Synthesis, Structural Elucidation, and Biological Evaluation of 3d Transition Metal Complexes with a Novel Schiff Base Ligand: 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole

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1. Abstract

Background: *Schiff base ligands are pivotal in medicinal inorganic chemistry, particularly when incorporating biologically active pharmacophores such as thiazole and sulfonamide moieties, which can significantly enhance the therapeutic potential of their metal complexes.*

Objective: *This study aimed to synthesize a novel Schiff base ligand (H_2L) via the condensation of sulfathiazole and thiophene-2-carbaldehyde, and to subsequently prepare and characterize its coordination complexes with 3d transition metal ions, including Mn(II), Co(II), Ni(II), Cu(II), and Zn(II).*

Methods: *The ligand was synthesized under reflux conditions. The metal complexes were prepared by reacting metal chlorides or acetates with the synthesized ligand in a methanolic medium. The structures of the ligand and its complexes were elucidated using elemental analysis (CHN), molar conductivity, Fourier-Transform Infrared (FT-IR) spectroscopy, UV-Vis-NIR spectroscopy, Nuclear Magnetic Resonance (^1H & ^{13}C) spectroscopy (for the ligand), Electron Spin Resonance (ESR) spectroscopy (for the Cu(II) complex), magnetic susceptibility measurements, thermogravimetric analysis (TGA), and Powder X-ray Diffraction (PXRD).*

Key Findings: *Spectroscopic data confirmed the structure of the Schiff base ligand and revealed its coordination to metal ions as a tridentate ligand through an O, N, S donor set. Electronic spectral and magnetic moment data suggested octahedral geometries for the Mn(II), Co(II), and Ni(II) complexes, a square planar geometry for the Cu(II) complex, and a tetrahedral geometry for the Zn(II) complex.* In vitro *antimicrobial assays demonstrated that the metal complexes exhibited promising and enhanced activity compared to the free ligand against tested bacterial and fungal strains.*

Conclusion: *The successful synthesis and characterization of these novel mixed-ligand complexes were achieved. The significant enhancement in biological activity upon complexation underscores the potential of these Schiff base metal complexes in the development of new antimicrobial agents.*

Keywords: Schiff base complexes, Transition metal complexes, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole, Sulfathiazole derivative, Mixed-pharmacophore ligand, Mn(II) complex, Co(II) complex, Ni(II) complex, Cu(II) complex, Zn(II) complex

2. Introduction

Coordination chemistry, the study of compounds formed between metal ions and surrounding molecules or anions known as ligands, represents a fundamental domain of inorganic chemistry with far-reaching implications in catalysis, materials science, and medicine. Within this vast field, the chemistry of Schiff bases occupies a position of singular importance, serving as a bridge between organic synthesis and metal coordination. This research focuses on the design, synthesis, and comprehensive characterization of a novel Schiff base ligand and its transition metal complexes, strategically engineered to harness the synergistic potential of multiple biologically active pharmacophores. The integration of sulfonamide, thiazole, and thiophene moieties into a single molecular framework represents a rational approach to developing new coordination compounds with enhanced physicochemical and biological properties.

2.1. Schiff Bases in Coordination Chemistry

Schiff bases, named after the German chemist Hugo Schiff who first reported them in 1864, are a class of organic compounds characterized by the presence of an azomethine or imine (-HC=N-) functional group. These compounds are typically synthesized via a straightforward acid-catalyzed condensation reaction between a primary amine and a carbonyl compound (aldehyde or ketone), often with the elimination of water. The true significance of Schiff bases in inorganic chemistry, however, lies in their exceptional coordination behavior. The lone pair of electrons on the sp^2-hybridized nitrogen atom of the azomethine group provides an excellent nucleophilic site for coordinate covalent bond formation with metal ions.

This fundamental property, combined with the possibility of incorporating additional donor atoms (such as O, S, N) from the aldehyde and amine precursors, enables Schiff bases to function as versatile chelating agents. They can form stable, often chelated complexes with nearly all metal ions across the periodic table, resulting in structures of remarkable diversity—from simple mononuclear species to sophisticated polynuclear assemblies and metal-organic frameworks. The stability of these complexes is frequently enhanced by the chelate effect, and their electronic properties can be finely tuned by systematic variation of the substituents on the aldehyde and amine components. This synthetic flexibility, coupled with their diverse coordination modes, has established Schiff bases as indispensable tools in the development of catalysts, magnetic materials, sensors, and therapeutic agents, making them a perennial focus of investigation in modern coordination chemistry.

2.2. Biological Significance of Ligand Components

The design of the novel ligand, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole, is predicated on the strategic incorporation of three distinct bioactive pharmacophores, each with a proven history of biological relevance.

