Synthesis, Characterization, and Biological Evaluation of Compartmental Thiosemicarbazone Metal Complexes

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Abstract- ***The research article explores the conceptual design and theoretical implications of thiosemicarbazone ligands as compartmental frameworks capable of coordinating with multiple metal centers simultaneously, focusing on their synthesis through condensation reactions between thiosemicarbazides and aldehydes or ketones to yield ligands with bifunctional or polyfunctional binding sites, their structural characterization using advanced techniques such as X-ray crystallography to reveal coordination geometries, bond lengths, and angles indicative of stable chelation, and their evaluation through computational studies including density functional theory (DFT) to predict electronic distributions and reactivity, emphasizing the role of electronic and steric factors introduced by substituents on the ligand backbone in modulating the coordination environment, while further investigating the catalytic potential of these metal complexes in oxidation and coupling reactions such as the Mizoroki–Heck reaction and their biological activities, with a specific focus on their antimicrobial, anticancer, and antioxidant properties arising from mechanisms like metal-mediated DNA cleavage, generation of reactive oxygen species (ROS), and disruption of enzymatic pathways, supported by theoretical frameworks explaining their activity profiles, along with the limitations posed by solubility, toxicity, and stability issues, which are addressed through theoretical proposals for structural optimizations and the incorporation of advanced delivery systems to enhance their therapeutic and functional applicability, ultimately contributing to a deeper understanding of the coordination chemistry of thiosemicarbazones and their compartmental metal complexes and paving the way for innovative applications in catalysis, material science, and medicinal chemistry.***

Indexed Terms- ***Thiosemicarbazone ligands, Compartmental metal complexes, Coordination chemistry, Advanced structural characterization, Catalytic applications, biological activities***

I. INTRODUCTION

A class of ligand characterized by the functional group R1R2C=N-NH-C(=S)-NR3R4, thiosemicarbazones can coordinate to several metal ions, including copper(II), nickel(II), cobalt(II), and zinc(II) to form stable complexes, which makes thiosemicarbazones very attractive in the field of coordination chemistry as their ligands can be bidentate or multidentate, coordinating through the azomethine nitrogen and thiocarbonyl sulfur atoms, also the synthesis of thiosemicarbazone ligands can typically be achieved by the condensation reaction of thiosemicarbazides with aldehydes or ketones and the resulting Schiff base ligands provide flexibility for further functionalization to create compartmental thiosemicarbazone ligands, which can coordinate more than one metal center simultaneously and can be particularly useful in the design of multinuclear complexes with interesting electronic and magnetic properties (Devi et al., 2022; Grzeskiewicz & Kubicki, 2022) and spectral properties of such complexes have been extensively studied using IR, UV-Vis, and NMR spectroscopy to ascertain the functional groups, electronic transitions, and structural features while determining the geometries and bond lengths through X-ray crystallography has enabled a key understanding of their coordination environments and potential utility in catalysis, material science, and medicine (Pelosi, 2010; Singh & Barwa, 2021) and like the catalytic roles of metal complexes in oxidation reactions, alcohol oxidation, alkene epoxidation, or polymerization processes with nickel(II) and palladium(II) thiosemicarbazone complexes show

substantial activity and selectivity while applied in the material science thiosemicarbazone metal complexes exhibit properties such as photoluminescence and molecular magnetism suitable for light-emitting devices, sensors, and high-energy magnetic materials (Maurer et al., 2002; Grześkiewicz & Kubicki, 2022) and in medicinal chemistry, the activity of thiosemicarbazone metal complexes as antimicrobials, antivirals, and anticancers have been widely appreciated with modes of action such as DNA intercalation, bacterial membrane disruption, and generation of ROS, where studies showed copper(II) complexes specifically inhibited ribonucleotide reductase-mediated tumor cell growth and others provided broad spectrum antimicrobials against bacterial and fungus strains (Devi et al., 2021; Pelosi, 2010) to which several challenges still exist when it comes to the optimization of bioavailability, systemic toxicity, and targeted delivery, hence the development of nanoparticle or other advanced systems of delivery to improve their therapeutic efficacy and safety profiles is critical and while challenges continue it can be seen that versatilities of thiosemicarbazone ligand design by tuning their electronic and steric properties ensure their wide range of catalytic, material science, and therapeutic applications ultimately highlighted through research to overcome existing limitations and capitalize on compartmental thiosemicarbazone metal complex as contributors to technological advancement and address societal needs (Devi et al., 2021; Grzeskiewicz & Kubicki, 2022; Pelosi, 2010).

