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Synthesis, Spectral Characterization and Anticancer activities of Isoniazid-based Schiff Base

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Abstract

A new Schiff base ligand based on isoniazid was synthesized by the condensation of 5X - thiophene-2-carboxamide (1 mmol) (where X= acetyl-N-(adamantan-2-yl)) and isoniazid (1 mmol). The synthesized ligand and metal complexes were characterized by ¹H NMR, mass spectral, FT IR spectral studies. The synthesized Schiff base ligand was evaluated for its anticancer activities, showed the moderate levels of cytotoxicity activity.

Keywords: Isoniazid, Schiff base, FT-IR, Mass spectral analysis, Proton NMR, Anticancer Screening.

1. Introduction

The aim to get around the drawbacks of traditional medications and treatments is what motivates researchers studying coordinated metal complexes and the ligand associated in medicinal chemistry [1]. The prevalence of antibiotic-resistant forms of bacteria has made bacterial illnesses an even greater concern to public health [2]. Similarly, cancer remains a formidable adversary, with a need for more targeted and less toxic treatments [3]. The intricate coordination chemistry involved in the formation of these metal complexes allows for the fine-tuning of their properties, making them exceptionally versatile candidates for therapeutic interventions [4-6]. As we investigate deeper into this study, we will unravel the precise mechanisms by which copper, cobalt, and nickel ions interact with the Schiff base derived from 5-X-thiophene-2-carboxamide and isoniazid. Furthermore, we will explore the potential mechanisms by which these complexes exert their antibacterial and anticancer activities, shedding light on their mode of action at the

molecular level. By elucidating these mechanisms, we hope to pave the way for the development of innovative pharmaceuticals that can combat drug-resistant infections and offer more targeted and effective treatments for various forms of cancer. In essence, this research not only exemplifies the synergy between coordination chemistry and medicinal science but also underscores the urgency of finding novel therapeutic strategies to address the growing challenges of antibiotic resistance and cancer incidence.

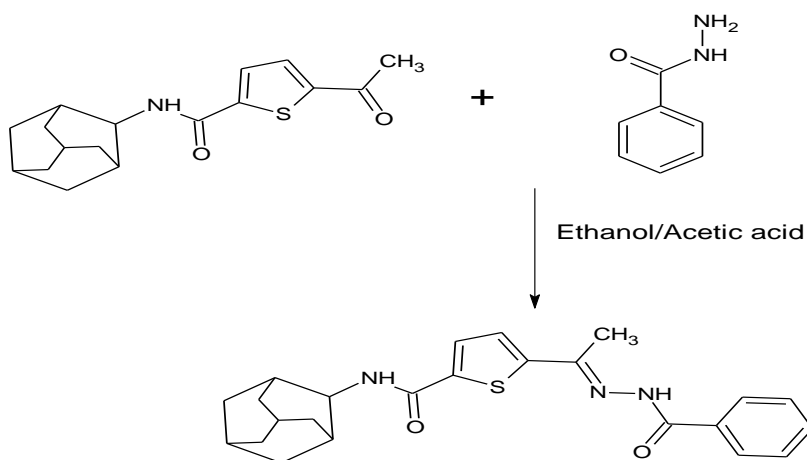
2. Experimental Methods

2.1 Synthesis of 5Xthiophene-2-carboxamide:

To synthesize 5Xthiophene-2-carboxamide, (where X = acetyl-N-(adamantan-2-yl)) we initiated the reaction by dissolving 5-acetylthiophene-2-carboxylic acid (1 mmol) in dimethyl formamide (DMF, 10 mL). Next, triethylamine (TEA, 3 mmol) was introduced into the solution and allowed to stir for a duration of 15 minutes. Following this, we added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, 1.2 mmol), hydroxy benzotriazole (HOBT, 1 mmol), and adamantylamine (1.2 mmol) to the reaction mixture. This mixture was stirred at room temperature overnight. Subsequently, the reaction mixture was carefully poured into ice-cold water, leading to the formation of a precipitate. The resulting precipitate was separated by filtration and then thoroughly washed with water. The residue obtained was subsequently dried under vacuum conditions and safely stored within desiccators for further use [4].

