# New Hexaaza Macro cyclic Complexes: Synthesis, Characterization and their Antimicrobial Activities

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**Abstract:**Template Condensation of diethylenetriamine, 2,4-pentanedione and metal salt in a 2:2:1 molar ratio results in the formation of a new series of hexaazamacrocyclic complexes: M[2,4,12,14-tetramethyl-1,5,8,11,15,18-hexaaza cycloeicosa-1,4,11,14-tetraene] where M = Ni(II), Co(II), Mn(II) and Zn(II). The Complexes were characterized by IR, $^{1}$HNMR, UV-Vis spectral studies, conductance 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 :**Macrocycle, Template synthesis, Transition metal, Antibacterial, Antifungal, Hexaaza

## 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. Macrocyclic species based on transition metal compounds and multidentate ligands is an interesting field in chemistry and has been the subject of extensive research due to their potential applications in building block macrocyclic-based chemistry [1,2] and environmental chemistry [3] and biomedical [4]. There are a number of important macrocyclic molecules which show biological activities including antibacterial, antifungal, antiviral, antiproliferative, anticancer, herbicidal and anti-inflammatory activities [5-12]. Microbial diseases have become a threat even in the current century being still very much problematic to diagnose. During the last century various types of antibacterial and antifungal drugs have been discovered [13-15]. To develop the more effective antimicrobial drugs is the need of an hour and the macrocyclic metal complexes are also known to act as promising antimicrobial agents [16,17].

Prompted by these facts, in the present paper we report the synthesis, characterization and antimicrobial activities of Ni(II), Co(II), Mn(II) and Zn(II) complexes of [$Me_4$(20)tetraene $N_6$] (Figure 1). The complexes were characterized with the help of various physico-chemical techniques, such as elemental analyses, IR, NMR, molar conductance, electronic spectral studies and magnetic susceptibility. These macrocyclic complexes were also screened for their *in vitro* antibacterial and antifungal activity.

## EXPERIMENTAL

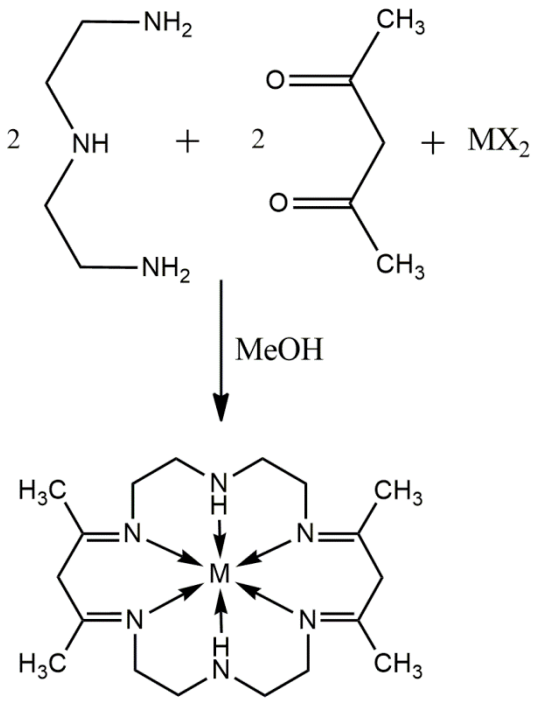
All the chemicals and solvents used in this study were of analytical grade.

### 1.1 Synthesis of complexes derived from [$Me_4$(20)tetraene$N_6$]

#### 1.1.1 Preparation of Ni(II),Co(II),Mn(II) and Zn(II) complexes(I-IV) of [2,4,12,14-tetramethyl-1,5,8,11,15,18-hexaaza cycloeicosa-1,4,11,14-tetraene)

All the reported macrocyclic complexes were prepared by the template method. Mixture of diethylenetriamine (2.16 ml; 0.02 mol) and 2,4-pentanedione (2.05 ml; 0.02 mol) in methanol was refluxed on a water bath for 2h. Ni(II) chloride (1.30g;0.01 mol) in methanol was added to the resulting solution with constant stirring and again refluxed for 1h. The solution was then allowed to cool at room temperature which gave a light purple colored precipitate. The product was filtered and washed several times with methanol. The crude product was recrystallised twice from methanol and dried in *vacuo*.A similar procedure was adopted for the preparation of Cobalt(II) nitrate hexahydrate (2.91g; 0.01 mol), manganese (II) chloride tetrahydrate (1.97g; 0.01 mol) and zinc (II) chloride (1.36g; 0.01 mol)were used to give bright yellow, black and cream colored products, respectively. All the complexes (I-IV) were soluble in water, methanol, dimethylformamide and dimethylsulfoxide.

