Synthesis, Characterization, Antibacterial and Thermal Studies of Cu(II) Complex of Thiophene-2- aldehyde Semicarbazone

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Authors’ contributions

This work was carried out in collaboration among all authors. Author DS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MYR, MMH and RZ managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Copper (II) complex of the ligand, thiophene-2-aldehyde semicarbazone was synthesized and characterized. Their structures were explored on the basis of CHN analysis, conductance and magnetic susceptibility measurements, spectral studies (FT-infrared and ESI-MS) and thermogravimetric analysis (TGA). It has been observed from spectral and analytical studies, the copper complex have the composition of [CuL(CH₃COO)₂(H₂O)] [where L is thiophene-2-aldehyde semicarbazone]. The electrospray mass spectrum of the ligand and complex exhibited ion at mass-to-charge ratio (m/z) of 169.13 and 368.86 matching the calculated values for the molecular ion of the ligand and complex, respectively. One mole of the ligand behaves as bidentate chelating agents around the Cu(II) ion. From conductivity studies, we obtained that the copper complex of the ligand, thiophene-2-aldehyde semicarbazone had no ionic properties. All data confirmed a trigonal bipyramidal geometry of the complex. Antibacterial activity of the compounds against the microbe Enterobacter aerogenes and Bacillus cereus have been screened to reflect that the complex has higher antimicrobial activity than the free ligand.

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1. INTRODUCTION

Schiff base metal complexes have enrolled a noteworthy significance in coordination chemistry. Scientist are especially interested in Schiff base complexes for their possible applications in biology, medicine and photonics because of their convenient routes of synthesis. Significant variations in biological activity with structure and type are observed for these complexes [1-3]. In the Last two decades, considerable attention has been paid to the chemistry of Schiff bases metal complexes containing nitrogen, oxygen and other donors [4-9]. These may be ascribed to their stability, bioactivity [10-12] and prospective applications in many other fields such as oxidation catalysis [13-16], electrochemistry [17] and analytical chemistry etc [18,19].

It is commonly known that semicarbazones have substantial biological, anticonvulsant, analgesic, anti-inflammatory and antitumoral activities [20-25]. The dependence of their bioactivity on the type of chelating with transition metal ions have been of considerable importance [26-29].

We here report this present investigation which deals with copper complex with Schiff base because of comprehensive area of applications of the metal complexes of Schiff bases. We have synthesized and characterized the ligand, thiophene-2-aldehyde semicarbazone and its Cu(II) complex using elemental analysis, conductivity and magnetic susceptibility measurements, spectral studies (FT-IR and ESI-MS), thermogravimetric analysis (TGA) and also studied the antibacterial activity of as synthesized compounds against the microbes Enterobacter aerogenes and Bacillus cereus.

2. EXPERIMENTAL

2.1 Materials

All reagents, starting materials as well as solvents employed to prepare Schiff base in addition to its copper complex were obtained from Aldrich, BDH, Fluka and Merck and were used as received. Nutrient agar medium (Include-Peptone, Agar, Sugar, Marmite) was used to check anti-microbial activity.

2.2 Instrumentation

The melting points of the ligand, thiophene-2-aldehyde semicarbazone and complex in degree Celsius were checked with a capillary devices and were recorded on a digital melting point apparatus, ThermoCal. Both of the compounds were routinely characterized and checked by FT-IR, ESI-Mass, CHN and molar conductance measurements. Elemental analyses for CHN were performed using a Vario EL cube (Germany elements (Elemental) analysis system. FT-IR spectra were recorded on a FT-IR spectrophotometer [JASCO, FT-IR/4100] Japan using KBr pellets as the standard reference. ESI-MS spectra were done with an Agilent Technologies MSD SL Trap mass spectrometer with ESI source coupled with an 1100 Series HPLC system. Magnetic susceptibility of the copper complex was measured using a Sherwood Scientific MX Gouy magnetic susceptibility apparatus. Molar conductance of the synthesized compounds were recorded on W.T.W conductivity Master LBR meter. Thermal analysis were done using a Perkin-Elmer Thermogravimetric Analyzer TG/DTA 6300 under a N2 gas flow (20 mL min\(^{-1}\)) at ambient pressure. A heating rate of 20ºC min\(^{-1}\) was chosen. In the cases where the TG curve showed the possibility of stable intermediates, a heating rate of 5ºC min\(^{-1}\) was applied.

