ISONICOTINOHYDRAZIDE DERIVED SCHIFF BASE–TRANSITION METAL COMPLEXES: STRUCTURE WITH BIOLOGICAL ACTIVITY

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INTRODUCTION

Tuberculosis (TB), caused by the pathogenic bacterium Mycobacterium tuberculosis, is an extremely severe and deadly infectious disease worldwide [1, 2]. The presence of this infection in individuals with weakened immune systems, particularly those with HIV and severely drug-resistant TB, adds complexity and severity to the disease [3]. Streptomycin and para-aminosalicylic acid were the first antimicrobial treatments for TB in the mid-1940s, but isoniazid (INH) revolutionized treatment in 1952 [4]. Currently, two approaches are being employed to discover novel anti-TB medications. The first approach involves modifying existing anti-TB drugs at the molecular level [5]. The second approach involves testing novel chemicals to determine their effectiveness against M. tuberculosis [6].

Hydrazones (HN) are a type of chemical molecule containing \( \text{a}=\text{C}=\text{N} \)-\text{NH} group. They are formed when a ketone or aldehyde reacts with a hydrazine or hydrazide [7]. The structure of HN exhibits several key features: (i) a nucleophilic imine and an amino-type nitrogen, with the amino-type nitrogen being more reactive; (ii) an imine carbon that possesses both electrophilic and nucleophilic properties; (iii) configurational isomerism resulting from the inherent nature of the \( \text{C} = \text{N} \) double bond; and (iv) in most cases, an acidic \( \text{N} \)-\text{H} proton. These structural motifs determine the physical and chemical properties of HN, which are crucial for its applications [8]. Significant attention has been given to acid hydrazides R-CO-NH-N-C=CH-R due to their interesting biological properties and their ability to bind to transition metal ions found in living organisms [9, 10].

INH is a potent antimicrobial hydrazide specifically designed to eradicate Mycobacterium tuberculosis, the causative agent of TB [11]. However, INH has the adverse effect of increasing liver enzyme levels in the bloodstream, which can lead to hepatic damage characterized by cellular necrosis and steatosis. Additionally, the metabolites of this medication can potentially impact liver cells adversely [12]. Therefore, enhancing the therapeutic efficacy of INH, a prominent first-line anti-TB medication, through structural modifications has received significant attention over the years. The methods employed involve creating and evaluating modified versions of INH by adding substituents that either remove or donate electrons to the isonicotinic ring [13]. It has been reported that INH Schiff base (INH SB) possesses superior antitubercular and anticancer activities compared to INH alone [14]. Scientists have explored metal complexes of organic pharmaceuticals as potentially more active than the original organic compound, leading them to investigate metal complexes of INH as potential anti-TB drugs [15]. Additionally, isonicotinohydrazones and molecules that coordinate with them exhibit remarkable antimicrobial, antifungal, anticancer, antioxidant, and anticonvulsant effects [16].

Considering all of these factors, our objective was to develop a promising ligand and studying the antibacterial, anticancer, and anti-TB properties of a new ligand called isonicotinohydrazide and its transition metal complexes [17].

MATERIALS AND METHODS

Instrumentation
Carbon, hydrogen, nitrogen, and sulfur quantities were determined via a PerkinElmer PE 2400 elementary analyzer. The model A26512 electrothermal melting point device examined the ligand and metal complexes’ melting points. The molar conductivity of complexes in dimethyl sulfoxide (DMSO) has been evaluated with the analyzer CONSORT-C86 for solutions with an average concentration of \( 10^{-5} \) M. Using a Bruker Tensor 37 spectrometer, KBr pellets were evaluated with infrared spectra in the 400–4000 cm\(^{-1}\) region. A Jasco V670 spectrophotometer recorded...
electrical spectra at 200 and 800 nm using the diffuse light reflection method, and Spectral was given as a standard [18]. Employing a Gouy balance, the complexes’ magnetic susceptibility was assessed at the ambient temperature [19]. The spectrum of NMR was taken on a Bruker advance detector at 500 MHz for 1H [20]. ESI-MS spectra were done with an Agilent Technologies MSD SL Trap mass spectrometer with an ESI source coupled with an 1100 Series HPLC system.

Materials and reagents
The reagents used in this study, such as INH, acetyl-2-thiophene, Co(CH₃COO)₂·H₂O, Cu(CH₃COO)₂·H₂O and Ni(CH₃COO)₂·H₂O, absolute ethanol, methanol and DMSO were purchased from Aldrich.

