Mycobiology 35(3): 150-153 (2007)

Synthesis and Spectral Characterization of Antifungal Sensitive Schiff Base Transition Metal Complexes

N. Raman*, A. Sakthivel and K. Rajasekaran

Research Department of Chemistry, VHNSN College, Virudhunagar-626 001, India
(Received July 12, 2007)

New N_2O_2 donor type Schiff base has been designed and synthesized by condensing acetoacetanilido-4-aminoantipyrine with 2-aminobenzoic acid in ethanol. Solid metal complexes of the Schiff base with Cu(II), Ni(II), Co(II), Mn(II), Zn(II), VO(IV), Hg(II) and Cd(II) metal ions were synthesized and characterized by elemental analyses, magnetic susceptibility, molar conduction, fast atom bombardment (FAB) mass, IR, UV-Vis, and ^{1}H NMR spectral studies. The data show that the complexes have the composition of ML type. The UV-Vis. and magnetic susceptibility data of the complexes suggest a square-planar geometry around the central metal ion except VO(IV) complex which has square-pyramidal geometry. The *in vitro* antifungal activities of the compounds were tested against fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Candida albicans*, *Rhizoctonia bataicola* and *Trichoderma harizanum*. All the metal complexes showed stronger antifungal activities than the free ligand. The minimum inhibitory concentrations (MIC) of the metal complexes were found in the range of 10~31 μg/ml.

KEYWORDS: Schiff base, Square-planar, Transition metal complexes

Drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria, fungi, parasite and virus. Infectious diseases like diarrhoea, dysentery, tuberculosis, acute respiratory tract infections, AIDS and recently SARS are global threat and their incidences are increasing significantly day by day. Although a number of chemotherapeutic agents are available in market places, the pathogenic organisms are developing resistance to these agents. So, it is important to find out safer, more effective and inexpensive chemotherapeutic agents. An extensive literature has developed in recent years in the field of chelate compounds with special reference to their antimicrobial activities (Al-Sha'alan *et al.*, 2007).

Recent years have witnessed a great deal of interest in the synthesis and characterization of transition Schiff base metal chelates of pyrazolone derivatives. Among these derivatives, 4-aminoantipyrine is a remarkable reagent due to its importance in biological, pharmacological, clinical and analytical applications (Raman *et al.*, 2007). Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists. In particular, heterocyclic Schiff base ligands containing an additional imine function could be applied variously. They have been of great importance due to their synthetic flexibility, selectivity and sensitivity towards the metal ions. The heterocyclic ring containing sulphur, nitrogen and oxygen import special biological activity to these Schiff bases and their metal complexes (Hankare *et al.*, 2001). Hence, in this paper we describe the synthesis and characterization of antifungal sensitive transition metal complexes of N_2O_2 donor type tetradentate Schiff base formed by condensing Acetoacetanilido-4-aminoantipyrine with 2-aminobenzoic acid. The *in vitro* antifungal activities of the investigated compounds were tested against fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Candida albicans*, *Rhizoctonia bataicola* and *Trichoderma harizanum*.

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

Materials and Methods

Apparatus and reagents. All reagents 4-aminoantipyrine, aceto-acetanilide, 2-aminobenzoic acid, vanadyl sulphate and various metal(II) chlorides were of Merck products and used as supplied. Anhydrous grade methanol and DMSO were purified according to standard procedures. Microanalytical data, ^{1}H NMR 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 Perkin-Elmer 783 spectrophotometer in 4000~200 cm^{-1} range using KBr pellet. The UV-Vis. spectra were recorded on a Shimadzu UV-1601 spectrophotometer using DMF as solvent. Magnetic susceptibility measurements of the complexes were carried out by Guoy balance using copper sulphate as the calibrant. The molar conductance of the complexes was measured using a Systronic conductivity bridge.

***In vitro* antifungal activity.** *In vitro* antifungal activity assay was performed by well diffusion method. DMSO was used as a solvent and nystatin (antifungal agent) as control. The Schiff base and its complexes were tested against fungi such as *A. niger*, *A. flavus*, *R. stolonifer*, *C. albicans*, *R. bataicola* and *T. harizanum*, cultured on potato dextrose agar as medium. In a typical procedure, a well was made on the agar medium inoculated with fungi. The well was filled with the test solution using a micropipette and the plate was incubated at 30°C for 72 h. During this period, the test solution diffused and the growth of the inoculated fungi was affected. The inhibition zone developed on the plate was measured. The MIC of the complexes was determined by serial dilution technique (Reiner, 1982).

