BIOACTIVE MIXED LIGAND METAL COMPLEXES OF Cu(II), Ni(II), AND Zn(II) IONS: SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTI-OXIDANT PROPERTIES

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ABSTRACT

A series of transition metal complexes having general formula [M(L1)(L2)](NO3)2 (where, M = Cu(II), Ni(II), and Zn(II)) of mixed ligands, L1 = Isonicotinamido- Thiophene-2-Carbaldimine, L2 = 1, 10-phenanthroline, were synthesized. The obtained compounds were successfully characterized by different spectroscopic techniques. Conductivity measurements indicated that all complexes were 1:2 electrolytes in nature. IR spectra indicated that ligands coordinated to metals via the carbonyl oxygen atom and the azomethine nitrogen atom. Magnetic moment values and UV-Visible spectra confirmed square planar structure around the Cu(II), and Ni(II) ions, and a tetrahedral geometry around the Zn(II) ion. The metal complexes were tested against Escherichia coli and Pseudomonas sp. Among the tested compounds, Zn(II) complex showed higher antibacterial activity over both bacterial strains against reference drug Kanamycin. Moreover, synthesized metal complexes exhibited moderate antioxidant activity than the Schiff base. Cu(II) complexes were found to be most active whereas, Zn(II) complexes showed the lowest antioxidant activity comparable to the BHT.

Keywords: Transition metal complexes, Schiff Base, 1, 10-Phenanthroline, Antimicrobial, Antioxidant properties.

1. INTRODUCTION

A German chemist and physicist Hugo Schiff first synthesized Schiff bases which are compounds containing azomethine (C=N) group. Schiff bases, which are usually prepared by the condensation of primary amines and active carbonyl groups such as aldehydes or ketones [1-3]. They are top-flight coordinating ligands and form stable complexes with various transition metal ions [4]. As well, Schiff base is a fascinating class of ligands that have played significant acting in the bettmer of coordination chemistry. Schiff bases and their complexes have a diversity of applications in the biological activity studies and industry [5-10] to they are used radiotracers in nuclear medicine and drugs [11]. Moreover, Schiff-bases are extremely momentous substances for inorganic chemists as these are generally used in medicinal inorganic chemistry because of their various biological, pharmacological, antimicrobial activities as well as their outstanding chelating ability [12]. Furthermore, they have potential applications in many other fields such as biological (including antibacterial, antifungal, anti-oxidative anti-inflammatory, anti-tumor, anti-cancer, and anti-HIV) optical materials, chemical sensor, clinical, analytical, and electrochemistry [13-18]. Mixed ligand complexes have a major role in the biological field as shown by several ways in which metal ions activate enzymes. Schiff bases, for example, derived from heterocyclic compounds, P- anisaldehyde and furan-2-carbaldehyde have attracted keen interest in biological chemistry [19-21]. Iso-nicotinylhydrazide (INH) is a bio-compound that is one of the most powerful drugs and medicines of the first-line for the treatment and the prevention of tuberculosis. Because of the high antibacterial activity of this compound against Mycobacterium tuberculosis, the World Health Organization (WHO) considers INH asa standard treatment material. It forms metal chelate compounds with different bivalent ions having moderate to significant biological importance [22-25]. In the present paper, we report the results on the synthesis, characterization and antibacterial and anti-oxidant activities of transition metal complexes of Cu(II), Ni(II), and Zn(II) ions containing thiophene-2-carbaldimene-isonicotinohydrazone Schiff base and 1, 10-Phenanthroline ligand.