The template condensation of diethylenetriamine and 2,4-pentanedione in the presence of divalent metal salts, in the molar ratio 2:2:1 is represented in scheme 1.




M= Ni(II), Co(II), Mn (II) and Zn(II)

X= $Cl^-$, $NO_3^-$

Scheme1. Representation of the template condensation of diethylenetriamine and 2, 4-pentanedione in the presence of divalent metal salts in the molar ratio 2:2:1

**1.2 In vitro antibacterial and antifungal assay**

**1.2.1 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 (I), Escherichia coli MTCC 1695 (II), Klebsiella pneumonia MTCC 2405 (III), Pseudomonas aeruginosa MTCC 2295 (IV), Staphylococcus aureus MTCC87 (V), Staphylococcus epidermidis MTCC 435 (VI) and Salmonella typhimurium MTCC 98 (VII) and three fungal strains i.e. Aspergillus niger-ORS-4 (VIII), Aspergillus flavus (IX) and Candida tropicalis (X). 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 $4^0$C 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 [18].

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 $121^0$C 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 coloured solid 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:$[M(C_{18}H_{34}N_6)]$**(I-IV)**, where M = Ni(II), Co(II), Mn(II) and Zn(II). The values of molar conductance for all the complexes in DMSO medium were indicative of non-electrolytic nature. All complexes give satisfactory elemental analyses results, as shown in Table 1and fit well the following structure.

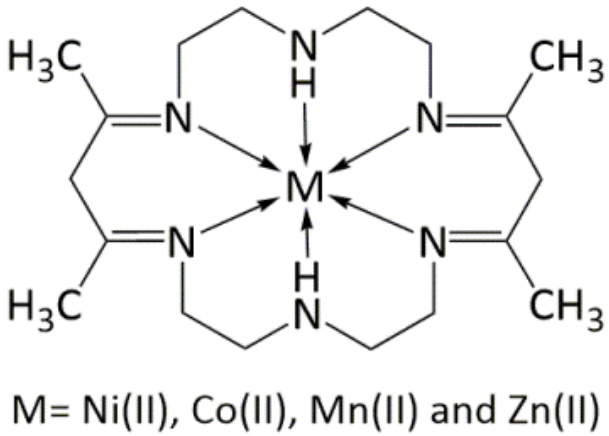



Fig.1 Proposed Structures of the synthesized complexes of [$Me_4$ (20) tetraeneN6]

**Table 1: Analytical, physical and spectral data of the complexes (I-IV) derived from [$Me_4$ (20) tetraene$N_6$]**

| Complex/Colour | M.Pt. / $^0$C | Yield% | $\mu_{eff}$ / B.M. | Analysis,%:Found (Calcd.) C | H | N | M | UV-Vis Spectra $\lambda_{max}$/cm$^{-1}$ | Molar Conductance / cm$^2$ohm$^{-1}$mol$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| [Ni($C_{18}H_{34}N_6$)] Light Purple (I) | 320 | 68 | 3.23 | 54.68 (54.95) | 8.26 (8.71) | 22.01 (21.35) | 14.80 (14.98) | 13436, 18921, 28761,31625 | 12 |
| [Co ($C_{18}H_{34}N_6$)] Bright Yellow (II) | 195 | 65 | 4.62 | 55.01 (54.95) | 8.63 (8.71) | 21.82 (21.36) | 15.03 (14.98) | 11220, 21505, 29161, 31224 | 14 |
| [Mn($C_{18}H_{34}N_6$)] Brown (III) | 168 | 48 | 5.58 | 55.62 (55.51) | 8.72 (8.80) | 21.31 (21.58) | 14.02 (14.10) | 18886, 22525, 30986 | 13 |
| [Zn($C_{18}H_{34}N_6$)] Cream(IV) | 150 | 52 | 0 | 54.11 (54.07) | 8.62 (8.57) | 21.22 (21.02) | 16.19 (16.3) | 31225 | 15 |