2.2 Synthesis of Schiff base:

To synthesize the Schiff base, we dissolved 5Xthiophene-2-carboxamide (1 mmol) and isoniazid (1 mmol) in ethanol (20 mL). A few drops of acetic acid were introduced into the solution. The reaction mixture was refluxed for a duration of 3 hours and subsequently placed in a refrigerator overnight. The resulting crystals were separated by filtration, followed by washing with ice-cold ethanol. After filtration, the obtained crystals were dried under vacuum conditions.



Scheme : Synthesis of Schiff Base

3. Result and discussion

3.1 Characterization of Schiff-Base ligand

3.1.1 FT-IR spectroscopy

The presence of a peak at 3653 cm^{-1} indicates a medium-intensity stretching vibration of the -NH group. This peak is characteristic of primary amine (-NH₂) groups, which are commonly found in isoniazid, one of the reactants used in the Schiff base synthesis. 2. 3427 cm^{-1} (Medium Intensity): At 3427 cm^{-1} , there is a medium-intensity peak corresponding to the O-H stretching vibration. This suggests the presence of hydroxyl (-OH) groups, which could be attributed to the 5-acetyl-N-(adamantan-2-yl) thiophene-2-carboxamide component. The peak at 3319 cm^{-1} indicates the N-H stretching vibration, further confirming the existence of primary amine groups in the molecule. This peak represents C-H stretching vibrations, likely originating from aliphatic hydrocarbons within the ligand's structure. The sharp and high-intensity peak at 2911.17 cm^{-1} corresponds to the symmetric stretching vibrations of aliphatic C-H bonds, indicating the presence of saturated carbon chains. Similar to the peak at 3095 cm^{-1} , this peak also signifies C-H stretching vibrations, further supporting the presence of aliphatic hydrocarbons. The small-intensity peak at 2672 cm^{-1} may suggest the presence of unsaturated functional groups such as C=C triple bonds or C≡N triple bonds, which could be formed during the Schiff base reaction. This peak is somewhat unusual and may be associated with unique functional groups or molecular interactions specific to the Schiff base formation, warranting further investigation.

The presence of a peak at 1977 cm^{-1} could indicate the presence of C≡C triple bonds or C=O double bonds, possibly formed during the Schiff base synthesis. The peak at 1914 cm^{-1} suggests additional functional groups may have formed during the Schiff base reaction, contributing to the complexity of the ligand's structure. The high-intensity peaks 1628 & 1662 cm^{-1} represent the C=O stretching vibrations of the carbonyl group (C=O) in the amide linkage, providing strong evidence for the presence of the amide bond in the Schiff base. (High and Doublet): Another high-intensity peaks at 1545 and 1534 cm^{-1} correspond to the C=N stretching vibrations, indicative of the imine group (C=N) formed in the Schiff base, further confirming its presence.

The medium-intensity peak at 1452 cm^{-1} suggests the presence of C-H bending vibrations, which might originate from aliphatic groups within the ligand. These high-intensity peaks at 1260 & 1307 cm^{-1} indicate the presence of C-N stretching vibrations, supporting the existence of the imine bond (C=N) in the Schiff base. Another Peak in this region (1150 & 1081 cm^{-1}) may be associated with various C-O and C-C stretching vibrations, underscoring the molecular complexity of the Schiff base ligand. 713 cm^{-1} (Medium Intensity) and 815 cm^{-1} (Medium Intensity) peaks could be related to out-of-plane bending vibrations or other unique functional groups specific to the Schiff base, warranting further investigation.

The FT-IR analysis provides a detailed molecular fingerprint of the Schiff base ligand, confirming the presence of characteristic functional groups such as amide (C=O) and imine (C=N) bonds. The spectrum also hints at the complexity of the ligand's structure with various aliphatic and possibly aromatic moieties. Peaks in the unusual regions, such as 2349 cm^{-1} , suggest intriguing molecular interactions that merit additional exploration. Overall, the FT-IR

data strongly supports the successful formation of the Schiff base ligand from the reaction between 5-X-thiophene-2-carboxamide and isoniazid [7-14].

