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Research Article

Synthesis, characterization and *in-vitro* biological activities of Co (II) complexes of 2-(4-Methaylbenzylideneamino) Pyrimidine-4, 6-Diol.

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ABSTRACT

The transition metal complex of Co (II) was prepared from bidentate Schiff base. The Schiff base ligand 2-(4-methaylbenzylideneamino) pyrimidine-4, 6-diol (L_4) derived from the condensation of P-methylbenzaldehyde and 2-amino -4,6 dihydroxypyrimidine in alcohol medium. These metal complexes were characterized on the basis of their micro analytical data, molar conductance, magnetic susceptibility, IR, UV-Vis, ^{1}H NMR, X-ray diffraction, thermogravimetric analysis. The ligand and their metal complexes were screened for fungicidal activity against various fungi like *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme and Aspergllus flavus* and antibacterial activity against various bacteria like *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aurious, B. subtilis*. The result indicated that the complexes exhibited good antifungal and antibacterial activities.

KEYWORDS

Schiff bases, Transition metal complexes, Thermal analysis, Powder X-ray diffraction, Antimicrobial activity.

1. INTRODUCTION

The design of ligands is an important part of the synthetic repertoire of chemists. It gets via subtle control coordination of ligands on a metal centre [1–3]. Ligands contain significantly different chemical functionalities, such as hard and soft donors, often called hybrid ligands that find utility in molecular chemistry [4–6]. The incorporation of pyrimidine moieties in multifunctional ligands of increasing complexity makes for excellent complexation; have rarely been documented in pyrimidine chemistry [7]. The coordination chemistry of pyrimidine derived ligands is of relevance due to their biological implications. In recent years, a number of studies have reported on synthesis and structural analysis of metal complexes of pyrimidine containing bi- and tri-dentate ligands (ONO donors) having microbial activity, i.e., from donor ligands and complexes [8,9]. Pyrimidines are endowed with a wide range of biological activities [10–13]. The chelation of metal ions with pyrimidine ring enhances their activities due to easy availability of potential sites for binding. The complexed metal ions give information on their coordination properties and insights towards understanding the role of metal ions in the biological systems [14]. A search of literature revels that no work has been done on the transition metal complexes of the Schiff bases derived from 2-amino -4,6 dihydroxypyrimidine and P-methylbenzaldehyde. In this communication we report the synthesis of bidentate Schiff bases formed by the condensation of 2-amino -4,6 dihydroxypyrimidine and P-methylbenzaldehyde (Fig.1). The solid complexes of Co (II) with these ligands have been prepared and characterized by different physico-chemical methods.

2. MATERIALS AND METHODS

2-amino-4,6 dihydroxypyrimidine(Aldrich sigma), P-methylbenzaldehyde, metal nitrate of AR grade was used for synthesis of ligand and metal complex . IR spectra were recorded on FTIR(ATR)-BRUKER -TENSOR37 spectrometer using KBr pellets in the range of 4000-400 cm^{-1}. ^1H- NMR 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. The carbon, hydrogen and nitrogen contents were determined on Elementar model vario EL-III. The UV-visible spectra of the complexes were recorded on model UV-1800, SHIMADZU spectrometer. Molar conductance of complexes was measured on Elico CM 180 conductivity meter using 10^{-4} M solution in DMSO. Magnetic susceptibility measurements of the metal chelates were done on a Guoy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrant.

2.1. Preparation of Ligand

The ligand was prepared by a modification of the reported methods [15-17]. The Schiff base ligand has been synthesized by refluxing a mixture of 0.01 mol (1.2015g) of P-methylbenzaldehyde and 0.01 mol (1.2710 g) of 2-amino-4, 6-dihydroxypyrimidine in 50 ml super dry ethanol refluxed for about 4h. 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:70%).

2.2. Preparation of Complexes

To a hot ethanol solution (25ml) of the ligand (2 mol) and (25ml) of metal Nitrate (1mol) was 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: 65%)

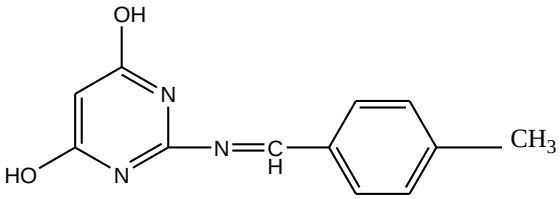

Fig. 1. Structure of ligand.