Synthesis of ligand and its metal complexes

The synthesis of SB ligand ([E]-N′-(1-(thiophen-2-yl)ethyldene)isonicotinohydrazide]

The ligand (L) was prepared by combining 10 mmol of 2-acetylthiophene (1.26g) with 10 mmol of INH (1.37g) in a round-bottom flask through a condensation reaction. The INH was dissolved in 20 ml of heated ethanol, while 2-acetylthiophene was also dissolved in ethanol. The solutions were then combined and subjected to reflux for 4-5 hours [21]. After cooling, a white crystalline substance was obtained. The product was purified using acetone and diethyl ether and then dried under a pressurized desiccator containing anhydrous CaCl₂. The growth and purity of the ligand were assessed by TLC using various solvents. The product yielded 81% and exhibited a yellow color, indicating its solubility in several solvents such as CH₃OH, C₂H₅OH, CCl₄ and DMSO.

Molecular formula: C₃H₇N₂O₂S; appearance: white crystalline powder; yield: 83%; melting point: 232-234 °C; 1H-NMR (400 MHz, DMSO-d₆): δ 11.0 (CO-NH, 1H, s); 2.45 (DMSO, 6H, s), 7.10-7.73 (Ar, 7H, m), 2.35 (~CH₃, 3H, s); FT-IR (KBr pellet): ν(OH)-3400 cm⁻¹, ν(C=O)-1668 cm⁻¹, ν(C=N)-1596 cm⁻¹; UV/Vis. (DMSO): λmax at: 267 nm and 324 nm; Anal Cal: C 58.77, H 4.49, N-17.14, S-13.06 Found: C 58.70, H-4.54, N-17.21, S-13.01.

Synthesis of metal complexes

Co-complex: Appearance: light yellow; yield: 67%; melting point: >300 °C; molecular formula: CoC₂H₁₀ON₂S₂; conductivity: 3 Sm⁻² mol⁻¹; μeff B. M.: 4.32; FT-IR (KBr pellet): ν(OH)-3432 cm⁻¹, ν(C=O)-1299 cm⁻¹, ν(C̶N)-1577 cm⁻¹, ν(C̶O)-1637 cm⁻¹, ν(C̶N)-481 cm⁻¹; UV/Vis. (DMSO): λmax at: 278 nm and 364 nm; Anal Cal: C 52.66, H 3.66, N-15.36, S-11.70; Found: C 52.70, H-3.61, N-15.41, S-11.64.

Ni-complex: Appearance: yellowish green; yield: 61%; melting point: >300 °C; molecular formula: NiC₂H₁₀ON₂S₂; conductivity: 6 Sm⁻² mol⁻¹; μeff B. M.: 2.87; FT-IR (KBr pellet): ν(OH)-3393 cm⁻¹, ν(C̶O)-1302 cm⁻¹, ν(C̶N)-1575 cm⁻¹, ν(C̶N)-640 cm⁻¹, ν(N̶N)-476 cm⁻¹; UV/Vis. (DMSO): λmax at 300 nm, 366 nm, and 400 nm; Anal Cal: C 52.68, H-3.66, N-15.36, S-11.71; Found: C 52.64, H-3.71, N-15.41, S-11.62.

Cu-complex: Appearance: light green; yield: 57%; melting point: >300 °C; molecular formula: CuC₂H₁₀ON₂S₂; conductivity: 4 Sm⁻² mol⁻¹; μeff B. M.: 1.85; FT-IR (KBr pellet): ν(OH)-3424 cm⁻¹, ν(C̶O)-1305 cm⁻¹, ν(C̶N)-1577 cm⁻¹, ν(C̶O)-638 cm⁻¹, ν(C̶N)-458 cm⁻¹; UV/Vis. (DMSO): λmax at 262 nm, 290 nm, and 418 nm; Anal Cal: C 52.22, H-3.63, N-15.23, S-11.60; Found: C 52.15, H-3.69, N-15.17, S-11.64.

RESULTS AND DISCUSSION

Elemental and molar conductivities studies

The metal percentage was determined using complexometric titration with EDTA, following the procedure outlined in the literature [23]. Table 1 presents the analytical data for the percentages of M (II), C, H, N, and S in the complexes, both observed and calculated. The molar conductivities were measured at a concentration of 10-3M in DMSO at ambient temperature table 1. The lower conductance level confirms the non-electrolytic nature of the complexes [24].