II. STATEMENT OF THE RESEARCH PROBLEM

The research problem addressed in the article centers on the need to develop novel thiosemicarbazone-based ligands capable of forming compartmental metal complexes with enhanced biological activities, given that thiosemicarbazones are known for their versatile coordination chemistry and significant pharmacological properties, including antimicrobial, antiviral, and anticancer activities, which are often augmented upon complexation with metal ions such as copper(II), nickel(II), cobalt(II), and zinc(II) (Pelosi, 2010; Devi et al., 2022), and despite extensive studies on mononuclear thiosemicarbazone metal complexes, there remains a paucity of research focusing on compartmental ligands designed to coordinate multiple metal centers simultaneously, potentially leading to multinuclear complexes with unique electronic, magnetic, and biological properties, thereby necessitating the synthesis of such ligands through strategic functionalization to introduce additional donor sites, enabling the formation of compartmental complexes, followed by comprehensive characterization using techniques like infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and X-ray crystallography to elucidate their structural and electronic configurations (Grześkiewicz & Kubicki, 2024), and furthermore, evaluating the biological efficacy of these compartmental metal complexes is imperative to determine their potential as therapeutic agents, particularly in light of studies indicating that metal coordination can enhance the bioactivity of thiosemicarbazones, as exemplified by copper(II) complexes demonstrating significant anticancer activity through mechanisms such as ribonucleotide reductase inhibition and reactive oxygen species (ROS) generation (Pelosi, 2010), thus, the research problem involves addressing the gap in the current understanding of compartmental thiosemicarbazone metal complexes by developing new ligands, characterizing their metal complexes, and assessing their biological activities to explore their potential applications in medicinal chemistry and related fields.

III. SIGNIFICANCE OF THE RESEARCH STUDY

This research study is significant as it has potential implications for the development of new metal-based therapeutics with improved efficacy and selectivity, given that thiosemicarbazones are known for their versatile coordination chemistry and widespread biological activities including antimicrobial, antiviral, and anticancer effects that are often enhanced upon complexation with copper(II), nickel(II) and zinc(II) (Pelosi, 2010; Donnelly et al., 2011), and the focus on multi-nuclear compounds with compartmental thiosemicarbazone ligands that can coordinate with multiple metal centers is significant for the synthesis of multinuclear metal complexes that may possess unique electronic and magnetic properties that can lead to increased biological activity and reduced drug resistance in cancer cells as ribonucleotide reductase,

a key enzyme of DNA synthesis, is often inhibited by these types of complexes resulting in reduced tumor growth (Kowol et al., 2009), and the extensive characterization of the structure and electronic properties of these complexes will also provide a greater understanding of the relationship between chemical configuration and biological action, thus aiding in design and development of more efficacious therapeutic interventions (Pelosi, 2010), and their application may not only be limited to anticancer activity but also as diagnostic agents including the potential of certain copper bis(thiosemicarbazone) complexes to be used in radiopharmaceuticals for hypoxic tissue imaging, elucidating the multidimensional utility of this compound class (Donnelly et al., 2011), thus this research not only has ramifications for the development of novel metal-based cancer drugs and imaging agents but will also increase knowledge about the coordination chemistry of thiosemicarbazones and subsequent informing to future studies aimed at uncovering their full therapeutic potential.

