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Antifungal Active Tetraaza Macrocyclic Transition Metal Complexes: Designing, Template Synthesis, and Spectral Characterization[1]

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Abstract—Eight novel macrocyclic complexes as candidates of antifungal agent were designed and synthesized by incorporating an N_4 donor site *via* the template condensation of 4,4'-diaminodiphenylmethane, formaldehyde. *p*-anisidine, and metal salts. The structural features were determined on the basis of their elemental analyses, magnetic susceptibility, molar conductance, FAB Mass, UV-Vis, and IR spectral data. Electronic absorption spectral data of the complexes suggest a square-planar geometry around the central metal ion except the VO(IV) complex, which shows square-pyramidal geometry. The stoichiometry of the complexes had been found to be 1 : 1 (metal : ligand). Electrolytic nature of the complexes is assessed from their high conductance data. Monomeric nature of the complexes is confirmed from magnetic susceptibility values. The X-band ESR spectra of the Cu(II) and VO(IV) complexes in DMSO at 300 and 77 K were recorded, and their salient features are reported. The antifungal activity of the macrocyclic metal complexes were screened *in vitro* against *Aspergillus niger*, *Aspergillus fluvus*, *Trichoderma harizanum*, *Trichoderma viridae*, and *Rhizoctonia solani*. The data showed that they possessed antifungal activity.

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INTRODUCTION

Synthetic tetraaza macrocycle (N_4) molecules are considered to be typically good models for oxygen carrier due to the presence of four nitrogen donor sites confined to a single fourfold or a slightly four fold plane in a ringed structure appropriate for metal ligand binding [1]. The interaction of transition metal complexes with nucleic acids is a major area of research due to the utility of these complexes in the design and development of synthetic restriction enzymes, chemotherapeutic agents, foot printing agents, spectroscopic probes, site-specific cleavers, and molecular photoswitches. Transition metals are essential for the normal functioning of living organisms. Therefore, it is not surprising that transition metal coordination compounds are of great interest as potential drugs. Nowadays interest is focused on the synthesis of macrocyclic complexes with potential applications, such as cancer diagnosis and treatment of tumor. Biological activities may be related to the redox properties of complexes. The study on electrochemical properties of some copper complexes indicates that the complexes having lower reduction potential enhance the biological activity [2]. The various organic ligands possess strong fungicidal, insecticidal, herbicidal, and antibacterial properties [2–7]. The antimicrobial activity of these compounds depends upon the nature of the microorganisms. In recent, much interest is focused on the synthesis of macrocyclic complexes with potential medicinal application [8, 9]. This prompted me to design and synthesize antifungal active macrocylic complexes formed by the template condensation of 4.4'-diaminodiphenylmethane, formaldehyde, and *p*-anisidine using metal salts. The in vitro antifungal activities of the investigated compounds were tested against fungi, such as *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harizanum*, *Trichoderma viridae*, and *Rhizoctonia solani*.

EXPERIMENTAL

Materials and methods. Formaldehyde, *p*-anisidine, and metal salts were purchased from Merck, and 4.4'-diaminodiphenylmethane was obtained from SDS fine chemicals. Anhydrous grade ethanol and DMSO were obtained from Fisher Scientific Company. Microanalytical data and FAB Mass spectra of the compounds were recorded at the Sophisticated Analytical Instrument Facility, Central Drug Research Institute (Lucknow). The FAB mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV, and the spectra were recorded at room temperature (RT) using *m*-nitrobenzyl alcohol the matrix. The IR spectra of the samples were recorded on a Shimadzu FTIR-8400S spectrophotometer in 4000–200 cm^{-1} range using pellet with KBr. The UV-Vis spectra were recorded on a Shi-

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Table 1. Physical characterization and analytical, molar conductance, and magnetic susceptibility data of the complexes

