and Candida tropicalis. In the whole series, complex 3 showed the highest percentage inhibition against all the fungal strains, but none of the tested complexes restricted the fungal growth excellently.

Table 3: Results of Antimicrobial activity of the metal complexes of [Me_2(12)dieneN_4]

Complex	Minimum Inhibitory Concentration (MIC) in mg/ml against									
	Bacteria							Fungi		
	I	II	III	IV	V	VI	VII	VIII	IX	X
$[Ni(C_{10}H_{20}N_4)].Cl_2$	0.123	-	0.210	0.175	0.090	0.165	-	-	0.080	0.110
$[Co(C_{10}H_{20}N_4)(NO_3)_2]$	0.102	-	0.202	0.180	0.085	0.173	-	0.128	0.096	0.098
$[Cu(C_{10}H_{20}N_4)(NO_3)_2]$	0.045	-	0.068	0.055	0.046	0.060	-	0.062	0.052	0.058
$[Cr(C_{10}H_{20}N_4)Cl_2]^+ \cdot Cl^-$	0.102	-	0.112	0.110	0.102	0.120	-	0.075	0.095	0.085
$[Fe(C_{10}H_{20}N_4)(NO_3)_2]^+ .NO_3^-$	0.098	-	0.088	0.099	0.098	0.095	-	0.082	0.090	0.099

CONCLUSION

Based on elemental analyses, magnetic measurements, electronic, IR and NMR spectral studies, the structure as shown in Fig. 1 may be proposed for all the prepared complexes. However, none of the synthesized macrocyclic metal complexes showed good antibacterial activities against the tested bacterial and fungal strains, but the copper iron complexes were reported to show good antibacterial activities against various bacterial strains as well as fungal strains. It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor group within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favors its permeation through the lipoid layer of membranes, 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 influenced by the metal ion may be the possible reasons for the antibacterial activities of these metal complexes.

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