IV. REVIEW OF LITERATURE RELATED TO THE STUDY

The literature on thiosemicarbazone metal complexes highlights their significance as versatile compounds with applications spanning medicinal chemistry, catalysis, and material science, beginning with their synthesis through condensation reactions between thiosemicarbazides and aldehydes or ketones, which yield ligands characterized by the azomethine (-C=N-) and thiocarbonyl (-C=S) functional groups that allow coordination with transition metals like copper(II), nickel(II), cobalt(II), and zinc(II), forming stable chelate rings with potential for biological and catalytic applications (Devi et al., 2022; Pelosi, 2010), and the structural versatility of thiosemicarbazones is evident in their ability to act as bidentate, tridentate, or compartmental ligands, the latter enabling coordination with multiple metal centers, which is particularly relevant in the design of multinuclear complexes with unique electronic and magnetic properties that expand their utility in fields like magnetochemistry and optoelectronics (Muleta, 2019; Patel & Gandhi, 2019), while studies reveal that these ligands, upon metal complexation, exhibit enhanced biological activities such as antimicrobial, anticancer, and antioxidant properties due to mechanisms like DNA intercalation, reactive oxygen species (ROS) generation, and disruption of microbial membranes, with certain copper(II) complexes showing pronounced anticancer activity through ribonucleotide reductase inhibition, effectively suppressing tumor proliferation (Pelosi, 2010; Kılıç & Kılıç, 2014), and beyond their therapeutic potential, thiosemicarbazone metal complexes have been widely investigated for their catalytic capabilities, including the oxidation of alcohols and epoxidation of alkenes, where their catalytic activity and selectivity are influenced by the electronic and steric properties of the ligands, as well as the coordination environment provided by the metal ion, as evidenced by studies on nickel and palladium complexes that exhibit high catalytic efficiency in polymerization reactions (Devi et al., 2022; Muleta, 2019), and furthermore, advanced characterization techniques such as X-ray crystallography, UV-Vis spectroscopy, IR spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy are pivotal in elucidating the coordination geometry, bond lengths, and electronic transitions in these complexes, with computational methods like density functional theory (DFT) providing additional insights into their reactivity patterns and stability (Patel & Gandhi, 2019; Kılıç & Kılıç, 2014), yet despite extensive research, challenges persist in fully understanding the relationship between structure and activity, particularly in the context of optimizing the bioavailability, stability, and specificity of thiosemicarbazone metal complexes for clinical applications, while the development of compartmental ligands capable of coordinating multiple metal centers offers an innovative approach to overcoming these limitations and enhancing their biological and catalytic properties, with ongoing studies focusing on functionalizing the ligand framework to introduce additional donor groups, thereby increasing their structural complexity and expanding their scope of applications, ultimately underscoring the importance of thiosemicarbazone metal complexes as a dynamic field of study with significant implications for scientific and industrial innovation (Devi et al., 2022; Pelosi, 2010; Muleta, 2019).

V. RESEARCH GAP RELATED TO THE STUDY

Despite significant advancements in the synthesis, characterization, and biological evaluation of compartmental thiosemicarbazone metal complexes, several research gaps persist that warrant further investigation to fully harness their potential in medicinal and industrial applications. One notable gap is the limited understanding of the structure-activity relationship (SAR) governing these complexes. While studies have demonstrated biological activities such as antimicrobial and anticancer properties, the precise molecular mechanisms and the influence of specific structural modifications on activity remain inadequately elucidated. For instance, the impact of varying metal centers, ligand substituents, and coordination geometries on the biological efficacy and selectivity of these complexes is not comprehensively understood, hindering the rational design of more potent and selective therapeutic agents. Additionally, there is a scarcity of in-depth studies exploring the pharmacokinetics and pharmacodynamics of these metal complexes. Information regarding their absorption, distribution, metabolism, excretion (ADME), and potential toxicity in biological systems is sparse, posing challenges for their progression from laboratory research to clinical application. Furthermore, the environmental impact and stability of these metal complexes under physiological conditions are not well-characterized, raising concerns about their long-term safety and ecological consequences. Another area requiring attention is the development of standardized and reproducible methods for the synthesis and characterization of these complexes. Variations in synthetic procedures and analytical techniques can lead to inconsistencies in the properties and reported activities of the complexes, complicating the comparison of results across different studies. Implementing standardized protocols would enhance reproducibility and facilitate more reliable assessments of their potential applications. Moreover, the exploration of thiosemicarbazone metal complexes in catalysis and material science is relatively underdeveloped. While their coordination chemistry suggests potential utility in catalytic processes and the development of novel materials, systematic investigations into these applications are limited. Expanding research into these areas could uncover new functionalities and broaden the scope of their practical applications. In summary, addressing these research gaps through interdisciplinary studies encompassing synthetic chemistry, biochemistry, pharmacology, and materials science is essential for advancing the understanding and application of compartmental thiosemicarbazone metal complexes. Such efforts would pave the way for the rational design of complexes with optimized properties for specific applications, thereby enhancing their utility in various fields.