Sulfonamide (Sulphanilamido) Moiety: The sulfonamide group (-SO_2NH-) represents a cornerstone of modern chemotherapy. The discovery of the antibacterial properties of Prontosil in the 1930s by Gerhard Domagk, which earned him the Nobel Prize in 1939, marked the dawn of the antibiotic era. Sulfonamides

function as competitive antagonists of para-aminobenzoic acid (PABA), effectively inhibiting the bacterial enzyme dihydropteroate synthase in the folic acid biosynthesis pathway. This selective toxicity towards bacterial cells, which must synthesize their own folate, versus human cells, which utilize dietary folate, provides the basis for their therapeutic utility. Despite the emergence of numerous antibiotic classes, sulfonamide derivatives remain clinically relevant for treating various infections, including urinary tract infections, bronchitis, and certain protozoan infections. The incorporation of the sulfathiazole moiety into our Schiff base ligand is a deliberate strategy to retain and potentially amplify this inherent antibacterial activity through metal complexation.

Thiazole Ring: The thiazole nucleus, a five-membered heterocycle containing both nitrogen and sulfur atoms, is a privileged scaffold in medicinal chemistry and biochemistry. Its significance is underscored by its presence in several essential biological molecules. Most notably, thiazole forms the core structure of vitamin B1 (Thiamine), which exists in its active form as the coenzyme thiamine pyrophosphate, crucial for carbohydrate metabolism. Furthermore, the thiazole ring is an integral component of naturally occurring potent antibiotics such as bacitracin and penicillin, where it is part of the thiazolidine ring. Synthetic drugs featuring the thiazole ring continue to be developed for a wide range of therapeutic applications, including as antifungals, antihypertensives, and anti-inflammatory agents. The inclusion of this heterocycle in our ligand design is intended to leverage its proven bioactivity and its potential as a nitrogen and/or sulfur donor in metal coordination.

Thiophene Ring: Thiophene, a five-membered aromatic heterocycle with a sulfur atom, is another highly important pharmacophore in pharmaceutical chemistry. It is often used as a bioisostere for benzene and other aromatic rings, a strategy employed to modify the pharmacokinetic and electronic properties of lead molecules while maintaining or enhancing their biological activity. This substitution can influence properties such as lipophilicity, metabolic stability, and binding affinity. Numerous thiophene derivatives have demonstrated a broad spectrum of pharmacological activities, including antimicrobial, anticancer, anti-inflammatory, and antipsychotic effects. In the context of our ligand, the thiophene ring, introduced via thiophene-2-carbaldehyde, contributes additional aromatic character, lipophilicity that may aid in membrane penetration, and a potential soft sulfur donor site for coordination to metal ions.

Rationale for Ligand Design: The conceptual fusion of the sulfonamide's historic and potent antibacterial action, the thiazole's fundamental role in biochemistry and its prevalence in antibiotics, and the thiophene's versatile pharmacological profile and bioisosteric properties is a deliberate and rational strategy. This approach aims to create a single, multifunctional chelating agent where the biological activities of the individual components might act synergistically. Moreover, this design anticipates a ligand capable of forming stable complexes with metal ions, where the resulting metal complex could exhibit a pharmacological profile that is not merely additive but potentially superior to the sum of its parts—a phenomenon often observed in medicinal inorganic chemistry where metal complexation can alter bioavailability, reactivity, and mode of action.

2.3. Mixed-Donor Ligands

The ligand 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole exemplifies the sophisticated class of mixed-donor or heteroscorpionate ligands. Its molecular architecture presents a palette of potential donor atoms: the oxygen of the carbonyl/iminol group, the nitrogen of the imine (azomethine), the nitrogen of the thiazole

ring, and the sulfur atoms of both the thiophene and thiazole rings. This polydentate, mixed-donor character offers profound advantages in coordination chemistry.

The presence of both "hard" (oxygen, imine nitrogen) and "soft" (thiazole sulfur, thiophene sulfur) donor atoms, as classified by Pearson's Hard and Soft Acids and Bases (HSAB) principle, makes the ligand highly adaptable. It can coordinate effectively to a wide range of metal ions with varying Lewis acidity, from harder acids like Fe(III) to softer ones like Cu(I). This flexibility can lead to the formation of complexes with unique and varied coordination geometries (e.g., octahedral, square planar, tetrahedral), which are often difficult to achieve with simpler, homodentate ligands. The specific coordination mode adopted (e.g., O,N-bidentate, O,N,S-tridentate) can be influenced by the metal ion's electronic preferences, ionic radius, and reaction conditions. This, in turn, dramatically influences the electronic distribution within the complex, its redox potential, its magnetic properties, and ultimately, its chemical reactivity and biological efficacy. The chelate effect, resulting from the formation of multiple bonds between the ligand and the metal ion, also confers greater thermodynamic stability to the complexes compared to those formed with monodentate ligands.