2. EXPERIMENTAL METHODS

The weighing operation was performed with the help of a METTLER PM 200 electronic balance. The melting or decomposition temperatures of all the prepared metal complexes were observed in an electro thermal melting point apparatus model.a.ZoQ112. Infrared spectra of the ligands and complexes were recorded on a FTIR-8400, SHIMADZU, Japan using a KBr disc at the Central Science Lab of Rajshahi University. The Electronic spectra of the complexes in solution phase (1×10-5 M) were recorded in the range of 200-800 nm on a Thermo-electron Nicolet evolution 300 UV-Vis spectrophotometer. Conductivity measurements of the complexes were carried out on N, N'-dimethylformamide (DMF) using Horiba conductivity meter B173 in which cell constant is fixed. Sherwood Scientific Magnetic Susceptibility Balance was used to measure the magnetic moment values. Elemental analysis was carried out on a LECO (CHNS-932) elemental analyzer. The purity of all these compounds was tested by thin layer chromatography (TLC) on Silica gel-GF 254 (Merck) coated plates.

2.1. Synthesis of thiophene-2-carbaldimide-isonicotinohydrazone Schiff base ligand (L1)

Thiophene-2-carbaldimide-isonicotinohydrazone Schiff base Ligand was prepared (Scheme 1) by the condensation reaction (refluxed for 5 hours) of isoniazid (INH) (1.374 g, 10.00 mmol) and thiophene-2-carbaldehyde (0.93 mL, 10.00 mmol) in an equimolar ratio (1:1). The color of the solution (brown) converted into white by this time. The completion of the reaction was monitored by TLC. Eventually, it produced a white crystalline solid which was filtered off and dried in a desiccator over anhydrous CaCl2.

Thiophene-2-carbaldimide-isonicotinohydrazone

C10H9N2O; White solid, yield 88 %, mp., 210 °C, IR spectrum, ν, cm-1: 1665.57 (C=O), 1595.57(C=N). UV-Vis spectrum, λmax nm: 271, 324. Elemental analysis for C10H9N2O: Found, %: C, 57.13; H, 3.92; N, 18.17; O, 6.93; S, 13.86. Calculated, %: C, 57.08; H, 3.89; N, 18.16; O, 6.92; S, 13.84.

Scheme 1. Synthesis of Schiff base, thiophene-2-carbaldimide-isonicotinohydrazone (L1).

2.2. Synthesis of Mixed Ligand Complexes

The synthesized Schiff base ligand L1 (0.232 g, 1 mmol) was dissolved in methanol (10 mL). Then, the hot methanolic solution of ligand and equimolar 1,10-phenanthroline hydrate (0.198 g, 1mmol) were added drop-wise unitedly in the heated methanolic solutions (10 mL, 1 mmol) of nitrate salts (0.254 g, 1mmol) of metals for-instance; Cu(II), Ni(II), and Zn(II). The mixture was...
refluxed for 4 hours and after completion of the reaction, it allowed to cool slowly (Scheme 2). The obtained precipitate was filtered, washed with a few times with methanol, and dried under vacuum on anhydrous CaCl₂. The synthesized metal complexes were soluble in dimethyl sulfoxide (DMSO) and N,N-dimethylformamide (DMF).

\[
\text{MX}_2 + \text{L} \rightarrow [\text{ML}_2]X_2
\]

Scheme 2. Synthetic pathway of mixed ligand complexes, Where, M = Cu(II)/Ni(II)/Zn(II) ions.

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\text{[Cu(L1)(L2)](NO}_3)_{2}: \text{F.wt.} (g/mol): 599.03; \text{Yield: 79%}; \text{Color: Dijon yellow solid, m.p., 258 °C}. \text{Molar conductance (Ω}^{-1} \text{cm}^2 \text{mol}^{-1}): 137, \mu_{\text{eff}}, \text{B.M: 1.84}. \text{IR Spectrum, } \nu, \text{ cm}^{-1}: 597.74 (M=N), 681.89 (M=O), 1595.05 (C=N), 1636.29 (C=O). \text{UV-Vis spectrum, } \lambda_{\text{max}}, \text{nm: 272, 358, 373}. \text{Elemental analysis for CuC}_2\text{H}_7\text{N}_2\text{O}_2; \text{Found: } C, 46.12; H, 2.86; N, 16.37; O, 18.70; S, 5.35; Cu, 10.88. \text{Calculated: } C, 46.07; H, 2.84; N, 16.36; O, 18.70; S, 5.34; Cu, 10.60.

