



Synthesis, spectral and antimicrobial studies of tetraazamacrocyclic complexes of bivalent transition metal ions of bioinorganic relevance

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ABSTRACT

Eight new tetraazamacrocyclic complexes of the types $[M(C_{32}H_{24}N_8O_4).2H_2O](CH_3COO^-)_2$ and $[M(C_{14}H_{20}N_8O_4).2H_2O](CH_3COO^-)_2$, where $M = Co(II) / Ni(II) / Cu(II) / Zn(II)$, have been synthesized by the reaction of oxaloyldihydrazide and benzyl / acetylacetone by adopting template method. All the synthesized compounds were characterized by running their TLC for single spot, elemental analyses, infrared spectral studies, 1H -NMR spectral studies, magnetic susceptibilities measurements and electronic spectral studies. The elemental analyses and spectral analysis results revealed their Metal: Ligand (1:1) stoichiometry. Magnetic moment values and electronic spectral studies suggested octahedral geometry for $Co(II)$, $Ni(II)$ complexes while distorted octahedral geometry for $Cu(II)$ complexes. Synthesized dihydrazide and all macrocyclic complexes were screened for antimicrobial activity against two bacteria *Staphylococcus aureus* and *Escherichia coli* and two fungi *Aspergillus niger* and *Aspergillus flavus*. ODH-AA $Cu(II)$ complexes was found most active against both bacteria while ODH-AA $Zn(II)$ complex was most active against both fungi.

Keywords: Macrocyclic complexes, IR, 1H -NMR and electronic spectral studies.

INTRODUCTION

In recent years chemistry of macrocyclic metal complexes has become a fascinating area of research due to their diversified applications in analytical and biological fields. Macrocyclic complexes of transition metal ions have received great attention due to their biological activities, including antiviral, anticarcinogenic [1], antifertile [2], antibacterial and antifungal [3]. Macrocyclic complexes are also important due to their use as dyes and pigments [4] as well as NMR shift reagents [5]. They have potential applications in fundamental and applied sciences [6] such as metal selective extraction [7], stabilization of unusual oxidation state [8], sensor technology [9-11], magnetic resonance imaging, catalysts and models for biological structures and functions [12-18]. Some macrocyclic complexes of lanthanides e.g. Gd^{3+} are used as MRI contrast enhancing agents [19-20]. Macrocyclic nickel complexes have been reported [21] as DNA recognition and oxidation while macrocyclic copper complexes find use in DNA binding and cleavage [22]. Macrocyclic metal chelating agents have been reported as detecting tumour lesions [23]. In continuation of previous research work [24-26] in this research paper we have reported the synthesis and antimicrobial studies of eight new tetraazamacrocyclic complexes of $Co(II)$, $Ni(II)$, $Cu(II)$ and $Zn(II)$ metal ions.

EXPERIMENTAL SECTION

All the chemicals used were of the AR grade. Acetylacetone, diethyl oxalate, benzil were procured from Merck and hydrazine hydrate was used from Sisco.

Synthesis of oxaloyldihydrazide (ODH): 1.3 ml (0.01M) diethyl oxalate was dissolved in 15 ml ethanol and 0.99 ml (0.02M) hydrazine hydrate, dissolved in 10 ml ethanol, was mixed with constant shaking. This mixture was refluxed

for 4 h and allowed to cool in a freezing mixture. The obtained white shiny product was filtered, washed with alcohol following by diethyl ether and dried in vacuo over anhydrous CaCl_2 .

Synthesis of [3, 4, 11, 12-tetraoxo-7, 8, 15, 16- Ph_4 [16]-1, 2, 5, 6, 9, 10, 13, 14- N_8 - 6, 8, 14, 16-tetraene] M(II) complexes: 10 ml acetic acid solution (30%) of oxaloyldihydrazide (0.59 g, 0.005M) was mixed with 20 ml ethanolic solution of benzil (1.05 g, 0.005M) with constant shaking. The contents were refluxed for 3 h and 15-20 ml aqueous solution (0.0025 M) of 0.62 g cobalt acetate tetrahydrate/0.62 g nickel acetate tetrahydrate /0.49 g copper acetate monohydrate /0.54 g zinc acetate dihydrate was added and again refluxed for 6-8 h. Coloured precipitates were obtained which were filtered, washed with water, ethanol followed by diethyl ether and recrystallized from acetonitrile. Finally, precipitates were dried over anhydrous CaCl_2 in vacuo.