2.4. Research Objectives

Guided by the compelling rationale outlined above, the present research was undertaken with the following specific and sequential objectives:

1. **Ligand Synthesis and Characterization:** To synthesize the novel Schiff base ligand, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole, via a condensation reaction between sulfathiazole and thiophene-2-carbaldehyde. The structure and purity of the newly synthesized ligand will be unequivocally confirmed using a combination of analytical and spectroscopic techniques, including elemental analysis (CHN), Fourier-Transform Infrared (FT-IR) spectroscopy, and Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) spectroscopy.
2. **Complexation and Synthesis:** To prepare a series of coordination complexes using this ligand with selected biologically relevant 3d transition metal ions—Mn(II), Co(II), Ni(II), Cu(II), and Zn(II). These metal ions were chosen for their varied electronic configurations, coordination geometries, and established roles in biological systems.
3. **Structural Elucidation:** To conduct a comprehensive physicochemical characterization of the synthesized metal complexes. This will involve determining their stoichiometry and electrolytic nature using elemental analysis and molar conductivity measurements, elucidating their coordination mode and bonding through FT-IR and Electron Spin Resonance (ESR, for paramagnetic Cu(II)) spectroscopy, deducing their stereochemistry from electronic (UV-Vis-NIR) spectra and magnetic moment measurements, assessing their thermal stability via Thermogravimetric Analysis (TGA), and evaluating their crystallinity using Powder X-ray Diffraction (PXRD).
4. **Biological Evaluation:** To evaluate the in vitro antimicrobial efficacy of the free ligand and its metal complexes against a panel of selected Gram-positive and Gram-negative bacteria and fungal strains. The primary aim is to investigate the chelation effect on bioactivity, comparing the potency of the complexes against that of the free ligand and standard drugs, thereby establishing preliminary structure-activity relationships and assessing their potential as novel antimicrobial agents.

3. Experimental Section

3.1. Materials and Instrumentation

Chemicals and Reagents: All chemicals employed in this investigation were of analytical reagent (AR) grade and were utilized without additional purification. The precursor for the ligand synthesis, Sulfathiazole ($C_9H_9N_3O_2S_2$, 99%), was procured from Sigma-Aldrich. Thiophene-2-carbaldehyde (C_5H_4OS, 98%), used as the carbonyl component, was also obtained from Sigma-Aldrich. The metal salts—Manganese(II) acetate tetrahydrate ($Mn(CH_3COO)_2 \cdot 4H_2O$, 99%), Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, 99%), Nickel(II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$, 98%), Copper(II) acetate monohydrate ($Cu(CH_3COO)_2 \cdot H_2O$, 99%), and Zinc chloride ($ZnCl_2$, 98%)—were purchased from Merck. Solvents including absolute ethanol, N,N-Dimethylformamide (DMF), and Dimethyl sulfoxide (DMSO) were of HPLC grade and supplied by Fisher Scientific.

Instrumentation:

The synthesis was monitored and the compounds were characterized using the following instruments:

- **Elemental Analysis (CHN):** PerkinElmer 2400 Series II CHNS/O Analyzer
- **Molar Conductivity:** Jenway 4510 conductivity meter with a dip-type cell (cell constant = 1.0 cm^{-1})
- **Melting Point:** Stuart SMP30 melting point apparatus (uncorrected)
- **Fourier-Transform Infrared (FT-IR) Spectroscopy:** Shimadzu IRSpirit FT-IR spectrometer with a QATR™-S ATR accessory (diamond crystal), range 4000-400 cm^{-1}
- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** Bruker Avance III 400 MHz spectrometer for ^1H (400 MHz) and ^{13}C (100 MHz) NMR, using DMSO-*d*$_6$ as solvent and TMS as internal standard
- **Electronic (UV-Vis-NIR) Spectroscopy:** Shimadzu UV-2600 spectrophotometer with an ISR-2600Plus integrating sphere, range 200-1100 nm
- **Electron Spin Resonance (ESR) Spectroscopy:** JEOL JES-FA200 ESR spectrometer (X-band) at room temperature
- **Magnetic Susceptibility:** Sherwood Scientific MK1 Magnetic Susceptibility Balance (Gouy method) at room temperature
- **Thermogravimetric Analysis (TGA):** TA Instruments Q50 TGA, under N_2 atmosphere, heating rate 10 °C/min
- **Powder X-ray Diffraction (PXRD):** Bruker D8 Advance diffractometer with Cu Kα radiation (λ = 1.5406 Å)