Synthesis of Schiff base. Acetoacetanilido-4-aminoantipyrine was prepared by the reported method (Raman *et al.*, 2001). Using this, the following Schiff base has been synthesized.

Acetoacetanilido-4-aminoantipyrinyl-2-aminobenzoic acid (H_2L). An ethanolic (50 *ml*) solution of acetoacetanilido-4-aminoantipyrine (3.62 g, 0.01 mol) and 2-amino benzoic acid (1.37 g 0.01 mol) were mixed at room temperature (27°C) and reflux for 36 h after the addition of 1 g of anhydrous potassium carbonate. The potassium carbonate was filtered off from the reaction mixture and the resulting solution was concentrated to 20 *ml* on a water bath. The solid product formed was separated by filtration and washed thoroughly with ethanol and then dried *in vacuo*.

Syntheses of metal complexes. A solution of $CuCl_2$/$NiCl_2$/$CoCl_2$/$MnCl_2$/$ZnCl_2$/$VOSO_4$/$HgCl_2$ and $CdCl_2$ (5 mmol) and the Schiff base H_2L (5 mmol) in ethanol (50 *ml*) was boiled under reflux for 1 h. The resulting solution was concentrated to 15 *ml* and the mixture was cooled to 0°C for *ca.* 12 h. The solid complex formed was removed by filtration, washed with ethanol and dried *in vaccuo*. Vanadyl complex was synthesized by the same procedure but in the presence of 5% aqueous sodium acetate solution (5 *ml*).

Results and Discussion

The analytical data for the Schiff base and its complexes together with some physical properties are summarized in Table 1. The analytical data of the complexes correspond well with the general formula ML where M = Cu(II), Ni(II), Co(II), Zn(II), Hg(II), Cd(II), VO(IV) and Mn(II); L = $C_{28}H_{27}N_5O_3$. The formulation of the complexes was based on IR data, molar conductivity values, elemental analyses, which were in close agreement with the values calculated for the assigned molecular formulae and on FAB mass spectral studies. The elemental analytical data were in good agreement with the molecular formulae arrived for the ligand and its complexes. The molar conductance values of the complexes in DMF lie in the range 0.8 to 2.36 ohm^{-} cm^2 mol^{-1} which is quite lower than that expected for an electrolyte and reveal their non-electrolytic nature. The magnetic susceptibilities of the complexes at room temperature are consistent with square-planar geometry around the central metal ion, except for the VO(IV) complex which exhibits square-pyramidal geometry.

Mass spectra. The FAB mass spectra of the ligand (H_2L) and its copper complex [CuL] were recorded and they are used to compare their stoichiometry composition. The Schiff base shows a molecular ion peak at m/z 481. The molecular ion peak for the copper complex, observed at m/z 542 confirms the stoichiometry of metal chelates as ML type. This composition is also supported by the mass spectra of other complexes. It is in good agreement with the microanalytical data.

^{1}H NMR spectrum. The ^{1}H NMR spectrum of H_2L in $CDCl_3$ solution shows the following signals: C_6H_5 as mul-

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

S. No	Compound	Colour	Yield %	Found (Cacld) (%)				Molar conductance Λ_m (ohm^{-}cm^2 mol^{-1})	μ_{eff} (BM)
				M	C	H	N		
1	H_2L	Yellow	73	----	69.85 (70.58)	5.61 (5.01)	14.55 (13.78)	----	----
2	CuL	Black	64	11.71 (11.25)	61.93 (62.45)	4.60 (5.20)	12.90 (12.34)	2.36	1.71
3	VOL	Green	54	9.33 (9.89)	61.54 (61.02)	4.57 (5.31)	12.82 (12.01)	1.90	1.75
4	MnL	Brown	71	10.28 (11.20)	62.92 (63.25)	4.68 (4.01)	13.11 (13.89)	1.20	5.97
5	CoL	Light pink	76	10.95 (10.07)	62.46 (63.34)	4.64 (4.11)	13.01 (12.78)	2.10	4.80
6	NiL	Green	58	10.91 (10.15)	62.48 (63.25)	4.64 (5.02)	13.01 (12.56)	1.61	----
7	ZnL	Colourless	32	12.01 (12.85)	61.72 (62.42)	4.59 (4.99)	12.85 (12.40)	1.82	
8	CdL	Colourless	57	19.00 (19.52)	56.81 (55.96)	4.22 (3.78)	11.83 (12.08)	0.80	----
9	HgL	Colourless	46	29.51 (29.98)	49.44 (50.51)	3.67 (4.56)	10.30 (9.86)	1.40	-----

