

Study on Synthesis, Structural Elucidation of Biologically Active Schiff Base Transition Metal Complexes

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Abstract— The synthesis and spectral characterization of new series of transition metal complexes of Cu(II), Ni(II), Co(II) and Zn(II) have been carried out from a Schiff base ligand (L), 2((4,6-dimethoxypyrimidin-2-ylimino)methyl)phenol. Structural study has been carried out from their elemental analysis, molar conductance, mass, IR, UV-Vis, ¹H-NMR and ESR spectral studies. The structural data reveal that the formed complexes have ML type composition and the complexes have square-planar geometry. The redox behaviour of metal complexes has been studied by cyclic voltammetry studies. The DNA cleavage and binding studies have also been investigated using calf thymus DNA (CT-DNA) by gel electrophoresis and absorption spectral studies.

Keywords- Schiff Base; Square planar geometry; DNA cleavage and DNA binding.

I. INTRODUCTION

The condensation reaction between an aromatic aldehyde and a primary amine leads to the formation of an imine known as Schiff base. The wide variety of possible structures for the ligand has made the field of Schiff base complexes fast developing [1]. Compounds containing pyrimidine ring systems play an important role in many biological systems. These provide potential binding sites for metal ions, and any information on their coordinating properties is important as a means of understanding the role of metal ions in biological systems. The flexibility of disposition of different donor sites is the secret behind their successful performance in mimicking peculiar geometries around the metal centers [2]. Many compounds of therapeutic importance are known to contain pyrimidine ring system. Pyrimidine compounds are also used as hypnotic drugs for nervous system [3]. In view of this context we have undertaken this present study.

II. EXPERIMENTAL

A. Materials and methods

All reagents 2-amino-4, 6-dimethoxypyrimidine and salicylaldehyde used for the preparation of ligand and complexes were sigma products. Spectroscopic grade solvents were used for spectral and cyclic voltammetric measurements. The carbon, hydrogen and nitrogen microanalysis content of each sample was determined at the STIC, CUSAT, Cochin. I.R. Spectra using KBr pellets were recorded on a FTIR Shimadzu spectrophotometer in 4000-400 cm⁻¹ range. Electronic absorption spectra of the complexes in DMSO were recorded on a Shimadzu UV-1800 spectrometer at the MSEC, Kilakarai. The X-band ESR spectra of the samples in methanol were obtained at 300K and 77K using a Varian E_{1/2} spectrometer, the field being calibrated with TCNE (Tetracyanoethylene) as the g-marker at the SAIF, IIT, Bombay. Electrochemical studies were carried out using EG & E Princeton Applied Research Potentiostat/galvanostat model 273 A, controlled by M270 software. Cyclic voltammetric measurements were performed using a glassy carbon working electrode (3mm dia), Pt wire auxiliary electrode and an Ag/AgCl reference electrode. All solutions were purged with N₂ for 30 min prior to each set of experiments. TBAP was used as the supporting electrolyte. The molar conductance values of the complexes in DMSO solution were measured using a type 305 systronic conductivity bridge. Solutions of CT DNA in 50 mM NaCl /5 mM tris-HCl (pH 7.2) gave a ratio of UV absorbance at 260 and 280 nm, A₂₆₀/A₂₈₀ of around 1.8 – 1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:100 dilutions. The Molar absorption coefficient was taken as 6600 M⁻¹ cm⁻¹. Stock solutions were kept at 4°C and used after no more than 4 days. Doubly distilled water was used to prepare the buffer.