Compound	Molecular formula	Color	M.p., °C	Contents (found/calcd), %				Λ_M, Ohm^{-1} cm^2 mol^{-1}	μ_{eff}, μ_B
				M	C	H	N		
$[CuL]Cl_2$	$CuC_{44}H_{46}N_6O_2Cl_2$	Brown	198	7.67/7.71	63.6/64.0	5.2/5.6	9.8/10.1	98	1.78
$[CoL]Cl_2$	$CoC_{44}H_{46}N_6O_2Cl_2$	Pink	120	7.15/7.19	64.1/64.4	5.3/5.6	9.8/10.2	105	3.90
$[NiL]Cl_2$	$NiC_{44}H_{46}N_6O_2Cl_2$	Green	155	7.12/7.16	64.1/64.4	5.0/5.6	9.9/10.2	102	–
$[ZnL]Cl_2$	$ZnC_{44}H_{46}N_6O_2Cl_2$	Colorless	160	7.87/7.92	64.0/64.0	5.1/5.5	9.7/10.1	120	–
$[MnL]Cl_2$	$MnC_{44}H_{46}N_6O_2Cl_2$	Brown	198	6.68/6.73	64.2/64.7	5.3/5.6	10.0/10.3	108	5.87
$[VOL]SO_4$	$VC_{44}H_{46}N_6O_7S$	Green	180	5.92/5.97	61.4/61.8	5.2/5.3	9.4/9.8	104	1.73
$[CdL]Cl_2$	$CdC_{44}H_{46}N_6O_2Cl_2$	Colourless	150	12.85/12.89	60.1/60.5	4.9/5.2	9.2/9.6	118	–
$[HgL]Cl_2$	$HgC_{44}H_{46}N_6O_2Cl_2$	Colourless	198	20.82/20.87	54.4/54.9	4.4/4.7	8.2/8.7	102	–

madzu UV-1601 spectrophotometer using DMSO as solvent. ESR spectra of the paramagnetic complexes were recorded at liquid nitrogen temperature (LNT) as a corresponding class in the X-band region on a Varian E-112 spectrometer equipped with 100 kHz field modulation at RSIC (IIT, Mumbai) using the tetracyanoethylene radical as the field marker. The LNT measurements were done by immersing the quartz tube in a liquid nitrogen Dewar, whose tail fitted into the ESR cavity. Magnetic susceptibility measurements of the complexes were carried out by a Guoy balance using copper sulfate as the calibrant. The values were corrected for diamagnetism by applying Pascal's constants. The molar conductance of the complexes was measured using a Systronic conductivity bridge.

Antifungal activity. National Committee for Clinical Laboratory Standards approved standard Potato dextrose agar (PDA) medium was used for antifungal activity by well diffusion method [10]. DMSO was used as the solvent and fluconazole (antifungal agent) as control. The PDA medium was prepared and inoculation was done inside the Laminar Air Flow. A well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution covered with Petri plates, and they were incubated at 35°C for 72 h. During the incubation period, the solution was diffused and affected the growth of the inoculated microorganisms. The inhibition zone was developed at which the concentration was noted. The minimum inhibitory concentration (MIC) of the complexes was determined by a serial dilution technique [11]. (The determination of the MIC involves a semiquantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. Serial dilution techniques (one part of the specimen with nine parts of diluent) was applied for the determination of MIC of the complexes. DMSO was used for our experiments to make dilutions of the above under test in MIC determination.)

Isolation and identification. The organisms (*A. niger, A. flavus, T. harizanum, T. viridae*, and *R. solani*) were isolated by primary selection from a sample of naturally contaminated cassava waste by serial dilution and courplate technique. The pure cultures were identified by their pharmacological and colony characteristics. The organisms were maintained on PDA slant stored at 4°C. The plants were freshly made once a month.

Synthesis of complexes. 4.4'-Diaminodiphenylmethane (0.02 M), formaldehyde (0.04 M), and *p*-anisidine (0.02 M) were slowly added to a stirred ethanol solution of metal salts (0.01 M). The mixture was heated under reflux for ~24 h. The solution was filtered under hot conditions, and the filtrate was allowed to stand at room temperature. The precipitate formed was filtered off, washed with ethanol, and further dried *in vacuo*.

RESULTS AND DISCUSSION

The synthesized complexes are found to be air stable and insoluble in water but soluble in DMSO and DMF. The analytical data of the complexes together with some physical properties are summarized in Table 1. The mass and microanalytical data of the complexes correspond well with the general formula $[ML]X_2$, where M = Cu(II), Ni(II), Co(II), Zn(II), Hg(II), VO(IV), Cd(II), and Mn(II); $X_2 = 2Cl^-$, SO_4^{2-}; $L = C_{44}H_{46}N_6O_2$. The magnetic susceptibilities and electronic spectral data of the complexes at room temperature are consistent with square-planar geometry around the central metal ion, except for the VO(IV) complex, which shows a square-pyramidal geometry, respectively. The high conductance values of the chelates support the electrolytic nature of the metal complexes (102–185 Ohm^{-1} cm^2 mol^{-1}). High molar conductance values of all the complexes in DMSO indicate that the chloride/sulfate ions are present outside of the coordination sphere, which is confirmed by the silver nitrate test and barium chloride test, respectively.

