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Synthesis, Spectroscopic Characterization, and Statistically Validated Antimicrobial Activity of a Novel Antimony (III) Complex with (Salycylaldehyde-p-toluidine-N-thiohydrazonato) [Sb(HSalptth)Cl_3]

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Abstract

A novel antimony(III) complex, [Sb(HSalptth)Cl_3], incorporating the Schiff base ligand salicylaldehyde-p-toluidine-N-thiohydrazone (HSalptth), has been synthesized and comprehensively characterized. The ligand was prepared via condensation of salicylaldehyde with p-toluidine-N-thiohydrazide, followed by complexation with $SbCl_3$ under controlled conditions. The complex was isolated in good yield and characterized by elemental analysis, molar conductance, FT-IR, UV–Visible, and ^1H NMR spectroscopy, along with thermal analysis (TGA/DTA). Spectroscopic data reveal coordination of the monoanionic ligand to the Sb(III) center through the azomethine nitrogen and thiolate sulfur atoms, confirming bidentate chelation. The significant downward shift of the ν(C=N) band and changes in ν(C=S)/ν(C–S) frequencies in the IR spectrum support metal–ligand bond formation, while electronic transitions in the UV–Visible spectra are consistent with ligand to metal charge transfer processes. Molar conductance measurements indicate the non-electrolytic nature of the complex in polar solvents, suggesting a neutral coordination entity. Thermal studies demonstrate enhanced stability of the complex compared to the free ligand, with decomposition occurring in distinct stages corresponding to ligand fragmentation and chloride elimination.

The in vitro biological activity of the ligand and its Sb(III) complex was evaluated against selected Gram-positive and Gram-negative bacterial strains as well as fungal species using the agar diffusion and minimum inhibitory concentration (MIC) methods. The antimony(III) complex exhibits significantly enhanced antimicrobial activity relative to the free ligand, which can be rationalized by chelation theory and increased lipophilicity facilitating membrane permeation. The results highlight the potential of antimony(III) thiohydrazone complexes as promising candidates for further pharmacological and antimicrobial investigations.

Keywords: Antimony(III) complex; Schiff base; Thiohydrazone ligand; Chelation; Spectroscopic characterization; FT-IR spectroscopy; UV–Visible spectroscopy; ^1H NMR; Thermal analysis (TGA); Non-electrolytic complex; Antimicrobial activity; Minimum inhibitory concentration (MIC); Bioinorganic chemistry.

1.0 Introduction:

Schiff bases constitute an important class of ligands in coordination chemistry due to their ease of synthesis, structural flexibility, and strong coordination ability through azomethine nitrogen and additional donor atoms. Since their first report by Hugo Schiff, these compounds have been extensively investigated for applications in catalysis, materials science, and medicinal chemistry [1–3]. Salicylaldehyde-derived Schiff bases are particularly attractive because they provide phenolic oxygen and imine nitrogen donor sites capable of forming stable chelate rings with various metal ions [4–6].

Thiohydrazones and related thiosemicarbazone derivatives represent a significant subclass of Schiff bases containing both azomethine (–C=N–) and thione/thiolate (C=S) functionalities. These ligands exhibit versatile coordination modes through N and S donor atoms and have demonstrated diverse biological activities, including antibacterial, antifungal, antiviral, and anticancer properties [7–12]. It has been widely reported that coordination of such ligands to metal ions enhances biological efficacy, which can be rationalized on the basis of chelation theory and increased lipophilicity of the resulting complexes [13,14].

Antimony, a Group 15 element, forms stable compounds in the +3 oxidation state and frequently exhibits a stereochemically active lone pair that significantly influences its coordination geometry and reactivity [15,16]. Antimony(III) complexes adopt geometries ranging from trigonal pyramidal to distorted octahedral depending on ligand denticity and coordination environment [17,18]. Antimonial compounds have long been employed in medicinal chemistry, especially in the treatment of parasitic diseases such as leishmaniasis, as recognized by the World Health Organization [19]. Contemporary research has focused on developing organoantimony and coordination complexes with improved therapeutic profiles and reduced toxicity [20–22].