B. Synthesis of Schiff base Ligand ,2-((4,6-dimethoxypyrimidin-2-ylimino)methyl)phenol

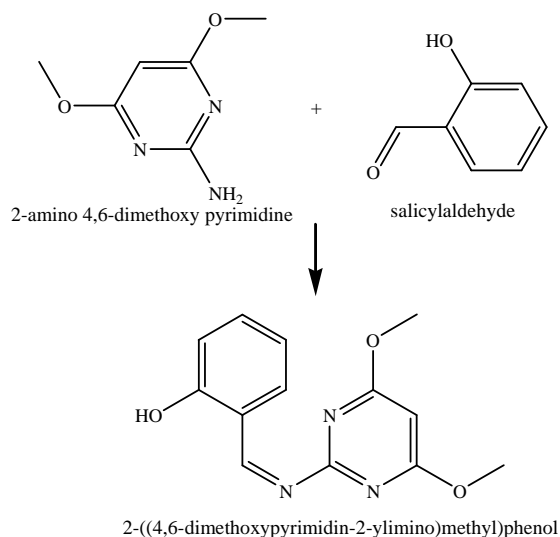
A methanolic solution (20 ml) of 2-amino-4,6-dimethoxypyrimidin (1.55g, 10 mmol) and salicylaldehyde (1.065 ml, 10 mmol) were mixed and refluxed for 5h, the course of reaction was followed by TLC. After the completion of the reaction the resulting yellow colored solution was allowed to evaporate slowly. Yellow solid separates out after complete solvent evaporation. The formed Ligand 2-((4,6-dimethoxypyrimidin-2-ylimino)methyl)phenol was washed with ether to remove excess methanol. The resulting powder was again purified by column chromatography.

C. Synthesis of complexes

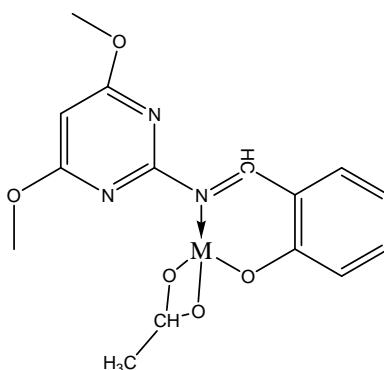
A solution of Schiff base ligand (L), (0.2592g, 1mmol) and a solution of metal salts like $\text{Cu}(\text{Ac})_2$, $\text{Co}(\text{Ac})_2$, $\text{Ni}(\text{Ac})_2$ and $\text{Zn}(\text{Ac})_2$ (1mmol), was refluxed for 6h, the course of the reaction was followed by TLC. After the completion of reaction the resulting reaction mixture was reduced to one third of its volume and the solute on was cooled to room temperature. The resulting solid was washed in ether. The solid was recrystallized from chloroform.

D. Scheme of the Reaction

a) Synthesis of Schiff base ligand



c) Proposed Structure of the complex



Where M = Cu(II), Co(II), Ni(II) and Zn(II)

III. RESULTS AND DISCUSSION

The Ligand and the prepared metal complexes were analyzed using various spectroscopic techniques.

A. Physical Characterization

Physical characterization, analytical and molar conductance studies were carried out (Table I). The complexes show low electrical conductance which reveals that these are non-electrolytes. The elemental analysis confirms the predicted structure of complexes.

B. Infrared Spectra of the Ligand and its Complexes

The free ligand gave broad band in the region of 3200cm^{-1} - 3600cm^{-1} indicating the presence of intramolecular hydrogen bonded $-\text{OH}$ groups. This band disappeared on complexation due to involvement of $-\text{OH}$ group on chelation. The IR spectrum of the ligand shows characteristic $-\text{C}=\text{N}$ bands in the region of 1566cm^{-1} , which is shifted to lower frequencies in all of the complexes, indicating the involvement of $-\text{C}=\text{N}$ nitrogen in coordination to metal ion. Hence from the IR spectra it is evident that ligand acts as a bidentate chelating agent, bonded to metal via azomethine nitrogen and phenolic oxygen atom of Schiff base ligand (L). The presence of new bands in all the complexes at 440cm^{-1} to 750cm^{-1} is probably due to formation of $(\text{M}-\text{O}$ and $\text{M}-\text{N})$ bonds (4) (Table II).

C. Electronic Absorption Spectra

The spectrum displayed high-energy bands at 274nm and 323 nm assignable to $\pi-\pi^*$ transitions corresponding to intraligand charge transfer. The spectrum of the complexes shows bands at 623nm-681nm which is in the region of d-d transitions. The Copper complex shows bands at 677nm assignable to ${}^3\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ corresponding to square planar geometry (5) (Table II).