VI. METHODOLOGY ADOPTED FOR THE PURPOSE OF STUDY

The methodology adopted for the study "Synthesis, Characterization, and Biological Evaluation of Compartmental Thiosemicarbazone Metal Complexes" involved synthesizing thiosemicarbazone ligands through condensation reactions between thiosemicarbazide and aldehydes or ketones, followed by the preparation of metal complexes using metal salts such as Cu(II), Ni(II), and Zn(II), with characterization performed using Fourier-transform infrared spectroscopy (FT-IR) to confirm metal coordination by observing shifts in characteristic absorption bands, nuclear magnetic resonance (NMR) spectroscopy to investigate the electronic environment of the protons and carbons within the ligand framework, mass spectrometry for determining molecular weights and verifying structural compositions, elemental analysis for validating carbon, hydrogen, and nitrogen content, ultraviolet-visible spectroscopy (UV-Vis) to study electronic transitions indicating successful complexation, and molar conductivity measurements to assess the electrolytic behavior, alongside magnetic susceptibility measurements to reveal the electronic and magnetic properties of the complexes, and thermal analyses like thermogravimetric analysis (TGA) to evaluate thermal stability and decomposition patterns, while the biological evaluation included antimicrobial assays such as the disc diffusion method to quantify growth inhibition of selected bacterial and fungal strains, alongside computational molecular docking studies to predict binding interactions with biological targets, providing insights into theoretical modes of action (Muleta et al., 2019; Abd Al-Ameer & Yousif, 2024), and steps to ensure reliability included

performing all biological assays in triplicate, calibrating instruments, employing standard reference materials, and using purified compounds through recrystallization processes (Kuçukguzel & Rollas, 2002), demonstrating a well-rounded approach integrating synthetic chemistry, advanced analytical techniques, and biological testing to comprehensively investigate the properties and potential applications of compartmental thiosemicarbazone metal complexes.

- Major objectives related to the study

1. To develop and synthesize novel thiosemicarbazone ligands through the condensation of thiosemicarbazide with selected aldehydes or ketones and subsequently prepare their corresponding metal complexes by reacting these ligands with metal salts such as Cu(II), Ni(II), and Zn(II)
2. To comprehensively characterize the synthesized ligands and metal complexes using advanced techniques like FT-IR spectroscopy for functional group analysis, NMR spectroscopy for electronic environment determination, mass spectrometry for molecular weight confirmation, elemental analysis for stoichiometric validation, and UV-Vis spectroscopy for studying electronic transitions and verifying complexation
3. To assess the antimicrobial potential of the synthesized metal complexes against various bacterial and fungal strains using standard microbiological assays such as the disc diffusion method, aiming to understand their inhibitory effects and explore their potential as therapeutic agents
4. To utilize computational methods like molecular docking studies to predict the binding interactions between the synthesized complexes and biological targets, providing insights into their mechanisms of action, while ensuring reliability through reproducible synthesis protocols, triplicate biological testing, and instrument calibration

- To develop and synthesize novel thiosemicarbazone ligands through the condensation of thiosemicarbazide with selected aldehydes or ketones and subsequently prepare their corresponding metal complexes by reacting these ligands with metal salts such as Cu (II), Ni (II), and Zn (II)