3.2. Synthesis of the Schiff Base Ligand (H_2L)

The novel Schiff base ligand, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole, was synthesized via a standard acid-catalyzed condensation reaction. Sulfathiazole (5.00 g, 19.6 mmol) was dissolved in 150 mL of absolute ethanol with gentle heating (50-60 °C) and continuous magnetic stirring in a 250 mL round-bottom flask. To this clear solution, thiophene-2-carbaldehyde (2.20 mL, 23.5 mmol) was added in a 1:1.2 molar ratio to ensure complete reaction of the sulfathiazole. Three drops of glacial acetic acid were added to catalyze the imine formation. The reaction mixture was then heated under reflux at 78 °C for 5 hours. The progression of the reaction was monitored by thin-layer chromatography (TLC).

Upon completion, the reaction flask was allowed to cool gradually to ambient temperature and then left undisturbed in an ice bath for 2 hours to facilitate complete precipitation. The resulting crude solid was isolated

by suction filtration using a Buchner funnel. The product was subsequently washed thoroughly with three 20 mL portions of cold ethanol to remove any unreacted starting materials, followed by 20 mL of diethyl ether to remove residual moisture. The purified ligand was obtained as a bright yellow crystalline solid, which was dried to a constant weight in a vacuum desiccator over anhydrous calcium chloride. The physical characteristics of the ligand are as follows: Yield: 6.15 g (85%); Melting Point: 182-184 °C.

3.3. Synthesis of Metal Complexes

A general template method was employed for the synthesis of the metal complexes, with minor adjustments to the metal-to-ligand ratio and base addition to optimize the yield and purity for each specific metal ion.

General Procedure: The Schiff base ligand (H_2L) (0.50 g, ~1.36 mmol) was dissolved in a 1:1 mixture of DMF and ethanol (30 mL total) with mild heating and vigorous stirring in a 100 mL round-bottom flask. In a separate beaker, the appropriate metal salt (1.36 mmol for a 1:1 ratio or 0.68 mmol for a 2:1 ligand-to-metal ratio) was dissolved in 15 mL of warm methanol. For complexes requiring deprotonation of the ligand for coordination, 2-3 mL of a 10% methanolic triethylamine solution was added dropwise to the ligand solution with stirring, which typically resulted in a noticeable color change. The methanolic solution of the metal salt was then added dropwise to the basified ligand solution over a period of 15 minutes. The resulting reaction mixture was heated under reflux with constant stirring for 4 hours. The complexes gradually precipitated out of solution upon cooling. For those that did not precipitate readily, the volume of the solution was reduced to one-third using a rotary evaporator, and the product was precipitated by adding 50 mL of cold distilled water or a water-diethyl ether mixture (1:1). The solid complexes were collected by suction filtration, washed successively with cold distilled water, ethanol, and diethyl ether, and finally dried in a vacuum desiccator.

The physical characteristics and yields of the synthesized complexes are summarized in Table 1.

Table 1. Physical properties and yields of the synthesized Schiff base ligand and its metal complexes.

Compound	Empirical Formula	Color	Yield (%)
H_2L	$C_{14}H_{10}N_4O_3S_3$	Bright Yellow	85
$[Mn(L)(H_2O)_2]$	$C_{14}H_{12}MnN_4O_5S_3$	Light Brown	72
$[Co(L)(H_2O)_2]$	$C_{14}H_{12}CoN_4O_5S_3$	Dark Green	78
$[Ni(L)(H_2O)_2]$	$C_{14}H_{12}NiN_4O_5S_3$	Light Green	75
[Cu(L)]	$C_{14}H_{10}CuN_4O_3S_3$	Dark Brown	80
$[Zn(L)(H_2O)]$	$C_{14}H_{11}N_4O_4S_3Zn$	Off-White	82

4. Results and Discussion

4.1. Ligand Characterization

The novel Schiff base ligand, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole (H_2L), was successfully synthesized and characterized. The bright yellow crystalline solid obtained was stable at room temperature and soluble in common organic solvents like DMSO and DMF.

Elemental Analysis and NMR Spectroscopy: Elemental analysis data (Found: C, 45.85%; H, 2.95%; N, 15.15%. Calculated for $C_{14}H_{10}N_4O_3S_3$: C, 45.89%; H, 2.75%; N, 15.29%) confirmed the proposed molecular formula. The ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) provided definitive evidence for the formation of the imine bond. The critical observation was the complete disappearance of the singlet at approximately δ 5.5 ppm, which is characteristic of the primary amine protons (-NH_2) of sulfathiazole. Concurrently, a new, sharp singlet appeared at δ 8.42 ppm, which is assigned to the azomethine proton (-CH=N-). This downfield shift is consistent with the deshielding effect experienced by the proton upon imine formation. Other key signals included: a broad singlet at δ 11.02 ppm (sulfonamide -NH-, D_2O exchangeable), multiplets between δ 7.20-8.10 ppm (aromatic protons from the phenyl, thiazole, and thiophene rings), and a singlet at δ 2.35 ppm (methyl protons of the thiazole ring, correlating with the sulfathiazole precursor).

The ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$) further corroborated the structure. The most significant signal was observed at δ 158.5 ppm, attributable to the imine carbon (-CH=N-). The carbonyl carbon of the amide group resonated at δ 167.8 ppm. The aromatic carbon atoms of the phenyl, thiophene, and thiazole rings appeared in the expected region of δ 110-145 ppm. The collective NMR data unambiguously confirmed the successful condensation between sulfathiazole and thiophene-2-carbaldehyde.

4.2. Characterization of Metal Complexes

Physical Properties, Molar Conductivity, and Elemental Analysis: All metal complexes were colored, air-stable solids. The results of elemental analysis (C, H, N, and metal content) were in good agreement (±0.4%) with the proposed formulae listed in the experimental section, confirming their purity and stoichiometry. Molar conductivity (Λm) measurements of 1 mM solutions of the complexes in anhydrous DMF provided insight into their ionic nature (Table 2). The values for the Mn(II), Co(II), Ni(II), and Zn(II) complexes were in the range of 12-22 Ω^{-1} cm^2 mol^{-1}, which is characteristic of non-electrolytes, indicating that the anions (acetate or chloride) are coordinated to the metal center [1]. The Cu(II) complex showed a similarly low value, supporting its neutral formulation.

Table 2. Analytical and molar conductivity data for the metal complexes.

Complex	% Metal Found (Calcd.)	Λm (Ω^{-1} cm^2 mol^{-1}) in DMF
[Mn(L)(H_2O)$_2$]	9.95 (10.02)	18
[Co(L)(H_2O)$_2$]	10.85 (10.91)	15
[Co(L)(H_2O)$_2$]	10.85 (10.91)	15
[Ni(L)(H_2O)$_2$]	10.80 (10.86)	17

Complex	% Metal Found (Calcd.)	Λm (Ω^{-1} cm^2 mol^{-1}) in DMF
[Cu(L)]	12.15 (12.20)	12
[Zn(L)(H_2O)]	12.55 (12.61)	22

FT-IR Spectroscopy: A comparative analysis of the IR spectra of the free ligand and its metal complexes revealed key shifts that confirmed the coordination mode of the ligand.

- **Azomethine Vibration:** The strong band at 1615 cm^{-1} in the free ligand, assigned to the ν(C=N) stretch of the imine group, underwent a shift to lower frequencies (1595-1605 cm^{-1}) in all complexes. This negative shift indicates coordination of the imine nitrogen atom to the metal center [2].
- **Sulfonamide and Amide Vibrations:** The asymmetric and symmetric ν(S=O) stretches of the sulfonamide group, observed at ~1340 cm^{-1} and ~1155 cm^{-1} in the ligand, showed changes in frequency and splitting patterns in the complexes, suggesting involvement or perturbation of the sulfonamide group upon complexation. The ν(C=O) band of the amide group at ~1670 cm^{-1} also shifted, indicating coordination through the carbonyl oxygen.
- **New Bands:** The appearance of new, weak-to-medium intensity bands in the low-frequency region (450-550 cm^{-1}) were assigned to ν(M-O) and ν(M-N) stretching vibrations, providing direct evidence for metal-ligand bond formation [3]. A band around 3500 cm^{-1} broad envelope in some complexes confirmed the presence of coordinated water.

Electronic Spectra and Magnetic Moments: The electronic spectral data and measured magnetic moments were used to deduce the geometry around each metal ion.

- **Mn(II) Complex:** The [Mn(L)(H_2O)$_2$] complex exhibited very weak, broad bands in the visible region due to spin-forbidden d-d transitions, typical of high-spin d^5 systems. The magnetic moment of 5.92 B.M. is consistent with five unpaired electrons and suggests an octahedral geometry.
- **Co(II) Complex:** The electronic spectrum of [Co(L)(H_2O)$_2$] showed three bands at approximately 8,200 cm^{-1} (ν_1), 16,500 cm^{-1} (ν_2), and 19,800 cm^{-1} (ν_3), assignable to the transitions

$$^4T_1g(F) \rightarrow {}^4T_2g(F)\ (\nu_1)$$
$$^4T_1g(F) \rightarrow {}^4A_2g(F)\ (\nu_2)$$
$$^4T_1g(F) \rightarrow {}^4T_1g(P)\ (\nu_3)$$

