

SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF THE 4-CHLORO-N'-(4-METHOXYBENZYLIDENE)-BENZOHYDRAZIDE SCHIFF BASE PROMOTED BY METAL(II) IONS

ABSTRACT

Metal complexes of novel Schiff base (HL) ligand, prepared via condensation of 4-chlorobenzohydrazide and 4-methoxybenzaldehyde, are synthesized. The ligand is characterized based on elemental analysis, mass, IR and ^1H NMR spectra. Metal complexes are reported and characterized based on elemental analyses, IR, ^1H NMR, UV-visible spectroscopy, magnetic moment, molar conductance, ESR spectra and thermal analyses. From the elemental analyses, 1:2 [M]:[ligand] complexes are prepared with the general formulae $[\text{M}(\text{HL})_2 \text{Cl}_2]$; (M = Co(II), Ni(II) and Cu(II)). The molar conductance data reveal that all the metal complexes are non-electrolytes. IR spectra show that HL is coordinated to the metal ions in a bidentate manner with NO donor sites of the azomethine-N, and carboxylic-O atom. From the magnetic and UV-visible spectra, it is found that the geometrical structures of these complexes are octahedral. The synthesized ligand, in comparison to its metal complexes is screened for its antibacterial activity against bacterial species. The activity data show that the metal complexes to be more potent/antimicrobial than the parent Schiff base ligand against one or more bacterial species.

Key Words: Schiff Base, Spectral studies and Biological properties

INTRODUCTION

There is a continuing interest in metal complexes of Schiff bases. Because of the presence of both hard nitrogen or oxygen donor atoms in the backbones of these types ligands. They readily coordinate with a wide range of transition metal ions yielding stable and intensely coloured metal complexes, some of which have been shown to exhibit interesting physical and chemical properties [1] and potentially useful biological activities [2]. Many reports are available for the preparation and properties of model copper complexes which mimic copper-containing metalloproteins such as hemocyanine and tyrosinase. Two noticeable properties of copper proteins are an intense absorption band [3] near 600 nm and relatively high copper(II)/copper(I) reduction potentials [1]. Attention was particularly focused on their correlation with the active site of metalloenzymes and metalloproteins containing dinuclear metalcenters in order to elucidate the factors that determine the reversible binding and activation of O_2 in various natural oxygen transport systems and mono- and dioxygenases and to mimic their activity [3]. Schiff bases [4] were still regarded as one of the most potential group of chelators for facile preparations of metallo-organic hybrid materials. In the past two decades, the properties of Schiff base metal complexes stimulated much interest for their noteworthy contributions to single molecule-based magnetism, material science [5], catalysis of many reactions like carbonylation, hydroformylation, oxidation, reduction and epoxidation [6], their industrial applications [7], complexing ability towards some toxic metals [8]. The interest in Schiff base compounds as analytical reagents is increasing since they enable simple and unexpensive determinations of different organic and inorganic substances [9]. The high affinity for the coordination of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes.

In this article, we synthesized the cobalt, nickel and copper metal(II) complexes containing Schiff base ligand. All the compounds were characterized by essential spectral and physicochemical studies. Antimicrobial studies have also been performed for all compounds.

2. EXPERIMENTAL

Materials and reagents

All the chemicals used in the present investigations were of the analytical reagent grade (AR). 4-chlorobenzohydrazide and 4-methoxybenzaldehyde (Sigma), metal salts and solvents were purchased from Qualigens Chemicals Company. They were used as

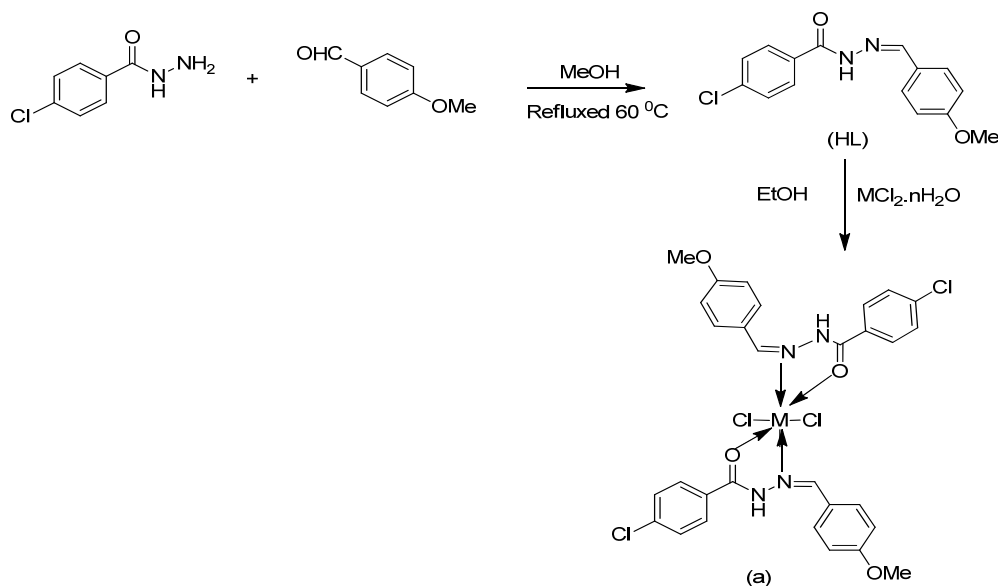
received. The elemental analysis (C, H, N) were performed using a Carlo-Erba 1106 Elemental Analyzer, IR spectra were recorded on a Shimadzu-160 Spectrometer using KBr discs in the range 4000-400 cm^{-1} . Electronic spectra were recorded on a Shimadzu-160 Spectrometer. The ^1H NMR spectra were obtained on a Bruker DPX-400 Spectrometer using DMSO-d_6 solvent and TMS as the internal reference at room temperature. The EPR spectra of the complexes were recorded as polycrystalline sample on a Varian E-4 EPR Spectrometer.

