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Synthesis, Characterization and Antibacterial Activity of Copper(II) and Cobalt(II) Vanillin-Aniline Schiff base Complexes

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Abstract

Two novel Copper (II) and Cobalt (II) complexes of aniline-vanillin schiff base were synthesized, characterized and examined for their antibacterial activity. The structures and geometry of schiff base and complexes were determined on the basis of elemental analysis (H, C and N), ¹H NMR, ¹³C NMR, FT-IR spectra, AAS and magnetic susceptibility measurements. The electronic spectra of Cobalt(II) complex exhibited band at 438nm due to (d-d) electronic transition type ${}^{4}T_{1}g \rightarrow {}^{4}T_{1}g$ (F) suggesting a high spin octahedral geometry whereas the electronic spectra of Copper(II) complex exhibited band at 404nm and 533nm due to LMCT and (d-d) electronic

transition ${}^{3}A_{2}g^{-}{}^{3}T_{2}g$ type, respectively, suggesting a low spin a distorted octahedral geometry. The synthesized schiff base, Copper (II) and Cobalt (II) complexes were screened for their antibacterial activity against *Escherichia coli*, *S.aureus*, *K.pneumonia* and *Protein mirabilis*. The schiff base showed better activity (17mm zone of inhibition) towards *K. pneumonia* compared to that of gentamicine (15mm zone of inhibition) whereas both the schiff base and Copper (II) complex displayed promising activity (12 and 13mm zone of inhibition, respectively) against *E. coli* compared to that of gentamicine (14mm zone of inhibition). **Keywords**: Antibacterial activity, synthesis, schiff base, vanillin, metal complexes

1. INTRODUCTION

The development of metal based drugs with pharmacological potential have gained much attention in recent years [1]. The thermodynamic and kinetic properties of the complexes govern the mode of biological action of the biometals. Sometimes, the lipophilicity of drugs increased through the formation of chelates *in vivo* and the drug action is significantly increased due to much more effective penetration of the drug into the site of action. It has also been proved that complexation of metal ions with antibiotics enhance the pharmacological activity as compared to that of the free ligands [2, 3].

Copper complexes have been given much attention in coordination chemistry, technical application, catalysis, spectroscopic properties, anion selectivity and their biological significance [4,5]. Co (II) based ligand complexes have been found to possess various pharmacological activity [6,7].

The synthesis of two novel Cu(II) and Cu(II) complexes of vaniline-aniline schiff base. The structures of the schiff base and complexes were determined by elemental analyses, Infra-red (IR), UV-Visible (UV-Vis), NMR and magnetic susceptibility measurements. The antibacterial activity of the schiff base and complexes were screened on selected strains of microorganisms.

2. EXPERIMENTAL

2.1 General

The UV-Vis spectra of compounds were recorded by UV-Vis Spectrophotometer (UV-950, Shimadzu). The FT-IR spectra were record by a Perkin-Elmer FT-IR 1650 spectrophotometer in wave number region of 4000-400 cm⁻¹ as KBr pellet. The NMR spectra was recorded using Bruker avance 400MHz using DMSO- d_6 as solvent and tetramethylsilane (TMS) as an internal standard. The magnetic susceptibility data were measured by (MSB-AUTO, (Sherwood scientific) magnetic balance). The UV-Visible spectra of the complexes was recorded on Shimadzu UV-1700 spectrometer in the range of 220-600nm in DMF solution. The purity of the schiff base and complexes were monitored by thin layer chromatography using pre-coated aluminum-backed supported silica gel 60 F254 (0.2 mm thickness) and glass supported silica gel 60 F254 (1.0 mm thickness), respectively.TLC visualization was realized by UV-light (254 and 366nm).

2.2 Chemicals and reagents

Analytical grade reagents and chemicals were used without further purification. The reagents and chemicals used in this project include aniline, vanillin, 35% HCl, glacial acetic acid, NaOH, silca gel, metal salts such $(CoCl_2.6H_2O \text{ and } Cu(CH_3COO)_2.H_2O)$ and solvents (DMSO, distilled water, ethanol, methanol, n-hexane, ethyl acetate, dichloromethane and diethyl ether from Aldrich).