**2.1 IR Spectra**

The IR spectra of all the complexes (Table 2) show a single sharp band in theregion 3210-3240 cm$^{-1}$ due to the presence of secondary amine group (-N-H) [19]. No stretching band corresponding to ketone group is observed in the IR spectra confirms the condensation of the ketone with amine resulting in the formation of >C=N-. The appearance of a strong absorption band in the region 1630-1640 cm$^{-1}$ further confirms the presence of>C=N- stretching frequency

[20]. A band appearing at 460-510 $cm^{-1}$ region can be ascribed [21] 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-IV) derived from [$Me_4$ (20) tetraene$N_6$]**

| Complex | C=N /$cm^{-1}$ | NH /$cm^{-1}$ | M-N /$cm^{-1}$ |
|---|---|---|---|
| [Ni($C_{18}H_{34}N_6$)] | 1634 | 3210 | 502 |
| [Co($C_{18}H_{34}N_6$)] | 1636 | 3212 | 528 |
| [Mn($C_{18}H_{34}N_6$)] | 1634 | 3235 | 500 |
| [Zn($C_{18}H_{34}N_6$)] | 1640 | 3220 | 515 |

**2.2 $^1$H NMR**

The $^1$H NMR spectrum of macrocyclic Zinc (II) complex in DMSO-$d_6$ shows singlets at δ2.12-2.20 ppm and 1.84-1.96 ppm attributable to $CH_3$(12H) and the methylene protons (4H) of acetylacetone moiety, respectively. Two multiplets in the region 3.20-3.34 and 2.68-2.88 ppm may be due to the non-equivalent methylene protons (C-$CH_2$-N=, 8H) and (C-$CH_2$-N, 8H) of the amine moiety. A multiplet in the region 6.38-6.44 ppm may be attributed to the secondary amino protons (C-NH-C, 2H).

**2.3 Magnetic measurements and electronic spectra**

The observed value of the magnetic moment (Table 1) for nickel (II) macrocyclic complex [21]indicates aoctahedral geometry around the metal ion. Which is being further confirmed by the appearance of bands in its electronic spectrum at 13436, 18921 and 28761 $cm^{-1}$, reasonably be assigned to $^3A_{2g}$ (F)→ $^3T_{2g}$ (F), $^3A_{2g}$ (F)→ $^3T_{1g}$ (F) and $^3A_{2g}$ (F)→ $^3T_{1g}$ (P) transitions [22], respectively. The electronic spectrum of Co(II) complex shows bands at 11220, 21505 and 29161 $cm^{-1}$ assignable to $^3T_{1g}$ (F) → $^4T_{2g}$ (F), $^4T_{1g}$ (F) → $^4T_{1g}$ (P) and $^4T_{1g}$ (F) → $^4A_{2g}$ (F) transitions, respectively, suggesting the octahedral environment around Co(II) ion which can be further confirmed from the magnetic moment value of 4.62 BM of the complex [23,24]. The electronic spectra of Mn(II) macrocycle exhibits two bands in the region 18886 and 22525$cm^{-1}$ which may reasonably be assigned to $^6A_{1g}$ →$^4T_{1g}$ and$^6A_{1g}$ → $^4T_{2g}$ transitions, respectively and are consistent with an octahedral geometry around Mn(II) ion [25]. All the complexes (I-IV) show the bands at ~31000 $cm^{-1}$, which may be due to charge transfer transition.

**2.4 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,Fig 2 & 3). All the complexes exhibited good activities against all the tested bacterial strains except II, IV and VII ranging from 0.060 to 0.349 mg/ml. Nickel complex showed the highest inhibition (0.060 mg/ml) against Staphylococcus aureus(Table 3, Fig 2). Based on the MIC values shown by these complexes against bacteria, all the complexes were found to be the most effective for Staphylococcus aureus. The antifungal activities of all the complexes were determined against three fungal strains, i.e., Aspergillus niger and Aspergillus flavus and Candida tropicalis (Table 3, Fig 3). In the whole series, all the complexes showed the highest percentage inhibition against IX and X fungal strains, but none of the tested complexes restricted the fungal growth excellently. Manganese complex found active against all the fungal strains.