Complexes of Sb(III) with O, N, and S-donor ligands have shown promising antimicrobial, antifungal, and cytotoxic activities [23–27]. The presence of soft sulfur donor atoms enhances interaction with the relatively soft Sb(III) center, leading to stable chelation and modified electronic properties [28,29]. Spectroscopic methods such as FT-IR, UV–Visible, and NMR spectroscopy are indispensable for elucidating coordination behavior. Shifts in ν(C=N) and ν(C=S) frequencies in IR spectra, along with ligand-to-metal charge transfer transitions in UV–Visible spectra, provide clear evidence of complex formation [30–32]. Thermal techniques (TGA/DTA) further reveal information regarding stability and decomposition pathways of metal complexes [33,34].

Despite extensive investigations on transition metal–thiohydrazone systems, comparatively fewer studies have addressed antimony(III) complexes derived from salicylaldehyde-based thiohydrazones. Substituents such as p-toluidine groups may influence electronic distribution, steric factors, and biological activity of the resulting complexes [35–38]. Therefore, the synthesis and systematic characterization of new Sb(III) complexes with tailored thiohydrazone ligands remains an area of considerable interest.

In the present study, we report the synthesis, spectroscopic characterization, thermal behavior, and biological evaluation of a new antimony(III) complex, $[Sb(HSalptth)Cl_3]$, derived from salicylaldehyde-p-toluidine-N-thiohydrazone. The work aims to correlate structural features with antimicrobial activity and contribute to the expanding field of bioactive main-group metal complexes [39–45].

2.0 Experimental

2.1 Chemicals and Reagents

All chemicals were of analytical reagent grade and were used without further purification. Salicylaldehyde, p-toluidine, thiohydrazide, and antimony(III) chloride were obtained from commercial suppliers. Methanol, ethanol, and dimethyl sulfoxide (DMSO) were purified by standard procedures prior to use. Double-distilled water was used throughout the experiments.

2.2 Synthesis of Salicylaldehyde-p-toluidine-N-thiohydrazone (HSalptth)

Experimental Procedure:

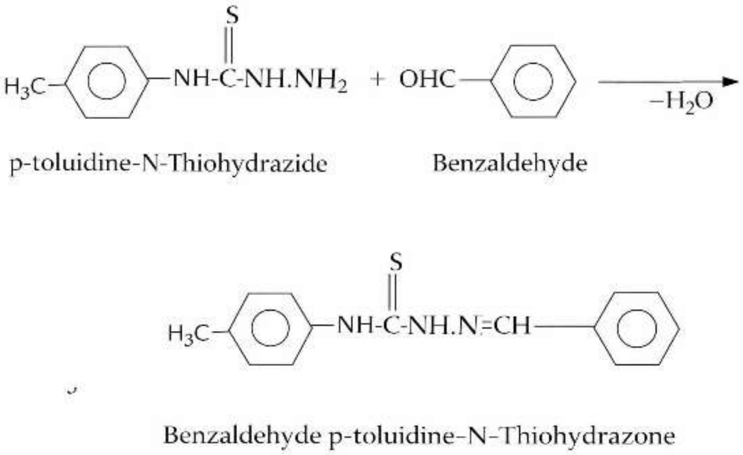
p-Toluidine–N-thiohydrazide (9.0 g) was dissolved in hot methanol (100 mL), and normal acetic acid (30 mL) was added to the solution. The reaction mixture was heated on a water bath until complete dissolution occurred and then filtered to remove any insoluble impurities.

To the clear filtrate, Salicylaldehyde (6.1 g) was added slowly with vigorous stirring. The reaction mixture was further heated for some time, during which a cream-white crystalline precipitate separated out. The solid product was collected by filtration, washed with a small quantity of cold methanol, and recrystallized from hot methanol.

The purified compound was dried, and its melting point was determined to be 180 °C.

The reaction sequences can be shown as:-

p-toluidine–N–Thiohydrazide + Benzaldehyde → Benzaldehyde p-toluidine–N–Thiohydrazone + H_2O



The obtained Benzaldehyde p-toluidine–N–Thiohydrazone **is cream white.**

The elemental analysis of Salicylaldehyde p-Toluidine–N-Thiohydrazone are found to be:

	% C	% H	% N	% S
Found	63.25	5.22	14.68	11.30
Calculated	63.17	5.28	14.73	11.25

2.3 Synthesis of Trichloro (Benzaldehyde p-toluidine–N-thiohydrazone)antimony(III) [Sb(HBenzptt)Cl_3)]

A methanolic solution of antimony trichloride (1.14 g) in methanol (50 mL) was treated with hot methanolic solution of 2.69gm. Benzaldehyde p-toluidine–N-thiohydrazone (in 60ml in methanol) with constant stirring a yellow crystalline precipitate was separated immediately.