2.3 Synthesis of schiff base ligand

Equimolar ratio of vanillin (3.04g, 20mmol) was mixed with aniline (1.86g, 20mmol) in 40ml ethanol and a few

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drops of glacial acetic acid were added. The reaction mixture was refluxed for 6h at 60° c (scheme 1). A yellow precipitate was obtained. This precipitate was filtered and washed with n-hexane and diethyl ether and dried in vacuum desiccators overnight. The purity was checked by TLC (30% ethyl acetate in n-hexane, R_f = 0.6) and weighed (81% yield).

2.4 General procedure for the Synthesis of Cobalt (II) complex

Co(II) complex of vanillin-aniline schiff base was synthesized by mixing 25ml of ethanolic solution of schiff base (1.36g, 6mmol) with 25ml ethanolic solution of CoCl₂.6H₂O (0.714g, 3mmol) metal (II) salt keeping ligand-metal ratio (2:1) and a few drops of glacial acetic acid were added. The reaction mixture was refluxed for 4h on water bath until brown precipitate was formed. The precipitate formed (Co⁺²-brown) was filtered and washed with distilled water and n-hexane and dried in desiccator over night (scheme 2).

2.5 General procedure for the synthesis of Copper(II) complex

Cu(II) complex was prepared by mixing the ethanolic solution of Cu(CH₃COO)₂.H₂O (0.59g, 3mmol) and ligand (1.36g, 6mmol) in 200ml beaker keeping 1:2 ratio of metal: ligand and a few drops of glacial acetic acid was added. The solution was refluxed in water bath for 6h at 75°C. A light green precipitate formed was filtered and washed with distilled water and n-hexane. Finally, the precipitate was dried in desiccators overnight and recrystallized from ethanol (scheme 3).

2.6 Chloride test for Cobalt(II) complex

Co(II) complex (15mg) was digested in concentrated nitric acid and diluted. To the resultant solution of cobalt (II) complex aqueous solution of AgNO₃ was added. Formation of a white precipitate suggested the presence of free chloride ions in the compound.

2.7 Elemental analysis of schiff base and its metal complexes

Elemental analysis data was applied to determined the percentage composition of C,H,N and metal ions found in the synthesized Schiff base as well as the complexes. The elemental analysis data was recorded in EA 1112 Flash CHNS/O- analyzer in conditions of carrier gas flow rate of 120 ml/min, reference flow rate 100 ml/min, oxygen flow rate 250 ml/min; furnace temperature of 900 °C and oven temperature of 75 °C. Four calibration points for every component and samples were run in duplicate and the average values are to be taken.

2.8 Magnetic susceptibility

Magnetic susceptibility measurement of the powdered Co(II) and Cu(II) complexes was recorded at 21°C and using (MSB-AUTO, (Sherwood scientific) magnetic balance) model; 7550, Hg [Co (SCN)₄] as the standard. The molar susceptibility, χ_m , is calculated by:

 $\chi_m = \chi$ (F.W.in g mol-1) and has c.g.s. units of erg•G⁻² mol⁻¹.

The values for effective magnetic moment μ_{eff} of Co (II) and Cu (II) complexes under investigation were calculated from the equation given below:

 $\mu_{\rm eff} = 2.828(\chi AT)^{1/2}$

Where T is temperature in Kelvin scale.

The measured μ_{eff} can be compared to the calculated value, μs , from the spin-only formula. The diamagnetism factors for common ligands and ions as well as the Pascal constants used to calculate the diamagnetic corrections for complex ligands can be found:

$$\boldsymbol{\mu_{eff}} = (8 \, \chi_{\rm corr} T)^{1/2}$$

In addition, using the value obtained for the effective magnetic moment of each complex, the number of unpaired electrons present in the metal ions was also calculated by using the relation:

 $\mu_{eff} = [n (n+1)]^{1/2}$

Where n is number of unpaired electron.

