Synthesis, Structural Characterization, Antibacterial Activity, DNA Cleavage and Binding Study of Pyrimidine Derivative Schiff Base Metal Complexes

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Abstract—**A new pyrimidine derivative Schiff base has been synthesized from 2-amino-4, 6-dimethylpyrimidine and 5-nitrosalicylaldehyde. Metal complexes of Schiff base have been prepared from acetate salts of Cu (II), Co (II), Ni (II) and Zn (II) and Sciff bases. The structures of the Schiff base ligand and its metal complexes were elucidated by elemental analyses, conductivity measurements, IR, ^{1}H-NMR, UV-Visible, ESR and Mass Spectroscopy. The redox behaviour of metal complexes was studied by cyclic voltammetry. The structural data reveal that the formed complexes have ML(OAc) type composition and the complexes have square planar geometry. The Schiff base and metal complexes have been screened for the antibacterial activity against some bacteria's. The results indicated mild antibacterial activity of some of the complexes. The interaction of the complexes with calf thymus DNA (CT-DNA) has been investigated by UV absorption method and the mode of binding to the complex has been investigated. UV studies of the interaction of the complexes with DNA have shown that they can bind to CT DNA.**

Key words: Pyrimidine derivative, Schiff base, Antibacterial activity

I. INTRODUCTION

Medicinal chemistry concerns with the discovery, development, identification and interpretation of the mode of action of biologically active compounds at molecular level. Synthetic drugs have resulted by simple changes in the structural alignment of a few novel heterocyclic compounds. Pyrimidine was first isolated by Gabriel and Colman in 1899(1). Pyrimidine represents one of the most active class of compounds possessing wide spectrum of biological activity viz. significant in vitro activity against unrelated DNA and RNA, viruses including polio herpes viruses, diuretic, antitumor, anti HIV, cardiovascular(2). The biodynamic property of the pyrimidine ring system prompted us to account for their pharmacological properties as antimicrobials acting against micro organisms (3). 2-amino-4,6-dimethylpyrimidine is the key to understand one step of enzyme reaction activity in the mechanism of the Vitamin B1 and mutation of DNA. The interactions of transition metal complexes with DNA have attracted considerable interest during recent decades. Metal complexes that exhibit interactions with DNA have been studied with the goals of developing both probes for nucleic acid structures and chemotherapy agents (4). Literature survey reveals that Schiff base using 2-amino-4 6-dimethyl pyrimidine and 5-nitrosalicylaldehyde have not yet been synthesised. We report here the synthesis, spectroscopic, antibacterial activity, DNA cleavage and binding studies of Schiff's base and its copper (II), cobalt (II), nickel (II) and Zinc (II) complexes.

II. EXPERIMENTAL

A. Materials and methods

All reagents 2-amino-4, 6-dimethylpyrimidine and 5-nitrosalicylaldehyde 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 N_2 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, A_{260}/A_{280} 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 (L) 2-(4,6-dimethylpyrimidin-2-ylimino)methyl)-4-nitrophenol

An equimolar amount of the methanolic solution (30 mL) solution of 2-amino-4, 6-dimethylpyrimidine and 5-nitrosalicylaldehyde was boiled under reflux for 10-11 h in presence of few drops of acetic acid as catalyst. At the end of the reaction, determined by thin layer chromatography, the reaction mixture was cooled to an ambient temperature. On slow evaporation of the mixture, yellow solid was isolated and washed with distilled water. Then the product was recrystallized with ethanol.

C. Synthesis of metal complexes

An equimolar amount of the methanolic solution (30 mL) of the acetate salts of metal and ligand was boiled under reflux for 7-8 h. The reaction mixture was cooled to room temperature. On slow evaporation of the mixture, the product was isolated and recrystallized with ethanol.

D. Antibacterial Activity

The in vitro biological screening effects of the investigated compounds were tested against the gram positive bacteria like as *Staphylococcus aureus, streptococcus pneumonia, staphylococcus pneumonia, bascillus subtilis* and gram negative bacteria like as *shigella flexneri, salmonella typi, klebsiella pneumonia, haemophyllus influenza* by the well diffusion method, using agar nutrient as the medium. The stock solutions (0.001 M) were prepared by the dissolving the compounds in DMSO. In a typical procedure, a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 h in the case of bacteria. During the period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone developed and the diameter of inhibition zone was measured.

