

Synthesis, spectral characterization and antimicrobial assessment of Schiff Base ligand derived from amino acid and its transition metal complexes

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ABSTRACT

A Schiff base ligand 2-[(5-Bromo-2-hydroxy-benzylidene)-amino]-5-methyl-pentanoic acid was prepared by condensation of leucine with 5-bromosalicylaldehyde. Cu(II), Co(II) and Ni(II) complexes of the above ligand was synthesized as well. The synthesized ligand and complexes were structurally characterized by spectral techniques like IR, UV, ¹H NMR and EPR. The analytical data of the complexes show the formation of 1:2 metal to ligand ratio with the formula [ML₂], where M represents Cu(II), Co(II) and Ni(II) ions, and L represents the Schiff base ligand respectively. IR spectra show that the ligand is coordinated to the metal ions in a tridentate manner through azomethine-N, phenolic-oxygen and carboxylic oxygen groups. The ligand and their metal chelates have been screened for their antimicrobial activities using disc diffusion method against the selected bacteria and fungi.

Key words: Schiff base, Amino acid, Transition metal Schiff base complexes, Antimicrobial activity.

1. INTRODUCTION

Schiff bases are some of the most widely used and easily synthesized organic compounds. The chelating nature, moderate electron donor property, easily tunable electronic and steric effects have proved Schiff bases are highly versatile in nature. They are widely employed in stabilizing rare earth, inner – transition and transition metals in various oxidation states [1]. The coordination between –NH₂ group of the amino acid and carbonyl group of the aldehyde or ketone is very difficult because of the Zwitter ion effect and the reaction needs special conditions. It has been observed that pH plays an important role in the process of condensation [2,3]. Due to the versatility of the Schiff base ligand, it has been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [4,5].

The coordination behaviour of Schiff base ligand with transition and rare earth metals plays a vital role in the recent days. Especially, transition metal complexes derived from amino acid Schiff bases have received much attention because of possible biological and pharmacological activities [6,7].

In view of diversified roles of Schiff base transition metal complexes, in this paper the metal complexes of Cu(II), Co(II) and Ni(II) with the Schiff base derived from leucine and 5-bromo salicylaldehyde have been synthesized. The isolated complexes have been characterized by spectral techniques like UV- Vis., IR, ¹H NMR and EPR. The ligand and their metal chelates have been screened for their antimicrobial activities using the disc diffusion method against the selected bacteria and fungi.