Cacld = Calculated values (Theoretical); Found = Experimental values; M = Metal, C = Carbon, H = Hydrogen and N = Nitrogen; BM = Bohr magnetron (unit of magnetic susceptibility).

tiplet at δ_H 6.9~7.7, -C-CH_3 at δ_H 2.1, =N-CH_3 at δ_H 3.1, Ph-NH- at δ_H 7.8, -C-CH at δ_H 4.8. The peaks observed at δ_H 11.2 and δ_H 10.2 are attributable to the acidic -OH group present in the 2-aminobenzoic acid and enolic -HC=O group present in acetoacetanilide moiety, respectively. The absence of these two peaks in the zinc complex indicates the deprotonation of the acidic -OH and enolic -HC=O group of acetoacetanilide moiety of the Schiff base on chelation. The azomethine proton signal in the zinc complex is shifted to down field compared to the free ligand, suggesting deshielding of the azomethine group due to the coordination with metal ion. There is no change in all of the other signals of this complex.

IR spectra. In order to study the binding mode of the Schiff base to the metal ion of the complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes. The IR spectrum of the ligand shows a weak broad band in the region 2900~3400 cm^{-1} assignable to intramolecular hydrogen bonding between enolisable -HC=O group of acetylacetone and acidic -OH group. Absence of this band in complexes indicates the deprotonation of the intramolecular hydrogen bonded enolic and acidic -OH groups upon complexation. The ligand shows its characteristic =C=N- bands at *ca.* 1640~1600 cm^{-1}, which are also shifted to lower frequencies in the spectra of the complexes (1620~1580 cm^{-1}). The metal chelates show some new bands in the region 480~450 cm^{-1} and 400~350 cm^{-1} which are due to the formation of M-O and M-N bonds respectively (Tabassum *et al.*, 2004). Hence, it is concluded that the coordination to the metal ion occurs through the enolisable carbonyl group of acetoacetanilide moiety, acidic group of 2-aminobenzoic acid and the azomethine nitrogen atoms of the Schiff base. In addition to other bands, the vanadyl complex shows an additional band at 990~970 cm^{-1} attributed to V=O frequency (Mishra *et al.*, 2005).

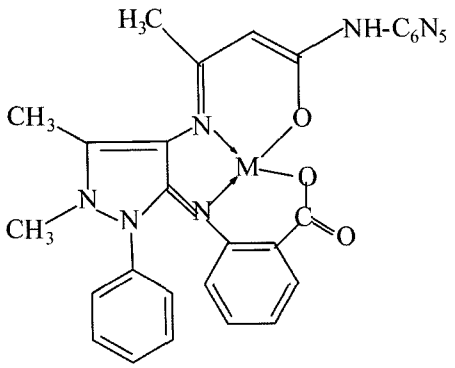

Fig. 1. The proposed structures of the Schiff base complexes [M = Cu(II), Ni(II), Co(II), Mn(II), Zn(II), VO(IV), Hg(II) and Cd(II)].

Electronic absorption spectra. The electronic absorption spectra of the Schiff base and its 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 that of the other reported complexes (Tabassum *et al.*, 2004; Lever, 1968; Mishra *et al.*, 2005) and consistent with square-planar geometry except VO(IV) complex which is having square-pyramidal geometry (Sharma *et al.*, 2003).

Antifungal activity. The *in vitro* antifungal activities of the compounds were tested against *A. niger*, *A. flavus*, *R. stolonifer*, *C. albicans*, *R. bataicola* and *T. harizanum* by

Table 2. Electronic absorption spectral data of the compounds

S. No	Compound	Solvent	Absorption (cm^{-1})	Band assignment	Geometry
1	H_2L	EtOH	45600	INCT	----
			3333	INCT	
2	CuL	DMF	47400	INCT	
			28730	INCT	Square
			18657	$^2B_1g \rightarrow {}^2A_1g$	Planar
3	NiL	DMF	42325	INCT	
			28800	INCT	
			23870	$^1A_1g \rightarrow {}^1A_2g$	Square
			17233	$^1A_1g \rightarrow {}^1B_1g$	Planar
4	CoL	DMF	47076	INCT	
			27900	INCT	Square
			17967	$^1A_1g \rightarrow {}^1B_1g$	Planar
5	VOL	DMF	46948	INCT	
			28730	INCT	
			21980	$^2B_2 \rightarrow {}^2A_1$	Square
			11630	$^2B_2 \rightarrow {}^2E$	Pyramidal

EtOH = Ethanol, DMF = Dimethylformide, INCT = Intra-ligand charge transfer transition.