D. ${}^1\text{H}$ NMR Spectra

The spectra showed $-\text{CH}=\text{N}$ signals at 8.1 ppm (s, 1H), phenyl multiplet at 6.66-7.26 ppm (m, 4H), $-\text{OCH}_3$ signal at 3.71ppm (m, 6H) and free $-\text{OH}$ peak at 5.0ppm (s, H), assigned to the Ligand (L). The azomethine proton of Zn(II) complex is obtained at (8.3ppm) suggesting deshielding of azomethine group due to coordination with metal ion. Also acetate peak appears at 1.8ppm (Table II).

E. Electron Spin resonance Spectra

The X-band powder EPR spectrum of Cu(II) complexes show good resolved nitrogen superfine structure at 77 K in perpendicular region due to interaction of odd electron with nitrogen atoms. The magnetic moment calculated from the spectrum was 1.88 BM for Cu(II) complexes, which corresponds to one unpaired electron, indicating the complex is mononuclear. The observed g-values are $g_{\parallel} > g_{\perp} > 2.0023$, indicating that Cu(II) metal has a $d_{x^2-y^2}$ ground state. The A value was 150G. The observed value for the exchange interaction parameter (G) for copper complex was 6.27 i.e ($G > 4$) suggests that local tetragonal axes are aligned parallel and the unpaired electron is present in the $d_{x^2-y^2}$ orbital and the exchange coupling effects are not operative in the present copper complex (6).

F. Cyclic Voltammetry

The cyclic voltammograms of Cu complex were examined in DMSO and found to be well behaved. The $E_{1/2}$ potentials were estimated from anionic and cathodic peak potentials. The ratio of cathodic to anodic current values indicates that the reaction is quasi-reversible. The data also confirms the redox process is corresponding to the formation of Cu (II)/Cu (I) couple.

G. Mass Spectra

The EI mass spectrum of the ligand and its copper complex were measured in room temperature and the data was used to compare their stoichiometric composition. The Schiff base ligand showed a molecular ion peak at m/z 259, which confirms the existence of odd number of nitrogen atoms in the ligand. The m/z peak of the complex were found to be 383, which is in accordance with the proposed structures.

H. DNA Cleavage Studies.

The cleavage reaction was monitored by gel electrophoresis. The electrophoresis revealed that the Schiff base ligand (L) and its metal complexes have acted on DNA as there was difference in bands of these lanes compared to control DNA, as seen in fig (I). The metal complexes were able to convert supercoiled DNA to open circular

DNA (7). The presence of smear in the gel diagram indicates a radical cleavage. The copper complex have higher cleavage efficiency compared with the other complexes. The DNA cleavage reaction of the complexes probably proceeds through oxidative hydroxyl radical pathway in a similar way proposed by Sigman and co-workers.

I. DNA Binding Studies

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. A variety of small molecules interact reversibly with double stranded DNA, primarily through three modes: (i) electrostatic interactions with negatively charged nucleic sugar –phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interaction with two grooves of DNA double helix and (iii) intercalation between the stacked base pairs of native DNA. Upon addition of CT-DNA, notable hyperchromic shift was observed, as seen in fig (II). The hyperchromic effect, characteristic of intercalation has been attributed to the interaction between electronic states of the compound chromophores and those of DNA bases. Thus the spectroscopic changes suggested that the complexes had strong interaction with DNA (8).

IV. CONCLUSION

A new pyrimidine based Schiff base ligand (L) was derived from the condensation of Salicylaldehyde and 2-amino 4,6-dimethoxy pyrimidine. This ligand was characterized using various spectrochemical techniques like IR, UV-Vis, NMR, ESR, Mass and elemental analysis. The spectral data suggest that the ligand coordinates through N and O donor atoms and hence acts as bidentate site. The complexes were predicted to be square planar in structure. The spectroscopic changes that occur in the binding studies confirm that the complex had strong interaction with DNA.