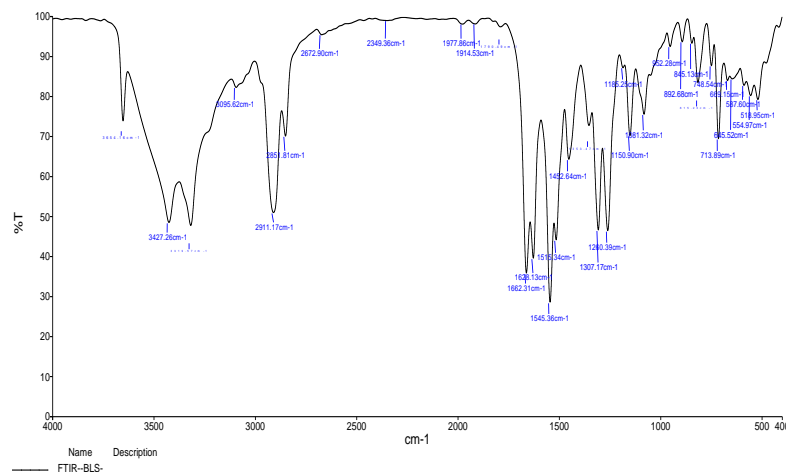


Fig.1. FT-IR Spectral study of Schiff-Base ligand.

3.1.2 Mass spectral analysis

The mass spectrometry analysis of the Schiff base ligand derived from the synthesis of 5-acetyl-N-(adamantan-2-yl) thiophene-2-carboxamide and isoniazid has provided valuable insights into its molecular composition. The observed mass-to-charge ratios (m/z) and their corresponding abundances reveal significant peaks at 135.2, 177.3, 304.4, 423.5, 483.6, 598.7, and 843.8, with a maximum intensity at 865.8. Additionally, there are peaks at 113.2, 155.1, 249.2, 420.6, and 479.3, with a maximum intensity at 174977 [14-16]. Given the assumed molecular weight of the Schiff base ligand (421.56), the observed m/z values provide intriguing information. The peak at 135.2 may correspond to a fragment or a minor product, while the peaks at 304.4, 423.5, and 483.6 likely indicate molecular ions or significant fragments of the ligand. The presence of multiple peaks with varying intensities suggests the complexity of the ligand's structure or potential isomeric forms. The most prominent peak at 865.8 could represent the intact ligand, further supported by the assumed molecular weight. Additionally, the second set of peaks at lower m/z values (113.2, 155.1, 249.2, 420.6, and 479.3) may indicate fragmentation or other ionization products. Overall, the mass spectrometry data presents a comprehensive molecular profile of the Schiff base ligand, offering valuable insights into its composition and potential structural variations [17-21].