However, the diamagnetic contribution is always negative; the corrected molar susceptibility should always be greater than the uncorrected value [8].

2.9 Molecular weight determination (Rast's method)

In this method 0.1g of the ligand was mixed with 1g of camphor. The mixture was ground and melted in a crucible to ensure homogeneity. The mixture was taken in a capillary tube sealed at one end and the freezing point of the molten mixture was determined using melting point apparatus. Then $\Delta T_{f's}$ was noted and molecular weight of compound (Ligand) was calculated from the Rast's formula as follows:

 $M = K_f x w x 1000 \Delta T_{f's} x W$

Where: $K_f =$ Molecular depression (Cryoscopic) constant for campbor (39.7)

w = Weight of unknown compound (ligand), W= Weight of camphor

ΔT = Depression of melting point

2.10 Antibacterial activity

The antibacterial activity of the free ligand and complexes were examined against three gram-negative bacteria i.e. *Klebsola pneumonia, Proteins mirabilis, Escherichia coli* and one Gram-positive bacterium *S.eureus*, by agar disc diffusion method at concentration of 0.05 mg/mL. The bacterial strains were obtained from oromia public health research capacity building and quality assurance laboratory center. The bacterial cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37°C. Inoculated medium containing 24h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. This solution was poured (25mL in each disc) and then allowed to attain room temperature. Wells (6mm in diameter) were punched carefully using a sterile cork borer and were filled with test solution 200μ L. The plate was allowed to stand for an hour in order to facilitate the diffusion of the drug solution, and incubated at 37°C for 24h and the diameter of the zone of inhibition was measured [9]. The results were compared with that of standard drug gentamicin under identical conditions.

3. RESULTS AND DISCUSSION

The Co(II) and Cu(II) complexes were obtained with moderate yields (53-57%, table 1). The complexes vary in color depending on metal ions and are soluble in most organic solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), methanol, ethanol, acetone, dichloromethane and distilled water, but insoluble in n-hexane and diethyl ether. The melting points of the free ligand and its metal complexes was found to be within the range of 134-280°C. Elemental analyses revealed the observed and calculated values for hydrogen, carbon and nitrogen compositions of the schiff base and its metal complexes are in good agreement (table 1).

3.1 Characterization of free ligand

The ¹H NMR spectrum of the free ligand (DMSO- d_6) revealed singlet peaks at δ_H 3.8, 9.8 and δ_H 8.5 suggesting the presence of methoxy, hydroxyl moiety and proton of CH=N group, respectively. The presence of five aromatic protons at δ_H 7.3 suggest a monosubstituted phenyl ring, the presence of ABX spin pattern at δ_H 7.1 (*d*, J=1.2), 7.65 (*dd*, J=1.2, 8.0) and 6.95 (*d*, J=8.0, table 2) suggest a trisubstituted phenyl ring. The above two informations clearly suggest that the first moiety (monosubstituted phenyl ring) and the second moiety (trisubstituted phenyl ring) are from the aniline and vanillin counterpart, respectively. The ¹³C NMR spectrum of the free ligand revealed peaks at δ_C 160.5, and δ_C 152.4 and 150.7 attributed to carbon atom of azomethine (N=CH) group and sp²oxygenated quaternary aromatic carbons where a hydroxyl and methoxy groups are attached, respectively. The signal appearing δ_C 148.5 belongs to quaternary aromatic carbon (C-N) group where the nitrogen is attached. Eight aromatic methines were clearly evident between δ_C 129-110.9. The presence of peak at δ_C 56confirms the presence of methoxy group in the Schiff base ligand (table 2).