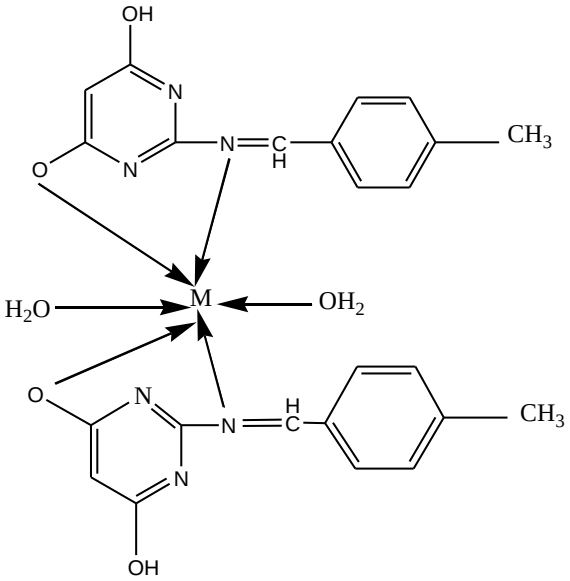

Fig. 2. The proposed Structure of the complexes. [When M= Co (II)]

3. RESULTS AND DISCUSSION

3.1. Elemental Analysis

Schiff bases of 2-amino- 4,6 dihydroxypyrimidine and its complexes have a variety of applications including biological, clinical and analytical. The coordinating possibility of 2-amino- 4,6 dihydroxypyrimidine 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 dihydroxypyrimidine with P-methylbenzaldehyde. Physical characteristics, micro analytical, and molar conductance data of ligand and metal complexes are given in (Table 1).The analytical data of complexes revels 2:1 molar ratio (ligand: metal) and corresponds well with the general formula $[ML(H_2O)_2]$ (where M= Co(II). The magnetic susceptibilities of Co(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 TG-DTA analysis. The metal chelate solutions in DMSO show low conductance and supports their non-electrolyte nature.(Table 1)

Table 1.Analytical and physical data of the ligand and its metal complexes

Com.	**M.F (F. Wt.)**	**Colour**	**mp ^{o}C**	**Contents (Found/Calc) %**				**Λ_M $\Omega^{-1}cm^2$ mol^{-1}**
				C	H	N	M	
L_4	$C_{12}H_{11}N_3O_2$ 229.24	Yellow	165	62.87 /62.87	4.84 /4.84	18.33 /18.33	-	-
Co-L_4	$C_{24}H_{26}N_6O_6Co$ 553.49	Brown	>300	52.08/ 52.00	4.73/ 4.69	15.18/ 10.65/	15.07 10.56	24.25

3.2.*^{1}H-NMR spectra*

The ^{1}H-NMR. Spectra of free ligand at room temperature show the following signals. 2.36 δ (s, 3H, Methyl hydrogen bonded to phenyl ring), 4.47 δ (s, 2H, Phenolic (OH) hydrogen of pyrimidine ring), 5.90 δ(s, 1H, Hydrogen bonded to pyrimidine ring), 7.83 δ (s, 1H, hydrogen bonded to azomethine carbon), 7.09-7.42 δ (D,4H, Aromatic Ha, Hb, protons of phenyl ring).

3.3. FT-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 3331, 1660, 1514, 1300, and 1211 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[16,17,23] 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 further supported by downward shift in υ C-O (phenolic) [24]with respect to free ligand. On complexation, the ν (C=N)[25]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 500-800 and 400-500 cm^{-1} regions which can be assigned to ν M-O and M-N[26] vibrations respectively The IR spectra of Co (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.

3.4. Electronic absorption spectra and magnetic studies

The electronic spectral studies of metal complexes of Co (II) with Schiff bases were carried out in DMSO solution. The absorption spectrum of the Co(II)complex shows bands at 19920 and 29239 cm^{-1} attributed to $^4T_{1g} \rightarrow {}^4A_{2g}(F)$ and charge transfer in an octahedral field[27].

3.5. X-ray diffraction study

The x-ray diffractogram of Co (II) complexes of L_4 was scanned in the range 20-80° at wavelength 1.540 Å(Figure. 3). The diffractogram and associated data depict the 2θ value for each peak, relative intensity and inter-planar spacing (d-values).. The diffractogram of Co(II) complex of L_4 had sixteen reflections with maxima at 2θ = 6.64° corresponding to d value 6.65Å. 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 [28] The above indexing method also yields Miller indices (hkl), unit cell parameters and unit cell volume. The unit cell of Co(II) complex of L_4 yielded values of lattice constants, a=9.567 Å, b=8.789 Å, c = 13.564Å and unit cell volume V=987.71955$Å^3$.In concurrence with these cell parameters, the condition such as $a \neq b \neq c$ and $\alpha = \beta = 90° \neq \gamma$ required for sample to be monoclinic. Hence it can be concluded Co (II) complex of L_4 has monoclinic crystal system. The experimental density values of the complexes were determined by using specific gravity method [27] and found to be 1.0956 gcm^{-3} for Co (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 Co (II) complexes respectively. With these values, theoretical density were computed and found to be 1.0846 gcm^{-3} for respective complexes. Comparison of experimental and theoretical density shows good agreement within the limits of experimental error [29].

