Synthesis and Spectroscopic studies on Manganese(II), Copper(II), Nickel(II), and Cobalt(II) Complexes derived from L-Valinol and pyridine aldehyde and their Antimicrobial studies

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Abstract- Novel metal complexes of the type$[M(L)_2]$(where M=Mn(II), Cu(II), Ni(II), Co(II); L= Schiff base derived from L-valinol and pyridine aldehyde) have been synthesized and characterized by elemental analysis, molar conductance , magnetic moments, FTIR,, ^1H and ^{13}C NMR and UV-visible techniques. On the basis of spectral studies, octahedral geometry has been assigned for all metal complexes. In vitro antimicrobial activity of ligand and metal complexes were also studied against bacteria (Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmona typhi) and fungi (Candia albicans and Candida parappsilosis) which show antimicrobial activity of ligand and metal complexes. Metal complexes showmore activity against bacteria and fungi as compare to pure ligand

Key Words: Schiff base, metal complexes, spectral studies, antifungal, antibacterial

1 INTRODUCTION

Metallo-drugs are becoming an interesting research area after the discovery of cisplatin[1]. Since then, many complexes have been synthesized and tested on a number of biological systems. New areas of research, which mainly focused on the specific synthesis, highly functional metal based drug complexes, have drawn considerable attention. Schiff bases and their complexes have a variety of applications in biological, clinical, analytical and pharmacological areas[2]. When ligands coordinate to transition metals, it is believed that the selectivity towards certain biological systems is improved[3-5]. The enormous interest in the field of coordination chemistry of transition metal ions with Schiff bases is due to use of these compounds as biological models, as oxygen carriers and as drugs[6]. Recently synthesized Cu(II), Ni(II), Co(II), Zn(II) and Fe(III) complexes show current interest of researchers in the field of coordination chemistry of these metal ions[7]. N and O coordinating ligand and their transition metal complexes have been found to possess important catalytic as well as biological activity[8]. Some other transition metal complexes have also been synthesized recently which show good biological activities *viz.* antimicrobial, toxicity, fluorescence quenching, DNA interaction and antitumor[9-14].In this article, we synthesized novel Schiff base and their metal complexes and were characterized by IR, NMR, Molar conductance, and Magnetic moments, elemental analysis, mass spectrum and UV-visible spectroscopy.

2 EXPERIMENTAL

All chemicals were of A.R. grade. MeOH, EtOH, diethyl ether and metal salts were purchased from Qualigens. 2-pyridine aldehyde and L-Valinol were purchased from (Sigma

Aldrich). All chemicals were used as obtained without further purification. Elemental analysis (C, H, N) were performed using a Carlo Erba 1106 elemental analyzer. Metals and chlorides were determined volumetricall[15] and gravimetrically[16] respectively. IR spectra were recorded using KBr discs (*4000-400* cm^{-1}) on a Shimadzu 8300 IR spectrophotometer. Electronic absorption spectra in the *200-900* nm range were obtained in DMF (10^{-4} M) on a Systronic UV-visible spectrophotometer. Molar conductance measurements were determined in DMSO ($\sim 10^{-3}$ M) at room temperature using a Jenway Model 4070 conductivity meter. Magnetic moment measurements were carried out by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant. ^1H and ^{13}C NMR spectra (at room temperature) (in DMSO-d_6, $\sim 10^{-3}$ M) were recorded on a Bruker AC 700L NMR spectrometer with reference to TMS (tetra methyl silane). Chemical shifts were reported on the δ scale. ESR spectra of Cu(II) complex was recorded as polycrystalline sample in acetonitrile solution, at room temperature on a E-112 ESR spectrophotometer employing DPPH as the g-marker.

2.1 Synthesis of ligand N-(2-Hydroxyethyl)-pyridine-2-aldimine

A mixture of pyridine-2- aldehyde and L-Valinol in 1:1 molar proportion in an alcoholic medium containing a few drops of concentrated HCl was refluxed for 3h. The product was separated , filtered, washed with alcohol and recrystallized from EtOH (Fig 1).

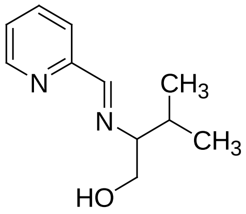


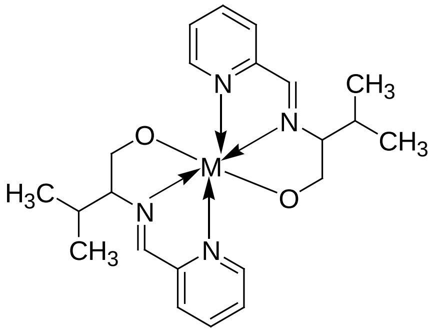
Fig 1. 3-Methyl-2-[(pyridin-2-ylmethylene)-amino]-butan-1-ol