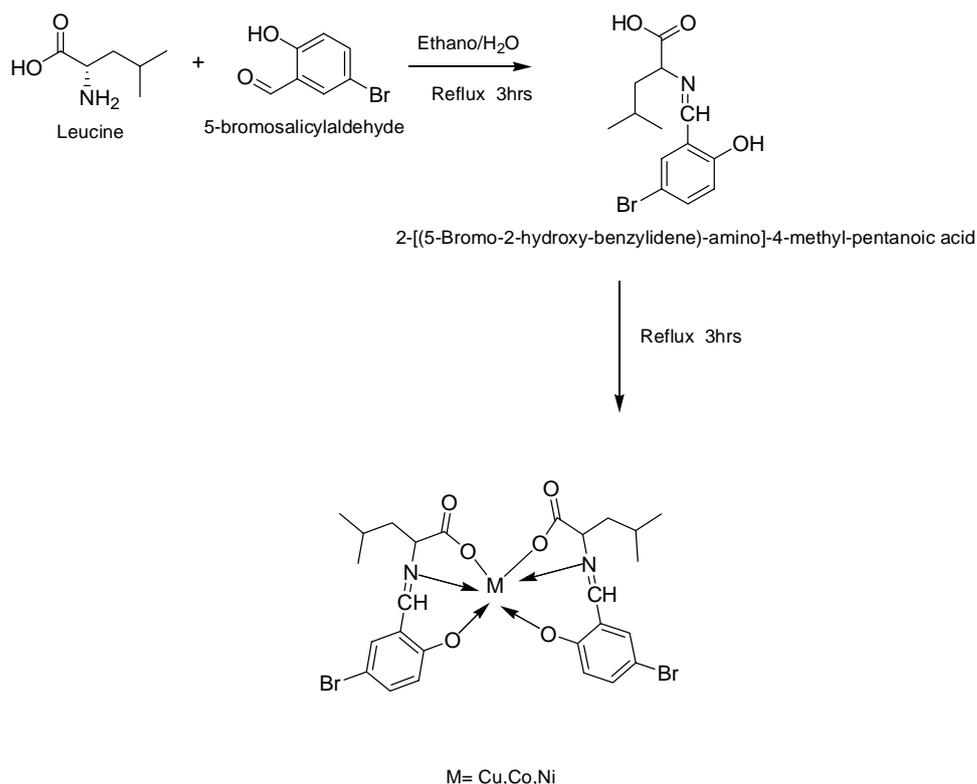
2. EXPERIMENTAL

2.1. Reagents

All the chemicals used were of analytical grade and used without further purification. All reagents were purchased from Aldrich and Sigma chemicals companies.

2.2. Technique

IR spectra (KBr pellets) were recorded in the region 4000 – 200 cm⁻¹ on a FT-IR spectrum BX-II spectrophotometer. ¹H NMR spectrum was recorded with a model Bruker Advance DPZ-300 spectrometer operating at 300 MHz using DMSO-d₆ as a solvent and TMS as an internal standard. Electronic spectra were recorded on Shimadzu UV mini-1240 spectrophotometer using DMF as a



Scheme - 1: Synthesis of Schiff base metal complexes

solvent. EPR spectra were recorded on an E4-EPR spectrometer. The measurements were done in the X-band, on microcrystalline powder at room temperature using DPPH as standard.

2.3. Synthesis of the Schiff base ligand

The Schiff base ligand was synthesized as per the following literature procedure [8]. To an aqueous solution of Leucine (0.001 mol) in 5 mL water containing NaOH (0.001 mol), 5-bromosalicylaldehyde in 5 mL ethanol was added drop wise with constant stirring and heated under reflux for 3-5 hrs. on a hot plate at 50°C. Then the reaction mixture was cooled to room temperature, fine shining yellow precipitate of the Schiff base ligand formed was filtered off, washed with ethanol-water mixture and stored in a vacuum desiccator over anhydrous calcium chloride.

2.4. Synthesis of Cu(II) Schiff base complex

The Cu(II) Schiff base metal complex was synthesized as per the following literature procedure [8]. To an ethanolic solution of the Schiff base ligand the metal ion (Copper (II) chloride) was dissolved in minimum water (5 mL), then added drop wise with constant stirring and finally heated under reflux for 3 – 5 hrs on a hot plate at 50°C. A fine green precipitate of the solid complex formed was filtered off, washed with ethanol-water mixture and stored in a vacuum

desiccator over anhydrous calcium chloride. All other transition metal complexes were synthesized using the same procedure.

3. RESULTS AND DISCUSSION

The analytical data and some physical properties of the metal complexes are collected in table 1 and the scheme of synthesis of complex is given in scheme 1.

The complex is stable in air, soluble in water and common organic solvents. The presence of leucine and 5 bromo- salicylaldehyde in the copper complex has been confirmed by the TLC after hydrolysis of the metal complex.

3.1. IR spectra

The most important IR assignments of the metal complexes have been determined by careful comparison with the spectra of Schiff base ligand derived from leucine and 5-bromo – salicylaldehyde. IR spectra of the Schiff base ligand shows band around 1640 cm^{-1} assigned to the azomethine group, this confirms the condensation between the amino group of leucine and the aldehyde group of 5-bromo-salicylaldehyde in formation of the Schiff base [9]. The medium intensity peak of absorption in the Schiff base ligand around 3432 cm^{-1} due to the phenolic hydroxyl group of the Schiff base ligand

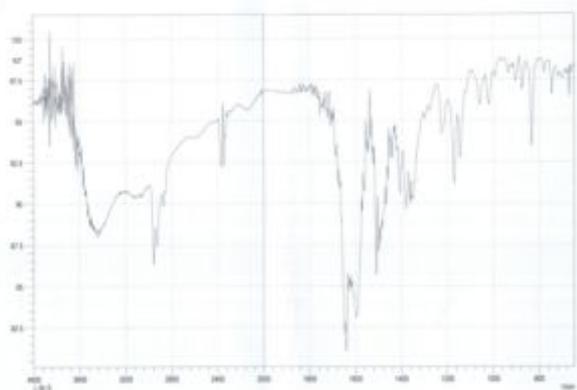


Figure - 1: IR Spectra of the Schiff Base Ligand [L]