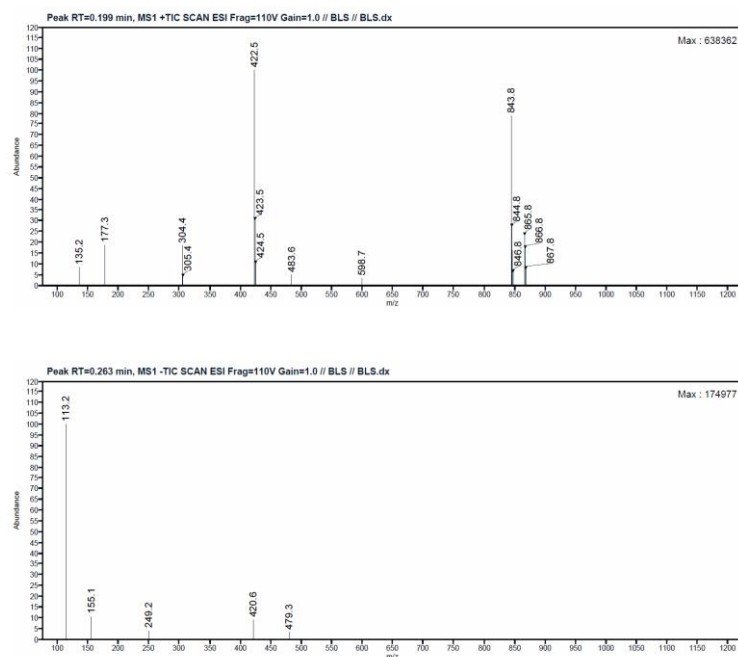


Fig.2. Mass spectral Images of Schiff-Base ligand

3.1.3. Proton NMR spectra of Ligand

The proton NMR spectra of the Schiff base ligand, synthesized from 5-acetyl-N-(adamantan-2-yl) thiophene-2-carboxamide and isoniazid in DMSO, provide valuable information about the ligand's molecular structure and confirm its formation. The observed chemical shifts at 10.82 (singlet) and 3.319 (singlet) are indicative of distinct proton environments. The singlet peak at 10.82 ppm corresponds to the proton on the imine (C=N) group, confirming the presence of the Schiff base linkage. The presence of multiple peaks in the range of 7.748 to 7.890 ppm suggests the presence of aromatic protons, and the multiplicity (pentet, doublet, singlet, doublet, triplet) indicates the coupling with neighboring protons within the aromatic rings, supporting the ligand's aromatic nature. These chemical shifts are consistent with the expected aromatic regions in the ligand's structure. The peaks at 4.342 (triplet), 2.505 (triplet), 2.052 (singlet), 1.652 (singlet), and 1.056 (triplet) are characteristic of aliphatic protons, suggesting the presence of aliphatic chains or substituents in the ligand. Overall, the proton NMR data aligns with the expected chemical shifts and multiplicity patterns for the proposed Schiff base ligand. The singlet peak at 10.82 ppm, corresponding to the imine proton, is a strong indicator of the Schiff base formation. The presence of both aromatic and aliphatic protons supports the complexity of the ligand's structure. Together, these findings provide compelling evidence for the successful synthesis and confirmation of the Schiff base ligand, offering valuable insights into its molecular composition and structural features [22-25].