Analytical data of 3-Methyl-2-[(pyridin-2-ylmethylene)-amino]-butan-1-ol

Ligand ($C_{22}H_{28}N_4O_2$): Yield: 52%; M.P. 230 ^{0}C, Mol. wt. 382, color: yellowish; analytical data for LIGAND found (calc.): C, 69.10 (69.24); H, 7.85 (7.92); N, 7.32 (7.43). IR (KBr, cm^{-1}): 3216 ν_{NH}, 1640 $\nu_{C=N}$, 3585 ν_{OH}, 2228 ν_{C-N}. ESI-MS, m/z Data found (calc.): 382.01 (381.11), ^{1}H NMR (DMSO-d_6) δ ppm: 10.4 (s, CH=N), 3.40 (s, CH_2), 3.29(s,Pyr.H),2.70(br,1 H, OH), ^{13}C NMR (DMSO-d_6) δ ppm: 161.4 (CH=N), 65.6 (OCH_2), 189.8(Pyri C).

2.1 Synthesis of Metal Complexes

An alcoholic solution of Schiff base (2mmol) was refluxed with 1mmol of $CoCl_2.6H_2O$/ $NiCl_2.6H_2O$/ $CuCl_2.2H_2O$/$MnCl_2.6H_2O$ in ethanol on water bath for 2 h; few drops of sodium acetate was added and refluxed for 4h. The separated complex was filtered, washed thoroughly with water, ethanol and ether and finally dried in vacuum over fused $CaCl_2$ (Fig2.).



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

Fig 2. Structure of metal complexes

Analytical data of Metal Complexes

Complex 1. ($C_{22}H_{28}CuN_4O_2$): Yield: 50%; M.P. 290 ^{0}C, Mol. wt. 443.54, color: blueish; found (calc.): C, 59.52 (59.61); H, 6.31 (6.43); N, 12.62 (12.69), Cu, 14.37(14.48). IR (KBr, cm^{-1}): 3216 ν_{NH}, 1640 $\nu_{C=N}$, 2228 ν_{C-N} ,465ν_{Cu-N}, 519$\nu_{Cu-O,}$. ESI-MS, m/z Data found (calc.): 443.54 (442.55), ^{1}H NMR (DMSO-d_6) δ ppm: 10.4 (s, CH=N), 3.40 (s, CH_2), 3.29(s,Pyr.H), ^{13}C NMR (DMSO-d_6) δ ppm: 161.4 (CH=N), 65.6 (OCH_2), 189.8(Pyri C)

Complex 2. ($C_{22}H_{28}NiN_4O_2$): Yield: 50%; M.P. 290 ^{0}C, Mol. wt. 438.69, color: light blue; found (calc.): C, 60.17 (60.40); H, 6.38 (6.49); N, 12.76 (12.70), Ni, 13.37(13.45). IR (KBr, cm^{-1}): 3216 ν_{NH}, 1640 $\nu_{C=N}$, 2228 $\nu_{C-N,}$ 467ν_{Ni-N}, 518$\nu_{Ni-O,}$.. ESI-MS, m/z Data found (calc.): 438.69 (437.69), ^{1}H NMR (DMSO-d_6) δ ppm: 10.4 (s, CH=N), 3.40 (s, CH_2), 3.29(s,Pyr.H), ^{13}C NMR (DMSO-d_6) δ ppm: 161.4 (CH=N), 65.6 (OCH_2), 189.8(Pyri C).

Complex 3. ($C_{22}H_{28}MnN_4O_2$): Yield: 45%; M.P. 305 ^{0}C, Mol. wt. 434.93, color: Pinkish; found (calc.): C, 60.69 (60.71); H, 6.43 (6.55); N, 12.87 (12.88), Mn, 12.62(12.69). IR (KBr, cm^{-1}): 3216 ν_{NH}, 1640 $\nu_{C=N}$, 2228 $\nu_{C-N,}$ 467ν_{Mn-N}, 519$\nu_{Mn-O,}$.. ESI-MS, m/z Data found (calc.): 434.93 (433.83), ^{1}H NMR (DMSO-d_6) δ ppm: 10.4 (s, CH=N), 3.40 (s, CH_2), 3.29(s,Pyr.H), ^{13}C NMR (DMSO-d_6) δ ppm: 161.4 (CH=N), 65.6 (OCH_2), 189.8(Pyri C).