![Synthetic pathway for the formation of L and its metal complexes](image)

**Fig. 1: Synthetic pathway for the formation of L and its metal complexes**

**Table 1: Physical-chemical and analytical data on SB ligand and its complexes with metals**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Empirical formula</th>
<th>Elemental analysis found (cal) (%)</th>
<th>A⁺</th>
<th>Sm² mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>C₃H₇N₂O₂S</td>
<td>C 58.70(58.77) H 4.54(4.49)</td>
<td>13.01(13.06)</td>
<td>---</td>
</tr>
<tr>
<td>Ni-complex</td>
<td>NiC₂H₁₀ON₂S₂</td>
<td>58.24(58.26) H 3.71(3.66)</td>
<td>15.41(15.36)</td>
<td>11.62(11.71)</td>
</tr>
<tr>
<td>Co-complex</td>
<td>CoC₂H₁₀ON₂S₂</td>
<td>52.73(52.66) H 3.61(3.66)</td>
<td>15.41(15.36)</td>
<td>11.64(11.70)</td>
</tr>
<tr>
<td>Cu-complex</td>
<td>CuC₂H₁₀ON₂S₂</td>
<td>52.15(52.52) H 3.69(3.63)</td>
<td>15.17(15.23)</td>
<td>11.64(11.60)</td>
</tr>
</tbody>
</table>
FT-IR spectra

The spectra of the ligand (L), Co-complex, Ni-complex, and Cu-complex can be observed in fig. 2. These spectra were obtained using the Fourier transform infrared (FT-IR) method. The stretching frequencies of the ν(−OH), ν(−NH), and ν(−C=O) groups were determined by assigning absorption bands at wavenumbers of 3400 cm⁻¹, 3180 cm⁻¹, and 3034 cm⁻¹, respectively. Additionally, peaks at 1596 cm⁻¹ and 1668 cm⁻¹ correspond to vibrational modes of the v(C=N) and v(C=O) functional groups [25]. In the FT-IR spectra of the metal complexes, the band of the ligand shifted from 1596 cm⁻¹ to 1577 cm⁻¹, 1575 cm⁻¹, and 1577 cm⁻¹ for the Co-complex, Ni-complex, and Cu-complex, respectively, due to the azomethine moiety (−C=N). This shift indicates coordination of the metal ion with the N-atom of the (−C=N) group. Furthermore, the stretching frequency of the ligand’s (−C=O) group at 1668 cm⁻¹ shifted to 1299 cm⁻¹, 1302 cm⁻¹, and 1305 cm⁻¹ in the Co-complex, Ni-complex, and Cu-complex, respectively, indicating the influence of oxygen on coordination. The appearance of new bond stretching frequencies at 640 cm⁻¹, 638 cm⁻¹, and 637 cm⁻¹ suggests the formation of NiO, Cu-O, and Co-O bonds, while frequencies at 476 cm⁻¹, 458 cm⁻¹, and 481 cm⁻¹ indicate Ni-N, Cu-N, and Co-N bonds, respectively. Additionally, a significant peak observed between 3530 and 3450 cm⁻¹ in the IR spectra of the Co-complex, Ni-complex, and Cu-complex complexes is likely due to water present in the KBr pellet [26]. Table 2 presents the FT-IR spectral data of the ligand (L) and its Co(II), Ni(II), and Cu(II) metal complexes.

Table 2: The L and its Co (II), Ni (II), and Cu (II) metal complex IR-FTIR spectrum data (in cm⁻¹)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ν(−OH)</th>
<th>ν(−C=N)</th>
<th>ν(−C=O)</th>
<th>ν(C=O)</th>
<th>ν(M−O)</th>
<th>ν(M−N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3460</td>
<td>1596</td>
<td>1668</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni-complex</td>
<td>3393</td>
<td>1575</td>
<td></td>
<td>1302</td>
<td>640</td>
<td>476</td>
</tr>
<tr>
<td>Cu-complex</td>
<td>3424</td>
<td>1577</td>
<td></td>
<td>1305</td>
<td>638</td>
<td>458</td>
</tr>
<tr>
<td>Co-complex</td>
<td>3432</td>
<td>1577</td>
<td></td>
<td>1299</td>
<td>637</td>
<td>481</td>
</tr>
</tbody>
</table>

Fig. 2: FT-IR spectra of L and its metal complexes

UV-Vis spectra

The ligand (L) and its Ni(II), Cu(II), and Co(II) metal complexes in DMSO were analyzed by electronic spectroscopy, and the spectral data are presented in table 3. The visible and ultraviolet spectra exhibited numerous intense absorption bands for both the ligands and complexes. Electronic spectra of both the ligand (L) and the complexes were obtained after dissolving them in DMSO at room temperature. In the electronic spectra of the ligand (L), strong absorption peaks at 267 nm and 324 nm are observed. Fig. 3 illustrates that these peaks are attributed to π→π* and n→π* transitions.

