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Synthesis, Characterization and Antimicrobial Activities of Aminopyrimidine Schiff Base Ligand and their Complexes of Cu(II) and Mn(II)

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ABSTRACT

The novel Schiff base ligand has been synthesized by the condensation of 2-amino-4,6-dimethylpyrimidine and 5-nitrosalicylaldehyde. Metal complexes of the Schiff base were prepared by the reaction of the Schiff base, copper nitrate and zinc nitrate in ethanol solvent. The complexes were isolated, washed and dried. The Schiff base was pale yellowish, while Cu(II) and Mn(II) complexes were light yellowish. The synthesized compounds were characterized using FT-IR, ^{1}H-NMR, UV-Vis techniques for the ligands and all the reactions were monitored by TLC, molar conductivity and magnetic susceptibility measurements for the corresponding complexes. The complexes were paramagnetic. The results of the molar conductivity measurements indicated that the complexes are non-electrolytes in DMSO with an octahedral geometry for all the complexes. The ligands are bidentate, (L_8) through phenolic (OH) and azomethine nitrogen. The ligand and its complexes were screened for their antifungal and antibacterial activities against *Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme, Aspergllus flavus* and *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *B. subtilis.* The results indicated that the complexes exhibited good antifungal and antibacterial activities.

Key words: Heterocyclic Schiff bases, 5-Nitrosalicylaldehyde, 2-amino-4,6-dimethylpyrimidine, Antimicrobial activity.

INTRODUCTION

Heterocyclic compounds are widespread in nature and important for many biochemical processes. These compounds are notable for their biological activity and clinical applications. Schiff bases have been the subject of extensive research because they form stable complexes with various transition metal ions and have applications in a wide range of fields, from materials science to life science [1-3]. The literature indicates that Schiff base ligands are excellent coordinating ligands.

Over the past three decades, considerable attention has been paid to the chemistry of Schiff base complexes containing nitrogen and other donors. This is due to their stability, biological activity, and potential applications in many areas, such as oxidation catalysis [4-6] and electrochemistry, making the behavior of transition metal–Schiff base complexes, a valuable research topic.

The coordination chemistry of amino acid Schiff base ligands is also of great interest due to their biological importance [7-9]. Due to their structure and physicochemical properties, Schiff base complexes of transition metals are often suitable as chemical nucleases. The interaction of these complexes with DNA has attracted considerable attention due to their potential application as new therapeutic agents. The presence and manipulation of the ligands can greatly enhance the interaction between the complexes and DNA, as the ligands affect the reactivity of the transition metal complexes with DNA and can be easily controlled and varied [10-12]. Many potent antibacterial and antifungal Schiff base compounds of heterocyclic compounds have been reported [13-15].

In recent years, numerous studies have reported the synthesis and structural analysis of metal complexes of pyrimidines with bidentate and tridentate ligands (ONO donors) with microbial activity, that is from donor ligands and complexes [16,17]. Pyrimidines are endowed with a wide range of biological activities [18-21]. Chelation of metal ions with pyrimidine rings increases their activity due to the ready availability of potential binding sites. Complexed metal ions provide information on their coordination properties and provide insights into understanding the role of metal ions in biological systems [22].

A literature survey indicates that no work has been done on Schiff base transition metal complexes derived from 2-amino-4,6-dimethylpyrimidine and 5-nitrosalicylaldehyde. In this communication, the synthesis of a bidentate Schiff base formed by condensation of 2-amino-4,6-dimethylpyrimidine with 5-nitrosalicylaldehyde are reported Solid complexes of these ligands with Cu(II) were prepared and characterized using various physicochemical methods.

MATERIALS AND METHODS

Reagents and solvents

2-Amino-4,6-dimethylpyrimidine (Aldrich sigma), and 5-nitrosalicylaldehyde, metal nitrate of AR grade were used for synthesis of ligand and metal complex.

Synthesis of ligand

The ligand was prepared by a modification of the reported methods [23-25]. The Schiff base ligand has been synthesized by refluxing a mixture of 0.01 mol (1.2015 g) of 5-nitrosalicylaldehyde and 0.01 mol (1.2710 g) of 2-amino-4,6-dimethylpyrimidine in 50 ml super dry ethanol refluxed for about 4 h. Schiff base thus formed was cooled to room temperature and collected by filtration, followed by recrystallization in ethanol and dried *in vacuo* over anhydrous calcium chloride (Yield:75%).