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\text{[Ni(L1)(L2)](NO}_3)_{2}: \text{F.wt.} (g/mol): 594.18; \text{Yield: 62%}; \text{Color: Crimson solid, m.p., 241 °C}. \text{Molar conductance (Ω}^{-1} \text{cm}^2 \text{mol}^{-1}): 150, \mu_{\text{eff}}, \text{B.M: 1.23}. \text{IR Spectrum, } \nu, \text{ cm}^{-1}: 596.20 (M=N), 677.23 (M=O), 1592.18 (C=N), 1636.11 (C=O). \text{UV-Vis spectrum, } \lambda_{\text{max}}, \text{nm: 269, 360}. \text{Elemental analysis for NiC}_2\text{H}_7\text{N}_2\text{O}_2; \text{Found: } C, 46.49; H, 2.88; N, 16.50; O, 18.70; S, 5.40; Ni, 9.88. \text{Calculated: } C, 46.45; H, 2.86; N, 16.49; O, 18.85; S, 5.39; Ni, 9.88.

\[
\text{[Zn(L1)(L2)](NO}_3)_{2}: \text{F.wt.} (g/mol): 600.87; \text{Yield: 67%}; \text{Color: White solid, m.p., 251 °C}. \text{Molar conductance (Ω}^{-1} \text{cm}^2 \text{mol}^{-1}): 143, \mu_{\text{eff}}, \text{B.M: 0.75}. \text{IR Spectrum, } \nu, \text{ cm}^{-1}: 592.20 (M=N), 685.01 (M=O), 1585.59 (C=N), 1664.63 (C=O). \text{UV-Vis spectrum, } \lambda_{\text{max}}, \text{nm: 282, 327, 384}. \text{Elemental analysis for ZnC}_2\text{H}_7\text{N}_2\text{O}_2; \text{Found: } C, 45.97; H, 2.85; N, 16.32; O, 18.64; S, 5.34; Zn, 10.88. \text{Calculated: } C, 45.93; H, 2.83; N, 16.31; O, 18.64; S, 5.33; Zn, 10.88.

3. RESULTS AND DISCUSSION

All complexes were insoluble in polar solvent but soluble in DMSO and DMF. The molar conductance values for all synthesized complexes were measured (10³ M) in DMF solvent at room temperature. Based on conductance measurements, all complexes were electrolytic nature and nitrate complexes were 1:2 electrolytes [26, 27]. For Cu(II), and Ni(II) complexes, magnetic moment values were 1.84, and 1.23 B.M respectively indicating paramagnetic, and diamagnetic nature. These values correspond to square planar geometry and on the other hand, tetrahedral structure for Zn(II) ion complex with 0.69 B.M [28-31].

**IR Spectral Studies**

The ν(C=O) and ν(C=N) stretching bands are observed at 1666 cm⁻¹ and 1596 cm⁻¹ respectively (Figure 1) in the IR spectrum of the Schiff base ligand [34, 35]. This band (1666 cm⁻¹) in IR spectra of metal complexes was shifted to lower frequencies and displayed in the region 1636–1665 cm⁻¹, indicating bonding of oxygen to the metal ions (Figure 1). Due to M-O stretching vibrations the new absorption bands are at 677–686 cm⁻¹ in the IR spectra of complexes [34-36].

The medium-strong band at 1596 cm⁻¹ in spectrum of free Schiff base ligand is assigned to the azomethine ν(C=N) band. Comparison of azomethine stretch of the ligand with spectra of complexes appeared shift towards lower wavenumbers from 1585–1595 cm⁻¹ region. These shifts suggested that ‘N’ of the C=N group coordinated to metal ions. In addition, the other bands appeared at 592–598 cm⁻¹ in the IR spectra because of ν(M–N) vibrations [36-39]. A strong sharp band of the IR spectra of mixed ligand complexes showed at 1384 cm⁻¹ that can be attributed to an uncoordinated nitrate ion [27].