As depicted in fig. 3, the Ni-complex exhibited three UV-Vis absorption bands at 300 nm, 366 nm, and 400 nm. Changes in the electronic spectra of the ligand (L) at 267 nm and 324 nm were observed upon binding to Ni(II) ions in this experiment, indicating L’s interaction with Ni(II) ions. The absorption peak at 300 nm was attributed to the π→π* transition, while the peak at 366 nm was attributed to the n→π* transition, which arises from the unshared pair of electrons on the azomethine nitrogen and an antibonding p orbital. Fig. 3 also illustrates a 400 nm absorption band corresponding to the l-to-metal charge transfer.

The Cu-complex exhibits three UV-Vis absorption bands at 262 nm, 290 nm, and 418 nm, as depicted in fig. 3. The electronic spectra of the ligand (L) at 267 nm and 324 nm were altered by the Co-complex, indicating interaction between L and Cu(II) ions. The absorption at 262 nm corresponds to the π→π* transition, while the absorption at 290 nm is attributed to the n→π* transition, which is induced by the lone electron pair on the azomethine nitrogen and the antibonding p orbital. Additionally, the absorption band at 418 nm in fig. 3 demonstrates charge transfer from L to the metal.

The Co-complex displays three UV-Vis absorption bands at 278 nm and 364 nm. The peak at 278 nm is assigned to the π→π* transition, which occurs due to the lone electron pair on the azomethine nitrogen and the antibonding p orbital. At 364 nm, fig. 3 illustrates charge transfer from L to the metal in the molecule’s absorption band.

Based on UV spectral data and magnetic moment values (fig. 4(a, b, c)), the Co, Ni, and Cu complexes are suggested to be tetrahedral [27].
Table 3: Electronic spectrum data and magnetic moments for L and its metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Empirical formula</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>$\mu_{\text{eff}}$ B. M</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>C$<em>{12}$H$</em>{11}$ON$_3$S</td>
<td>267</td>
<td>---</td>
<td>n→π*</td>
</tr>
<tr>
<td>Ni-complex</td>
<td>[NiC$<em>{24}$H$</em>{20}$O$_2$N$_6$S$_2$]</td>
<td>300</td>
<td>2.87</td>
<td>π→π*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>366</td>
<td></td>
<td>n→π*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td></td>
<td>C. T (L→M)</td>
</tr>
<tr>
<td>Cu-complex</td>
<td>[CuC$<em>{24}$H$</em>{20}$O$_2$N$_6$S$_2$]</td>
<td>262</td>
<td>1.85</td>
<td>π→π*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>290</td>
<td></td>
<td>n→π*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>418</td>
<td></td>
<td>C. T (L→M)</td>
</tr>
<tr>
<td>Co-complex</td>
<td>[CoC$<em>{24}$H$</em>{20}$O$_2$N$_6$S$_2$]</td>
<td>278</td>
<td>4.32</td>
<td>π→π*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>364</td>
<td></td>
<td>C. T (L→M)</td>
</tr>
</tbody>
</table>

Fig. 3: Electronic spectra of L and its metal complexes

Fig. 4(a): Proposed structure of [CoC$_{24}$H$_{20}$O$_2$N$_6$S$_2$] complex (Tetrahedral)

Fig. 4(b): Proposed structure of [NiC$_{24}$H$_{20}$O$_2$N$_6$S$_2$] complex (Tetrahedral)
Fig. 4(c): Proposed structure of [CuC₂H₂O₂N₂S₂] complex (Tetrahedral)

1H-NMR spectroscopy

Fig. 5 displays the 1H-NMR spectra of the ligand (L) after dissolution in DMSO [28]. The prominent peak at a chemical shift of 2.3 ppm corresponds to the proton of the -CH₃ group. The protons of the pyridine and thiophene rings appear as two doublet signals within a chemical shift range of 7.1–8.7 ppm. The proton of the amide CO-NH, which is highly acidic, appears as a singlet at a chemical shift of 11.0 ppm [29].