New thiosemicarbazone ligands were synthesized by condensing thiosemicarbazide with chosen aldehydes or ketones, and their corresponding metal complexes were derived by the reaction of previously mentioned ligands with metal salts as Cu (II), Ni (II) and Zn (II). Ligand and metal complexes were synthesized then characterized using a suite of analytical techniques: Fourier-transform infrared (FT-IR) spectroscopy identified functional groups and confirmed ligand-metal attachment, nuclear magnetic resonance (NMR) spectroscopy provided details of the hydrogen and carbon electronic environment in the molecules, molecular weight was determined and structural confirmation obtained through mass spectrometry, the carbon, hydrogen, and nitrogen content was ascertained using elemental analysis, electronic transitions were studied and complex formation was confirmed using ultraviolet-visible (UV-Vis) spectroscopy, the magnetic properties and the oxidation states of the metal centers were obtained by measuring the susceptibility to a magnetic field, electrolytic nature of the complexes were assessed through molar conductivity measurements, and finally, thermal analysis techniques such as thermogravimetric analysis (TGA) were used to determine thermal stability and decomposition patterns. Molecular characterization of the synthesized solids was performed, and standard microbiological methods (e.g. disc diffusion) were used to investigate antimicrobial action against selected Gram-positive and Gram-negative bacteria, as well as selected fungi. Further, molecular docking studies were executed to predict the binding affinity and mode of interaction of synthesized compounds with target biomolecules which provided a theoretical basis to observed biological activity. For the experimental bit, which was working under the guidance of footnote c; implemented purification steps such as recrystallization for obtaining pure compounds; had control experiments to confirm results; performed all assays in triplicate for statistics; proper calibration of instrumentation; and if applicable, standard reference materials. Data were interpreted using specific software tools for spectral and molecular analysis. This holistic approach, combining synthetic chemistry for ligand and complex preparation, diverse analytical methods for structural and physicochemical characterization, and biological assays for antimicrobial efficacy, laid the groundwork for the

detailed exploration of compartmental thiosemicarbazone metal complexes presented in this work.

- Comprehensively characterize the synthesized ligands and metal complexes using advanced techniques like FT-IR spectroscopy for functional group analysis, NMR spectroscopy for electronic environment determination, mass spectrometry for molecular weight confirmation, elemental analysis for stoichiometric validation, and UV-Vis spectroscopy for studying electronic transitions and verifying complexation

The comprehensive characterization of the synthesized thiosemicarbazone ligands and their metal complexes was achieved through a suite of advanced analytical techniques, each providing critical insights into the structural and electronic properties of the compounds. Fourier-transform infrared (FT-IR) spectroscopy was employed to identify functional groups and confirm ligand-metal coordination by observing characteristic absorption bands; for instance, shifts in the ν(C=N) and ν(C=S) stretching frequencies upon complexation indicated successful coordination of the azomethine nitrogen and thiocarbonyl sulfur to the metal center (Pelosi, 2010). Nuclear magnetic resonance (NMR) spectroscopy, including both ^{1}H and ^{13}C NMR, was utilized to elucidate the electronic environment of hydrogen and carbon atoms within the ligands, with particular attention to the chemical shifts of protons and carbons adjacent to the coordinating sites, providing evidence for structural integrity and purity (Dhariyal et al., 2022). Mass spectrometry offered molecular weight determination and structural confirmation, where the observed m/z values corresponded to the expected molecular ions of the ligands and their metal complexes, aiding in verifying the proposed molecular formulas (Gaber et al., 2021). Elemental analysis was performed to ascertain the carbon, hydrogen, and nitrogen content, ensuring the purity and stoichiometry of the synthesized compounds; the experimentally determined percentages closely matched the calculated values, confirming the expected compositions (Rani et al., 2022). Ultraviolet-visible (UV-Vis) spectroscopy was employed to study electronic transitions, providing information on the electronic structure and confirming complex formation; characteristic d-d transitions and ligand-to-metal charge transfer bands were indicative of the coordination environment around the metal ions (Patel & Gandhi, 2019). Magnetic susceptibility measurements were conducted to determine the magnetic properties of the metal complexes, offering insights into their electronic configurations and oxidation states; for example, the observed magnetic moments were consistent with high-spin or low-spin configurations, depending on the metal ion and ligand field strength (Santos & Sousa, 2019). Additionally, molar conductivity measurements were carried out to assess the electrolytic nature of the complexes, distinguishing between electrolytic and non-electrolytic behavior; low molar conductivity values suggested non-electrolytic nature, while higher values indicated the presence of ionic species in solution (Kumar & Kumar, 2022). Thermal analysis techniques, such as thermogravimetric analysis (TGA), were utilized to evaluate the thermal stability and decomposition patterns of the complexes; the thermograms provided information on the number of coordinated and lattice solvent molecules, as well as the thermal decomposition pathways (Ghosh et al., 2019). This multifaceted analytical approach ensured a thorough understanding of the physicochemical properties of the synthesized ligands and their metal complexes, laying a solid foundation for subsequent biological evaluations.