Synthesis of [3, 4, 12, 13-tetraoxo-7, 9, 16, 18- Me_4 [18]-1, 2, 5, 6, 10, 11, 14, 15- N_8 - 6, 9, 15, 18-tetraene] M (II) complexes : 0.51 ml acetylacetone (0.005M), dissolved in 20 ml of ethanol, was added to 20 ml 30% acetic acid solution of 0.59 g oxaloyldihydrazide (0.005M) and refluxed for 2 h. 10-15 ml aqueous solution (0.0025M) of 0.62 g cobalt acetate tetrahydrate / 0.62 g nickel acetate tetrahydrate 0.49 g copper acetate monohydrate /0.54 g zinc acetate dihydrate was added and again refluxed for 5-7 h and allowed to cool. Obtained coloured precipitates were filtered washed with water followed by ethanol then diethyl ether and recrystallized from a mixture of acetonitrile: DMF (2:1) and dried in vacuo .

Antimicrobial Activity: The Antimicrobial screening of the synthesized compounds were carried out against the bacteria *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve) and fungi *Aspergillus niger* and *Aspergillus flavus* by adopting Serial Dilution Method [27] in suitable nutrient medium (6.0 g peptone, 3.0 g yeast extract, 1.5 g beef extract, 1.5 g agar only for slant and 1.0 g dextrose in one litre distilled water for bacteria and 10.0 g peptone, 20.5 g agar only for slant 20.0 g dextrose in one litre distilled water for fungi). Graded diluted solutions of the test compounds, with the micro organisms under examination using aseptic condition, were incubated at 37 °C for 24 h in case of bacteria and at 28 °C for 96 h in case of fungi in a B.O.D. incubator. The test tube having the highest dilution, i.e lowest concentration showing no visible turbidity was chosen for the MIC value.

Physical and analytical measurements

The purity and formation were ascertained by determining the melting points of recrystallized samples on electrothermal melting temperature apparatus in open capillaries, by running single spot on TLC and carbon, hydrogen and nitrogen analyses (Reported in Table 1) carried out by micro analytical technique on Elemental Analyser Euro-E 3000. Infrared spectra were recorded in the range of 4000-400 cm^{-1} on 'Bruker spectrophotometer' by using KBr pellets. ^1H - NMR spectra of the synthesized dihydrazide was recorded in acetone solvent on NMR spectrophotometer Bruker DRX 300(MHz FT- NMR with low and high temperature facility (-90°C to +80°C) and electronic spectra of complexes were recorded on Lab-India UV-Visible spectrophotometer UV 3000⁺ in DMSO at room temperature. The magnetic susceptibilities were measured at room temperature on a Gouy balance using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ as calibrant.

RESULTS AND DISCUSSION

IR spectral studies: In the IR spectra of dihydrazide a band appeared at 3442.21 cm^{-1} which may be attributed due to $-\text{NH}$ stretching vibrations of $-\text{NH}_2$ group [28]. In the IR spectra of dihydrazide and all macrocyclic complexes the band due to $>\text{NH}$ stretching vibrations has been observed in the regions 3323.63-3267.74 cm^{-1} . Macrocyclic complexes of ODH-BENZIL exhibited a band in the regions 3064.52-3000.65 cm^{-1} which may be attributed due to C-H stretching vibrations [29] of aromatic ring. In the IR spectra of ODH-AA two medium sharp intensity bands appeared in the region 2927.45-2918.04 cm^{-1} and 2852.32-2813.88 cm^{-1} which may be attributed due to $-\text{CH}$ stretching vibrations of $-\text{CH}_3$ group and $-\text{CH}_2$ moiety respectively. A medium sharp intensity band in the region 1720.75-1697.65 cm^{-1} in dihydrazide and all metal complexes may be attributed due to $>\text{C}=\text{O}$ stretching vibrations [29]. IR spectra of all macrocyclic complexes exhibited two bands in the regions 3457.30-3411.77 cm^{-1} and 899.92-800.65 cm^{-1} which indicated the presence of co-ordinated water molecules in the coordination sphere of macrocyclic complexes. The co-ordinated water molecules in co-ordination sphere have been further confirmed by heating the metal complexes in vacuum at 100 °C over P_2O_5 . IR spectra of the all complexes exhibited a medium sharp intensity band in the region 1599.53-1557.61 cm^{-1} respectively which may be attributed due to $>\text{C}=\text{N}$ - stretching vibrations of azomethine linkage [30]. This region is lower region as compared to normal region i.e. 1620-1590 cm^{-1} which indicating that the N atom of azomethine linkage has participated in the co-ordination. The IR spectra of all macrocyclic complexes exhibited medium sharp intensity bands in the region 527.60-483.02 cm^{-1} and 463.55-440.20 cm^{-1} which may be attributed due to the formation of M-N and M-O stretching vibrations [31] respectively.

¹H-NMR spectral studies: The ¹H-NMR spectra of ODH exhibited a singlet at δ10.094 ppm due to proton of –CONH group. A singlet at δ 2.896 ppm due to –NH₂ protons [32] was also observed in the spectra of synthesized dihydrazide (ODH).