Synthesis of metal complexes

To a hot ethanol, 25 ml of the ligand (2 mol) and 25 ml of metal nitrate (1 mol) were added with constant stirring. The pH of reaction mixture was adjusted to 7-8 by adding 10% alcoholic ammonia solution and refluxed for about 3 h. The precipitated solid metal complex was filtered off in hot condition and washed with hot ethanol and dried over calcium chloride in vacuum desiccators. (Yield: 70%)

Physical measurement

IR spectra were recorded on FTIR(ATR)-BRUKER -TENSOR37 spectrometer using KBr pellets in the range of 4000-400 cm^{-1}. ^{1}HMR Varian mercury 300MHZ spectra of ligand were measured in $CDCl_3$ using TMS as internal standard. X-RD were recorded on BRUKER D8 Advance. TGA-DTA were recorded on SHIMADZU thermogravimetric analyser. The carbon, hydrogen and nitrogen contents were determined on Elemental analyser (model vario EL-III). The UV-visible spectra of the complexes were recorded on model Jasco V-530 UV-Vis spectrometer. Molar conductance of complexes was measured on Elico CM 180 conductivity meter using 10^{-4} M solution in DMSO. The magnetic susceptibility measurements of the metal chelates were done on a Guoy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrate.

RESULTS AND DISCUSSION

The schiff base ligand of 2-amino-4,6-dimethylpyrimidine and its complexes have a variety of applications including biological, clinical and analytical. The coordinating possibility of 2-amino-4,6-dimethylpyrimidine has been improved by condensing with a variety of carbonyl compounds. An attempt has been made to synthesize Schiff bases from 2-amino-4,6-dimethylpyrimidine with 5-Nitrosalicylaldehyde.

Physical characteristics, micro analytical, and molar conductance data of ligand and metal complexes are given in Tables 1 and 2.

Table 1: Physical characterization, analytical and molar conductance data of compounds

Compound Molecular formula	Mol.Wt.	M.P. Decomp temp. ^{0}C	Colour	Molar Conduc. Mho Cm^2mol^{-1}
L_8	227.27	175	Yellow	----
Cu- L_8	554.10	>300	Dark Gray	20.10
Mn- L_8	560.10	>300	Faint pink	23.10

Table 2: Elemental analysis of Cu(II) complex

Compound	Found (Calculated)			
	C	H	N	M
L_8	68.71 (68.71)	5.77 (5.77)	18.49 (18.49)	--
Cu-L_8	56.35 (56.23)	5.45 (5.33)	15.16 (15.03)	11.46 (11.43)
Mn-L_8	56.32 (56.23)	5.40 (5.33)	15.19 (15.03)	11.44 (11.43)

The analytical data of complexes reveal 2:1 molar ratio (ligand: metal) and correspond with the general formula $[ML(H_2O)_2]$ (where M= Cu(II) and Mn(II)). The magnetic susceptibilities of Cu(II) complexes at room temperature are consistent with high spin octahedral structure with two water molecules coordinated to metal ion. The presence of two coordinated water molecules was confirmed by TGA-DTA analysis. The metal chelate solutions in DMSO show low conductance and supports their non-electrolyte nature.(Table 1)

^{1}H-NMR spectra of ligand

The ^{1}H-NMR. spectra of free ligand at room temperature show the following signals: 2.35 δ (s, 3H, Methyl hydrogen bonded to pyrimidine ring), 2.35 δ (s, 3H, Methyl hydrogen bonded to phenyl ring), 5.47 δ (s, 1H, Phenolic (OH) hydrogen of pyrimidine ring), 6.77 δ(s, 1H, Hydrogen bonded to pyrimidine ring), 7.84 δ (s, 1H, hydrogen bonded to azomethine carbon), 7.2-7.42 δ (D,4H, Aromatic Ha, Hb, protons of phenyl ring).

IR spectra

The IR spectra of the complexes are compared with that of the ligand to determine the changes that might have taken place during the complexation. The bands at 3363, 1678, 1516, 1309, and 1186 cm^{-1} assignable to ν OH (intramolecular hydrogen bonded), ν C=C(aromatic), ν C=N (azomethine), ν C-N (aryl azomethine) and ν C-O (phenolic) stretching modes respectively[26-28]. The absence of a weak broad band in the 3200-3400 cm^{-1} region, in the spectra of the metal complexes suggests deprotonation of the intramolecular hydrogen bonded OH group on complexation and subsequent coordination of phenolic oxygen to the metal ion. This is supported by downward shift in υ C-O (phenolic) [29] with respect to free ligand. On complexation, the ν (C=N)[30] band is shifted to lower wave number with respect to free ligand, denoting that the nitrogen of azomethine group is coordinated to the metal ion. The ν C-N band is shifted to lower wave number with respect to free ligand, The IR spectra of metal chelates showed new bands in between the 500-600 and 400-500 cm^{-1} regions which can be assigned to ν M-O and M-N[31] vibrations respectively