Synthesis of Schiff base

The reaction mixture of 4-chlorobenzohydrazide (0.01 mmol, 1.70 g) and 4-methoxybenzaldehyde (0.01 mmol, 1.36 mL) in methanol (20 mL) were heat (50 $^{\circ}\text{C}$) and stirring about 2 hrs. The resulting solution was cooled at room temperature and after the completion of this reaction; the reaction mixture was monitored by thin layer chromatography. The crude yellowish product was obtained, taken in chloroform and washed with water (3 \times 20 mL) and then with brine solution (3 \times 20 mL), evaporate the solvent under reduced pressure to afford the product (**Scheme 1**).

Synthesis of Metal complexes

The metal complexes of Schiff base HL was prepared by the addition of hot solution (60 $^{\circ}\text{C}$) of the appropriate metal chloride (1.0 mmol) in an ethanol-water mixture (1:1, 25 mL) to the hot solution (60 $^{\circ}\text{C}$) of the Schiff base (0.290 g HL, 2.0 mmol) in the same solvent (25 mL). The resulting mixture was stirred under reflux for 1 h whereupon the complexes precipitated. They were collected by filtration and purified by washing with an ethanol-water mixture (1:1) and diethyl ether. The analytical data for C, H and N were repeated twice.



Where HL= 4-chloro-N'-(4-methoxybenzylidene)-benzohydrazide Schiff base;

M=Cobalt(II), Nickel(II) and Copper(II) metal ions

Scheme 1 Synthesis of Schiff base ligand and their metal(II) complexes

3. RESULTS AND DISCUSSION

The novel Schiff base is synthesized and subjected to elemental analyses, mass and IR spectral analyses. The results of elemental analyses (C, H, N) with molecular formula and the melting point are presented in **Table 1**.

Compound	Color (yield) %	M.p. (⁰ C)	% Found (calcd)			μ_{eff} . (B.M.)	λ (mho cm^2 mol^{-1})
			C	H	N		
HL ($\text{C}_{15}\text{H}_{13}\text{ClN}_2\text{O}_2$)	Yellow (89)	180	62.40 (62.25)	4.54 (4.45)	9.70 (9.63)	-	-
[Co(2L)Cl ₂] ($\text{C}_{30}\text{H}_{26}\text{Cl}_4\text{N}_4\text{O}_4\text{Co}$)	Orange (71)	260	51.55 (51.50)	4.05 (3.90)	7.76 (7.71)	4.71	8.05
[Ni(2L)Cl ₂] ($\text{C}_{30}\text{H}_{26}\text{Cl}_4\text{N}_4\text{O}_4\text{Ni}$)	Green (75)	265	51.56 (51.53)	4.05 (4.05)	7.76 (7.65)	1.10	9.65
[Cu(2L)Cl ₂] ($\text{C}_{30}\text{H}_{26}\text{Cl}_4\text{N}_4\text{O}_4\text{Cu}$)	Blue (70)	265	51.22 (51.20)	4.02 (4.00)	7.71 (7.70)	1.78	12.15

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

The results obtained are in good agreement with those calculated for the suggested formula and the melting point is sharp indicating the purity of the synthesized Schiff base ligand. The structure of HL is also confirmed by IRE and ¹H NMR spectra, which will be discussed in detailed manner with metal complexes latter.