The FAB mass spectrum of the copper complex shows a molecular ion peak at *m*/*z* 824, which confirms the stoichiometry of metal chelates as $[ML]X_2$ type. It is also supported by the nitrogen rule, since the compound possesses even number of nitrogen atoms. Further, the

Table 2. Electronic absorption data of the complexes at 300 K

Complex	Absorption, cm^{-1}	Transition*	Solvent	Geometry
[CuL]Cl$_2$	17241	$^2B_1g \longrightarrow {}^2A_1g$	DMSO	Square-planar
	38610	INCT		
	31055	INCT		
[NiL]Cl$_2$	17094	$^1A_1g \longrightarrow {}^1B_1g$	DMSO	″
	22222	$^1A_1g \longrightarrow {}^1A_2g$		
	32573	INCT		
	40322	INCT		
[CoL]Cl$_2$	18115	$^1A_1g \longrightarrow {}^1B_1g$	DMSO	″
	32258	INCT		
	46456	INCT		
[VOL]SO$_4$	11862	$^2B_2 \longrightarrow {}^2E$	DMSO	Square-pyramidal
	21929	$^2B_2 \longrightarrow {}^2A_1$		
	35200	INCT		

* INCT is intraligand charge transition.

mass spectra of the other complexes support the above stoichiometry. It is in good agreement with the microanalytical data.

The preliminary identification of the macrocyclic complexes was done from their IR spectra. The complexes give no bands assignable to carbonyl group stretching modes. The sharp band observed around 3195 cm^{-1} region corresponds to ν(CH). The bands observed at 1400–1420, 1040–1080, and 700–740 cm^{-1} region can be assigned to phenyl ring vibrations [12]. In all of the metal complexes, a band seen in the 455–500 cm^{-1} region is probably due to the formation of M–N bonds [13, 14]. In addition to the other bands, the vanadyl complex shows an additional band at 970 cm^{-1} attributed to the V = O frequency [15].

The electronic spectra often provide quick and reliable information about the ligand arrangement in transition metal complexes. The electronic absorption spectra of Cu(II), Ni(II), Co(II), and VO(IV) complexes were recorded at 300 K. The absorption region assigned and the proposed geometry of the complexes are given in Table 2. These values are comparable with those of the other reported complexes [16–18] and consistent with square-planar geometry, except the VO(IV) complex having square-pyramidal geometry [19].

The ESR spectrum of the copper complex recorded in DMSO at 77 (LNT) and 300 K (RT) provides useful information, which is important in studying the metal ion environment. The RT spectrum shows one intense absorption band in the high-field region and it is isotropic due to the tumbling motion of the molecule. The LNT spectrum shows four well resolved peaks in the low-field region and one intense peak in the high-field region. The magnetic moment of the copper complex is 1.78 μ_B, which corresponds to one unpaired electron, indicating that the complex is mononuclear. This fact was also evident from the absence of the half-field signal at 1600 G due to $m_s = \pm 2$ transitions, ruling out any Cu–Cu interaction. The complex exhibits a $g_\parallel$ value of 2.34 and $g_\perp$ value of 2.06. The g-tensor values of the copper complex can be used to derive the ground state. In square-planar complexes, the unpaired lies in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_\parallel > g_\perp > 2$, while the unpaired electron lies in the d_z^2 orbital giving $^2A_{1g}$ as the ground state with $g_\perp > g_\parallel > 2$. From the observed values, it is clear that $A_\parallel = 145 > A_\perp = 36$; $g_\parallel = 2.34 > g_\perp = 2.06 > 2$ coinciding well with related systems, which suggests that the complex has square-planar geometry and system is axially symmetric. This is also supported by the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital. In the axial spectra, the g-values are related to the exchange interaction coupling constant (G) by the expression [20, 21]