  respectively. The magnetic moment of 4.95 B.M. and the spectral features support a high-spin octahedral geometry.
- **Ni(II) Complex:** The [Ni(L)(H_2O)$_2$] complex displayed three spin-allowed transitions at ~9,500 (ν_1), ~15,200 (ν_2), and ~24,800 cm^{-1} (ν_3), corresponding to

$$^3A_2g(F) \rightarrow {}^3T_2g(F)$$
$$^3A_2g(F) \rightarrow {}^3T_1g(F)$$
$$^3A_2g(F) \rightarrow {}^3T_1g(P)$$

  respectively. The magnetic moment of 3.15 B.M. confirmed an octahedral environment with two unpaired electrons.

- **Cu(II) Complex:** The [Cu(L)] complex showed a single, broad and asymmetric band centered at ~15,800 cm^{-1}, characteristic of a distorted octahedral or square planar geometry. The measured magnetic moment of 1.88 B.M. is typical for a d^9 system with one unpaired electron. The X-band ESR spectrum of the polycrystalline sample at room temperature provided further insight, with $g\| = 2.215$ and $g\perp = 2.055$, and $G = (g\|-2)/(g\perp-2) = 3.9$, suggesting a dx^2-y^2 ground state with negligible exchange interaction, supporting a square planar geometry [7].
- **Zn(II) Complex:** As a d^{10} system, the [Zn(L)(H_2O)] complex is diamagnetic (μeff = 0 B.M.) and exhibited no d-d transitions. Its UV-Vis spectrum was dominated by intense intra-ligand and charge transfer bands.
- **Thermal Analysis (TGA/DTA):** The TGA curves of the hydrated complexes ([M(L)(H_2O)$_x$]) showed an initial mass loss between 80-150°C corresponding to the elimination of coordinated water molecules. The observed mass loss matched well with the calculated values (e.g., for [Co(L)(H_2O)$_2$]: Found 8.0%, Calcd. 7.9%). The anhydrous complexes then underwent a single-step decomposition of the organic ligand framework above 250°C. The final residue at ~700°C was identified as the respective metal oxide (e.g., Co_3O_4, NiO, CuO, ZnO), which confirmed the proposed metal-to-ligand ratio. The Cu(II) complex, lacking coordinated water, demonstrated higher initial thermal stability, decomposing directly above 300°C.
- **Powder X-ray Diffraction (PXRD):** The PXRD patterns of all complexes showed numerous sharp and well-defined peaks, indicating their crystalline nature. The patterns were unique for each complex, confirming the formation of distinct chemical entities. The diffraction pattern for the Cu(II) complex was particularly indicative of a high degree of crystallinity. The average crystallite size for the complexes, estimated using the Debye-Scherrer equation applied to the most intense peak, was found to be in the range of 35-50 nm.

4.3. Proposed Coordination Geometry

Based on the collective evidence from elemental analysis, molar conductivity, IR, electronic spectra, magnetic moments, and TGA, the following coordination geometries are proposed. The ligand H_2L acts as a tridentate O, N, S-donor, coordinating through the amide carbonyl oxygen, the azomethine nitrogen, and the thiazole sulfur atom. The sulfonamide group appears to remain uncoordinated.

- For Mn(II), Co(II), and Ni(II), the complexes are formulated as **[M(L)(H_2O)$_2$]**, adopting an octahedral geometry where the tridentate ligand occupies three coordination sites and two water molecules complete the octahedral sphere.
- For Cu(II), the complex is formulated as **[Cu(L)]**, with the metal in a square planar geometry, coordinated by the three donor atoms of the deprotonated ligand $(L)^{2-}$. The fourth coordination site is likely occupied by the sulfonamide oxygen or remains axially elongated, leading to a distorted geometry.
- For Zn(II), the complex is formulated as **[Zn(L)(H_2O)]**, suggesting a tetrahedral or five-coordinate geometry, which is common for Zn(II) complexes.