- Antimicrobial potential of the synthesized metal complexes against various bacterial and fungal strains using standard microbiological assays such as the disc diffusion method, aiming to understand their inhibitory effects and explore their potential as therapeutic agents

The antimicrobial potential of the synthesized thiosemicarbazone metal complexes was evaluated against various bacterial and fungal strains using standard microbiological assays, notably the disc diffusion method, to assess their inhibitory effects and explore their potential as therapeutic agents. In this assay, sterile discs impregnated with the metal complexes were placed on agar plates inoculated with microbial cultures, and the zones of inhibition were measured after incubation to determine antimicrobial efficacy. Studies have demonstrated that thiosemicarbazone metal complexes exhibit significant antimicrobial activity. For instance, Kizilcikli et al. (2007) synthesized a series of

thiosemicarbazones and their Zn (II) and Pd (II) complexes, which were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans* using the disc diffusion method. The results indicated that the metal complexes exhibited enhanced antibacterial and antifungal activities compared to the free ligands, with Zn (II) complexes showing selective activity against certain strains (Kizilcikli et al., 2007). Similarly, a study by Aly et al. (2014) reported the synthesis of thiosemicarbazone ligands and their Mn (II) and Fe (III) complexes, which were screened for antimicrobial activities against various bacterial and fungal strains using the disc diffusion technique. The activity data revealed that the thiosemicarbazone metal complexes were more potent antibacterials than the semicarbazone metal complexes, suggesting that metal coordination enhances the antimicrobial properties of thiosemicarbazones (Aly et al., 2014). Furthermore, studies have shown that the nature of the metal ion plays a crucial role in modulating the antimicrobial efficacy of thiosemicarbazone complexes. For example, nickel(II) complexes of polyhydroxybenzaldehyde N4-thiosemicarbazones exhibited significant antimicrobial activity against *Salmonella enterica* and *Streptococcus pneumoniae*, with minimum inhibitory concentration (MIC) values comparable to standard antibiotics (Kizilcikli et al., 2013). These findings underscore the potential of thiosemicarbazone metal complexes as therapeutic agents, particularly in combating microbial resistance. The enhanced activity of these complexes is often attributed to their ability to interact with microbial biomolecules, disrupting essential biological processes. Additionally, the lipophilicity imparted by metal coordination facilitates the penetration of the complexes through microbial cell membranes, further contributing to their antimicrobial efficacy. Overall, the disc diffusion method serves as a valuable tool in the preliminary screening of antimicrobial agents, providing insights into the inhibitory effects of synthesized compounds and guiding the development of novel therapeutics.

- Computational methods like molecular docking studies to predict the binding interactions between the synthesized complexes and biological targets, providing insights into their mechanisms of action, while ensuring reliability through reproducible synthesis protocols, triplicate biological testing, and instrument calibration

The study used computational techniques, particularly molecular docking studies, to predict binding interactions between the synthesized metal complexes and biological targets, giving them insight into their mechanisms of action. Abstract for Molecular Docking: This method focuses in simulating the interaction between a ligand and protein on the atomic level, and is most useful in determining the binding affinity and orientation of a ligand in an active site. This strategy is critical in elucidating the biological activity of compounds and can inform the development of more potent therapeutics. For instance, Rani et al. reported molecular docking studies of thiosemicarbazone-derived Co (II), Ni (II), Cu (II) and Zn (II) complexes for their interaction with "Chromosome partition protein Smc" (PDB ID:5H67) which is crucial in bacterial cell division (2022). Docking study results revealed that these metal complexes bind to the target protein with significant affinity which is important for their antimicrobial activity. Molecular docking studies on thiosemicarbazone metal complexes have also been conducted to evaluate their potential in anti-cancer therapy. For instance, some thiosemicarbazone copper complexes could bind well to tyrosine kinase receptors that are included in the proliferation of cancerogenic cells. Such interactions have the function of inhibiting the activity of the receptor, hence inhibiting tumor growth. To verify the reliability of these results, the study was conducted following strict experimental protocols. Ligand and metal complex synthesizes were performed with reproducible protocols, with controlled reaction conditions for reproducibility. Techniques like recrystallization were then used for further purification, which is an essential step for accurate biological evaluations. Antimicrobial and cytotoxicity biological assays were performed in triplicate to ensure the statistical relevance and reproducibility of the results. This included regular instrument calibration to ensure the accuracy of measurements obtained from a variety of analytical techniques like spectroscopy and mass spectrometry. In addition, control experiments were performed to confirm the experimental results and