**Table 3: Results of Antimicrobial activity of the metal complexes of [$Me_4$ (20) tetraeneN6]**

| Complex | Minimum Inhibitory Concentration (MIC) in $mgml^{-1}$ against | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bacteria | | | | | | | Fungi | | |
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| $[Ni(C_{18}H_{34}N_6)]$ | 0.225 | - | 0.325 | - | 0.060 | - | - | - | 0.088 | 0.126 |
| $[Co(C_{18}H_{34}N_6)]$ | 0.214 | - | 0.250 | - | 0.075 | - | - | - | 0.080 | 0.115 |
| $[Mn(C_{18}H_{34}N_6)]$ | 0.349 | - | 0.225 | - | 0.080 | 0.275 | - | 0.090 | 0.092 | 0.275 |

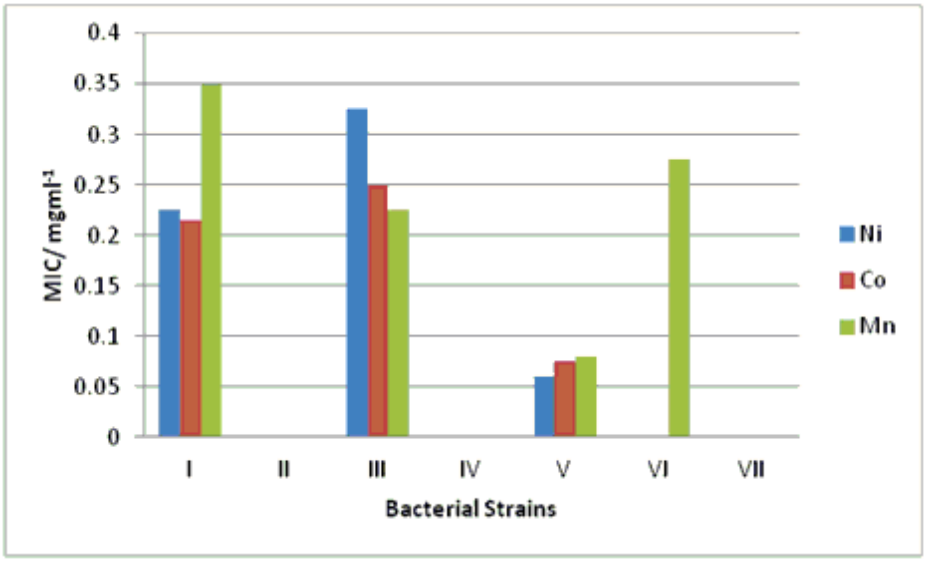



**Fig 2: Results of Antibacterial activity of the metal complexes of [$Me_4$ (20) tetraene$N_6$]**

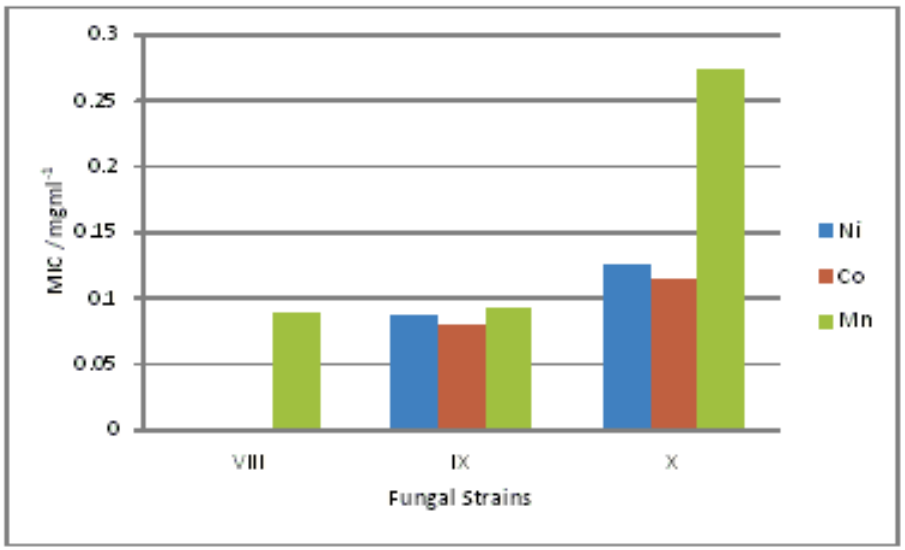



**Fig 3: Results of Antifungal activity of the metal complexes of [$Me_4$(20)tetraene$N_6$]**

## CONCLUSION

Based on elemental analyses, molar conductance, magnetic measurements, electronic, IR and NMR spectral studies, the structure as shown in Fig. 1 may be proposed for all the prepared complexes. All the synthesized macrocyclic metal complexes showed good antibacterial activities against the tested bacterial and 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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