Table 3. Minimum inhibitory concentration of the synthesized compounds against growth of six fungi ($\mu g/ml$)

S. No	Compound	*A. niger*	*A. flavus*	*R. stolonifer*	*C. albicans*	*R. bataicola*	*T. harizanum*
1	H_2L	60	50	85	75	57	69
2	CuL	18	19	22	24	27	31
3	VOL	10	12	19	10	15	17
4	NiL	14	24	17	21	24	26
5	CoL	22	23	27	15	28	25
6	MnL	29	27	22	24	21	23
7	CdL	18	15	18	20	16	18
8	ZnL	16	14	21	14	19	15
9	HgL	20	17	19	17	20	22
10	Nystatin	10	8	16	12	14	13

the serial dilution method. The minimum inhibitory concentration (MIC) values of the compounds are summarized in Table 3. A comparative study of the ligand and its complexes (MIC values) indicates that complexes exhibit higher antifungal activity than the free ligand. From the MIC values, it was found that the compound VOL is more potent among the other investigated complexes and the Schiff base. 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 (Dharmaraj *et al.*, 2001). 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. Furthermore, the mode of action of the compound may involve formation of a hydrogen bond through the azomethine group with the active centre of cell constitutes resulting in interference with the normal cell process.

Acknowledgments

The authors express their sincere thanks to the University Grants Commission, New Delhi for financial support. They also express their sincere thanks to the Managing Board, Principal and Head of the Department of Chemistry, VHNSN College, Virudhunagar for their constant encouragement and providing research facilities.

References

Al-Sha'alan, N. H. and Riyadh, P. O. 2007. Antimicrobial activity and spectral, magnetic and thermal studies of some transition metal complexes of a Schiff base hydrazone containing a quinoline moiety. *Molecules* **12**: 1080-1091.

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.

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

Hankare, P. P., Patil, R. K., Chavan, S. S., Jagtap A. H. and Battase, P. S. 2001. Synthesis, characterization and biological studies of bivalent metal complexes of Schiff bases derived from aminothiazole and substituted azosalicylaldehyde. *Ind. J. Chem.* **40A**: 1326-1329.

Lever, A. B. P. 1968. *Inorganic Electronic Spectroscopy*, Elsevier, New York.

Mishra, A. P. and Pandey, L. R. 2005. Synthesis, structure and reactivity of oxovanadium (IV) Schiff base complexes. *Ind. J. Chem.* **44A**: 1800-1805.

Raman, N., Raja, S. J., Joseph, J. and Raja, J. D. 2007. Molecular designing, structural elucidation, and comparison of the cleavage ability of oxovanadium (IV) Schiff base complexes. *Russ. J. Coord. Chem.* **33**: 1-5.

Raman, N., Kulandaisamy, A., Shunmugasundaram, A. and Jeyasubramanium, K. 2001. Synthesis, spectral, redox and antimicrobial activities of schiff base complexes derived from 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one and acetoacetanilide, *Transition Met. Chem.* **26:** 131-135.

Reiner, R. 1982. *In Antibiotics-AnIntroduction.* Roche Scientific Services, Switzerland. **1**: 21-25.

Sharma, P., Chand, M. and Nagar, M. 2003. Oxovanadium (IV) and Diaxouranium (VI) complexes of some acyclic monoterpenic constituents of essential oil. *Ind. J. Chem.* **42A**: 2778-2780.

Tabassum, S., Parveen, S. and Arjmand, F. 2004. New binuclear Oxygen carriers of the type [M.-Mac. M' $(L)_4$] Cl_2 $(ClO_4)_2$ where M-Mac=Co(II) and Ni(II),1,8-dihydro 1,3,6,8,10,13-hexaazacyclotetradecane, M'=Mn(II), Ni(II), Cu(II) and L=tetra (3-acetyl pyridine) and there kinetic studies with H_2O_2. *Ind. J. Chem.* **43A**: 270-277.