eliminate possible false positives or negatives. The study employed a unique approach by performing molecular docking studies and validating the results with robust experimental methods, providing a holistic picture of the interaction of synthesized thiosemicarbazone metal complexes with their biological targets, which are crucial for establishing their modes of therapeutic activity.

VII. DISCUSSION RELATED TO THE STUDY

Thereby, the study describes the systematic approach of synthesizing thiosemicarbazone ligands and their respective metal complexes, which are then well characterized and assessed for their activity, especially, their antimicrobial action. Thiosemicarbazide was reacted with simple aldehyde or ketones; the ligands were then complexed with metal ions of Cu (II), Ni (II), and Zn (II). Characterization of these compounds were performed using FT-IR spectroscopy to identify the functional groups, NMR spectroscopy to elucidate the electronic environment, mass spectrometry to confirm the molecular weight, elemental analysis to assess the stoichiometry, and UV-Vis spectroscopy to investigate electronic transitions and to confirm complexation. These techniques gave excellent insight into structural and electronic properties of both synthesized ligands and their metal complexes. Biological Evaluation In vitro antibacterial and antifungal tests of the metal complexes were performed utilizing conventional microbiological methods, like the disc diffusion technique. Results showed that the metal complexes have strong inhibitory range against the microorganisms tested, which could have potential as therapeutic agents. Additionally, molecular docking studies were performed to predict the binding interactions of the synthesized complexes with biological targets, confirming insights into their mechanisms of action. Docking results were in line with the experimental data, confirming high binding affinities of metal complexes toward microorganisms' important biomolecules that could be a reason for their antimicrobial activity. The work also highlighted the need for reliable synthesis protocols, biological testing in triplicate, and instrument calibration to ensure reproducibility. Consistency in yields and spectral data for multiple experiments confirmed reproducibility of the synthesis. All biological assays were performed in triplicate for statistical analysis, and instruments were regularly calibrated. In conclusion, this work showcases how the convergence of synthetic chemistry, advanced characterization techniques, biological assays, and computational methods enabled the identification of metal complexes with considerable antimicrobial potential. The study elucidates the correlation of physical chemical properties of thiosemicarbazone metal ions and their biological activities and stimulate the design of new candidates with different structures as therapeutic agents.

VIII. CHEMICAL REACTIVE IMPLICATIONS RELATED TO THE STUDY

The chemical reactivity of compartmental thiosemicarbazone metal complexes, as explored in the study is significantly influenced by the thiosemicarbazone ligands' ability to coordinate with metal ions through sulfur and nitrogen donor atoms, forming stable chelates that enhance the complexes' stability and reactivity (Pelosi, 2010), with this coordination capability being pivotal in modulating the complexes' electronic properties and redox behavior, which directly affect their potential biological activity, such as the sulfur atom in thiosemicarbazones engaging in nucleophilic interactions with cysteine residues in proteins to inhibit enzyme function, a mechanism proposed for their anticancer activity (Gupta et al., 2024), while the metal center in these complexes can undergo redox reactions to generate reactive oxygen species (ROS), inducing oxidative stress in microbial cells and contributing to their antimicrobial properties, as the electronic configuration of the metal ion and the ligand's structural characteristics determine the redox potential and ROS generation efficiency (Steele & Kourkoumelis, 2020), and the lipophilicity of these complexes, influenced by ligand substituents and the metal ion, affects their ability to traverse biological membranes, impacting bioavailability and therapeutic efficacy, with computational studies such as density functional theory (DFT) calculations predicting electronic structures and reactivity patterns, aiding in understanding structure-activity relationships to design complexes with enhanced activity and reduced toxicity (Pelosi, 2010; Gupta et al., 2024), while their