Figures and Tables

TABLE I

Compounds	Physical Characterization								
	Color	Molecular Formula	Mol. Wt	Mt.Pt °C	Found (Cal)%			MOLAR CONDUCTANCE	
					M	C	H	N	
HL	Yellow	C ₁₃ H ₁₃ N ₃ O ₃	259.2	228	--	63.6 (63.4)	5.5 (5.3)	20.5 (19.0)	--
Cu(OAc)L	Green	C ₁₅ H ₁₅ N ₃ O ₅ Cu	381.5	178	16.6 (16.4)	36.4 (36.5)	2.1 (2.3)	9.5 (9.2)	14.9
Co(OAc)L	Brown	C ₁₅ H ₁₅ N ₃ O ₅ Co	376.2	173	15.6 (15.5)	47.8 (47.7)	4.0 (3.8)	11.1 (11.0)	12.9
Ni(OAc)L	Green	C ₁₅ H ₁₅ N ₃ O ₅ Ni	375.9	180	15.5 (15.6)	47.9 (47.8)	4.2 (4.0)	11.1 (11.0)	11.0
Zn(OAc)L	Yellow	C ₁₅ H ₁₅ N ₃ O ₅ Zn	382.6	220	17.0 (16.9)	47.0 (47.1)	3.9 (4.0)	10.9 (11.0)	32.0

TABLE II

Compounds	Spectral characterization								
	UV-Vis (cm ⁻¹)			IR (cm ⁻¹)				¹ H NMR (ppm)	
	Absorption (cm ⁻¹)	Band Assignment	Geometry	-OH	-C=N	M-N	M-O	C=N	-OH
HL	30959	INCT	--	3200-3500	1566	--	--	8.1	5.0
Cu(OAc)L	14598	² B _{1g} → ² A _{1g}	Square-planar	--	1547	756	462	--	--
Co(OAc)L	14880	¹ A _{1g} → ¹ B _{1g}	Square-planar	--	1549	756	518	--	--
Ni(OAc)L	20040 14771	¹ A _{1g} → ¹ A _{2g} ¹ A _{1g} → ¹ B _{1g}	Square-planar	--	1554	759	441	--	--
Zn(OAc)L		---		--	1543	758	447	8.3	--

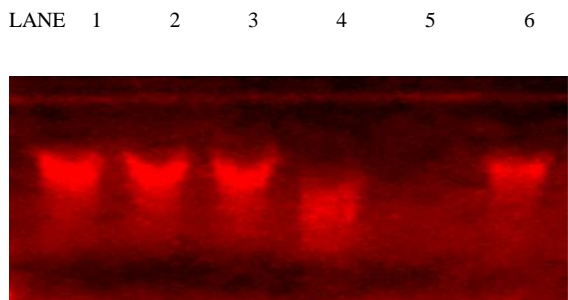


FIGURE 1
DNA CLEAVAGE

LANE 1: DNA alone, LANE 2: DNA + Ligand, LANE 3: DNA+ [CoL] +H₂O₂, LANE 4: DNA + [NiL] + H₂O₂, LANE 5:DNA + [CuL] + H₂O₂, LANE 6 : DNA +[ZnL] + H₂O₂

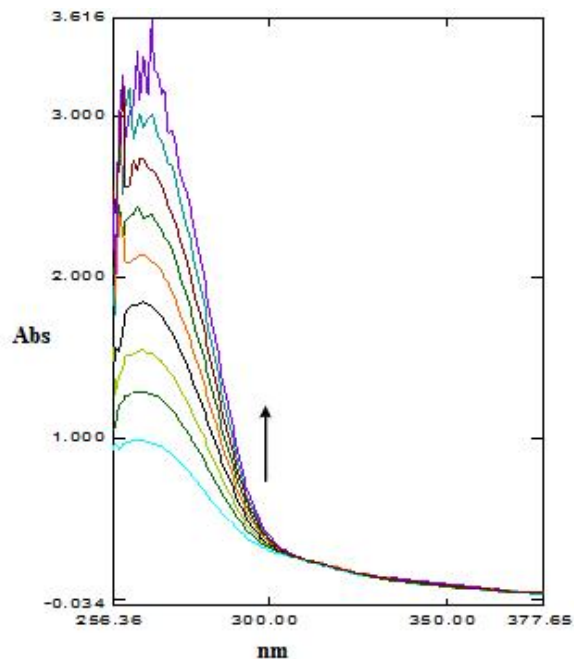


Figure 2: UV spectrum for [Cu(OAc)L] in the presence of CT-DNA

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