Mycobiology 35(2): 65-68 (2007)

Designing and Synthesis of Antifungal Active Macrocyclic Ligand and Its Complexes Derived from Diethylphthalate and Benzidine

N. Raman* and S. Parameswari

Research Department of Chemistry, VHNSN College, Virudhunagar-626001, India

(Received February 12, 2007)

Three novel complexes of Cu(II), Co(II) and Zn(II) using a macrocyclic ligand derived by the condensation of diethylphthalate and benzidine have been designed, synthesized and characterized by UV-Vis., IR, Mass and Elemental analyses data in order to find out their antifungal activities. The stoichiometry of the complexes has been found to be 1 : 1 (Metal : Ligand). The analytical data indicate that the complexes exhibit square-planar geometry. The antifungal activity of the macrocyclic ligand and its metal complexes has been screened *in vitro* against fungi such as *Aspergillus niger, A. flavus, Trichoderma harizanum, T. viridae* and *Rhizoctonia solani.*

KEYWORDS: Antifungal activity, Metal complexes, Macrocyclic ligands, Square planar

In recent, much interest is focused on the synthesis of macrocyclic complexes with potential medicinal application (Kong *et al.*, 1999). It is well known that various organic ligands possess strong fungicidal, insecticidal, herbicidal and antibacterial properties (Ashu Chaudhary and Singh, 2001; Chitra Gupta and Goutam, 2002; Glory *et al.*, 2006; Biyala *et al.*, 2006; Singh *et al.*, 2003; Venkateswar Rao and Venkata Narasaiah, 2003). It has been reported that the activity of biometals is very often altered through the formation of chelates with different bioligands. In recent years, an extensive literature has been published in the field of chelate compounds with antimicrobial activities. Metal coordination complexes have been widely studied for their antimicrobial (Kamalakannan *et al.*, 2002) and anticancer (Treshchalina *et al.*, 1979) properties. Many drugs demonstrated modified pharmacological and toxicological properties when administered in the form of metallic complexes. Transition metals form some specific and important drugs. These drugs showed antiserothenine, antihistaminic, anticonvulsant and antifungal activities. It has been well established that certain platinum and palladium complexes demonstrated carcinostatic activity (Biyala *et al.*, 2004). It is suggested that the compounds exert antimicrobial activity either by killing the microbe or by inhibiting the growth of the microbe. The antimicrobial activity of these compounds depends upon the nature of the microorganisms. This prompted us to design and synthesize antifungal active macrocylic complexes formed by the condensation of diethylphthalate and benzidine using metal salts. The *in vitro* antifungal activities of the investigated compounds were tested against fungi such as *Aspergillus niger*, *A. flavus*, *Trichoderma harizanum*, *T. viridae* and *Rhizoctonia solani*.

*Corresponding author <E-mail: drn_raman@yahoo.co.in>

Materials and Methods

All chemical reagents were purchased from Merck. Lithium perchlorate ($LiClO_4$) was purchased from Sigma. Anhydrous grade ethanol and DMSO were obtained from Fisher Scientific Company. Micro analytical data and FAB Mass spectra of the compounds were recorded at the Regional Sophisticated Instrumentation Center, Central Drug Research Institute (RSIC, CDRI), 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 using *m*-nitrobenzylalcohol (NBA) as the matrix. The IR spectra of the samples were recorded on a Shimadzu FTIR-8400S spectrophotometer in 4,000~200 cm^{-1} range using KBr as solvent. The UV-Vis. spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Magnetic susceptibility measurements of the complexes were carried out by Guoy balance using copper sulphate 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. NCCLS approved standard potato dextrose agar medium was used for antifungal activity by well diffusion method (Irobi *et al.*, 1996). 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) values of the compounds against the growth of microorganisms are summarized in Table 3.