Complex 4. ($C_{22}H2_8CoN_4O_2$): Yield: 55%; M.P. 298 ^{0}C, Mol. wt. 438.93, color: pinkish; found (calc.): C, 60.14 (60.80); H, 6.37 (6.40); N,. 12.75(12.79), Co, 13.42(13.52). IR (KBr, cm^{-1}): 3216 ν_{NH}, 1640 $\nu_{C=N}$, 2228 $\nu_{C-N,}$ 464ν_{Co-N}, 520 $\nu_{Co-O,}$.. ESI-MS, m/z Data found (calc.): 438.93 (437.93), ^{1}H NMR (DMSO-d_6) δ ppm: 10.4 (s, CH=N), 3.40 (s, CH_2), 3.29(s,Pyr.H), ^{13}C NMR (DMSO-d_6) δ ppm: 161.4 (CH=N), 65.6 (OCH_2), 189.8(Pyri C).

3 In *Vitro* Antibacterial and Antifungal Studies

Antibacterial and antifungal study of newly synthesized ligand (L) and all metal complexes were done *in vitro* by reported method[15]. The stock solution (*1* mg ml^{-1}) of the test chemical was prepared by dissolving *10* mg of the test chemical in *10* ml of DMSO. The stock solution was suitably diluted with sterilized distilled water to get *500* and *100* µg ml^{-1}. Control for each dilution was prepared by diluting *10* ml of solvent instead of stock solution with sterilized distilled water. All compounds were evaluated for their *in vitro* antibacterial activity against *Bacillus subtilis* and *Escherichia coli* and antifungal activity against *Aspirgillus niger* and *Aspirgillus flavus* by the agar-well diffusion method. Bacteria were inoculated into Nutrient Broth (Difco) and incubated for *30* h and the fungi studied incubated in Malt Extract Broth (Difco) for *54* h. In the agar-well diffusion method, Mueller Hinton Agar (Oxoid) for bacteria and Malt Extract Broth (Difco) sterilized in a flask and cooled to ~ *48* °C was distributed (*20* ml) to sterilized petri dishes after injecting *0.01* ml culture of bacterium prepared as mentioned above and allowed to solidify. The dilution plate method was used to enumerate microorganism (10^5 bacteria per ml and fungi 10^3-10^4 per ml) for *24* h. By using a sterilize cork borer (*7* mm diameter), wells were dug in the culture plates. Compounds dissolved in DMSO were added (*0.2* µ) to these wells. The petri dishes were left at *4* °C for *2* h and then the plates were incubated at *30* °C for bacteria (*24-28* h) and at *25* °C for fungi (*78* h). At the end of the period, inhibition zones formed on the medium were evaluated as millimeters (mm) diameter. Biological activity data of all compounds were expressed as inhibition level % over control calculated from the size of inhibition zone. The percent inhibition was calculated using the formula:

$$\text{Inhibition level, \%} = (C - T)100 / C$$

Where *C* is the diameter of the microbial colony in the control plate and *T* is the diameter of the microbial colony in the tested plate after same incubation period.

4 RESULTS AND DISCUSSION

The newly synthesized ligand and its metal complexes are stable at room temperature in solid state. The ligand is soluble in common organic solvents but metal complexes are soluble in DMF and DMSO. The analytical data are in good agreement with the proposed stoichiometry of the complexes.

4.1 IR Spectral Studies

The IR spectra of ligand shows a sharp band at 3585 cm^{-1} and 1576 cm^{-1} which show the presence of o*f* υ(OH) and υ(C=N) vibrations respectively. In the IR spectra of metal complexes, no υ(OH) vibration was found and υ(C=N) are shifted by 1598-1626 cm^{-1} which shows the coordination of (C=N)[17]. A new band appeared in the range *460-466* cm^{-1} and 515- 530 cm^{-1}may be assigned υ(M-N) and υ(M-O) vibrations respectively[18].