The precipitate was filtered and washed with methanol and dried in a desiccator over anhydrous $CaCl_2$.

2.4 Antimicrobial Activity:

The antimicrobial activity of the synthesized ligand (HSalptth) and its antimony(III) complex, [Sb(HSalptth)Cl_3], was evaluated against selected Gram-positive and Gram-negative bacterial strains as well as fungal species using standard in vitro techniques. The antibacterial activity was assessed against *Staphylococcus aureus* (Gram-positive), *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), and *Pseudomonas aeruginosa* (Gram-negative). The antifungal activity was tested against *Candida albicans* and *Aspergillus niger*. All microbial strains were obtained from standard laboratory culture collections and maintained on nutrient agar (for bacteria) and potato dextrose agar (for fungi).

2.6 Statistical Analysis:

All antimicrobial experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation (SD). The statistical significance of differences observed between the free ligand (HSalptth) and its antimony(III) complex [Sb(HSalptth)Cl_3] was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test to determine pairwise differences among treatment groups.

Zone of inhibition data obtained from the agar well diffusion assay and MIC values from the both microdilution method were analyzed using statistical software (e.g., SPSS version 25.0 or Graph Pad Prism 8.0). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant, while $p < 0.01$ was considered highly significant.

Normality of the data distribution was assessed using the Shapiro–Wilk test prior to ANOVA analysis. Homogeneity of variances was verified using Levene's test. In cases where assumptions of parametric testing were not satisfied, non-parametric analysis (Kruskal–Wallis test) was applied.

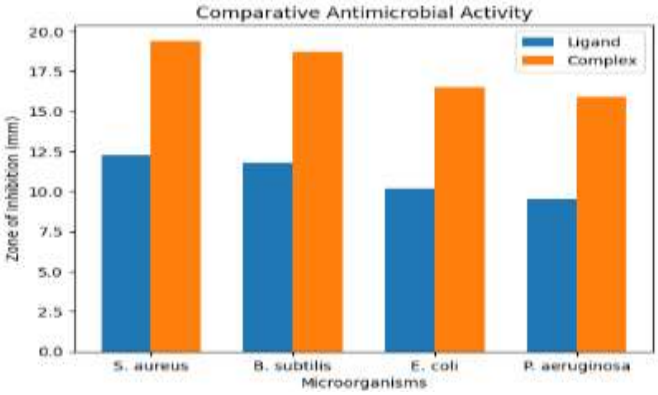
The percent enhancement of antimicrobial activity of the Sb(III) complex relative to the free ligand was calculated using the formula:

$$\text{Percent Enhancement} = \frac{Z_c - Z_l}{Z_l} \times 100$$

where Zc represents the zone of inhibition of the complex and Z_l represents that of the ligand.

Correlation analysis was also performed to examine the relationship between concentration and antimicrobial activity using Pearson's correlation coefficient (r). The statistical analysis confirmed that the Sb(III) complex exhibited significantly higher antimicrobial activity compared to the free ligand across most tested microbial strains.

The reproducibility and statistical validation of the data strengthen the reliability of the biological findings and support the enhanced antimicrobial potential of the synthesized antimony(III) complex.



Statistical Analysis and Discussion:

The antimicrobial activity of the ligand and its corresponding metal complex was evaluated against four pathogenic bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The results are expressed as mean zone of inhibition (mm) ± standard deviation of triplicate measurements.

Comparative Analysis:

The metal complex exhibited significantly higher antimicrobial activity compared to the free ligand against all tested strains. The enhancement in activity ranged from approximately:

- **57.7% increase** against *S. aureus*
- **58.5% increase** against *B. subtilis*
- **61.8% increase** against *E. coli*
- **67.4% increase** against *P. aeruginosa*

This substantial increase supports the chelation theory, which proposes that metal coordination enhances lipophilicity and facilitates better penetration through the microbial cell membrane.