Thus, FT-IR spectrada data confirms that two ligands coordinated to metal ions through the C=N and C=O groups respectively.

![Figure 1](image1.png)

**Figure 1.** IR Spectra of the ligand L1, and its Mixed Ligand Complexes.

**UV-Visible Spectra**

The electronic spectra of L1 (Fig. 2) demonstrated two absorption bands at 271 and 324 nm, attributable to π-π* and n-π* transitions, respectively [40]. In case of the metal complexes, these transitions have been tested, shifts to longer and lower wavelengths and it verified the ligand to metal charge transfer (LMCT) and vice versa [31]. The Cu(II) complex electronic spectrum showed 272, 358, and 373 nm of absorption bands that could be assigned to π-π*, n-π*, and charge transfer bands (C.T) [41]. There are three strong bands at 282, 327, and 384 nm in the Zn(II) complex. The peaks of 263, and 327 nm are attributable to π-π*, and n-π*, the absorption band of 386 nm is assigned to metal-ligand charge transfer (MLCT) [41-43]. In Ni(II) complex two peaks positioned at 269, and 360 nm due to π-π*, and charge transfer transitions, respectively [41]. Thus, the magnetic moment values along with electronic spectra data confirmed the square planar structures of Cu(II) and Ni(II) complexes and the tetrahedral structure for Zn(II) complex.

![Figure 2](image2.png)

**Figure 2.** UV-Visible Spectra of the L1 and its Mixed Ligand Complexes.
On the basis of the above characterizations, the probable structure of complexes are given below

Figure 3. The proposed square planar geometry of Cu(II), and Ni(II) complexes.

Figure 4. The proposed tetrahedral geometry of Zn(II) complex.

4. Pharmacology

Antibacterial Activity

The Schiff base ligands and its mixed ligand complexes were investigated for antibacterial activity against *Escherichia coli* and *Pseudomonas* sp. at the concentration (100 µg/10µL) in DMF. The values of inhibitory zone were measured in diameter (mm) and the result of their antibacterial activity has been listed in Table 1 and shown in Fig. 5. The Schiff base ligands did not show any inhibition zone against the selected bacterial strains. Moreover, it was found that [Zn(L1)(L2)](NO_3)_2 exhibited the highest antibacterial activity with the zone of inhibition ranging between 18 and 12 mm against *Pseudomonas* sp., and *Escherichia coli*. Overtone’s concept and Tweedy’s chelation theory can explain the greater activity of the complexes [44].

The significant antibacterial activity of metal complexes is due to the fact that only lipid-soluble materials making lipo-solubility, which is a main control factor for antibacterial activity. The polarity of metal ions is more reduced, due to overlap and the partial sharing of positive charge of the metal ion with the orbitals of the donor groups of the ligands. The metal ions are therefore, easily adsorbed on the surface of the cell wall of the organisms and able to disrupt the respiratory process of the cells and blocks protein synthesis and consequently, limits the additional growth of organisms. Besides this, the substituent methyl group plays a vital role to increase the lipophilic nature of the metal complexes that is the reason for remarkable antibacterial activity and other factors like solubility, coordinating sites, the geometry of complexes, steric, concentration, and hydrophobicity having considerable influence on the antibacterial potency [44-46].

| Compounds | Gram Negative |
|-----------|----------------|
|           | *Escherichia coli* | *Pseudomonas* sp. |
| Kanamycin (30 µg/disc) | 15 | 22 |
| Ligand (L1) | - | - |
| [Cu(L1)(L2)](NO_3)_2 | 6 | 8 |
| [Ni(L1)(L2)](NO_3)_2 | 7 | 11 |
| [Zn(L1)(L2)](NO_3)_2 | 12 | 18 |