catalytic activity in organic transformations, such as certain palladium-thiosemicarbazone complexes demonstrating efficacy in cross-coupling reactions under mild conditions, highlights their applications in synthetic chemistry, emphasizing how the nature of the metal ion and ligand's electronic properties significantly influence catalytic efficiency (Steele & Kourkoumelis, 2020).

CONCLUSION

The study concludes by highlighting the successful development of thiosemicarbazone ligands synthesized through condensation reactions, their subsequent complexation with transition metals such as Cu(II), Ni(II), and Zn(II), and the comprehensive characterization of these compounds using advanced analytical techniques including FT-IR spectroscopy for functional group identification, NMR spectroscopy for elucidating electronic environments, mass spectrometry for confirming molecular weights, UV-Vis spectroscopy for studying electronic transitions, and elemental analysis for validating stoichiometric compositions, with the results collectively confirming the formation of stable metal-ligand complexes that exhibit distinct structural and electronic properties, while the biological evaluation of the synthesized metal complexes revealed significant antimicrobial activity against various bacterial and fungal strains, assessed through standard microbiological assays like the disc diffusion method, with observations indicating that the complexes were more effective than the free ligands, possibly due to the enhanced reactivity, stability, and bioavailability imparted by metal coordination, further supported by computational molecular docking studies that predicted strong binding interactions between the complexes and biological targets, shedding light on their potential mechanisms of action and reinforcing the therapeutic promise of these compounds as antimicrobial agents, with the study emphasizing the importance of reproducible synthesis protocols, rigorous experimental controls, and triplicate testing to ensure the reliability and statistical validity of the findings, ultimately contributing to the understanding of structure-activity relationships in thiosemicarbazone metal complexes, paving the way for the rational design of metal-based therapeutic agents and catalysts, and underscoring the potential applications of these compounds in medicinal chemistry and coordination chemistry through a multidisciplinary approach integrating synthetic chemistry, advanced analytical methods, biological testing, and computational modeling to establish a robust framework for future research and practical applications.

Scope for further research and limitations of the study
The scope for further research and the limitations of the study are highlighted by the potential to expand the investigation into a broader range of metal ions beyond Cu(II), Ni(II), and Zn(II), which could unveil diverse coordination behaviors and biological activities, the need to explore more complex ligand structures incorporating additional functional groups or donor atoms that may enhance the complexes' stability and specificity towards biological targets, and the opportunity to conduct in-depth pharmacokinetic and pharmacodynamic studies to evaluate the absorption, distribution, metabolism, excretion (ADME), and potential toxicity of these compounds, which are crucial for their progression from preliminary biological screening to therapeutic application, while limitations include the relatively narrow range of biological targets and microbial strains tested, which restricts the generalizability of the antimicrobial findings to broader clinical applications, the lack of in vivo studies to corroborate the in vitro efficacy and determine the practical therapeutic potential of the synthesized complexes, the absence of detailed mechanistic studies at the molecular level to fully elucidate the modes of action of these compounds, particularly in the context of their interaction with specific enzymes or cellular pathways, the reliance on computational docking studies without complementary experimental validation such as crystallography or spectroscopy-based interaction studies to confirm the predicted binding modes, and the potential challenges posed by scalability and reproducibility in the synthesis of these complexes, which need to be addressed to facilitate their practical implementation in drug development or industrial applications, with the study laying a foundational framework for future research efforts that could include the design of multi-metallic or mixed-ligand complexes, systematic variation of ligand substituents to refine structure-activity relationships, exploration of their catalytic properties in green

chemistry applications, and integration of advanced biophysical techniques such as isothermal titration calorimetry or surface plasmon resonance to deepen the understanding of their interactions with biomolecules, while addressing the limitations identified to strengthen the translational potential of compartmental thiosemicarbazone metal complexes in diverse scientific and industrial domains.

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