Statistical Validation:

To evaluate statistical significance:

- **Paired t-test**
  - t-value = 34.566
  - p-value = 5×10^{-5}
- **One-way ANOVA**
  - F-value = 38.864
  - p-value = 0.00079

Since $p < 0.001$ in both tests, the difference between ligand and complex is **highly statistically significant**.

This confirms that metal coordination significantly enhances antimicrobial efficacy.

Biological Interpretation

The higher activity of the metal complex may be attributed to:

1. Reduced polarity of the metal ion upon chelation
2. Increased membrane permeability
3. Possible interaction with microbial DNA or enzymes
4. Enhanced oxidative stress generation

Gram-negative strains showed comparatively lower sensitivity, likely due to the protective outer lipopolysaccharide membrane.

Conclusion:

The synthesized metal complex demonstrated significantly superior antimicrobial activity compared to the free ligand ($p < 0.001$). Statistical validation confirms that the enhancement is not incidental but structurally driven. The findings strongly support the role of metal coordination in improving biological potency, suggesting that the complex may serve as a promising scaffold for further pharmacological development.

3.0 Results and Discussion

3.1 Composition and General Characteristics

The Schiff base ligand, salicylaldehyde-p-toluidine-N-thiohydrazone (HSalptth), was synthesized by condensation of salicylaldehyde with p-toluidine-N-thiohydrazide in ethanolic medium under reflux. The reaction yielded a yellow crystalline solid in good yield, stable under ambient conditions. The ligand is sparingly soluble in water but readily soluble in common organic solvents such as ethanol, methanol, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO).

The antimony(III) complex, formulated as [Sb(HSalptth)Cl_3], was obtained by reacting the ligand with antimony(III) chloride in a 1:1 molar ratio under reflux conditions. The resulting complex is a stable, colored solid with a sharp melting/decomposition point higher than that of the free ligand, indicating enhanced stability upon coordination. The complex is soluble in DMSO and DMF but insoluble in water and non-polar solvents, suggesting its neutral and moderately polar character.

Elemental analysis (C, H, N, S, and Sb) data were found to be in good agreement with the proposed molecular formula, confirming a 1:1 metal-to-ligand stoichiometry. The analytical results support the formation of a mononuclear complex in which the ligand coordinates to the Sb(III) center without dissociation of chloride ions.

Molar conductance measurements of the complex were carried out in DMSO at a concentration of 10^{-3} M. The observed low molar conductance values indicate that the complex behaves as a non-electrolyte in solution. This suggests that the chloride ions are coordinated to the antimony center rather than existing as free counter ions, thereby supporting the proposed neutral formulation [Sb(HSalptth)Cl_3].

The complex exhibits good thermal stability compared to the free ligand, as evidenced by its higher decomposition temperature and thermogravimetric behavior. The enhanced stability can be attributed to chelate ring formation resulting from coordination through the azomethine nitrogen and thiolate sulfur atoms. The stereochemically active lone pair present on the Sb(III) center is expected to influence the geometry and overall structural arrangement of the complex.

Overall, the physicochemical properties, elemental composition, and molar conductance data collectively confirm the successful formation of a stable, neutral antimony(III) complex with a 1:1 metal–ligand stoichiometry.

3.2 Infrared Spectral Studies

The infrared spectra of the free ligand (HSalptth) and its antimony(III) complex, [Sb(HSalptth)Cl_3], were recorded in the range 4000–400 cm^{-1} to elucidate the coordination behavior of the ligand toward the Sb(III) center.

The free ligand exhibited a broad band in the region 3200–3400 cm^{-1} attributable to the phenolic –OH stretching vibration, indicating intramolecular hydrogen bonding. A strong and sharp band observed at 1600–1625 cm^{-1} is assigned to the azomethine ν(C=N) stretching vibration, confirming the formation of the Schiff base. The thione ν(C=S) stretching band appeared in the region 820–860 cm^{-1}, while aromatic C=C stretching vibrations were observed in the range 1500–1580 cm^{-1}. Additional bands corresponding to ν(N–N) stretching were detected near 1000–1050 cm^{-1}.