IR Spectra

The data of the IR spectra of Schiff base ligand (HL) and its complexes are listed in **Table 2**. The IR spectra of the complexes are compared with those of the free ligand in order to determine the coordination sites that may be involved in chelation. There are some guide peaks, in the spectra of the ligand which are good help for achieving this goal. This behavior is confirmed by the appearance of intense peaks at 3242 cm^{-1} , a characteristic feature of the NH stretching frequency indicating the existence of the NH groups. The IR spectrum of the HL ligand shows a broad band at 1685 cm^{-1} which attributed to C=O group [10]. The participation of C=O group confirmed by the shifted of this band from 20-40 cm^{-1} . Moreover, the downfield shift of the carboxylic group vibrations of the Schiff base suggest that the oxygen atom take part in the coordination to the metal atom, This observation has been further confirmed [11] by the appearance of new bands around the range 520-522 cm^{-1} assignable to (M-O).

In the spectrum of Schiff base a band is found at 1621 cm^{-1} due to azomethine group. The lower shift 15-20 cm^{-1} of the azomethine group of the ligand on complexation supports that the coordination occurs through the nitrogen atom of the azomethine group This is consistent with the presence of new bands at 412-422 cm^{-1} assignable to (M-N) vibration [12]. The second most important features of the infrared spectra of some complexes are the coordination behavior of chloride anions. The IR spectra of the complexes show strong absorption bands which are consistent with bidentate mode.

¹H NMR Spectra

¹H NMR spectra of the ligand, the signals of the -NH proton was observed as singlets at δ 11.42. The signals of the -CH=N proton which appears as singlets at δ 8.03 in the spectrum of ligand [13]. The signals of the aromatic protons of the ligand appeared at δ 7.21-

7.91, and the resonance lines found correspond to the calculated multiplicity. The -CH₃ proton signals appears at δ 1.02 in the spectrum of ligand.

S. No.	Complexes	ν (C=N)	ν (C=O)	ν (NH)	ν (M-O)	ν (M-N)	ν (M-Cl)
1	HL	1621	1685	3247	-	-	-
2	[Co(HL) ₂ Cl ₂]	1601	1646	3245	521	412	386
3	[Ni(HL) ₂ Cl ₂]	1600	1647	3247	522	422	385
4	[Cu(HL) ₂ Cl ₂]	1602	1640	3246	520	420	385

Table 2 Key FT-IR Bands (cm⁻¹) of ligand and transition metal complexes

Mass spectra

Mass spectra provide a vital clue for elucidating the structure of compounds. The spectrum shows the molecular ion peak at $m/z = 288$ (C₁₅H₁₃ClN₂O₂, calculated atomic mass 287 amu due to ¹³C and ¹⁵N isotopes). The different competitive fragmentation pathways of ligand give the peaks at different mass numbers at 288. The intensity of these peaks reflects the stability and abundance of the ions.

EPR spectrum

The powder EPR spectrum was carried out for copper complex at room temperature with frequency at 9.723 Ghz and magnetic field set of 3459 G. For the Cu(II) complex, the spectrum reveals a broad line with poorly resolved hyperfine structure. The g tensor values of the Cu(II) complex can be used to derive the ground state. The EPR spectra of the Cu (II) complexes were recorded as polycrystalline sample at LNT since the rapid spin lattice relaxation of Cu (II) broaden the lines at higher temperature. The $g_{||}$ value for metal complex is less than 2.3 suggesting a small amount of ionic character of the metal-ligand bond. The trend $g_{||} > g_{\perp} > 2.0023$, suggests that the unpair electron lie predominantly in the dx^2-y^2 orbital characteristic of octahedral geometry [14] in Cu (II) complexes.

Electronic Spectra, Magnetic Moments molar conductance measurements

It is possible to draw up the electronic transitions and predict the geometry with the aid of magnetic moments of most metal ions (**table 3**). The main absorption band of the ligand is also cited in the table. The bands in all metal complexes located at 380 nm and the shoulder at 396 nm were assigned to $\pi \rightarrow *$ transitions within the aromatic rings in the ligand. The absorption band in the range 383 nm could be assigned to both the C=N groups.

The electronic spectrum of the Co (II) complex consists of three spin allowed transitions. Since the ${}^4T_{2g}(F) \leftarrow {}^3T_{1g}(F)$ (ν_1) transition is essentially a two electron transition and the usual assignment observed are ${}^4A_{2g}(F) \leftarrow {}^4T_{1g}(F)$ (ν_2) and ${}^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$ (ν_3) transitions corresponding to 563 and 473 nm, respectively. These transitions are in agreement with the formation of a high spin octahedral geometry. Also, the magnetic moment provides a complementary means of octahedral geometry, the measured magnetic moment for this complex is 5.09 B.M which lies in the range of octahedral complexes [15].