Electronic spectral studies: The electronic spectra of Co(II) complexes displayed three bands in the region 783.37-770.18, 663.42-628.21, 535.26-518.14 nm corresponding to transitions ⁴T_{1g}→⁴T_{1g}(P), ⁴T_{1g}→⁴A_{2g}(F) and ⁴T_{1g}→⁴T_{2g}(F) respectively. Appearance of these bands indicating octahedral geometry [33] for these complexes. The magnetic moment values of these complexes were found 4.71-4.60 BM which further indicated the octahedral environment around the metal ion.

In the electronic spectra of Ni(II) complexes three bands in the region 780.32-775.27, 575.45-525.16, 449.32-384.52 nm have appeared which may correspond to the transitions ³A_{2g}→³T_{1g}(P), ³A_{2g}→³T_{1g}(F) and ³A_{2g}→³T_{2g} respectively which suggested that these complexes have octahedral geometry [33]. The magnetic moment values for these complexes were found 3.20-3.12 BM, which is further in conformity for distorted octahedral geometry

The electronic spectra of Cu (II) complexes exhibited three bands in the region 778.12-772.17, 630.45-592.25, 395.57-388.37 nm corresponding to transitions ²B_{1g}→²E_{1g}, ²B_{1g}→²B_{2g}, and 2B_{1g}→²A_{1g}. These transitions as well as magnetic moment values of complexes (1.87-1.84 BM) are in close agreement with the distorted octahedral geometry [34] for these complexes.

In the electronic spectra of Zn(II) complexes no significant band was noticed due to diamagnetic nature of Zn(II).

On the basis of elemental analyses, IR, electronic spectral data and magnetic moment values the probable structure of complexes may be given as (Figs.1 and 2)

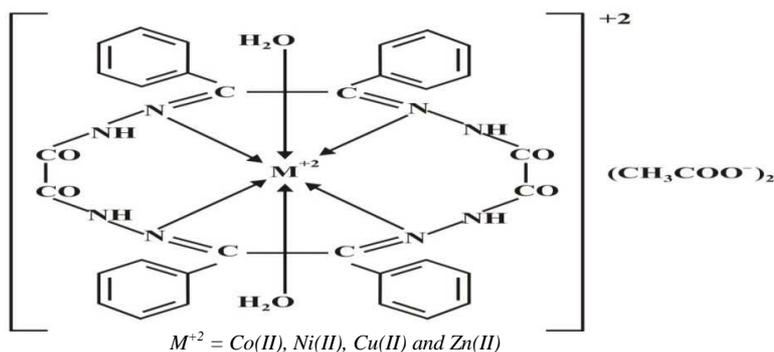


Fig.1: [3, 4, 11, 12-tetraoxo-7, 8, 15, 16-Ph₄[16]-1, 2, 5, 6, 9, 10, 13, 14-N₈- 6, 8, 14, 16-tetraene] M(II) complexes

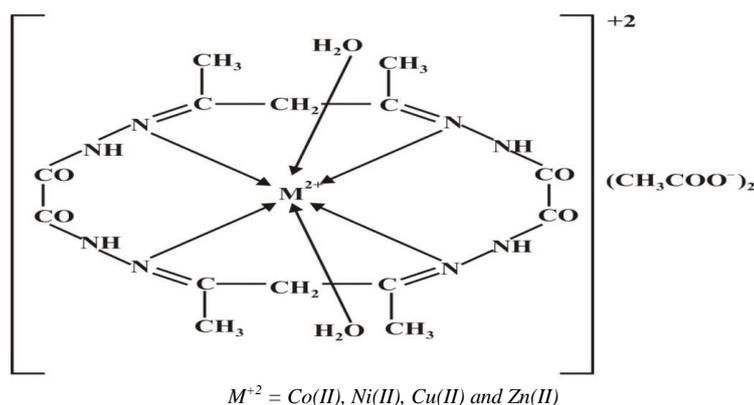


Fig.2: [3, 4, 12, 13-tetraoxo-7, 9, 16, 18-Me₄[18]-1, 2, 5, 6, 10, 11, 14, 15-N₈- 6, 9, 15, 18-tetraene] M(II) complexes

TABLE I : Physical and analytical data of oxaloyl dihydrazide (ODH) and macrocyclic complexes