E. DNA Cleavage Experiment

The nuclease activity of present complexes, investigated on CT DNA by agarose gel electrophoresis in the presence of H_2O_2 as oxidant. The gel electrophoresis experiments were performed by incubation at 35℃ for 2h as follows: CT DNA each complex and H_2O_2 in tris-HCl buffer (pH=7.2) were eletrophoresed for 2h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer, pH=8.3. After electrophoresis, the gel was stained using 1µg/cm^3 EB (ethidiumbromide) and photographed under UV light.

F. DNA BindingExperiment

All the experiments involving the interaction of the complexes with calf thymus (CT) DNA were carried out in Tris – HCl buffer at room temperature. Absorption titration experiments were performed by maintaining the metal complex concentration as constant, while measuring the absorption spectra, equal quantity of CT DNA and Tris-HCl buffer was added to both the complex solution and the reference solution to eliminate the absorbance of CT DNA itself. From the absorption data, the intrinsic binding constant K_b was determined from a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] using the following equation

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} + [K_b(\varepsilon_b - \varepsilon_f)]^{-1}$$

Where, [DNA] is the concentration of CT DNA in base pairs. The apparent absorption coefficients ε_a, ε_f and ε_b correspond to $A_{obs.}/[M]$, the extinction coefficient for the free metal (II) complex and extinction coefficient for the metal (II) complex in the fully bound form respectively. K_b is given by the ratio of slope to the intercept.

III. RESULTS AND DISCUSSION

The Schiff base ligand and its Cu(II), Co(II), Ni(II) and Zn(II) complexes have been synthesized and characterized by spectral and elemental analytical data given in Table I. They are found to be air stable and pure in nature. The analytical data of the complexes correspond well with the general formula ML where M=Cu(II), Co(II), Ni(II) and Zn(II) and L=ligand. The molar conductance values clearly indicated non-electrolytic nature of the metal complexes.

A. Electronic Absorption Spectroscopy

The electronic absorption spectra of ligand (L) and its metal complexes, Cu(II), Co(II), Ni(II) and Zn(II) were recorded in DMSO at room temperature. The spectrum displayed high-energy bands at 298nm and 362 nm assignable to π -π^* transitions corresponding to intra ligand charge transfer. The spectrum of the complexes shows bands at 618nm-670nm which is in the region of d-d transitions. The Copper complex shows band at 19685 cm^{-1} assignable to $^2B_1g \rightarrow {}^2A_1g$ corresponding to square planar geometry (5). The absorption data are given in Table II.

B. IR Spectroscopy

The free ligand shows broad $\upsilon_{(OH)}$ band in the region of 3200cm^{-1}-3500 cm^{-1} indicating the presence of intramolecular hydrogen bonded –OH groups. This band disappeared on complexation due to involvement of –OH group on chelation. The IR spectrum of the ligand shows characteristic –CH=N band in the region of 1658cm^{-1}, which is shifted to lower frequencies in all of the complexes (1600-1651 cm^{-1}), indicating the involvement of –CH=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 460 $cm^{-1}$to 680cm^{-1} is probably due to formation of υ (M-O and M-N) bonds(6). The important IR bands of the ligand (L) and its complexes are given in Table II.

TABLE I. Physical characterization, analytical and molar conductance data of the ligand and its complexes

S.No	Compound	Formula	Colour	Molecular Weight (g)	Yield (%)	Melting Point (°C)	C Calculated (Found)	H Calculated (Found)	N Calculated (Found)	M Calculated (Found)	Λ_m (mho,c m^2mol^{-1})
1	HL	$C_{13}H_{12}N_4O_3$	Yellow	272	84	170-172°C	57.34 (57.32)	4.44 (4.40)	20.58 (20.51)	-	-
2	Cu(OAc)L	$C_{15}H_{14}N_4O_5Cu$	Deep Brown	393	93	168-170°C	45.80 (45.78)	3.56 (3.58)	14.25 (14.31)	16.03 (15.98)	17.5
3	Co(OAc)L	$C_{15}H_{14}N_4O_5Co$	Pale Brown	358	83	182-184°C	50.28 (50.30)	3.94 (3.92)	15.64 (15.58)	7.82 (7.85)	31.6
4	Ni(OAc)L	$C_{15}H_{14}N_4O_5Ni$	Pale Green	389	83	166-168°C	46.33 (46.40)	3.60 (3.63)	14.40 (14.37)	15.09 (15.1)	32.6
5	Zn(OAc)L	$C_{15}H_{14}N_4O_5Zn$	Orange	396	75	180-182°C	45.53 (45.50)	3.57 (3.58)	14.16 (14.13)	16.52 (16.43)	21.0