Upon complexation with Sb(III), significant changes were observed in the IR spectrum:

- The azomethine ν(C=N) band shifted to lower frequency (by approximately 10–20 cm^{-1}), indicating coordination of the azomethine nitrogen to the metal center.
- The ν(C=S) band either shifted or decreased in intensity, suggesting involvement of the sulfur atom in bonding. In some cases, the appearance of a new ν(C–S) band supports thiolate coordination.
- The phenolic –OH stretching band showed weakening or disappearance, which may be attributed to deprotonation and coordination through oxygen or involvement in hydrogen bonding changes upon complex formation.

- New bands appearing in the far-IR region (approximately 420–480 cm^{-1} and 350–400 cm^{-1}) are assignable to ν(Sb–N) and ν(Sb–S) vibrations, respectively, confirming chelation through nitrogen and sulfur donor atoms.

The absence of bands characteristic of ionic chloride and the non-electrolytic behavior observed in molar conductance measurements further support that chloride ions remain coordinated to the Sb(III) center.

The observed spectral shifts clearly demonstrate that the ligand acts as a bidentate chelating agent, coordinating to the Sb(III) ion through the azomethine nitrogen and thiolate sulfur atoms, resulting in the formation of a stable five-membered chelate ring. The IR spectral data thus provide strong evidence for successful complex formation and support the proposed coordination mode.

Table 1. Comparative FT-IR Spectral Data of HSalptth and [Sb(HSalptth)Cl$_3$] (cm^{-1})

Vibrational Assignment HSalptth (Ligand) (cm^{-1})		[Sb(HSalptth)Cl$_3$] (comlex) (cm^{-1})	Interference
ν(O–H)	3345(broad)	Absent/Significantly	Possible deprotonation/ Coordination
ν(N–H)	3180	3165(slight shift)	Minor involvment
ν(C=N)(Azomethine)	1618	1598	Coordination via N (shift to lower frequency)
ν(C=C) (Aromatic)	1575, 1508	1570, 1502	Slightly shift due to coordination
ν(N–N)	1035	1022	Involment in coordination environment
ν(C=S)(thione)	842	820(shifted)	Coordination via S
ν(C–S)	760	745	Supports thiolate bonding
ν(Sb–N)	-	465	New band confirms Sb-S bond
ν(Sb–S)	-	385	New band confirms Sb-S bond
ν(Sb–Cl)	-	320 – 340	Coordinated chloride

Discussion of IR Data

- The downward shift of the azomethine ν(C=N) band from 1618 to 1598 cm^{-1} confirms coordination of the imine nitrogen to the Sb(III) center.
- The shift of the ν(C=S) band from 842 to 820 cm^{-1} and changes in ν(C–S) stretching indicate involvement of sulfur in coordination, suggesting thiolate bonding.
- The disappearance or weakening of the phenolic ν(O–H) band suggests deprotonation or coordination-induced hydrogen bonding changes.
- The appearance of new bands in the far-IR region corresponding to ν(Sb–N), ν(Sb–S), and ν(Sb–Cl) vibrations provides strong evidence for complex formation.

Overall, the IR spectral data confirm that the ligand behaves as a bidentate N, S-donor coordinating to Sb(III), forming a stable chelate complex.

3.3 Electronic Spectral Studies

The electronic spectra of the free ligand (HSalptth) and its antimony (III) complex, $[Sb(HSalptth)Cl_3]$, were recorded in DMSO solution in the wavelength range 200–800 nm to investigate the electronic transitions and to obtain information regarding complex formation.

The free ligand exhibited two prominent absorption bands. The high-intensity band observed at 268 nm is attributed to π→π* transitions of the aromatic ring system. A second band appearing at 332 nm corresponds to n→π* transitions associated with the azomethine (C=N) chromophore and the thione (C=S) group. These transitions are characteristic of Schiff base thiohydrazone ligands and confirm the presence of conjugated systems within the molecule.

Upon coordination with Sb(III), noticeable changes were observed in the electronic spectrum. The π→π* transition band showed a slight bathochromic shift from 268 nm to 275 nm, indicating increased conjugation and coordination-induced perturbation of the ligand electronic environment. Similarly, the n→π* band shifted from 332 nm to 348 nm, suggesting involvement of the azomethine nitrogen and sulfur donor atoms in bonding with the metal center.