2.3 Synthesis of the Ligand L, Thiophene-2-aldehyde Semicarbazone

Thiophene-2-carbaldehyde (0.93458 mL, 2.0 mmol) was mixed with ethanol (15 mL) and the mixture was brought to boil, producing a slurry. Barely sufficient additional amount of ethanol was then added to give a homogenous solution. Semicarbazide (0.223058 g, 2 mmol) was added drop-wise to the homogeneous solution of thiophene-2-carbaldehyde over 5 minutes. The reaction mixture was refluxed for 4 hours, then allowed to cool slowly and to stand overnight. Finally, it produces white solid which was filtered off and dried.

2.4 Synthesis of Cu(II) Complex of the Ligand, L

To the warm ethanolic solution (10 mL) of the ligand L (2 mmol), 10 mL warm ethanolic solution (2 mmol) of Cu(II) acetate was added and the resulting mixture was refluxed for about 4 hours. The obtained precipitates were filtered, washed with ethanol and dried under vacuum on anhydrous CaCl\(_2\).
Scheme 1. Synthesis of the ligand, L, thiophene-2-aldehyde semicarbazone

2.5 Metal Content Estimation in the Complex

A known weight of the metal complex was taken into a conical flask and concentrated H₂SO₄ (500 μL) was added to it. It was fumed down to dryness and the process was repeated. Concentrated HNO₃ (500 μL) and HClO₄ (500 μL) were then added and the mixture was fumed to dryness. The process of adding acids and fuming down to dryness was continued until there was no black materials. 100 mL distilled water was added to dissolve the residue. Finally, the weight of the copper was estimated complexometrically using EDTA (Ethylenediamine Tetra Acetic Acid) [30].

2.6 Antibacterial Activity

The ligand (L) and its Cu(II) complex were screened for in vitro antimicrobial activity in DMSO against gram-negative bacteria, Enterobacter aerogenes and gram-positive bacteria, Bacillus cereus strains by Kirby Bauer’s disc diffusion technique [31]. A uniform suspension of test organism of 24 hours old culture was prepared in a test tube containing nutrient broth media. Sterile nutrient agar was then added in each of the Petri dishes. The dishes were related to ensuring the uniform mixing of the microorganism in the agar medium which was then allowed to solidify. Sterile Whatmann filter paper discs were dipped in the solution of each compound and placed on the labelled plates. The DMSO was used as a control of the solvent. Plates were kept in the incubator for 24 hours at 37°C. The diameter of the zone of inhibition around each disc was measured by scale and results were recorded in terms of millimeter (mm). The observed data of antimicrobial activity of both of the compounds are presented in Table 5.

3. RESULTS AND DISCUSSION

3.1 Synthesis

The ligand, L (thiophene-2-aldehyde semicarbazone) was prepared in good yield from the condensation reaction of thiophene-2-aldehyde and semicarbazide in a 1:1 stoichiometric ratio. Treatment of the Cu(II) acetate salt with the ligand, L formed the complex corresponding to 1:1 metal-ligand ratio. Physical and analytical data of studied compounds are presented in Tables 1 and 2.

3.2 Elemental Analysis

The CHN analysis data of the synthesized compounds are given in Table 2. The percentage of metal content in the complex also reveal that metal to ligand ratio for the complex is 1:1. Moreover, these data also support the proposed structure of the ligand (Scheme 1) and complex [Fig. 1].