3.6. Thermogravimetric studies

The dynamic TGA with the percentage mass loss at different steps have been recorded. The simultaneous TGA/DTA analysis of Co(II) was studied from ambient temperature to 1000 ^0C in nitrogen atmosphere using α-Al_2O_3 as reference. In TG curve of Co (II) complex, of ligand L_4 (Figure 4) shows two step decomposition. The first weight loss 5.74%, in between temp. 50-170^{0}C could be correlated with the loss of two lattice water (calcd. 6.50 %). The anhydrous compound does not remain stable at higher temperature, it undergoes rapid decomposition in the range 170-550°C, with 80.46 % mass loss corresponds to decomposition of the complex (calcd. 79.94%) in second step. The decomposition is completed leading to the formation of stable residue of metal oxide CoO obs. 12.93 % (calcd. 13.54 %). 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 [30] values are given in Table 2. 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 [31].

Table 2. Thermal decomposition kinetic parameters of metal complexes.

Complex	Steps	Decompo-sition Temp(0C)	n	Activation energy (Ea) kJmol-1	Free energy change (ΔG), (kJmol-1)	Entropy change (ΔS) Jmol-1 K-1	Frequency factor (Z), S-1	Correlation coefficient
Co-L4	I	370	0.1	11.88	2.26 ×104	-167.97	24.86	0.968

3.7. Molar Conductivity Measurements

The metal complexes of Co (II) 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.

3.8. Antibacterial activity

Antifungal activity and Antibacterial activity 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. Aureus And Bacillus subtlis* by paper disc plate method [18-21]. The compounds were tested at the concentrations 1% and 2% in DMSO and compared with known antibiotics *viz Griseofulvin* and *Penicilin*. (Table 3 and 4)., it is 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 [22].

3.9. Antifungal Activity

Antifungal activity was performed by poison plate method [32]. The medium used was potato dextrose agar (Himedia). The medium was prepared and sterilized at 10 Psi in autoclave for 15 minutes. Then the compound to be tested is added to the sterile medium in aseptic condition so as to get final concentration as 1%. A plate with DMSO was prepared as standard reference plate (positive control).

Aspergillus niger, Penicillium chrysogenum, Fusarium moneliforme, Aspergillus flavus were selected as test fungal culture. They were allowed to grow on slant for 48 hours so as to get profuse sporulation. 5 ml of 1:100 aqueous solution of Tween 80 was added to the slant and spores were scraped with the help of nicrome wire loop to form suspension. The fungal suspension was spot inoculated on the plates prepared using. Compound with the help of nicrome wire loop. The plates were incubated at room temperature for 48 hours. After incubation plates were observed for the growth of inoculated fungi. Results were recorded as growth of fungi (no antifungal activity), reduced growth of fungi (moderate antifungal activity), and no growth of inoculated fungi (antifungal activity).

3.10. Antibacterial Activity

The antibacterial activity was measured by agar cup method [32]. Nutrient agar (Himedia) was prepared and sterilized at 15 Psi for 15 minutes in the autoclave. It was allowed to cool below 45^0 C and seeded with turbid suspension of test bacteria separately, prepared from 24 hours old slant culture. 3% inocula were used every time. The bacterial culture selected were, two gram negative culture viz. *Escherichia coli, Salmonella typhi* and two gram positive culture viz. *Staphylococcus aureus, Bacillus subtilis*. This seeded preparation was then poured in sterile Petri plate under aseptic condition and allowed it to solidify.

Cup of 10mm diameter were borered in the agar plate with sterile cork borer.100 µl of compound solution prepared in Dimethyl Sulphoxide (1%) was added in the cup under aseptic condition with the help of micropipette. 100 µl 0f DMSO was also placed in one of the cup as blank (negative control). A standard antibiotic disk impregnated with 10 units of penicillin was also placed on the seeded nutrient agar surface as standard reference antibiotic (positive control).

The plates were kept in refrigerator for 15 minutes to allow diffusion of the compound from agar cup into medium. Then the plates were shifted to incubator at 37^0C and incubated for 24 hours.

After incubation plates were observed for the zone of inhibition of bacterial growth around the agar cup. Results were recorded by measuring the zone of inhibition in millimeter (mm) using zone reader.

Table 3. Antifungal activity of ligands and their metal complexes.