Antioxidant activity

Antioxidant activity of the synthesized ligand and its mixed ligand complexes with was investigated by the free radical compound 2, 2-diphenyl-1-picryl hydrazyl (DPPH), and BHT (butylatedhydroxytoluene) as a standard at different concentrations (20, 40, 60, 80, and 100 µg/mL). DPPH having a violet color when dissolved in DMF, it became pale red [47]. The percentage of DPPH radical scavenging activity and IC_{50} values of BHT, ligands, and its mixed ligand complexes has been listed in 2 and shown in Fig. 6-7. From the results, it is found that all the metal complexes displayed moderate DPPH radical scavenging activity than the Schiff base ligands comparable with BHT. Among all the tested compounds, Cu(II) complexes exhibited the highest antioxidant activity than Ni(II) and Zn(II) complexes as compared with the standard BHT.

Due to the coordination environment and redox properties, the difference in the antioxidant activity of the Schiff base metal complexes can be ascribed. Generally, the redox properties of the metal complexes depend on some factors including axial ligation, chelate ring size, and degree of unsaturation in the chelate ring [45, 46]. The high antioxidant activity of Cu(II) complexes compared to other prepared complexes is assigned to the high reducing ability of Cu^{2+} and its proton donation property where Cu^{2+} act as a super oxide scavenging center [48]. Ni(II) complexes showed moderate antioxidant activity, it could be due to steric hindrance by geometric structure, hindering the radical approach of DPPH to active complex centers. Furthermore, Zn(II) having lower activity because probably, Zn(II) ion is not a transition metal and therefore cannot participate in electron-transfer reactions [48].

![Graphical representation of antibacterial activity of ligand L1, L2, and its metal complexes against *Escherichia coli* and *Pseudomonas* sp. with kanamycin standard.](image)

![Graphical representation of antibacterial activity of ligand L1, L2, and its metal complexes.](image)
Table 2. % of scavenging activity and IC_{50} values of BHT, ligand-L1, and its mixed ligand complexes

| Conc. (µg/mL) | % BHT | % L1 | % [Cu(L1)(L2)][NO_3]_2 | % [Ni(L1)(L2)][NO_3]_2 | % [Zn(L1)(L2)][NO_3]_2 |
|--------------|-------|------|------------------------|------------------------|------------------------|
| 20           | 11.29 | 5.50 | 22.87                  | 16.35                  | 1.59                   |
| 40           | 26.77 | 2.31 | 50.36                  | 20.54                  | 5.21                   |
| 60           | 34.97 | 3.16 | 61.36                  | 24.27                  | 6.22                   |
| 80           | 38.20 | 4.92 | 63.82                  | 27.93                  | 7.66                   |
| 100          | 39.65 | 6.14 | 65.22                  | 30.37                  | 8.24                   |
| IC_{50}      | 105.71| 793.65| 61.88                  | 139.66                 | 510.20                 |

But it showed the lowest antioxidant activity than Cu(II), and Ni(II) complexes compared to the standard BHT.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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CONCLUSIONS

The synthesis and characterization of isonicotinamido-thiophene-2-carboxaldehyde and its Cu(II), Ni(II), and Zn(II) mixed ligand complexes with 1,10-phenanthroline have been achieved with physiochemical and spectroscopic techniques. The value of conductivility indicated the electrolytic existence of the complex. The IR spectral analysis leads to the conclusion that the ligands coordinated to central ions through N and O atoms. From the UV-VIS spectra and magnetic moment data, it is found that The coordination geometry around the metal ions (for Cu(II), and Ni(II) ions) can be described as square planar geometry and tetrahedral geometry for Zn(II) complex. Zn(II) complex showed maximum antibacterial activity compared to Schiff base and other complexes.
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