The IR spectra of Cu (II) show a strong band in the 3050-3600 cm^{-1} region, suggesting the presence of coordinated water in these metal complexes. The presence of coordinated water is further confirmed by the appearance of non-ligand band in 830-840 cm^{-1} region, assignable to the rocking mode of water.

The presence of coordinated water is also established and supported by TGA/DTA analysis of these complexes. Hence it is concluded that the coordination takes place via phenolic oxygen and azomethine nitrogen of ligand molecule.

Thermogravimetric analysis

The dynamic TGA with the percentage mass loss at different steps have been recorded in Fig. 1.

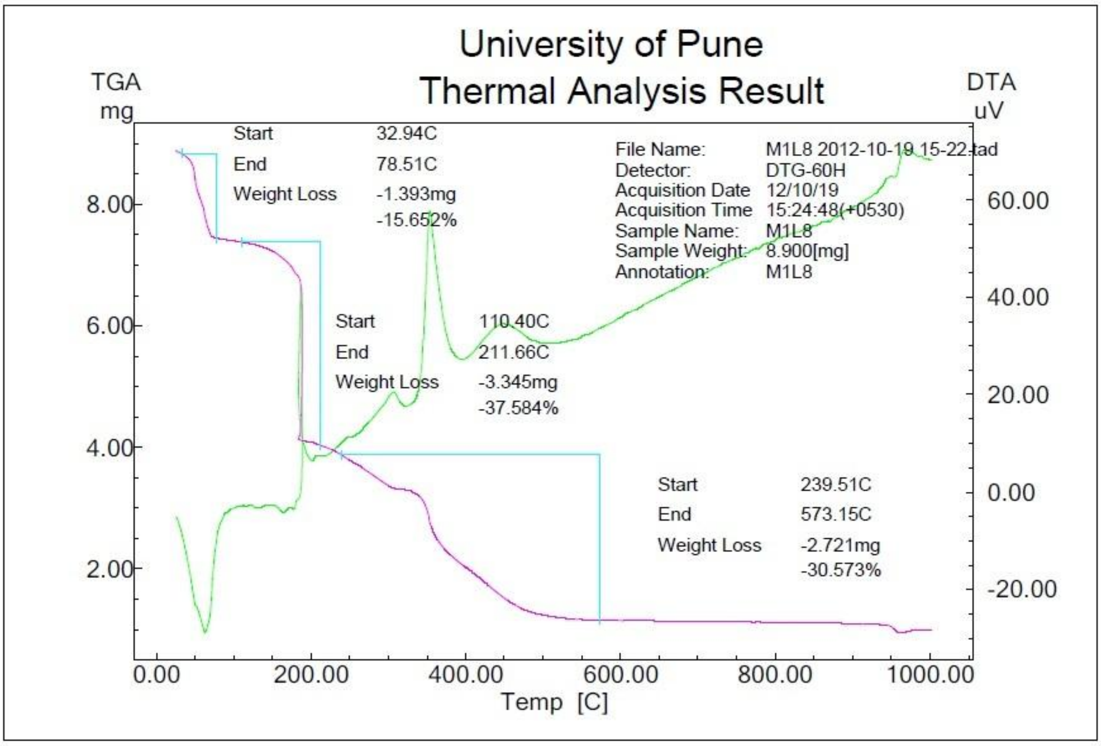

Fig. 1: TGA-DTA curve of Mn(II) complex of Ligand L_8

The simultaneous TGA/DTA analysis of copper was studied from ambient temperature to 1000 ^{0}C in nitrogen atmosphere using α-Al_2O_3 as reference. An analysis of the thermogram of the complexes indicated that copper complexes ligand L_8 (Fig.1) show two step decomposition. The first weight loss is 5.61%, in between temp. 50-195^{0}C which could be correlated with the loss of two molecules of lattice water (calcd 6.50 %). The anhydrous compound does not remain stable at higher temperature. It undergoes rapid decomposition in the range 195-570 °C, with 79.45% mass loss, corresponding to decomposition of the complex (calcd. 79.14%) in second step. The decomposition is completed leading to the formation of stable residue of metal oxide CuO (obs. 11.23%, calcd. 14.35%).