Isolation and identification. The organisms (*A. niger*, *A. flavus*, *T. harizanum*, *T. viridae* and *Rhizoctonia 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 phar- macology and colony characteristics. The organisms were maintained on PDA slant (potato dextrose agar) stored at 4°C. The plants were freshly made once a month.

Synthesis of macrocyclic ligands. An ethanolic solution of diethylphthalate (10 mmol) was added to the ethanolic solution of benzidine (10 mmol) and refluxed for 3h. Then the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered and washed thoroughly with ethanol and dried *in vacuo*.

Synthesis of metal complexes. A solution of macrocyclic ligand (5 mmol) in ethanol (20 *ml*) was added to a solution of MCl_2 (5 mmol) in ethanol (10 *ml*) and the mixture was refluxed for 3 h and concentrated to one-third volume on a water bath after cooling. The solid product formed was filtered, washed with ethanol and dried *in vacuo*.

Results and Discussion

All the complexes are stable at room temperature, insoluble in water and partially soluble in acetonitrile but soluble in DMF and DMSO. The physical properties and analytical data of the complexes are enlisted in Table 1. The elemental analysis data of the complexes are in good agreement with theoretical values. These complexes showed high conductance values (105~136 ohm^{-1} cm^2 mol^{-1}) indicating their electrolytic nature. High molar conductance values of all the complexes in DMSO indicate that the chloride ions are present outside of the coordination sphere which is confirmed by silver nitrate test. The magnetic moment (Table 1) of the Cu(II) complex under the present study was 1.81 B.M. at room temperature, suggesting a square-planar geometry around the copper ion (Harikumaran Nair *et al.*, 2005).

Mass spectra. A fast atom bombardment mass spectrum was obtained for the macrocyclic ligand (L). In this spectrum was seen the ligand molecular ion peak at m/z 628, as expected for a monomeric formulation of the respective ring. Also the fast atom bombardment mass spectrum of copper $[Cu(L)]Cl_2$ complex shows molecular ion peak at m/z 761 [M^+], which confirms the 1 : 1 stoichiometric ratio of the complex formation. Further, the peak at m/z 71 is due to the presence of two chloride ions which reinforces the conclusions drawn from the conductance studies.

IR spectra. The infrared spectra gave some important information regarding to the skeleton of the complexes. The IR spectra of the macrocyclic ligands show characteristic bands for ν(N-H) at 3327 cm^{-1} and amide (C = O) at 1619 cm^{-1}. In the complexes, ν(N-H) bands were shifted to lower frequencies (104 cm^{-1}), due to coordination of the NH groups. On the other hand, the stretching vibration of ν(C = O) was not affected in all the complexes, which indicates that the carbonyl groups are not involved in coordination to the metal cation. The coordination of nitrogen to the metal atom is supported by the appearance of a new band in the region 436 cm^{-1} assignable to ν(M-N) vibration.

Electronic spectra. The electronic absorption spectra of ligand and its complexes were recorded at 300 K using suitable solvent. The solvent, absorption region, assignment of the absorption bands and the proposed geometry of the complexes are given in Table 2. From the table, we concluded that all the complexes are having square-planar geometry.

Based on the above spectral data, the proposed structure of the macrocyclic ligand and its metal complexes is shown in Fig. 1 and 2.

Antifungal activity.

Disc diffusion method: The *in vitro* antifungal activity of the compounds was tested against filamentous fungi

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

S. No	Compound	Found (calc)%				Λ_m mhocm^2 mol^{-1}	Magnetic moment μ_{eff} (BM)
		M	C	H	N		
1	L	–	75.9(76.0)	4.0(4.4)	8.7(8.9)	–	–
2	$[CuL]Cl_2$	8.1(8.3)	62.7(63.0)	3.5(3.6)	7.4(7.3)	115	1.81
3	$[CoL]Cl_2$	7.6(7.7)	63.0(63.4)	3.4(3.6)	7.0(7.3)	136	3.92
4	$[ZnL]Cl_2$	8.4(8.5)	62.4(62.8)	3.4(3.6)	7.1(7.3)	105	–