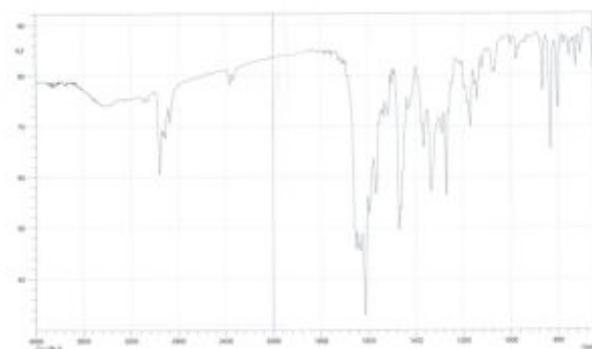


Figure - 2: Representative IR spectrum of the Copper Complex [CuL₂]

and this peak of absorption disappears while chelation with metal ion. This confirms the coordination through phenolic -OH and the central metal ion [10]. The azomethine absorption band undergoes a negative shift of 1612 cm⁻¹ in the complex, which may be attributed to the

coordination of the nitrogen atom of azomethine group to the metal ion [11]. The IR spectrum of the ligand and the Cu complex is given in Figure 1 and 2.

3.2. Electronic Spectra

The electronic spectrum of the ligand and the representative Cu(II) Schiff base complex is given in Fig. 3 and Fig. 4 respectively and the spectral data has been given in Table 2. The absorption of the ligand L is characterized by four main absorption bands in the regions 200-500 nm. The band at $\lambda_{\max} = 213$ nm and $\lambda_{\max} = 251$ nm is attributed to $\pi \rightarrow \pi^*$ transition [12,13].

The band at $\lambda_{\max} = 334$ nm corresponds to the $\pi \rightarrow \pi^*$ transition of the azomethine group and $\lambda_{\max} = 424$ nm is attributed to the $n \rightarrow \pi^*$ transition associated with the azomethine group with intra molecular charge transfer [14]. The electronic spectrum of the [CuL₂] complex shows the shoulder of charge transfer transition at 255 nm and broad d-d absorption band centered at 367 nm, due to ${}^2B_{1g} \rightarrow {}^2E_{1g}$. This transition suggest the distorted octahedral geometry for the metal complex [15,16].

The electronic spectrum of [CoL₂] showed bands at 258, 273 and 452nm which may be assigned to intra ligand charge transfer, ligand-metal charge transfer and $4T_1g(F) \rightarrow 4T_2g(P)$ transitions, respectively. The electronic spectrum of Ni(II) complex also displayed three bands at 254, 269 and 463 nm, of which the band at 463nm assigned to $3A_2g(F) \rightarrow 3T_1g(P)$ d-d transitions. The band at 269 nm, attributed to ligand to metal charge transfer transition.

Table - 1: Analytical data and some physical properties of the ligand and metal complexes

| Compound | M.P (°c) | Colour | Yeild% | m/z | % Found (calc.) | | | |
|------------------|----------|---------------|--------|-----|-----------------|------------|------------|--------------|
| | | | | | C | H | N | M |
| L | 160 | Bright Yellow | 86 | 313 | 49.70(49.65) | 5.13(5.09) | 4.46(4.45) | - |
| CuL ₂ | >300 | Green | 82 | 687 | 51.23(51.18) | 5.53(5.48) | 4.27(4.26) | 16.07(16.09) |
| CoL ₂ | 230 | Orange red | 76 | 683 | 45.70(45.72) | 4.13(4.12) | 4.10(4.09) | 8.63(8.65) |
| NiL ₂ | 175 | Light yellow | 79 | 682 | 45.72(45.71) | 4.13(4.12) | 4.09(4.10) | 8.59(8.57) |

3.3. ¹H NMR Spectra

The ¹H NMR Spectra of Schiff base ligand in DMSO-d₆ shows signal of multiplets at δ 0.842-0.888 ppm equivalent to the 6H of two methyl groups. Singlet at δ 1.698 ppm corresponds to the

methine protons associated with two methyl groups. Multiplets at δ 2.435-2.567 ppm are due to the α , β methylene hydrogen atom associated with azomethine group. The multiplets in δ 6.597-7.495 ppm region are assigned to the protons of benzylideneimine ring groups. The singlet at δ

3.329 ppm is due to the free hydroxyl group and strong singlet at δ 8.347 ppm is assigned for the azomethine proton. The ^1H NMR Spectra of Schiff base ligand is shown in Figure 5.