Compound	***Aspergillus Niger***		***Penicillium chrysogenum***		***Fusarium moneliforme***		***Aspergillus Flavus***	
	1%	2%	1%	2%	1%	2%	1%	**2%**
L	-ve	-ve	-ve	-ve	-ve	-ve	RG	-ve
Co-L_4	-ve	-ve	-ve	-ve	-ve	-ve	RG	+ve
DMSO	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-ve control (Griseofulvin)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 4. Antibacterial activity of ligands and their metal complexes. Diameter of inhibition zone (mm)

Compound	***E.Coli***		***Salmonella typhi***		***Staphylococcu saureus***		***Bacillus subtlis***	
	1%	2%	1%	2%	1%	2%	1%	**2%**
HL	13mm	13mm	-ve	-ve	14mm	17mm	11mm	14mm

$Co(L)_2$	12mm	13mm	12mm	14mm	18mm	20mm	11mm	14mm
DMSO	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Penicillin	14mm	14mm	17mm	17mm	30mm	30mm	19mm	19mm

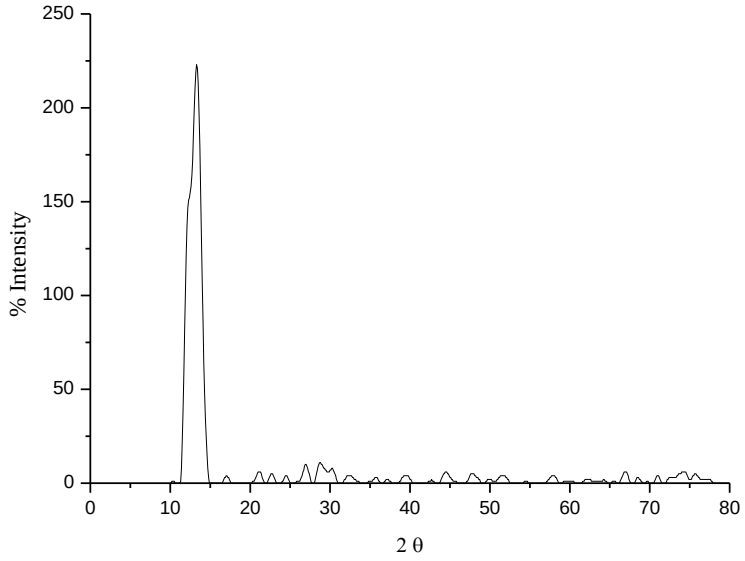

Fig. 3. X-ray Diffractogram of Co (II) complex of L_4.

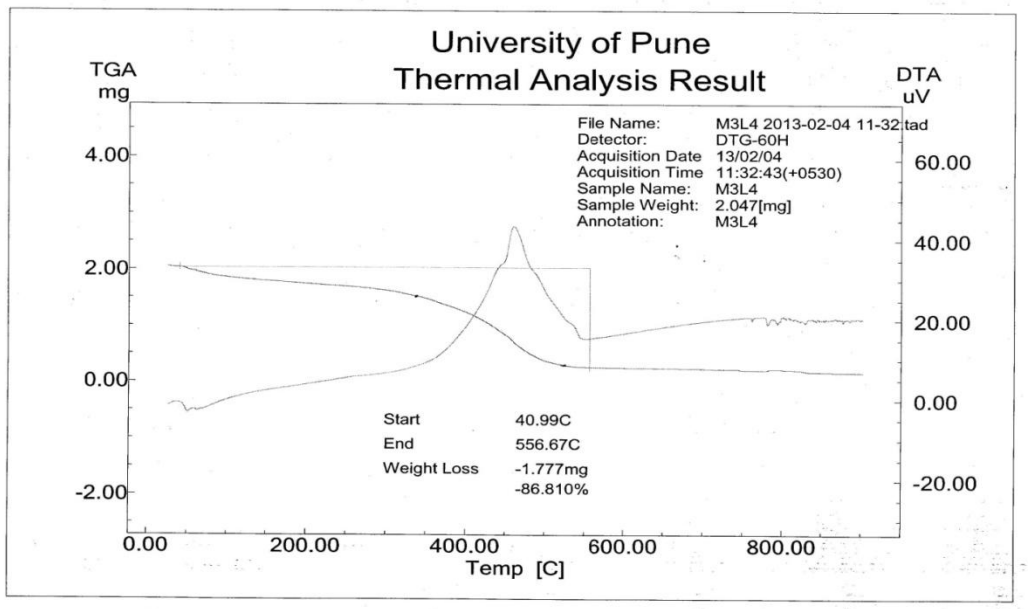

Fig. 4. TGA-DTA Curve of Co (II) Complex of Ligand L_4.

4. CONCLUSION

In the light of above discussion we have proposed octahedral geometry for Co (II) complexes. On the basis of the physico-chemical and spectral data discussed above, one can assume that the ligand behave as dibasic, NO bidentate, coordinating via phenolic oxygen and imino nitrogen as illustrated in Fig.2. 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 monoclinic crystal system for Co (II) complexes.

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