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

S.No	Compound	Solvent	Absorption (cm^{-1})	Band Assignment	Geometry
1	L	C_2H_5OH	30769	INCT	---
2	$[CuL]Cl_2$	DMSO	36764 22471	INCT $^2B_{1g} \rightarrow {}^2E_g$	Square-planar
3	$[CoL]Cl_2$	DMSO	32362 16339	INCT $^1A_1g \rightarrow {}^1B_1g$	Square-planar

Table 3. Antifungal activity of the synthesized compounds MIC (minimum inhibitory concentration $\times 10^{-4}$ M) [standard error: ± 0.00001 M]

S. No	Compound	*A. niger*	*A. flavus*	*T. viridae*	*T. harizanum*	*R. solani*
1	L	2.0	1.3	1.3	1.3	1.3
2	$[Cu\ L]Cl_2$	2.6	2.0	2.6	2.6	2.0
3	$[Co\ L]Cl_2$	1.3	1.3	2.0	1.3	1.3
4	$[Zn\ L]Cl_2$	1.3	1.3	2.6	2.0	1.3
5	Fluconazole	1.3	1.3	1.3	1.3	1.3

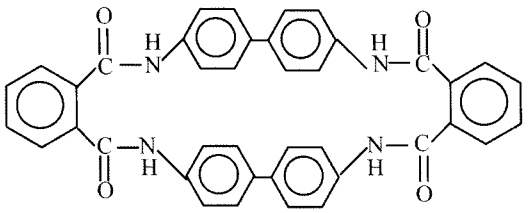

Fig. 1. Structure of macrocyclic ligand.

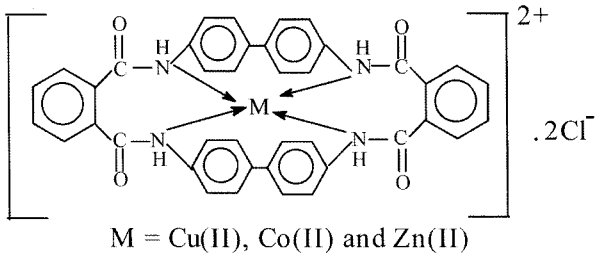

Fig. 2. Structure of macrocyclic complexes.

such as *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harizanum*, *Trichoderma viridae* and *Rhizoctonia solani*, by serial *Dilution method*. The minimum inhibitory concentration (MIC) values of the compounds against the growth of microorganisms are summarized in Table 3. A comparative study of the ligand and its complexes (MIC values) indicates that most of the metal chelates show higher antibacterial activity than the free ligand. (Mishra and Singh, 1993; Dharmaraj *et al.*, 2001). The increased activity of the metal chelates is due to increased lipophilicity of the metal ions.

Such increased activity of the complexes can be explained on the basis of Overtone's concept (Anjaneyula and Rao, 1986) and Tweedy's Chelation theory (Srivastava, 1981). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours 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.

Ackowledgements

The authors express their sincere thanks to the Head, Managing Board and Principal of VHNSN College, Virudhunagar for providing research facilities. NR thanks to the UGC, New Delhi for financial assistance.

References

Abellana, M., Torres, L., Sanchis, V. and Ramos, A. J. 1997. Caracterizacion de diferentesproductos de bolleria industrial. II, Estudio de la microflora. *Alimentaria* **287**: 51-56.

Anjaneyula, Y. and Rao, R. P. 1986. Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetylacetone and various salicylic acids. *Synth. React. Inorg. Met.-Org. Chem.* **16**: 257-272.

Ashu, C. and Singh, R. V. 2001. Synthesis and characterization of 18-to 22-membered tetraazamacrocycles and their tin complexes. *Indian J. Chem.* **40A**: 1330-1333.

Biyala, M. K., Fahmi, N. and Singh, R. V. 2004. Preparation,characterization and antimicrobial properties of some palladium and platinum complex with active Schiff base ligands. *Indian J. Chem.* **43A**: 2536-2541.