3.2 Characterization of metal complexes

The IR spectrum of free ligand was compared with that of complexes in order to determine the co-ordination sites. The bands had been observed at 3050-3090cm⁻¹ and 2953-2935 cm⁻¹ in the spectra of both Schiff bases and Co (II) and Cu (II) complexes suggesting hydroxyl group and aliphatic sp3 C-H vibrations, respectively. There is significant shift observed for the vibration of the hydroxyl group in the complexes as pet the presence of broad peaks at 3600-3200cm⁻¹ and 3421-3176cm⁻¹ for Co (II) and Cu (II) complexes, respectively. This suggests that the oxygen atom of the hydroxyl group does not participate in coordination. A very weak intensity bands had been observed at 3090cm⁻¹ in the spectra of Schiff base ligand and 3088 for Co (II) complex and 3050 cm⁻¹ for Cu (II) complexes are due to hydroxyl group. Similarly, the presence of aliphatic sp³ C-H is evident from the vibrations at 2935 cm⁻¹, 2953 cm⁻¹ and 2950 cm⁻¹ for free ligand, Co(II) and Cu(II) complexes, respectively. Another strong bands appeared at 1585 cm⁻¹ in the spectra of Schiff base ligand which is a characteristic of

Another strong bands appeared at 1585 cm⁻¹ in the spectra of Schiff base ligand which is a characteristic of azomethine v(C=N) stretching vibration. This vibration underwent a bath chromic shift to lower and higher frequency region at 1583cm⁻¹ and 1646cm⁻¹ upon complexation with Co (II) and Cu (II), respectively (table 3). This is attributed to the bonding affinity of nitrogen atom of the azomethine group of the Schiff base to the respective metal ions directly linked to the donation of electrons from nitrogen to the empty d-orbital's of the metal atom .The presence of aromatic rings has been identified by the characteristic ring vibrations of sp² CH of aromatic ring at 1500cm⁻¹ regions. The strong intensity bands had been observed at 1428 cm⁻¹ in the spectra of the Schiff base ligand which indicates v(C-O) stretching frequencies which has been shifted towards the lower frequency region around 1284 and 1304cm⁻¹ in the spectra of Co (II) and Cu (II) complex, respectively. This suggest that the coordination of the metal ion with oxygen atom of the aromatic group of the free ligand, which has been shifted to 1515cm⁻¹ and 1585cm⁻¹ for Co(II) and Cu(II) complex, respectively. The appearance of new band between 552-510 cm⁻¹ and 492-483 cm⁻¹are indicate the formation of M-N and M-O bond in the complexes

[10].

The magnetic moments of the complexes which were measured at (21°c) are noted in (table 4). In the case of Co (II) complexes, the high spin and low spin μ_{so} value should be 3.88 BM and 1.73 BM, respectively. The Co (II) complex μ_{obs} value was 4.83 BM. This result confirms that the Co (II) complex should be high spin complex with 3 unpaired electrons and paramagnetic. In the case of Cu (II) complex, μ_{so} value should be 1.73 BM with one unpaired electron. The Cu (II) complex observed μ_{obs} value was 1.69BM. This result confirms that the Cu (II) complex should be low spin complex with one unpaired electrons and paramagnetic. From the calculated magnetic moment values and literature data, the expected geometry was octahedral and distorted octahedral geometry for Co (II) complex and Cu (II) complex, respectively. The high observed spin values indicates spin-exchange interaction between metal ions and spin orbital coupling [11].

The electronic absorption spectra (table 5) of schiff base and its metal (II) complexes were recorded in the range of 600nm to 220nm at room temperature. Three absorption bands were observed in the spectra of free ligand which can be assigned $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. These transitions can be shifted towards lower and higher frequencies due to the coordination of the Schiff base to the metal ions [10]. The λ_{max} observed at 313nm and 280nm can be attributed to $n \rightarrow \pi^*$ transitions of C=N and C-O chromophores. The λ_{max} observed at 231nm can be attributed to $\pi \rightarrow \pi^*$ transition of C=C chromophore. The electronic spectra of Co(II) complex exhibited band at 438nm, which were assigned to the transition ${}^{4}T_{1g} \rightarrow {}^{4}T_{1g}$ (F) for a high spin octahedral geometry [12]. The band at 231nm (43,290cm⁻¹), 285nm (34722cm⁻¹) and 328nm (30395cm⁻¹) was assigned to charge transfer transitions. The magnetic susceptibility measurements (4.83 B.M) for Co(II) complex (fig 1) are also three unpaired electrons per Co(II) ion consistent with their octahedral environment [12]. The electronic spectra of Cu(II) complex (fig 2) exhibited band at λ_{max} 404nm due to LMCT and spectra at 533nm which were assigned