TABLE II. UV-Visible IR and ^{1}H-NMR Spectral Data

S.NO	Compound	UV		IR			^{1}H-NMR		Geometry
		Λ_{max} (cm^{-1})	Band Assignment	$\upsilon_{(C=N)}cm^{-1}$	$\upsilon_{(M-N)}cm^{-1}$	$\upsilon_{(M-O)}cm^{-1}$	-CH=N-	-OH	
1	Ligand	33112 23201	INCT	1658	-	-	8.4	5.7	-
2	Cu(OAc)L	19685	$^2B_1g \rightarrow {}^2A_1g$	1651	671	498	-	-	Square Planar
3	Co(OAc)L	20202	$^1A_{1g} \rightarrow {}^1B_{1g}$	1647	678	462	-	-	Square Planar
4	Ni(OAc)L	20080 14925	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1B_1g$	1643	669	518	-	-	Square Planar
5	Zn(OAc)L	-	-	1600	648	503	9.8	-	Square Planar

C. ^{1}H NMR Spectroscopy

The ^{1}H NMR spectra of the ligand and complexes were recorded in DMSO-d_6. The spectra showed –CH=N signals at 8.4ppm (s, 1H), phenyl multiplet at 6.2-8.2ppm (m, 4H), -CH_3 signal at 2.1ppm (m, 6H) and peak obtained at 5.7ppm (s, 1H) is assigned to free –OH group of the Ligand (L). The absence of phenolic peak at 5.7 ppm in Zn(II) complex indicates that –OH proton of the ligand is involved in chelation with the metal. The azomethine proton of Zn(II) complex is shifted to lower frequency (9.8ppm) suggesting deshielding of azomethine group due to coordination with metal ion. Also acetate peak appears at 1.8ppm (s, 3H). There is no appreciable change in all the other signals of the complex.

D. Mass Spectroscopy

The EI mass spectrum of the ligand and its copper complex was 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 272, which confirmed the existence of odd number of nitrogen atoms in the ligand. And the copper complex showed a molecular ion peak at m/z at 393, which confirmed the molecular formula of the complex, ML(OAc).

E. EPR Spectroscopy

The X-band EPR spectra of copper complex in methanol recorded at 300K and 77K. The spectrum of the complex at 300K shows one intense absorption band in the high field region and is isotropic due to dumpling motion of the molecules. The magnetic moment (1.89 BM) calculated from the equation $\mu_{eff} = g[s(s+1)]^{1/2}$ using the experimental g_{iso} values (2.18) indicating that the solid structure is retained in methanol solution. The EPR spectrum of the copper complex at 77 K indicates a poorly resolved nitrogen super hyperfine structure in the perpendicular region due to the interaction of the Cu (II) odd electron with nitrogen atoms. The copper complex has a magnetic moment 1.89 BM, corresponding to the one unpaired electron, indicating that the complex is mononuclear. The frozen methanol solution is axial with $g_{||} > g_{\perp} > 2.0023$, indicating a $d_{x^2-y^2}$ ground state (7) which is in agreement with the electronic absorption spectroscopic assignments. From the observed values, it is clear that $A_{||}$ (135) $> A_{\perp}$ (26); $g_{||}$ (2.38) $> g_{\perp}$ (2.09) > 2.0023 and the EPR parameters of the complex coincide well with related systems which suggest that the complex has square planar geometry and the system is axially symmetric. This is also supported by the fact the unpaired electron lies predominately in the $d_{x^2-y^2}$ orbital. In the axial spectra the g-values are related with exchange interaction coupling constant (G) by the expression, $G = (g_{||} - 2.0023) / (g_{\perp} - 2.0023)$. According to Hathaway (8), if the G value is larger than four, the exchange interaction is negligible because the local tetragonal axes are aligned parallel or are slightly misaligned. If the G value is less than four, the exchange interaction is considerable and the local tetragonal axes are misaligned. The observed value for the exchange interaction parameter for the copper complex (G=4.3) suggests that the ligand forming complex is regarded as a weak field, and the local tetragonal axes are aligned parallel or slightly misaligned, 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.

F. Cyclic Voltammetry

The cyclic voltammograms of Cu complex (Fig.2) in DMSO exhibit two cathodic waves at -0.2V (E_{pc1}) and at -0.048 (E_{pc2}) followed by two anodic waves at -0.02 (E_{pa1}) and at 0.36 ($_{Epa2}$) found to be well behaved. The data shows well defined redox process (9) corresponding to the formation of Cu (III) → Cu (II) and Cu (II) → Cu (I) couple. Based on the spectral data the following structure given in Fig.1.have been proposed for the ligand and its complexes.