S. No	Compounds	Molecular Formula	Colour	% Analysis						M.P./ D.T. (±2) °C
				Carbon		Hydrogen		Nitrogen		
				C	F	C	F	C	F	
1.	ODH	C ₂ H ₆ N ₄ O ₂	Shiny white	20.33	22.83	5.08	4.37	47.45	49.21	240
2.	ODH-BENZIL Co(II)	[Co(C ₃₂ H ₂₄ N ₈ O ₄).2H ₂ O]Ac ₂	Dark brown	54.20	55.32	4.26	5.69	14.05	15.56	250
3.	ODH-BENZIL Ni(II)	[Ni(C ₃₂ H ₂₄ N ₈ O ₄).2H ₂ O] Ac ₂	Red	54.22	55.92	4.26	5.39	14.05	15.21	220
4.	ODH-BENZIL Cu(II)	[Cu(C ₃₂ H ₂₄ N ₈ O ₄).2H ₂ O] Ac ₂	Dark green	53.89	54.88	4.24	5.87	13.97	12.11	240
5.	ODH-BENZIL Zn(II)	[Zn(C ₃₂ H ₂₄ N ₈ O ₄).2H ₂ O] Ac ₂	Brown	53.77	55.56	4.23	3.15	13.94	12.56	280
6.	ODH-AA Co(II)	[Co(C ₁₄ H ₂₀ N ₈ O ₄).2H ₂ O] Ac ₂	Dark brown	37.43	38.90	5.19	6.46	19.41	18.33	230
7.	ODH-AA Ni(II)	[Ni(C ₁₄ H ₂₀ N ₈ O ₄).2H ₂ O] Ac ₂	Dark green	37.45	38.63	5.20	6.37	19.42	20.60	250
8.	ODH-AA Cu(II)	[Cu(C ₁₄ H ₂₀ N ₈ O ₄).2H ₂ O] Ac ₂	Dark green	37.14	39.31	5.15	4.02	19.25	18.46	240
9.	ODH-AA Zn(II)	[Zn(C ₁₄ H ₂₀ N ₈ O ₄).2H ₂ O] Ac ₂	Brown	37.02	38.79	5.14	4.69	19.19	18.35	280

Abbreviation : F = Found, C = Calculated, M.P. = Melting Point, D.T. = Decomposition Temperature

Table 2: The Minimum Inhibitory Concentration (X10⁻³ mole) values of oxaloyl dihydrazide (ODH) macrocyclic complexes

S. No	Compounds	Bacteria		Fungi	
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
1.	ODH	211.8	211.8	211.8	211.8
2.	ODH-BENZIL Co(II)	31.3	31.3	31.3	31.3
3.	ODH-BENZIL Ni(II)	15.6	15.6	31.3	31.3
4.	ODH-BENZIL Cu(II)	15.5	15.5	15.5	15.5
5.	ODH-BENZIL Zn(II)	31.1	31.1	31.1	31.1
6.	ODH-AA Co(II)	43.3	43.3	43.3	43.3
7.	ODH-AA Ni(II)	21.6	21.6	43.4	43.4
8.	ODH-AA Cu(II)	10.7	10.7	21.5	21.5
9.	ODH-AA Zn(II)	42.9	42.9	10.7	10.7
10.	Ciprofloxacin	9.4	9.4	-	-
11.	Griseofulvin	-	-	8.8	8.8

A comparative study of MIC values (Table.2) indicated that dihydrazide has moderate antimicrobial active against both bacteria and fungi its activity increased in the form of its macrocyclic complexes. The enhanced biological activity of the macrocyclic complexes can be explained on the basis of Overtone's concept [35] and Tweed's Chelation theory [36]. According to this theory it has been suggested that coordination reduces the polarity of the metal ion to a greater extent because of partial sharing of the positive charge of the metal ion [37] with donor groups. Further, this coordination process also increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes which subsequently enhances the penetration through the lipid layer of cell membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also perturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism. The comparison of antimicrobial activity results showed that all the synthesized compounds were less active as compared to standard drug ciprofloxacin (Antibiotic drug) and griseofulvin (Antifungal drug). However, complex ODH-AA Cu (II) and ODH-AA Zn(II) exhibited very close antibacterial and antifungal activity to the standard Ciprofloxacin and Griseofulvin respectively

CONCLUSION

In the present research work all the compounds were synthesized by adopting template method. Based on various physicochemical studies such as elemental analyses, IR spectral studies, electronic studies and magnetic susceptibilities measurements it was noticed that Co (II) and Ni (II) complexes have octahedral geometry while Cu(II) complexes have distorted octahedral geometry. The antimicrobial screening of the synthesized compounds has been carried out against the bacteria *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve) and fungi *Aspergillus niger* and *Aspergillus flavus* by adopting Serial Dilution Method. ODH-AA Cu(II) complexes was found most active against both bacteria while ODH-AA Zn (II) complex was most active against both fungi. However, All the synthesized compounds were found less active as compared to standard drug ciprofloxacin (Antibiotic drug) and griseofulvin (Antifungal drug).

Acknowledgements

Authors are thankful to Head, SAIF, CDRI, Lucknow (India) for C, H, N analyses and NMR spectral studies and Head, Department of Chemistry Dr. B.R. Ambedkar University, Agra for providing necessary facilities like IR and UV-Visible spectrometer for spectral analysis.

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