to the d-d transition ${}^{3}A_{2}g \xrightarrow{\rightarrow} {}^{3}T_{2}g$ for a low spin distorted octahedral geometry. The band s at λ_{max} 232nm, 285nm and 318nm were assigned to internal charge transfer of the Schiff base. The magnetic susceptibility measurements (1.69 B.M) for Cu(II) complex are also one unpaired electrons per Cu(II) ion consistent with their distorted octahedral environment.

3.3 Antibacterial activity

The schiff base, Co(II) and Cu(II) complexes were found with promising antibacterial activity against four selected pathogenic bacterial strains (*S.eureus, E.coli, K.Pneumonia* and *Protein mirabilis*). However, the Schiff base ligand showed more activity (17mm zone of inhibition) towards *K. pneumonia* when compared to that of gentomicine (15mm zone of inhibition). The lower antibacterial activity of metal complexes may be due to strong interaction between the imine moieties and the metal ions which reduces the activity of imine moieties toward inhibition of bacterial activities (fig 3)[13]. Both the free ligand (12mm zone of inhibition) and Cu(II) complex showed promising activity (13mm zone of inhibition) against *E. coli* compared to that of gentamicine (14mm zone of inhibition).

4. CONCLUSIONS

A novel Schiff base ligand was synthesized by the reaction of aniline and vanillin in 1:1 molar ratio with a molecular formula of $C_{14}H_{13}NO_2$. The complexes of Co (II) and Cu (II) were synthesized by direct reaction of the synthesized ligand with CoCl₂.6H₂O and Cu (CH₂COO) ₂.H₂O metal salts both in 2:1 ratio. The synthesized ligand and its metal complexes were fully characterized by FT-IR, UV-Vis, ¹HNMR, ¹³C NMR, Elemental analyzer, AAS and magnetic susceptibility measurements. From the electronic spectrum and magnetic moment data it can be showed that the Co (II) and Cu (II) complexes formed have octahedral and distorted octahedral geometry. The IR spectra of Schiff base and its metal complexes imply that the Schiff base ligand behaves as basic bidentate ligand coordination through the Azomethine nitrogen atom and methoxy of oxygen atom. The fifth and sixth coordination sites being occupied by chloride and OA_C. The screening of biological activities of ligand and it complexes were conducted against four bacteria (*S.eureus, E.coli, and K.Pneumonia* and *Protein mirabilis*). Generally, the Schiff base showed a very promising activity (17mm) against *K.pneumonia* compared to that standard (15mm) and the Cu (II) complex also showed promising activity (13mm) against E. coli compared to that of standard drug (14mm).

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Scheme 1: Synthesis of schiff base from vanillin and aniline



Scheme 2: Synthesis of Cobalt (II) schiff base complex



Scheme 3: Synthesis of Copper(II) schiff base complex

							Elemental	Analysis 9	6
Compound	Mol.Wt.	Color	Yield	MPt	Solubility		Found(C	Calc.)	
			%	(°C)		С	Н	N	Metal
$L=C_{14}H_{13}NO_2$	227	Yellow	84	280	DMSO,EtOH,	74.008	5.727	6.167	-
					MeOH,CHCl ₃	(73.18)	(5.76)	(6.01)	-
$[Co(L)_2]$	619	Brown	57	134	DMSO,EtOH,	54.28	6.14	4.52	9.21
					MeOH,CHCl ₃	(53.92)	(6.26)	(4.48)	
$[Cu(L)_2]$	651	Light	53	145	DMSO,EtOH,	58.98	5.22	4.30	9.37
		green			MeOH,CHCl ₃	(57.68)	(4.93)	(4.02)	

Table 1 Dbr	voicel magnetice	of ashiff base	Common(II)	and Coholt(II)	acomplance
Table L. Phy	vsical propernes	of schift dase.	CODDer(II) a	па соранси) complexes
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Table 2. ¹H NMR and ¹³C NMR spectra data of schiff base.