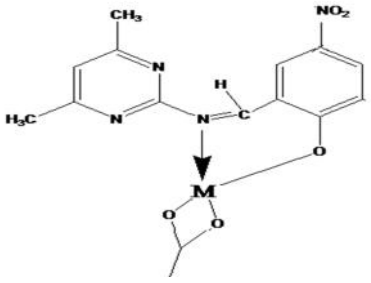

Figure 1: Structure of complexes where M = Cu(II),Co(II),Zn(II) and Ni(II)

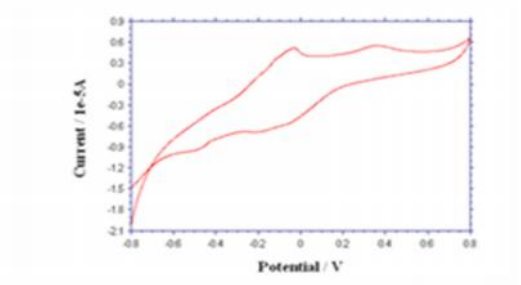

Figure 2. Cyclic Voltammatogram for [CuL(OAc)]

G. Antibacterial Activity

The newly synthesized pyrimidine derivatives were screened for antibacterial activity using Kirby Bauer method against gram positive bacteria like as *staphylococcus aureus, streptococcus pneumonia, staphylococcus pneumonia, bascillus subtilis* and gram negative bactria like as *shigella flexneri, salmonella typhi, klebsiella pneumonia, haemophyllus influenza*. Sparfloxacin was used as the standard. The results indicated mild antibacterial activity of

cobalt complex against *Bascillus subtilis* only. And also there was no antibacterial activity of other complexes in all other bacterium (10).

H. DNA Cleavage Study

The cleavage reaction was monitored by gel electrophoresis. The gel diagram is shown in the figure 3.The results indicated that there was no cleavage in the complexes (11).

I. DNA Binding study

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 posses selectivity; (ii) binding interaction with two grooves of DNA double helix and (iii) intercalation between the stacked base pairs of native DNA (12). Upon addition of CT-DNA, notable hypo chromic shift and hyper chromic shift were observed which is shown in Fig.4. The hypo chromic effect and hyper chromic 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.

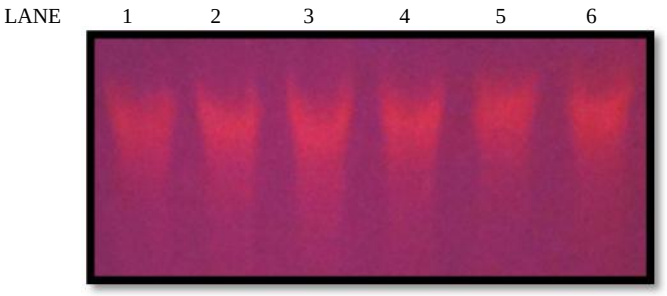

Figure 3.DNA Cleavage activity. LANE 1: DNA alone, LANE 2: DNA + Ligand, LANE 3: DNA+ [Cu(OAc)L] +H_2O_2, LANE 4: DNA + [Zn(OAc)L] + H_2O_2, LANE 5:DNA + [Co(OAc)L] + H_2O_2, LANE 6 : DNA +[Ni(OAc)L] + H_2O_2

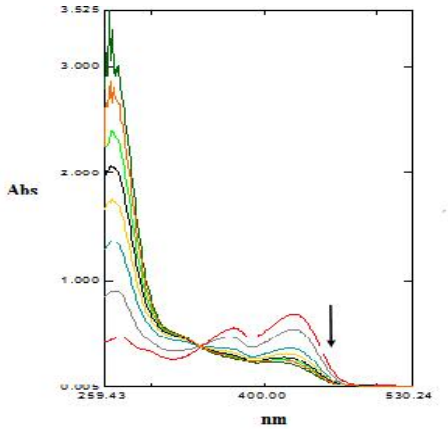

Figure 4 UV spectrum for [Cu(OAc)L] in the presence of CT-DNA

IV. CONCLUSION

A new pyrimidine based Schiff base ligand (L) was derived from the condensation of 5-nitrosalicylaldehyde and 2-amino 4, 6-dimethyl pyrimidine. 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.

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