Table - 2: Electronic spectra of the metal complexes

| Complex | λ_{max} | Band assignment | Geometry |
|---------------------|------------------------|--|----------------------|
| [CuL ₂] | 255 | INCT | Distorted octahedral |
| | 272 | LMCT | |
| | 367 | 2B _{1g} → 2E _{1g} | |
| [CoL ₂] | 258 | INCT | Octahedral |
| | 273 | LMCT | |
| | 452 | 4T _{1g} (F) → 4T _{2g} (P) | |
| [NiL ₂] | 254 | INCT | Octahedral |
| | 269 | LMCT | |
| | 463 | 3A _{2g} (F) → 3T _{2g} (P) | |

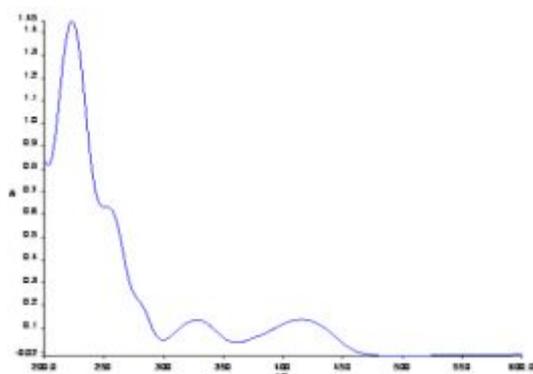


Figure - 3: Electronic Spectrum of the Schiff Base Ligand

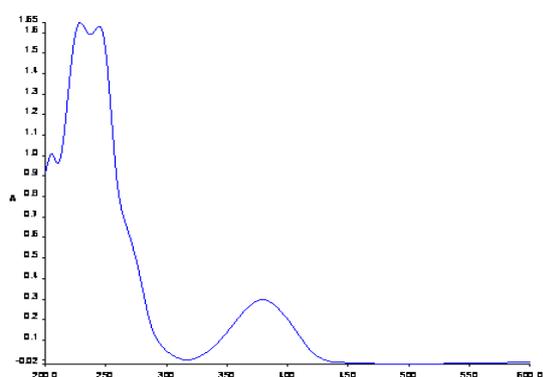


Figure - 4: Representative electronic Spectrum of Copper Complex [CuL₂]

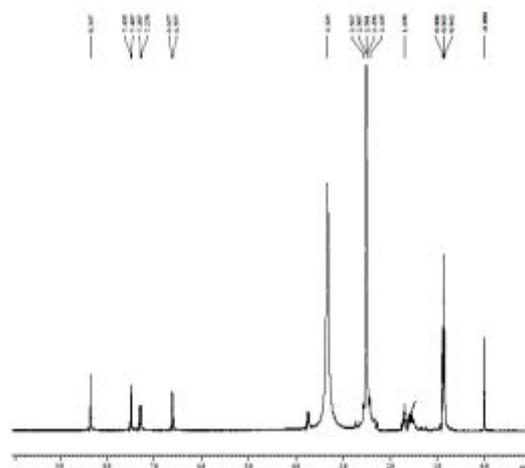


Figure - 5: ^1H NMR Spectra of the Schiff Base Ligand [L]

3.4. EPR Spectra

The X-band EPR spectra of copper complex was recorded at room temperature in polycrystalline form. The spectra show only one broad signal at $g_{\text{iso}} = 2.019$ (2.17 – 2.26) [17-20]. The magnetic moment μ effect by using the equation $\mu_{\text{eff.}} = 1.7444 (g [S(S+1)]^{1/2})$ with the help of g_{iso} value agree very well with the measured values [1.87-1.95 BM].

The spectral studies reveal that Cu (II) ion in the present complex is in octahedral field. "g" tensor values of copper complex can be used to calculate ground state. The calculated values of $g_{\parallel} = 2.0486$ and $g_{\perp} = 2.0042$ for the complex show the order as $g_{\parallel} > g_{\perp} > 2.0023$, which is consistent with the $d_{x^2-y^2}$ ground state [21]. The odd electron is located in the $^2B_{1g}$ anti bonding orbital. This represents the geometry may axially elongated octahedral with $^2B_{1g}$ ground state.