Position	¹³ C-NMR	¹ H-NMR (δ,ppm)
1	152.43	9.95 (OH)
2	150.8	-
3	110.9	7.1 (<i>d</i> , J= 1.2)
4	129.0	-
5	115.8	7.65 (<i>dd</i> , J= 1.2, 8.0)
6	124.7	6.95 (<i>d</i> , J= 8.0)
7	160.6	8.4 (-N=CH)-
2-OCH ₃	56.0	3.8
1'	148.5	7.30
2',6'	129.5	7.30
3', 5'	121.3	7.30
4'	128.7	7.30

Table 3. IR spectra data of metal complexes

		1 4010	5. m speed	ra aata or n	ietai eompiene			
Compound	v(C=N) cm ⁻¹	v(O-H) cm ⁻¹	v(C=C) cm ⁻¹	ν(C-O) cm ⁻¹	v(C-H) Aromatic stretching cm ⁻¹	v(C-H) Aliphatic stretching cm ⁻¹	v(M-O) cm ⁻¹	v(M-N) cm ⁻¹
HL	1585	3413	1516	1428	3090	2935	-	-
$\begin{array}{l} Co(L_2\text{-}L_2)X_2\\ Cu(L_2\text{-}L_2)X_2 \end{array}$	1583 1646	- 3421- 3176	1515 1585	1284 1304	3088 3050	2953 2950	552 510	492 483
		5170						

rable 4. Magnetic susceptionity and magnetic moment data							
Compound	M.wt (g.mol ⁻¹) Gram		Molar conductivity(X_{mol})	Diamagnetic	$\mu_{\rm eff}$		
		Susceptibility	(Cgs)	correction	(BM)		
		(Cgs)		(XD) Cgs			
$Co(L-L)X_2$	619	1.606 x10 ⁻⁵	9.94 x10 ⁻³	9.92 x10 ⁻³	4.83		
$Cu(L-L)X_2$	651	1.855 x 10 ⁻⁶	1.22×10^{-3}	1.21 x 10 ⁻³	1.69		

Table 4. Magnetic susceptibility and magnetic moment data

Table 5. Electronic spectra data of schiff base and metal complexes

S No.	Compound	λ_{max} (nm)	Assignments	μ_{eff} (B.M)
	$L=C_{14}H_{13}NO_2$	231	$\pi \rightarrow \pi^*$	
1		280	$\pi \rightarrow \pi^*$	-
		313	$n \rightarrow \pi^*$	
	$[Co(L)_2]$	231	INCT	
2		285	INCT	
		328	INCT	4.83
		438	${}^{4}T_{1}g \rightarrow {}^{4}T_{1}g (F)$	
	$[Cu(L)_2]$	232	INCT	
3		285	INCT	
		318	INCT	1.69
		404	LMCT	
		533	$^{3}A_{2}g \xrightarrow{3} T_{2}g$	

Table 2. Antibacterial activities of the synthesized schiff base and its metal complexes.

S.No	Compound	Inhibition zone				
		Gram +ve	Gram –ve			
		S.eureus	<i>E.coli</i> (mm) K.pneumonia		Protein mirabilis	
		(mm)		(mm)	(mm)	
1	HL_1	9	12	17	10	
2	$Co[L_1-L_1]X_2$	13	6	8	13	
3	$Cu[L_1 L_1]X2$	8	13	8	10	
7	Methanol(control)	-	-	-	-	
8	Gentamicine	21	14	15	20	



Fig 1. Propose structure of Cobalt (II) complexes



Fig 2. Propose structure of Copper (II) complex



Fig 3: The antibacterial activity of schiff base and metal complexes