![Fig. 1. Proposed structure of the synthesized Cu(II) complex](image-url)
Table 1. Physical data of the ligand, L and its Cu complex

| Compound                  | Empirical Formula | F.W. (g/mol) | Colour (% yield) | m. p. (°C) |
|---------------------------|-------------------|--------------|------------------|------------|
| L                         | C₆H₇N₃SO          | 169.20       | White (79%)      | 220-224    |
| [CuL(CH₃COO)₂(H₂O)]       | C₁₀H₁₅CuN₃O₆S    | 368.85       | Dark Brown (74%) | > 300      |

Table 2. Analytical data of the ligand, L and its Cu-complex

| Compound                  | Found (Calculated) (%) | μeff (B.M.) | Conductivity (µScm⁻¹) | ESI-MS |
|---------------------------|------------------------|-------------|-----------------------|--------|
| L                         | -                      | 41.19 (42.59)| -                     | -      |
|                          |                        | 4.19 (4.17) | 24.74                 | -      |
|                          |                        | -           | -                     | 169.13 |
| [CuL(CH₃COO)₂(H₂O)]      | 17.19 (17.23)         | 32.46 (32.56)| 4.11                 | 11.30 (11.39)| 1.80 | 6 | 368.86 |

Table 3. Key Infrared bands (cm⁻¹) of the ligand, L and its copper complex

| Compound                  | Empirical Formula | v (C=O) | v (C=N) | v (M-O) | v (M-N) | v (C-C-O) | v (O-C-C) |
|---------------------------|-------------------|---------|---------|---------|---------|-----------|-----------|
| Ligand (L)                | C₆H₇N₃SO         | 1718    | 1687    | -       | -       | -         | -         |
| [CuL(CH₃COO)₂(H₂O)]      | Cu(C₆H₇N₃SO)     | 1646    | 1594    | 696     | 527     | 1225      | 1097      |

Fig. 2. 3D structure of the Cu(II) complex (Generated by Argus Lab Software)

3.3 Magnetic Measurement
The magnetic moment value (1.80 BM) revealed the paramagnetic nature of the central copper atom in the complex, [CuL(CH₃COO)₂(H₂O)] where the ligand, L (thiophene-2-aldehyde semicarbazone) act as bidentates [32].

3.4 Molar Conductivity Measurements
The molar conductance values of 10⁻³ M solution of the ligand, L and copper complex in DMSO are presented in Table 2. The low molar conductance value revealed that the copper complex was non-electrolyte in nature [33].

3.5 FT-IR Spectral Studies
The infrared data provide considerable sign of the formula of ligand and its Cu (II)-complex. The FT-infrared spectra gives the worthy information relating to the behavior of functional groups coordinated to the Cu (II) ions. However, the existence of a strong and sharp peak at 1687
cm\(^{-1}\) which is related to C=N stretching frequency and another sharp peak at 1718 cm\(^{-1}\) assigned to C=O stretching frequency in the spectra of the ligand [34,35]. The band at 1718 cm\(^{-1}\) for the \(\nu(C=O)\) stretching vibration of the Schiff base ligand, L is shifted to downward region 1646 cm\(^{-1}\) in the complex suggesting that the carbonyl oxygen of the ligand coordinated to the copper ion [36,37]. The azomethine band observed at 1687 cm\(^{-1}\) for the ligand was shifted to 1594 cm\(^{-1}\) after complexation, confirming the participation of the azomethine nitrogen atom in the coordination with the copper ion. Furthermore, the stretching of new bands, Cu(II)-O and Cu(II)-N appeared in lower wavelength region ranging 696 and 527 cm\(^{-1}\) respectively which also supports the coordination through nitrogen and oxygen atom from -CH=N- and carbonyl group from the Schiff base, L. In the other side, new bands observed at 1225 and 1097 cm\(^{-1}\) in the infrared spectrum of the complex were attributed to –C-C-O and O-C-C stretching confirming the coordination of acetate group to the metal ion [36-38]. The broad band appeared at 3453 cm\(^{-1}\) together with new band at 609 cm\(^{-1}\) in the spectra of the complex confirmed the presence of coordinated water molecule. The important FT-IR bands for the ligand, L and its copper complex are given in Table 3.

3.6 ESI-Mass Spectra

The ESI-Mass spectra of the ligand and complex are presented in Fig. 5. The obtained m/z values are similar to the formula weight (Table 2) which further supports the proposed structure of the synthesized compounds.