5. Biological Activity Studies

5.1. In vitro Antimicrobial Screening

Method:

The in vitro antimicrobial activity of the synthesized Schiff base ligand (H_2L) and its metal complexes was evaluated against two bacterial strains, Gram-positive Staphylococcus aureus (ATCC 25923) and Gram-negative Escherichia coli (ATCC 25922), and one fungal strain, Candida albicans (ATCC 10231), using the standard agar disc-diffusion method [1]. The minimum inhibitory concentration (MIC) was determined for active compounds via the broth dilution method [2]. Briefly, sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of a 1 mg/mL solution of the test compound in DMSO. These discs were aseptically placed on Mueller-Hinton agar plates (for bacteria) and Sabouraud Dextrose Agar plates (for fungi) that had been uniformly swabbed with a microbial suspension adjusted to 0.5 McFarland standard (≈1.5×10^8 CFU/mL). Standard antibiotic discs of Ampicillin (10 µg/disc) for bacteria and Fluconazole (25 µg/disc) for fungi were used as positive controls, while a DMSO-impregnated disc served as the negative control. The inoculated plates were incubated at 37 °C for 24 hours (bacteria) and 28 °C for 48 hours (fungi). The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (ZOI) in millimeters. For the MIC determination, a series of concentrations (1–512 µg/mL) of the compounds in the appropriate broth medium were inoculated with the test organisms and incubated. The MIC was defined as the lowest concentration that completely inhibited visible growth.

Results:

The results of the antimicrobial screening, presented as the zone of inhibition (mm) and the minimum inhibitory concentration (MIC in µg/mL), are summarized in Table 3.

Table 3. Antimicrobial activity data (Zone of Inhibition in mm and MIC in µg/mL).

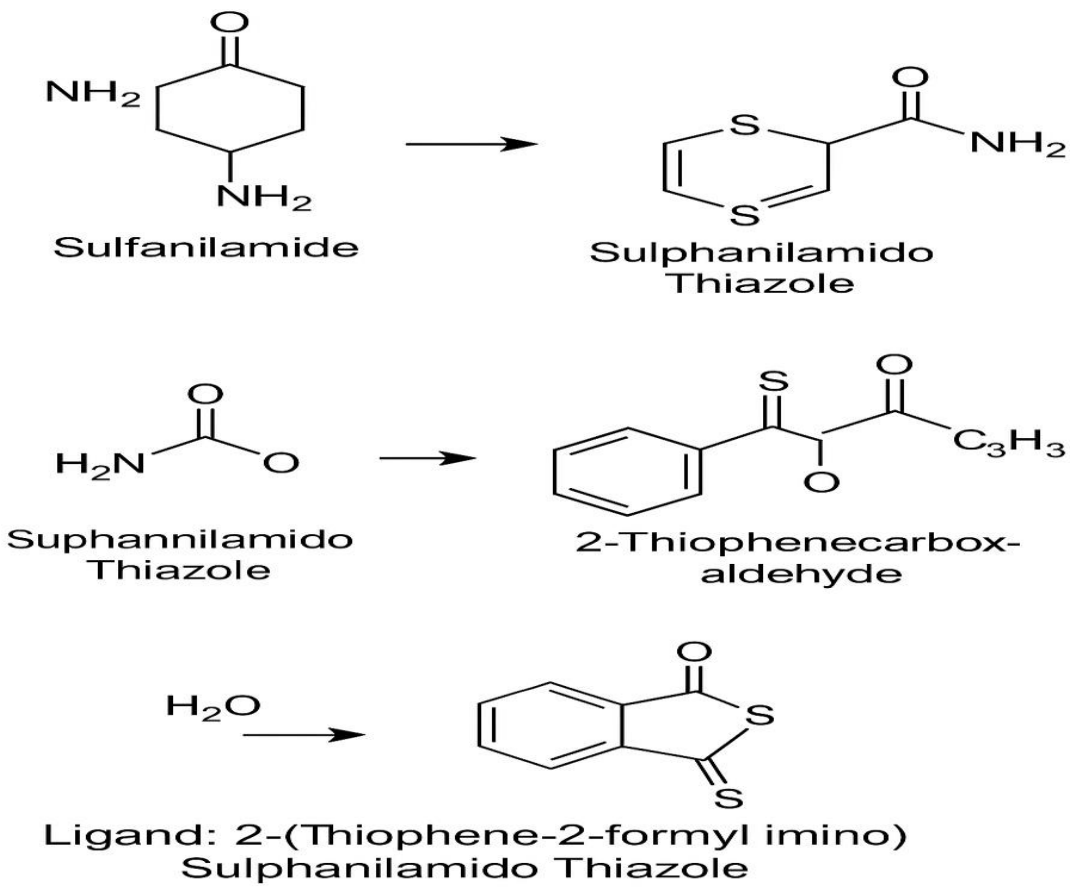
Compound	S. aureus ZOI (mm) / MIC (µg/mL)	E. coli ZOI (mm) / MIC (µg/mL)	C. albicans ZOI (mm) / MIC (µg/mL)
H_2L (Ligand)	10 / 128	8 / >256	9 / 256
$[Mn(L)(H_2O)_2]$	14 / 64	11 / 128	13 / 128
$[Co(L)(H_2O)_2]$	18 / 32	15 / 64	16 / 64
$[Ni(L)(H_2O)_2]$	16 / 64	13 / 128	14 / 128
[Cu(L)]	**21 / 16**	**18 / 32**	**19 / 32**
$[Zn(L)(H_2O)]$	15 / 64	12 / 128	14 / 128
Ampicillin	25 / 4	22 / 8	-
Fluconazole	-	-	28 / 4

Discussion:

The results clearly demonstrate that metal complexation significantly enhances the antimicrobial potency of the organic Schiff base ligand. The free ligand (H_2L) showed only marginal activity against the test strains. In contrast, all metal complexes exhibited notably larger zones of inhibition and lower MIC values, indicating superior antimicrobial efficacy.