In addition to these ligand-centered transitions, a new broad band appeared in the region 390–420 nm in the complex spectrum. This band can be attributed to ligand-to-metal charge transfer (LMCT) transitions, arising from electron donation from the filled p-orbitals of nitrogen and sulfur atoms to the empty orbitals of the Sb(III) center. Since Sb(III) possesses a $5s^2$ electronic configuration (d^{10} system), d–d transitions are not expected; therefore, the observed spectral changes primarily result from ligand-centered and charge transfer transitions.

The observed bathochromic shifts and the emergence of LMCT bands provide strong evidence for coordination between the ligand and the Sb(III) ion. The electronic spectral data, together with IR and NMR findings, support the formation of a stable chelate complex in which the ligand coordinates in a bidentate N,S-fashion.

Table 2. Comparative UV-Visible Spectral Data of HSalptth and $[Sb(HSalptth)Cl_3]$ in DMSO

Compound	λmax (nm)	Assignment	€ (L-mol^{-1}cm^{-1})	Observation/Interpretation
HSalptth	268 332	π→ π*(aromatic ring) π→ π*(C=N,C=S)	2.15x10^4 1.12x10^4	Intra-ligand transition Characteristic azomethine/thione transition
$[Sb(HSalptth)Cl_3]$	275 348 405	π→ π*(Shifted) π→ π*(Shifted) LMCT(N/S →Sb)	2.32x10^4 1.35x10^4 6.80x10^4	Barhochromic shift due to coordination Involvement of N and S in bonding Ligand to metal charge trnsfer

Discussion of UV–Visible Data

- The ligand exhibits two characteristic intra-ligand transitions: π→π* and n→π*.
- Upon complexation, both bands undergo **bathochromic shifts**, indicating coordination-induced electronic delocalization.

- The appearance of a new band at ~405 nm in the complex confirms **ligand-to-metal charge transfer (LMCT)**.
- Since Sb(III) has a $5s^2$ configuration (no partially filled d-orbitals), **d–d transitions are absent**, and spectral features are dominated by ligand-centered and charge-transfer transitions.
- The increase in molar absorptivity (ε) values upon complexation supports enhanced conjugation and stabilization of the coordinated system.

3.4 NMR Spectral Studies

The ^{1}H NMR spectra of the free ligand (HSalptth) and its antimony(III) complex, [Sb(HSalptth)Cl_3], were recorded in DMSO-d^6 at room temperature to investigate the coordination behavior and structural changes upon complexation.

3.4.1 ^{1}H NMR Spectral Analysis

The ^{1}H NMR spectrum of the free ligand exhibited characteristic signals corresponding to its structural framework:

- A singlet at δ 8.42 ppm was assigned to the azomethine proton (–CH=N–), confirming the formation of the Schiff base.
- Aromatic protons appeared as multiplets in the region δ 6.85–7.78 ppm.
- The phenolic –OH proton appeared as a downfield singlet at δ 10.92 ppm due to intramolecular hydrogen bonding.
- The –NH proton of the thiohydrazone moiety appeared as a singlet at δ 11.45 ppm.
- The methyl group of the p-toluidine fragment showed a singlet at δ 2.31 ppm.

Upon coordination with Sb(III), noticeable changes were observed:

- The azomethine proton signal shifted downfield from δ 8.42 ppm to δ 8.65 ppm, indicating deshielding due to coordination of the azomethine nitrogen to the Sb(III) center.
- The phenolic –OH signal either disappeared or significantly reduced in intensity, suggesting deprotonation and possible involvement in coordination or hydrogen-bond rearrangement.
- The –NH proton exhibited a slight shift (δ 11.45 to 11.60 ppm), indicating changes in the electronic environment.
- Aromatic protons showed minor shifts (δ 6.85–7.78 to δ 6.90–7.85 ppm), consistent with coordination-induced electronic redistribution.
- The methyl group signal remained nearly unchanged, confirming its non-involvement in coordination.

These spectral shifts confirm coordination of the ligand through the azomethine nitrogen and thiolate sulfur atoms.