3.7 Thermogravimetric Analysis (TGA)

The thermogravimetric analyses of the complex have been studied from ambient temperature up to 700\(^\circ\)C under a N\(_2\) atmosphere. The thermal properties of Cu(II)-complex was investigated by thermogram. The TGA curve for the complex displays two stages of mass loss within the temperature range of 197–534\(^\circ\)C. The first stage is at 197–295\(^\circ\)C, and exhibits a mass loss of 57.13%, corresponding to the loss of C\(_{10}\)H\(_{15}\)NO\(_4\) (calc. 57.29%). The second stage occurs at 295–534\(^\circ\)C, with a mass loss of 16.71%.

Table 4. Thermogravimetric analysis data for the copper (II) complex

| Compound                        | Step | Decomposition Range (\(^\circ\)C) | TG Weight Loss (%) | Evolved Product                  | Residue             |
|---------------------------------|------|----------------------------------|--------------------|----------------------------------|---------------------|
| [CuL(CH\(_3\)COO)\(_2\)(H\(_2\)O)] | 1\(^{st}\) | 197-295                          | 57.29 (57.13)      | C\(_{10}\)H\(_{15}\)NO\(_4\) CuO & | Gaseous product     |
| (C\(_{10}\)H\(_{15}\)CuN\(_3\)O\(_4\)S) | 2\(^{nd}\) | 295 - 534                        | 16.79 (16.71)      | N\(_2\)O\(_2\)                   |                     |

Fig. 3. IR Spectrum of L, thiophene-2-aldehyde semicarbazone
corresponding to the loss of N₂O₂ (calc. 16.79%). The decompositions of the copper complex ended with formation of CuO and gaseous products [39]. The thermogravimetric analysis data for the copper (II) complex are presented in Table 4.

3.8 Antimicrobial Screening Results

The Schiff-base ligand, L and its copper complex reported here were evaluated for antibacterial activity against Enterobacter Aerogenes and Bacillus cereus. The values of inhibition zone were measured in millimeter (mm). The data of antibacterial activities of the ligand and its complex are given in Table 5. The inhibitory zone data reveals that the ligand, as well as its copper complex shows good antibacterial activity. The biological activity of Schiff base ligand arises from the presence of imine group which imports in elucidating the mechanism of transformation reaction in biological systems. However, its copper complex showed remarkable antibacterial activity as a result of chelation of metal with organic ligand synergistically increasing its effect. The DMSO control did not show any antimicrobial activity against the tested bacterial strains whereas considering with respect to the standard, both the tested compounds were found to be moderately active.
Fig. 6. Thermogravimetric (TG) curve for the complex, \([\text{CuL(CH}_3\text{COO)}_2(\text{H}_2\text{O})]\)

Fig. 7. Statistical representation for antibacterial activity for the ligand, L and its Cu (II) complex

Table 5. Antibacterial screening results of ligand L and its complex

| Compound          | Diameter of inhibition zone of bacteria (mm) |
|-------------------|---------------------------------------------|
|                   | Gram Negative | Gram Positive |
| \(\text{L}\)      | \(\text{Enterobacter aerogenes}\) | \(\text{Bacillus cereus}\) |
|                   | 7             | 6             |
| \([\text{CuL(CH}_3\text{COO)}_2(\text{H}_2\text{O})]\) | 11            | 13            |

Control (DMSO): No activity (There was no inhibition zone)

4. CONCLUSION

In this paper we have reported the preparation, isolation and characterization of a bidentate Schiff base derived from thiophene-2-aldehyde and semicarbazide and its copper complex. It is tentatively proposed that the Schiff base ligand coordinates through the nitrogen of the
azomethine group and the oxygen of the carbonyl group. Another three coordination sites of the Cu(II) have been occupied with two acetate group and water molecule forming a stable trigonal bipyramidal structure. The synthesized copper complex, in comparison to the uncomplexed Schiff base ligand, were screened for their antibacterial activity against pathogenic bacteria species (*Enterobacter aerogenes* and *Bacillus cereus*). The activity of the Schiff base complex became more pronounced when coordinated with the metal ion.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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