S. No.	Complexes	λ_{\max} (nm)	Assignments
1.	[Co(HL) ₂ Cl ₂]	236, 275, 335, 366, 425, 580	n- π^* , π - π^* , d-d
2.	[Ni(HL) ₂ Cl ₂]	270, 330, 350, 375, 610	n- π^* , π - π^* , d-d
3.	[Cu(HL) ₂ Cl ₂]	231, 282, 371, 490, 559	n- π^* , π - π^* , d-d

Table 3 Electronic spectral data (nm) of the metal complexes

On the other hand, the electronic spectra of the Ni(II) complex showed a band at 633 nm due to the ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P), transition, which could occur [16] in a octahedral d_8 arrangement with another one at 483 nm owing to ${}^3T_1(P) \leftarrow {}^3A_2(F)(v_3)$. The magnetic moment of 3.88 B.M., lies in the range reported for tetrahedral compounds. The electronic spectrum of the bluish Cu(II), exhibit two bands at 580 and 499 nm corresponding to ${}^3T_{2g} \leftarrow {}^2E_{2g}$ transitions in a octahedral geometry. The magnetic moment value (1.89 B.M) recorded for Cu (II) complex [17] lies within the permissible range recorded for one unpaired electron.

4. BIOLOGICAL PROPERTIES

Schiff base (HL) and its metal complexes were evaluated for their antimicrobial activity against one strain Gram-positive bacteria *S. aureus* ATCC 29253, *S. aureus* ATCC 3160 as Gram-negative bacteria as well as one pathogenic fungus, as *S. cerevisiae* MTCC 316, *Candida albicans* (227). The results of the biological studies [18, 19] of the ligand and the complexes are shown in **Table 4**. The data are compared with standard antibiotics, Gentamycin as Gram-negative and Amphotericin-B as standard reference for Gram-positive bacteria was used as antifungal standard reference. The in vitro antibacterial and antifungal activities demonstrated that the complexes have higher antimicrobial activity in comparison with that of the ligand

Compounds	Time Hrs	S. aureus MTCC 3160		S. aureus MTCC 25923		C. albican MTCC 227		S. cerevisiae MTCC 361	
		Diameter of zone of inhibition (mm)		Diameter of zone of inhibition (mm)		Diameter of zone of inhibition (mm)		Diameter of zone of inhibition (mm)	
		100 μ g	50 μ g	100 μ g	50 μ g	100 μ g	50 μ g	100 μ g	50 μ g
HL	24	40	41	41	42	23	12	13	15
	48	42	45	43	43	25	15	15	18
[Co(HL) ₂ Cl ₂]	24	10	12	13	16	19	17	19	18
	48	11	14	14	19	11	10	12	19
[Ni(HL) ₂ Cl ₂]	24	19	18	20	28	25	27	21	18
	48	-	-	-	-	11	29	12	19
[Cu(HL) ₂ Cl ₂]	24	-	-	-	-	13	10	12	10
	48	14	10	13	12	14	13	14	11
Gentamycin	24	32	34	32	34	-	-	-	-
	48	32	34	32	34	-	-	-	-
Amphotericin-B	24	-	-	-	-	17	21	17	21
	48	-	-	-	-	17	21	17	21

Table 4. Antimicrobial activity of Schiff base ligand and their metal complexes

According to Tweedy's theory, chelation reduces the polarity of the metal atom because of partial sharing of its positive charge with a donor group and the possible π -electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganism. There are other factors which also increase the activity, such as solubility, conductivity and bond length between the metal and the ligand. It is worth to mention that the ligand showed moderate biological activity against the tested strains and the complexes show higher bacterial activity as compared to the fungus. The result revealed also that complex of copper displayed the maximum (significant) inhibition against the growth of the selected Gram-positive, Gram-negative bacteria and fungi followed by Co(II) and Ni(II). From the data, conclusive evidence of structure activity relationships showed that the mononuclear complex enhances the antimicrobial activity rather than the acyclic complexes. It is of interest to note that the mononuclear Co(II) complex exhibit approximately equal inhibition to the standard antibiotic which reveals the biological efficiency of these complexes and showed the possibility to be useful as new drug.

CONCLUSIONS

Schiff base ligand derived from the condensation between 4-chlorobenzohydrazide and 4-methoxybenzaldehyde is a versatile ligand since it afforded a series of mononuclear complexes of octahedral geometries. From the infrared spectra, the chelation of the binuclear complexes occurs through the oxygen and the nitrogen atoms of the carboxylic and azomethine group respectively, of the chelated ligand. The oxygen atom, chloride ions, satisfies the other coordination sites to complete the geometry around the central metal ion. The spectral, magnetic studies and molar conductivity measurements of the metal complexes were used to determine the type of coordination and the geometry around the central metal ion. The synthesized ligand and its corresponding metal complexes were tested for the growth inhibitory activity against phytopathogenic bacteria and fungi. Some of them can act as practical use as antimicrobial agents.

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