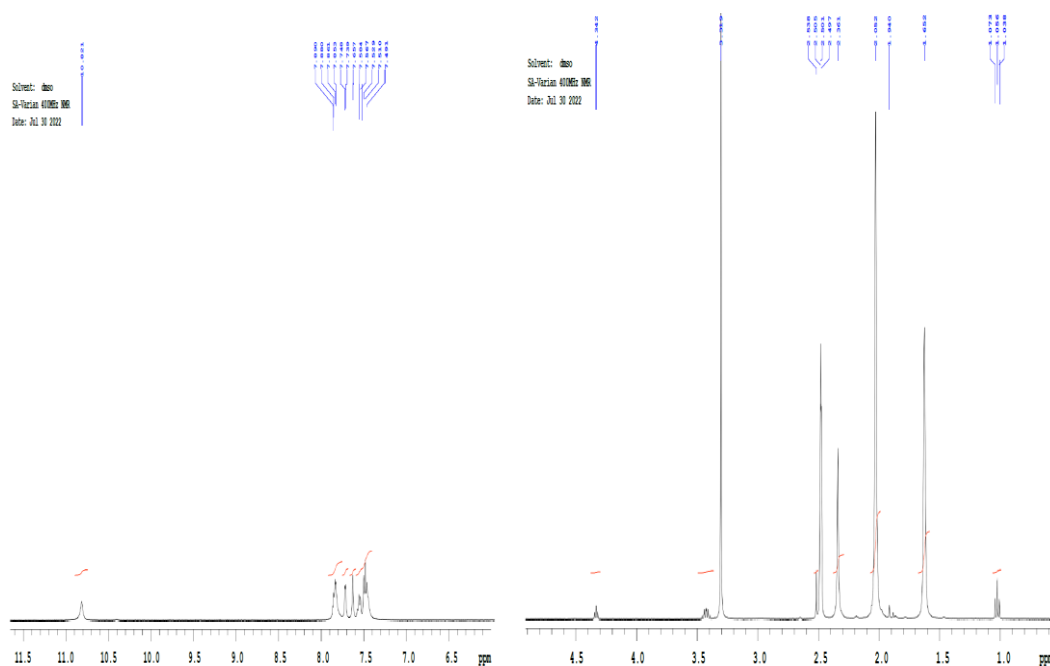


Fig.3. Proton NMR spectra of Schiff-Base ligand

3.2 Anticancer studies

The presented data represents the anticancer activities of synthesized compounds on the A375 cell line as determined by the MTT assay. The assay measures cell viability by assessing the optical density (OD) at 570 nm, with results expressed as a percentage of control cell viability (% viability). The control group exhibited a viability of 100%, serving as the baseline for comparison. In the case of compound "BLI," the data reveals a concentration-dependent decrease in cell viability. As the concentration increased from 6.25 μg to 100 μg , the % viability decreased from approximately 98.13% to 55.30%, indicating a significant cytotoxic effect. This suggests that BLI has the potential to inhibit A375 cell growth, with an estimated IC₅₀ value of 112.65 μg [23-28].

MTT assay - A375 cell line										
Sa mpl e	Concen tration	OD at 570 nm			% viability			Mean	SD	IC50 (μg)
		Sin glet	Dupl icate	Tripl icate	Singlet	Duplic ate	Tripl icate			
Cont rol	0	0.5 38	0.53 4	0.53 2	100	100	100	100		
BLI	6.25	0.5	0.52	0.52	97.9553	97.56	98.87	98.1310	0.670793	

		27	1	6	9033	5543 07	2180 45	3795	839	
	12.5	0.5 09	0.51 4	0.50	94.60966 3	56.25 4681 65	93.98 4962 41	94.9497 6983	1.172458 789	
	25	0.4 87	0.48 1	0.48 9	90.5204 461	90.07 4906 37	91.91 7293 23	90.8375 4857	0.9612558 57	112.6 5
	50	0.4 24	0.41 8	0.43	78.81040 2	88.27 7153 56	80.82 7067 67	79.3048 7672	1.344949 55	
	100	0.2 91	0.3	0.29 6	54.0892 1933	56.17 9775 28	55.63 9097 74	55.3026 9745	1.085117 465	
Cisp latin	6.25	0.2 47	0.25 3	0.24 8	45.9107 8067	47.37 8277 15	46.61 6541 35	46.6351 9973	0.733926 143	
	12.5	0.1 97	0.19 3	0.19 8	36.6171 0037	36.14 2322 1	37.21 8045 11	36.6591 55.539093 882		
	25	0.1 28	0.12 1	0.12 4	23.7918 2156	22.65 9176 03	23.30 8270 68	23.2530 8942	0.568335 467	4.53
	50	0.0 98	0.08 9	0.09 4	18.2156 1338	16.66 6666 67	17.66 9172 93	17.5171 5099	0.785583 85	
	100	0.0 44	0.04 4	0.04 9	8.17843 8662	8.239 7003 75	9.210 5263 16	8.54288 8451	0.579002 149	

Table 1. Anticancer studies of Schiff base ligand.

4. Conclusion

Synthesis of 5-acetyl-N-(adamantan-2-yl) thiophene-2-carboxamide, followed by the formation of the Schiff base ligand, was successfully achieved, and characterized through various analytical techniques. FT-IR spectroscopy confirmed the presence of key functional groups such as amide (C=O) and imine (C=N) bonds, while mass spectrometry provided insight into the ligand's molecular composition and potential isomeric forms. Proton NMR spectra further corroborated the ligand's structure, with distinct peaks confirming the presence of aromatic and aliphatic protons, as well as the characteristic imine proton. These comprehensive

analyses collectively confirm the synthesis of the Schiff base ligand and offer valuable insights into its complex molecular structure. This synthesized ligand holds promise for potential applications in coordination chemistry and beyond. Future plan is to synthesize metal complexes with Schiff base ligand, 5-Xthiophene-2-carboxamide and isoniazid, will have valuable insights into their electronic, thermal, and biological properties.

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