Chitra, G. and Goutam, R. K. 2002. Bioinorganic studies on Co(II), Ni(II), and Cu(II) tellurite complexes with 2, 2'-bipyridyl ligand. *Indian J. Chem.* **41A**: 763-766.

Dharamaraj, N., Viswanathamurthi, P. and Natarajan, K. 2001. Ruthenium (II) complexes containing bidendate Schiff bases and their antifungal activity. *Transition Met. Chem.* **26**: 105-109.

Glory, M., Suseelan, M. S. and Krishnan, R. 2004. Synthesis and Characterization of Cobalt (II) Complexes of chromen-2-one-3-carboxyhydrazide and 2-(chromen-2'-only)-5-(aryl)1,3,4-oxiadiazole derivatives. *Indian J. Chem.* **45A**: 2040-2044.

Harikumaran Nair, M. L., Mathew, G. and Sudarasana Kumar, M. R. 2005. Synthesis and characterisation of some new Cu(II) complexes of azo dyes derived from 1,2-dihydro-1,5-dimethyl-2-phenyl-4-amino-3H-pyrazol-3-one. *Indian J. Chem.* **44A**: 85-89.

Irobi, O. N, Moo-Young, M. and Anderson, W. A. 1996. Antimicrobial activity of Annatto (Bixaorellana) extract. *Int. J. Pharm.* **34**: 87-90.

Kamalakannan, P. and Venkappayya, D. 2002. Synthesis and characterization of cobalt and nickel chelates of 5-dimethylaminomethyl-2-thiouracil and their evaluation as antimicrobial and anticancer agents. *J. Inorg. Biochem.* **21**: 22-37.

Kong, D., Meng, L., Song, L. and Xie, Y. 1999. Synthesis, structure and antitumour activities of a new macrocyclic ligand with four natural pendent groups: 1,4,7,10-tetrakisbenzyl-1,4,7,10-tetraazacyclododacane (L) and its Co. Ni and Cu complexes. *Transition Met. Chem.* **24:** 553-557.

Mishra, L. and Singh, V. K. 1993. Synthesis and structural and antifungal studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with new Schiff bases bearing benzimidazoles. *Indian J.Chem.* **32A**: 446-449.

Mukesh, K. B., Nighat, F. and Singh, R. V. 2006. Antifertility and antimicrobial activities of palladium and platinum complexes of 6-nitro-3-(indolin-2-one) hydrazine carbothioamideand6-nitro-3-(indolin-2-one)hydrazinecarboxamide. *Indian J. Chem.* **45A**: 1999-2005.

Singh, N. K., Singh, S. B., Singh, D. K. and Chauhan, V. B. 2003. Synthesis, characterization and biological properties of N-nicotinoyl-N'-thiobenzoyl-hydrazine complexes of Co(II), Ni(II), Cu(II) and Zn(II). *Indian J. Chem.* **42A**: 2767-2771.

Srivastava, R. S. 1981. Pseudotetrahedral Co(II), Ni(II) and Cu(II) complexes of N'-(o-chlorophenyl)-2-(2', 4'-dihydroxyphenyl)-2-benzylazomethine, their fungicidal and herbicidal activity. *Inorg. Chim. Acta* **56**: L65-L67.

Treshchalina, E. M., Konovalova, A. L., Presnov, M. A., Chapurina, L. F. and Belichuk, N. I. 1979. Antitumor properties of mixed coordination compounds of copper (II) and alpha-amino acids. *Dokl. Akad. Nauk.* **248**: 1273-1276.

Venkateswar Rao, P. and Venkata Narasaiah, A. 2003. Synthesis, characterization and biological studies of oxovanadium(IV), manganese(II), iron(II), cobalt(II), nickel(II) and copper(II) Complexes derived from a quadridentate ligand. *Indian J. Chem.* **42A**: 1896-1899.