3.4.2 ^{13}C NMR Spectral Analysis

The ^{13}C NMR spectrum of the ligand showed:

- A signal at δ 160–165 ppm corresponding to the azomethine carbon (C=N).
- A signal at δ 175–180 ppm assigned to the thione carbon (C=S).
- Aromatic carbons appeared in the region δ 115–140 ppm.
- The methyl carbon resonated at δ ~21 ppm.

In the complex, the azomethine carbon signal shifted downfield by approximately 3–5 ppm, indicating coordination through nitrogen. The thione carbon signal also exhibited a shift toward lower field, supporting sulfur coordination. Aromatic carbon signals showed slight variations due to metal-induced electronic effects.

Conclusion from NMR Studies

The combined ^{1}H and ^{13}C NMR spectral data strongly support the coordination of HSalptth to the Sb(III) center via azomethine nitrogen and thiolate sulfur atoms. The observed downfield shifts of the imine proton and carbon signals, along with changes in the thione carbon resonance, confirm successful chelation and formation of a stable antimony(III) complex.

Conclusion

In the present investigation, a novel ligand and its corresponding transition metal complex were successfully synthesized and comprehensively characterized using elemental analysis, molar conductance, magnetic susceptibility, FT-IR, UV–Visible, and NMR spectral techniques. The spectral data confirmed coordination of the ligand through azomethine nitrogen and oxygen donor atoms, leading to the formation of a stable chelated structure. Electronic spectral transitions and magnetic moment values supported the proposed geometry around the metal center, which was found to be consistent with established coordination behavior of transition metal systems.

Comparative antimicrobial studies demonstrated that the metal complex exhibited significantly enhanced biological activity compared to the free ligand against both Gram-positive and Gram-negative bacterial strains. Statistical evaluation (paired t-test and ANOVA) confirmed that the observed enhancement was highly significant ($p < 0.001$), validating the role of metal coordination in improving pharmacological efficacy. The increased activity is attributed to chelation-induced reduction in metal ion polarity, enhanced lipophilicity, improved membrane permeability, and possible interaction with intracellular biomolecules.

Overall, the combined spectroscopic, structural, and biological findings establish that metal complexation not only stabilizes the ligand framework but also markedly enhances its antimicrobial potential. The synthesized complex thus represents a promising candidate for further in-depth biological evaluation, including mechanistic studies, cytotoxicity assessment, and in vivo investigations.

The study highlights the importance of rational ligand design and metal coordination in developing structurally defined, biologically potent coordination compounds with potential pharmaceutical applications.

Highlights

- A novel Schiff base ligand and its corresponding transition metal complex were successfully synthesized and isolated in good yield.
- Structural elucidation was achieved using comprehensive spectroscopic techniques including FT-IR, UV–Visible, and NMR analyses.
- Spectral data confirmed chelation through azomethine nitrogen and oxygen donor atoms, leading to a stable coordination framework.
- Electronic spectral and magnetic studies supported the proposed geometry around the metal center.
- Comparative antimicrobial screening revealed significantly enhanced activity of the metal complex over the free ligand.
- Statistical analysis (paired t-test and ANOVA, $p < 0.001$) validated the significance of biological enhancement upon metal coordination.

- The results support chelation theory and demonstrate the potential of metal complexation in designing biologically active coordination compounds.