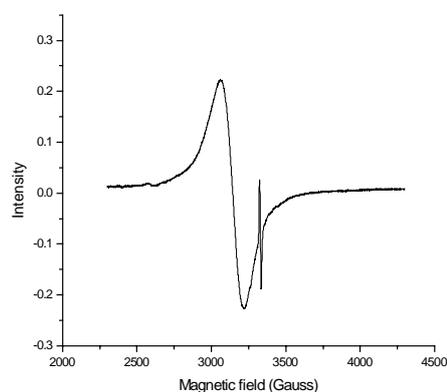


Figure - 6: EPR Spectrum of Copper Complex [CuL₂]

The geometric parameter G, which is a measure of exchange interaction between the copper centres in the polycrystalline compounds,

is calculated by using the expression. $G = g_{\parallel} - 2.0023 / g_{\perp} - 2.0023$. The complex show the G value greater than 4, which indicate that the interaction between metal centres is negligible. EPR spectra is given in Figure 6.

3.5. Antimicrobial activity

The Schiff base ligand and its Cu(II),Co(II) and Ni(II) Schiff base complexes were tested for their inhibitory effects on the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococci* bacteria because bacteria can achieve resistance to antibiotics through biochemical and morphological modifications [22]. They have also been tested on *Candida* and *Aspergillus* fungi. The antimicrobial activity was tested by using the disc diffusion method. The antimicrobial activity of the new compounds is presented in Table 3. The presence of hydroxyl group in the Schiff base ligand plays an important role for its antimicrobial activity [23], and also the presence of imine group which imports in elucidating the mechanism of transformation reaction in biological systems [24]. The metal complexes were found to inhibit more

compared with their parent Schiff base ligand against the same micro-organisms and under the identical experimental conditions. The increase in biological activity of the metal chelates may be due to the effect of the metal ion on the normal cell process. A possible mode of toxicity increase may be considered in the light of Tweedy's chelation theory [25]. Chelation considerably reduce the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π electron delocalization within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence increasing the hydrophobic character and lipo solubility of the complex favoring its permeation through the lipid layers of the cell membrane thereby deactivates respiration process in the microorganisms. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine nitrogen atom ($>C=N$) with the active centers of cell constituents, resulting in interference with the normal cell process [26].

Table - 3: Antimicrobial activity

| Compounds | Diameter of inhibition zone (in mm) | | | | | | | | | | | | | | | | | |
|--------------------------------|-------------------------------------|----|----|------------------------|----|----|-----------------------|----|----|--------------|----|----|---------|----|----|-------------|----|----|
| | Escherichia coli | | | Pseudomonas aeruginosa | | | Staphylococcus aureus | | | Streptococci | | | Candida | | | Aspergillus | | |
| Conc.($\mu\text{g ml}^{-1}$) | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 |
| L | 5 | 8 | 12 | 3 | 7 | 11 | R | 4 | 7 | 4 | 6 | 11 | 10 | 14 | 19 | 4 | 9 | 16 |
| [CuL ₂] | 7 | 11 | 19 | 6 | 11 | 18 | 4 | 9 | 12 | 8 | 14 | 19 | 9 | 16 | 23 | 6 | 11 | 17 |
| [CoL ₂] | 6 | 10 | 16 | R | R | 5 | 3 | 7 | 11 | 7 | 12 | 17 | 7 | 13 | 18 | 5 | 9 | 12 |
| [NiL ₂] | R | 3 | 7 | R | 6 | 11 | 2 | 5 | 9 | 5 | 10 | 14 | 8 | 15 | 19 | 4 | 11 | 16 |
| Control | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| Standard | 8 | 15 | 21 | 8 | 15 | 21 | 9 | 17 | 24 | 9 | 17 | 24 | 8 | 14 | 21 | 8 | 15 | 21 |

4. CONCLUSION

The present work reveals the synthesis of the Schiff base ligand and its Cu(II),Co(II) and Ni(II) Schiff base complexes. The synthesized ligand and the complexes were structurally characterized by spectral techniques like IR, UV, ¹H NMR and EPR. The analytical data of the complexes show the formation of 1:2 Metal to Ligand ratio of the general formula ML₂, where M represents Cu(II), Co(II) and Ni(II) ions, while L represents the Schiff base. The spectral studies show that the ligand is coordinated to the metal ions in a tridentate manner through azomethine-N phenolic-oxygen and carboxylic oxygen groups. The metal complexes have been assigned octahedral geometry. The ligand and their metal chelates have been screened for their

antimicrobial activities, the result shows that the metal complexes has enhanced inhibitory activity than the Schiff base ligand under identical conditions.

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