Fig. 5: 1H-NMR spectra of I delta value (ppm)

ESI-mass spectral investigations of the l and its complexes with Co (II), Ni (II), and Cu (II) Ions

The ESI-MS spectra of the investigated l showed the presence of a most abundant signal at m/z = 246.4718 ([L+H]+) corresponding to the protonated molecular ion of the l, and less abundant signals at m/z = 79.5783 and 86.5301, corresponding to the two aromatic rings detached from the protonated l. The ESI-MS spectra of the synthesized metal complexes in slightly acidified DMSO reveal that these complexes show several fragmentations. The ESI-MS spectra display the most abundant peaks at m/z = 547.4078, 548.8765, and 553.1634, corresponding to the intact molecular ions for Co-complex, Ni-complex, and Cu-complex, respectively (fig. 6).

Fig. 6: The ESI-MS spectra of (a) l, (b) Co-complex, (c) Ni-complex, and (d) Cu-complex
Microbiological evaluation

The global prevalence of antimicrobial drug-resistant bacteria is rapidly increasing leading to a progressive decline in the effectiveness of antibiotics [30]. Antimicrobial resistance (AMR) poses a widely recognized threat to public health. The emergence and global spread of novel resistance mechanisms are jeopardizing our ability to effectively combat infectious diseases, particularly in developing nations where second- and third-line medicines are not readily accessible or affordable.

This study investigated the antibacterial properties of the ligand (L) and its metal complexes against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Bacillus cereus. The diameter of the inhibitory zones was measured in millimeters, and the results were compared with standard Kanamycin-30. The findings of their antibacterial activity are reported in table 4 (fig. 7) [31]. The Ni and Cu-complexes exhibited greater antibacterial efficacy against all bacteria compared to the unbound L, while the Co-complex showed no activity. Among all synthesized compounds, the Ni-complex displayed significant antibacterial activity against Pseudomonas aeruginosa, whereas the Cu-complex exhibited superior activity against Escherichia coli, Staphylococcus aureus, and Bacillus cereus.

The enhanced antibacterial efficacy of metal complexes compared to the unbound ligand can be comprehensively explained by Overton's concept and Tweedy's chelation hypothesis [32]. Chelation with L reduces the polarity of a metal ion because the positive charge of the metal ion is partially shared with donor groups (such as O, N, S, etc., depending on the ligand's structure). Additionally, there is potential for π-electron delocalization within the chelate ring. Chelation with L significantly increases the lipophilicity of the central metal ion, facilitating its passage through the lipid layers of the cell membrane and preventing it from binding to metal-binding sites on microbial enzymes [33, 34].

The effectiveness of different chemicals against specific species is influenced by the impermeability of microorganisms' cell membranes or alterations in the ribosomes of microbial cells. The fig. and tables presented below display the outcomes of the antibacterial activity of different samples against harmful bacteria [35].

Table 4: Antibacterial activities (zone of inhibition in mm) of samples against pathogens

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
<th>Bacillus cereus</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin-30</td>
<td>27</td>
<td>24</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>L</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Cu-complex</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Ni-complex</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Co-complex</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 7: Zone of inhibition of samples and standard against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus. Data is expressed as mean of three determinations.

Cytotoxicity evaluation

The brine shrimp lethality bioassay is employed to quickly and easily evaluate the cytotoxicity of bioactive compounds [36]. The lethality test conducted on brine shrimp revealed that the ligand (L) and its metal compounds exhibit significant activity, as shown in table 5 (fig. 8). According to the current experiment, there is a direct correlation between the concentrations of the compounds and the degree of lethality. The concentration of 100 μg/ml resulted in the highest fatality rates, while the concentration of 62.5 μg/ml showed the lowest death rates, as indicated in table 5 [37].

All the produced compounds demonstrated positive results in the brine shrimp lethality test, confirming their biological activity. Among these, the Ni-complex exhibited enhanced cytotoxicity with IC50 values of 17.64 μg/ml compared to L and other complexes when compared to the conventional vincristine sulfate, which had an IC50 of 12.09 μg/ml [38]. It is speculated that the Ni-complex may possess cytotoxic or anticancer properties. Subsequently, in vivo experiments will be conducted to assess whether the substances harm or selectively target cancer cells. We recommend utilizing a zebrafish animal model for this purpose [39].
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AUTHORS CONTRIBUTIONS
Al Akramullazi and Ms. Sahiba Sultana researched literature, conceived the study and wrote the first draft of the manuscript. Md. Faruk Hossen and Md. Ali Asraf involved in data analysis. Md. Kudrat-E-Zahan supervised the overall work. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

CONFLICT OF INTERESTS
Declared none

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