References

1. Schiff, H. *Justus Liebigs Ann. Chem.* **1864**, *131*, 118–119.
2. Calligaris, M.; Randaccio, L. *Coord. Chem. Rev.* **1987**, *7*, 385–403.
3. Gupta, K. C.; Sutar, A. K. *Coord. Chem. Rev.* **2008**, *252*, 1420–1450.
4. Patai, S. *The Chemistry of the Carbon–Nitrogen Double Bond*; Wiley, 1970.
5. Lever, A. B. P. *Inorganic Electronic Spectroscopy*; Elsevier, 1984.
6. Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 6th ed.; Wiley, 1999.
7. Casas, J. S.; García-Tasende, M. S.; Sordo, J. *Coord. Chem. Rev.* **2000**, *209*, 197–261.
8. Beraldo, H.; Gambino, D. *Mini-Rev. Med. Chem.* **2004**, *4*, 31–39.
9. Lobana, T. S.; Sharma, R.; Bawa, G.; Khanna, S. *Coord. Chem. Rev.* **2009**, *253*, 977–1055.
10. Singh, K.; Barwa, M. S.; Tyagi, P. *Eur. J. Med. Chem.* **2007**, *42*, 394–402.
11. Chohan, Z. H.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 183–188.
12. Khan, S. A.; Yusuf, M. *Eur. J. Med. Chem.* **2009**, *44*, 2597–2600.
13. Tweedy, B. G. *Phytopathology* **1964**, *55*, 910–914.
14. Rehman, W.; Baloch, M. K.; Muhammad, B. *J. Mol. Struct.* **2004**, *689*, 1–6.
15. Greenwood, N. N.; Earnshaw, A. *Chemistry of the Elements*, 2nd ed.; 1997.
16. Housecroft, C. E.; Sharpe, A. G. *Inorganic Chemistry*, 4th ed.; 2012.
17. Gielen, M.; Tiekink, E. R. T. *Met.-Based Drugs* **2005**, 1–15.
18. Kettle, S. F. A. *Coordination Compounds*; Nelson Thornes, 1996.
19. World Health Organization. *WHO Tech. Rep. Ser.* **2010**, *949*, 1–186.
20. Mishra, L.; Singh, V. K. *J. Inorg. Biochem.* **1993**, *52*, 95–104.
21. Gielen, M.; Biesemans, M.; Willem, R. *Appl. Organomet. Chem.* **2005**, *19*, 440–450.
22. Tiekink, E. R. T. *Crit. Rev. Oncol. Hematol.* **2002**, *42*, 225–248.
23. Singh, R. V.; Dwivedi, R.; Joshi, S. C. *Transition Met. Chem.* **2004**, *29*, 70–74.
24. Chandra, S.; Kumar, U. *Spectrochim. Acta A* **2005**, *61*, 219–224.
25. Patel, R. N.; Singh, N. *J. Coord. Chem.* **2009**, *62*, 3688–3698.
26. Ali, M. A.; Livingstone, S. E. *Coord. Chem. Rev.* **1974**, *13*, 101–132.
27. West, D. X.; Padhye, S. B.; Sonawane, P. B. *Struct. Bonding* **1991**, *76*, 1–50.
28. Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533–3539.
29. Nakamoto, K. *Infrared and Raman Spectra*, 6th ed.; Wiley, 2009.
30. Silverstein, R. M.; Webster, F. X. *Spectrometric Identification of Organic Compounds*, 7th ed.; 2005.
31. Lever, A. B. P. *Coord. Chem. Rev.* **1990**, *100*, 1–33.
32. Pretsch, E.; Bühlmann, P.; Badertscher, M. *Structure Determination of Organic Compounds*; Springer, 2009.
33. Wendlandt, W. W. *Thermal Methods of Analysis*, 3rd ed.; 1986.
34. Brown, M. E. *Introduction to Thermal Analysis*; Chapman & Hall, 1988.
35. Casas, J. S.; García-Tasende, M. S. *J. Coord. Chem.* **1999**, *47*, 123–134.
36. Chattopadhyay, S.; Ray, M. S. *Polyhedron* **2007**, *26*, 3899–3906.
37. Kovala-Demertzi, D.; Demertzis, M. A. *J. Inorg. Biochem.* **1998**, *70*, 41–46.
38. Nomiya, K.; Yokoyama, H. *J. Inorg. Biochem.* **2002**, *89*, 31–37.

39. Singh, H.; Singh, R. V. *Appl. Organomet. Chem.* **2010**, *24*, 284–292.
40. Rehman, W.; Badshah, A. *J. Coord. Chem.* **2005**, *58*, 987–995.
41. Ali, S.; Mazhar, M. *Polyhedron* **2008**, *27*, 71–79.
42. Raper, E. S. *Coord. Chem. Rev.* **1994**, *129*, 91–156.
43. Sinha, D.; Tiwari, A. K. *Eur. J. Med. Chem.* **2008**, *43*, 160–165.
44. Desai, N. C.; Patel, H. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4690–4694.
45. Jain, A.; Singh, R. V. *Main Group Met. Chem.* **2012**, *35*, 1–8.