4.2 Electronic Spectra

In the spectrums of UV-Vis spectroscopy, the electronic spectra of Mn(II) complexes show two bands in the regions 22500 - 22650 and 18500 - 18850 cm^{-1} which may be assigned to $^6A_{1g} \rightarrow {}^4T_{2g}$ and $^6A_{1g} \rightarrow {}^4T_1g$ (G) transitions respectively suggesting octahedral environment around the Mn(II) ion[29] . The magnetic moment 4.88 BM is an additional evidence for an octahedral structure. The electronic spectra of the cobalt (II) complex showed three bands at 8780-8810, 17475-17775 and 30235-30270 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4T_{1g}$(P), and $^4T_{1g} \rightarrow {}^3A_{2g}$ (F) transitions, respectively, and suggested octahedral geometry around the cobalt ion.. The electronic spectra of the copper(II) complex display a broad band at 14920 cm^{-1} due to $^2B_{1g} \rightarrow {}^2E_g$ and two bands at 16390 and 27250 cm^{-1} assigned to d–d transitions and a charge transfer band respectively, of an octahedral environment. The moments in such complex, as in apparent, lie appreciably above the spine-only value (1.73 BM), but as the electronic ground states are non-degenerate this cannot arise from inherent angular momentum in the ground state. It arises due to mixing in of some orbital angular momentum from excited states via spin orbit coupling. The copper (II) complex exhibit magnetic moments of 1.78 B.M., respectively, at room temperature. These values are quite close to the spin-allowed values expected for an S =1/2 system and may be indicative of a distorted octahedral geometry around copper (II) ion. The nickel(II) complex was insoluble in common organic solvents and water. The compound was soluble in coordinating solvents, pyridine and DMSO, presumably by displacing water molecules from the coordination sphere. The nickel(II) complex exhibited three bands at 9960-10165, 15850-16155, and 29940-29985 cm^{-1} assignable, respectively to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ($\nu 1$) , $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (P) (ν_3), which are characteristic of nickel(II) in octahedral geometry. Magnetic behavior of octahedral nickel(II) complex is relatively simple. From both the simple 'd' orbital splitting diagram and the energy level diagram, all the octahedral complexes of the divalent nickel should have two unpaired electrons. The experimental magnetic moment values usually lie in rang 2.9 to 3.4 BM, a little higher than spin only value (2.83). The electronic spectra of the cobalt (II) complex showed three bands at 8780-8810, 17475-17775 and 30235-30270 cm^{-1}, which may be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4T_{1g}$(P), and $^4T_{1g} \rightarrow {}^3A_{2g}$ (F) transitions, respectively, and suggested octahedral geometry around the cobalt ion. The cobalt(II) complex shows magnetic moment values[18] of 4.85 B.M. at room temperature. This high value of the magnetic moments and the stoichiometries suggest a coordination number of six for the central cobalt(II) ion and an octahedral geometry.

4.3 ^{1}H and ^{13}C NMR Spectra

^{1}H- NMR and ^{13}C-NMR of ligand in DMSO-d_6 solution show that they are NMR active. The ^{1}H-NMR spectrum of free ligand show a singlet at 10.4 ppm due to imine proton , 3.40 ppm due to CH_2 proton, 3.29 ppm due to pyridine proton respectively. ^{13}C-NMR spectrum of free ligand show at 161.4 ppm in the range due to imine carbon ,65.6 ppm due to OCH_2 and 189.8 ppm due to pyridine carbon. Thus, ^{1}H- and ^{13}C- NMR spectral data support proposed structure of ligand and metal complexex and as well coordination behavior of ligand.

4.4 Molar Conductance Measurement

The molar conductance (Λ_M) values of all metal complexes were measured in DMSO and non electrolyte nature[19].

4.5 ESR spectrum of Cu(II) metal complex

The ESR spectrum of Cu(II) complex shows $g_{\parallel}$ and $g_{\perp}$ values are 2.02 and 2.16 with g av calculated to be 2.11. The Cu(II) complex, shows a compressed octahedron with $g_{\parallel} < g_{\perp}$ indicating the electron is delocalized in the dz^2. The parameter G, determined as G =($g_{\parallel}$ -2/ $g_{\perp}$- 2) is.126 suggesting considerable interaction in the solid state.

4.6 In *vitro* antimicrobial activity

In vitro antimicrobial activity of newly synthesized ligand and metal complexes have been tested against the bacteria *Bacillus subtilis* and *Escherichia coli* and fungi *Aspirgillus niger* and *Aspirgillus flavus* and are summarized in Table1.

Table 1. Antibacterial and antifungal activity data of ligand and metal complexes (% inhibition)

Compound	*Conc.	a	b	C	d
L	*100*	*40*	*51*	*62*	*65*
	500	*48*	*58*	*74*	*78*
$[Cu(L)_2]$	*100*	*48*	*54*	*71*	*72*
	500	*53*	*55*	*83*	*85*
$[Co(L)_2]$	*100*	*52*	*73*	*82*	*80*
	500	*61*	*82*	*89*	*86*
[Ni(L)2]	*100*	*46*	*59*	*69*	*70*
	500	*52*	*64*	*77*	*79*
$[Mn(L)_2]$	*100*	*51*	*70*	*81*	*78*
	500	*61*	*82*	*89*	*86*

* = (*μg ml-1*), a = *Bacillus subtilis*, b = *Escherichia coli*, c = *Aspirgillus niger* , d = *Aspirgillus flavus.*

The values indicate that all complexes have higher antimicrobial activity than the free ligand. Among all tested metal complexes Co(II) metal complex show highest biological activity against all microbes. Such increased activity of the metal chelates can be explained on the basis of chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhance the penetration of the complexes into lipid membranes and blocking of the metal binding sites in enzymes of microorganism. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of microorganism[20].

5 Conclusions

Newly synthesized Ligand and all the metal complexes have been characterized by analytical and spectral studies. Octahedral geometry and non electrolyte nature has been assigned to all metal complexes. Biological investigations show that metal complexes are more biologically active than ligand.

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