The kinetic and thermodynamic parameters for decomposition of metal complexes are presented in Table 3.

Table 3: The kinetic and thermodynamic parameters for decomposition of metal complexes

Complex	Step	Decomp. Temp. (^{0}C)	n	Ea (kJmole^{-1})	Z (S^{-1})	ΔS (JK^{-1}mole^{-1})	ΔG (kJmole^{-1})	Correlation coefficient
Mn-L$_8$	I	150	0.1	3.77	4.77×10^4	-177.78	12.81	0.994
	II	490	0.8	12.26	1.48×10^4	-172.88	28.12	0.987

Kinetic and thermodynamic viz the energy of activation (Ea), frequency factor (Z), entropy change (-ΔS) and free energy change (ΔG) for the non-isothermal decomposition of complexes have been determined by employing Horowitz-Metzger method [32]. The values are given in Table 3. The calculated values of the given activation energy of the complexes are relatively low, indicating the autocatalysis effect of metal ion on the thermal decomposition of the complex. The negative value of activation entropy indicates that the activated complexes were more ordered than the reaction was slow. The more ordered nature may be due to the polarization of bonds in the activated state, which might occur through charge transfer transitions [33].

Magnetic measurements and electronic absorption spectra

The electronic spectral studies of metal complexes of copper with Schiff bases were carried out in DMSO solution. The absorption spectrum of the copper complex shows bands at 13812 cm^{-1} and 30030 cm^{-1} are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and charge transfer respectively in an octahedral field [34]. The copper complexes were diamagnetic in nature.

Molar conductivity measurements

The metal (II) complexes were dissolved in DMSO and the molar conductivity of

10^{-4}M of their solution at room temperature was measured. The lower conductance values of the complexes support their non-electrolytic nature of the compounds.

Powder x-ray diffraction

The x-ray diffractogram of Cu (II) complexes of L_8 was scanned in the range 20-80° at wavelength 1.543 Å (Fig. 2).

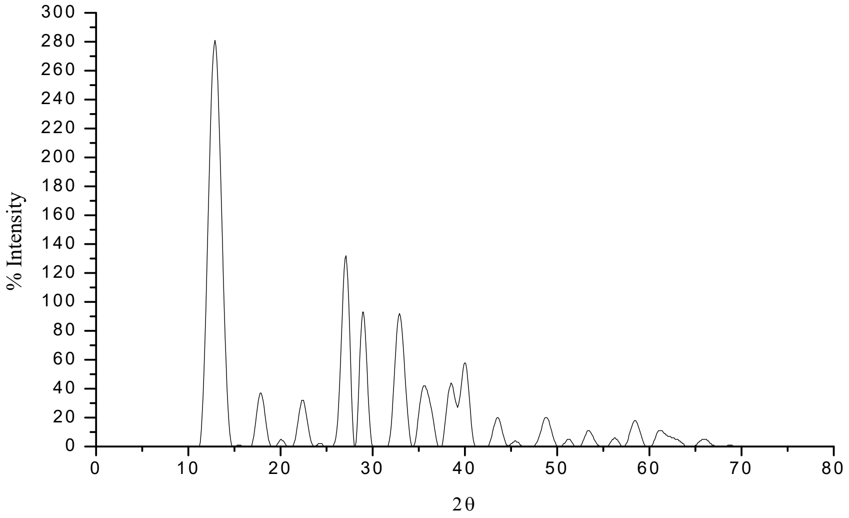

Fig. 2: X-ray Diffractogram of Mn complex of L_8

The diffractogram and associated data depict the 2θ value for each peak, relative intensity and inter-planar spacing (d-values). The diffractogram of Cu(II) complex of L_8 had fifteen reflections with maxima at 2θ = 12.89° corresponding to d value 6.86Å. The x-ray diffraction pattern of these complexes with respect to major peaks of relative intensity greater than 10% has been indexed by using computer programmed [35]. The above indexing method also yields Miller indices (hkl), unit cell parameters and unit cell volume. The unit cell of Cu(II) complex of L_8 yielded values of lattice constants, a= 9.76 Å, b=10.24 Å, c = 27.24 Å and unit cell volume V=2722.43096 Å^3. In concurrence with these cell parameters, the condition such as a = b = c and α = β = γ = 90^0 required for sample to be Monoclinic were tested and found to be satisfactory. Hence it can be concluded that Cu(II) complex has Orthorhombic crystal system. Hence it can be concluded Cu (II) complex of L_8 has monoclinic crystal system. The experimental density values of the complexes were determined by using specific gravity method [31] and found to be 0.8968 gcm^{-3} for Cu (II) complexes respectively. By using experimental density values, molecular weight of complexes, Avogadro's number and volume of the unit cell were calculated. Number of molecules per unit cell were calculated by using equation $\rho = nM/NV$ and was found Cu (II) complexes respectively. With these values, theoretical density were computed and found to be 0.8858 gcm^{-3} for respective complexes. Comparison of experimental and theoretical density shows good agreement within the limits of experimental error [33].