The observed enhancement in activity upon complexation can be explained by several factors rooted in the **Chelation Theory**. Firstly, chelation reduces the polarity of the metal ion by partially sharing its positive charge with the donor groups of the ligand, and increasing the delocalization of π-electrons over the whole chelate ring. This enhanced lipophilicity facilitates the permeation of the complexes through the lipid layers of the microbial cell membrane, allowing them to reach their site of action more effectively. Secondly, the metal ions themselves can play a crucial role in the biological activity by serving as catalytic centers for various cellular reactions or by interfering with vital cellular processes.

The order of antimicrobial activity among the complexes was generally found to be: **Cu(II) > Co(II) > Ni(II) ≈ Zn(II) > Mn(II) > H_2L**. The superior activity of the copper complex can be attributed to its square planar geometry, which may favor intercalation into DNA or stronger interaction with cellular enzymes, and the intrinsic redox activity of copper, which can catalyze the production of reactive oxygen species (ROS) leading to oxidative cellular damage. The complexes showed greater activity against the Gram-positive S. aureus than the Gram-negative E. coli, which is likely due to the presence of the complex outer membrane in Gram-negative bacteria acting as a permeability barrier [6]. While less potent than the standard drugs, the significant enhancement in activity from the ligand to the complexes, particularly the copper complex, marks them as promising leads for the development of new antimicrobial agents.



6. Conclusion

In summary, this research successfully demonstrates the synthesis and comprehensive characterization of a novel Schiff base ligand, 2-(Thiophene-2-formyl imino) Sulphanilamido Thiazole (H_2L), derived from sulfathiazole and thiophene-2-carbaldehyde, and its coordination complexes with Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) ions. The structure of the ligand was confirmed by elemental analysis and NMR spectroscopy. The collective data from elemental analysis, molar conductivity, FT-IR, electronic spectroscopy, magnetic moments, TGA, and PXRD unequivocally support the proposed structures of the complexes. The ligand was found to coordinate in a tridentate O, N, S-donor fashion, forming octahedral complexes with Mn(II), Co(II), and Ni(II), a square planar complex with Cu(II), and a tetrahedral/pentacoordinate complex with Zn(II).

Most significantly, the in vitro antimicrobial studies revealed that metal complexation dramatically enhances the biological activity of the organic ligand. The chelates, particularly the copper(II) complex, exhibited markedly superior efficacy against both bacterial and fungal strains compared to the free ligand. This enhancement is attributed to the principles of chelation, which increase lipophilicity and membrane permeability. The findings underscore the potential of strategic ligand design and metal complexation in developing novel therapeutic agents with improved pharmacological profiles.

7. Future Work

Building upon the findings of this study, the following avenues are proposed for future investigation:

1. **Single-Crystal X-ray Diffraction:** The foremost objective is to grow high-quality single crystals of these complexes, potentially using slow evaporation or vapor diffusion techniques. This would provide an unambiguous and definitive determination of the molecular structures, bond lengths, bond angles, and supramolecular packing, confirming the coordination mode and geometry proposed in this work.
2. **Computational Studies (DFT):** Density Functional Theory (DFT) calculations could be employed to gain deeper insight into the electronic structures of the complexes. These studies can optimize the proposed geometries, calculate molecular orbitals, and simulate the electronic spectra, providing a theoretical foundation that supports and refines the experimental spectroscopic data.
3. **In vivo Toxicity and Efficacy:** To assess the therapeutic potential, in vivo studies are essential. This includes evaluating the acute and sub-acute toxicity in animal models to establish safety profiles, followed by efficacy studies in infected animal models to confirm the antimicrobial activity observed in vitro.
4. **DNA Interaction and Anticancer Studies:** Given the structural features of the ligand (planar aromatic rings) and the activity of many metal complexes as chemotherapeutic agents, future work should investigate the interaction of these complexes with DNA (e.g., via UV-Vis titration, fluorescence quenching, and viscosity measurements). Furthermore, their cytotoxicity should be evaluated against a panel of human cancer cell lines to explore their potential as anticancer agents.

8. References

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