$$G = \frac{g_\parallel - 2.0023}{g_\perp - 2.0023}.$$

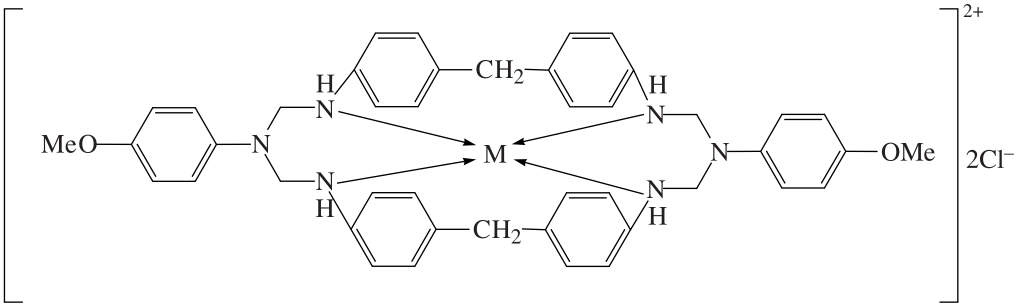
The ESR spectrum of the vanadyl complex recorded in DMSO solution at 300 and 77 K shows a typical eight lines and sixteen lines pattern, respectively. The isotropic ESR parameters $g_{\text{iso}} = 1.97$ and $A_{\text{iso}} = 106$ can be calculated from the position spacing of the resonance lines from the RT solution spectrum of the complex. The spectrum is like a typical eight-line pattern, which shows that a single vanadium is present in the molecule, i.e., it is a monomer. In the frozen solid state the spectrum shows two types of resonance components: one set due to the

Table 3. Antifungal data of the investigated complexes (MIC × 10^{-2} M)

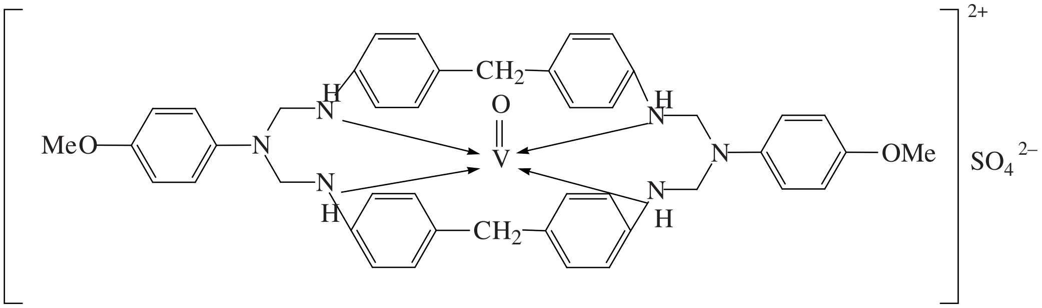
Compound	*A. niger*	*A. flavus*	*T. viridae*	*T. harizanum*	*R. solani*
$[CuL]Cl_2$	3.9	4.2	3.8	3.8	3.5
$[CoL]Cl_2$	4.2	3.8	3.8	3.8	4.1
$[Zn]Cl_2$	3.8	4.6	4.0	4.6	4.2
$INiL]Cl_2$	2.5	3.0	1.8	4.8	3.0
$[VOL]SO_4$	3.0	3.0	2.2	3.0	1.8
$[HgL]Cl_2$	3.2	3.8	3.2	2.5	2.0
$[CdL]Cl_2$	2.6	2.6	3.8	3.0	3.2
$[MnL]Cl_2$	2.8	2.8	2.0	2.5	2.5
Fluconazole	2.0	2.6	1.8	2.3	2.0

parallel features and the other set due to the perpendicular features, which indicates axially symmetric anisotropy with well resolved sixteen line hyperfine splitting characteristic of an interaction between the electron and vanadium nuclear spin. From the anisotropic spectrum, the anisotropic parameters were calculated. The observed order ($A_{\parallel} = 178 > A_{\perp} = 76$; $g_{\perp} = 1.99 > g_{\parallel} = 1.97$) indicates that the unpaired electron is present in the d_{xy} orbital with square-pyramidal geometry around the VO(IV) chelate [22].

Based on the above spectral and analytical data, the proposed structures of the Cu(II), Ni(II), Co(II), Zn(II), Hg(II), and Mn(II) complexes are given below:



Proposed structure $[VOL]SO_4$ macrocyclic complex is the following:



The in vitro antifungal activity of the compounds was tested against filamentous fungi, such as *A. niger, A. flavus, T. harizanum, T. viridae*, and *R. solani*, by the serial dilution method. MIC values of the compounds against the growth of microorganisms are summarized in Table 3. The data of MIC values indicated that the complexes showed high antifungal activity. The activity of the metal chelates is due to increased lipophilicity of the metal ions. The activity of the complexes can be explained on the basis of Overtone's concept [23] and Tweedy's chelation theory [24]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid-soluble materials due to which liposolubility is an important factor, which controls the antifungal activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism.

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