ANTIBACTERIAL ACTIVITY

Antifungal and antibacterial activities of ligand and metal complexes were tested *in vitro* against fungal such as *Aspergillus niger ,Penicillium chrysogenum,Fusarium moneliforme, Aspergillus flavus* and bacteria such as *E. Coli, B.Subtilis*, *S. Aurious and Bacillus subtlis* by paper disc plate method [36-39]. The compounds were tested at the concentrations 1% and 2% in DMSO and compared with known antibiotics *viz Griseofulvin* and *Penicilin* (Table 4 and 5).

Table 4: Antifungal activity of ligands

Test Compound	Antigungal growth							
	Aspergillus niger		*Penicillium chrysogenum*		*Fusarium moneliforme*		*Aspergillus flavus*	
	1%	2%	1%	2%	1%	2%	1%	2%
L_8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Cu- L_8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Mn- L_8	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
+ve control	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-ve control (Griseofulvin)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Ligand & Metal : +ve – Growth (Antifungal Activity absent)

-ve - Growth (Antifungal Activity present)

RG - Reduced Growth (More than 50% reduction in growth observed)

Table 5: Antibacterial activity of ligands and their metal complexes

Test Compound	Diameter of inhibition zone (mm)							
	E. Coli		*Salmonella typhi*		*Staphylococcu saureus*		*Bacillus subtlis*	
	1%	2%	1%	2%	1%	2%	1%	2%
L_8	14 mm	18 mm	16 mm	18 mm	20 mm	25 mm	19 mm	22 mm
Cu- L_8	11 mm	16 mm	15 mm	16 mm	14 mm	22 mm	15 mm	18 mm
Mn- L_8	14 mm	16 mm	13 mm	16 mm	17 mm	22 mm	11 mm	18 mm
DMSO	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Penicillin	14 mm	14 mm	17 mm	17 mm	30 mm	30 mm	19 mm	19 mm

Ligand & Metal: - ve ; - No Antibacterial Activity; Zone of inhibition - --mm

It was found that the inhibition by metal chelates is higher than that of a ligand and results are in good agreement with previous findings with respect to comparative activity of free ligand and its complexes [40]

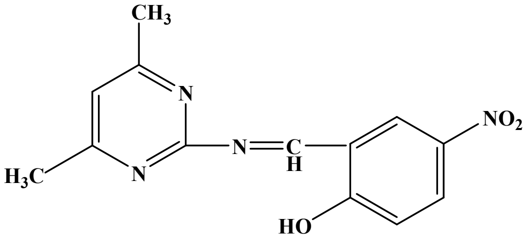

Figure 3: Structure of the ligand

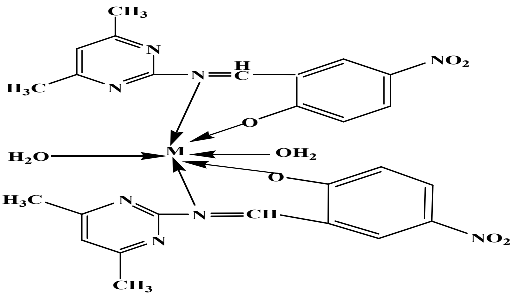

Figure 4: The proposed Structure of the complexes

where M= Cu and Mn.

CONCLUSION

In the light of above discussion we have proposed octahedral geometry for Cu and Mn complexes. On the basis of the physico-chemical and spectral data discussed above, it is assumed that the ligand behaves as dibasic, NO bidentate, coordinating via phenolic oxygen and imino nitrogen as illustrated in Fig.4. The complexes are biologically active and show enhanced antimicrobial activities compared to free ligand. Thermal study reveals thermal stability of complexes. The X-ray study suggests orthorhombic crystal system for Cu and Mn complexes.

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