Mechanistic Insights into the Antioxidant and Antimicrobial Activities of Transition Metal-Thiosemicarbazone Complexes

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Abstract- ***This study explores the mechanistic underpinnings of the antioxidant and antimicrobial activities of transition metal-thiosemicarbazone (TSC) complexes through a detailed conceptual framework combining theoretical analyses and literature-driven insights, where the antioxidant potential is hypothesized to arise from the ability of the complexes to participate in redox cycling via reversible one-electron transfer processes facilitated by the metal center, while the TSC ligand provides stabilization of the reactive intermediates through resonance and inductive effects, and the antimicrobial activity is attributed to the disruption of microbial cell membrane integrity by the complexes via enhanced lipophilicity due to metal coordination that enables facile penetration and binding to intracellular targets such as DNA, proteins, or enzymes, disrupting essential metabolic pathways and cellular replication mechanisms, and the theoretical analysis emphasizes the role of electronic, steric, and geometric factors in dictating the activity, supported by computational data indicating that complexes with certain transition metals, such as Cu(II), Zn(II), and Fe(III), exhibit superior reactivity due to optimal orbital overlap and charge transfer interactions, while the ligand scaffold modulates the metal ion reactivity through electron-donating or withdrawing substituents, thereby influencing the redox potential, antimicrobial spectrum, and selectivity of the complexes, and the work integrates density functional theory (DFT) studies to predict the electronic distribution and binding affinity of the complexes with microbial DNA and reactive oxygen species (ROS), correlating these properties with experimental data from previous studies reporting minimum inhibitory concentration (MIC) values in the range of 1-10 μg/mL against gram-positive and gram-negative bacteria, alongside DPPH radical scavenging activity reaching 90% inhibition at micromolar concentrations, and the theoretical framework also considers the influence of solubility, stability, and pH on the activity profile, proposing a dual mechanistic model involving ROS generation for oxidative damage and direct interaction with microbial biomolecules, culminating in a comprehensive understanding that establishes transition metal-thiosemicarbazone complexes as promising candidates for therapeutic applications targeting oxidative stress and multidrug-resistant infections, with a focus on future strategies for optimizing their efficacy through rational ligand design and precise metal selection.***

Indexed Terms- ***Transition Metal Complexes, Thiosemicarbazones (TSC), Antioxidant Activity, Antimicrobial Mechanism, Density Functional Theory (DFT), Reactive Oxygen Species (ROS)***

I. INTRODUCTION

Thiosemicarbazones (TSCs), a subclass of Schiff base ligands formed as a result of the condensation of thiosemicarbazone with aldehydes or ketones, exhibit diverse coordination chemistry, and high biological activities, over the past 10 years, including antioxidant and antimicrobial activity (Patra et al., 2012; Singh et al., 2020) that have been fully reported (up to December 2022). The coordination of TSCs with transition metals improves their biological activity, owing to the formation of stable metal–TSC complexes that can interact with a range of biological targets (Abele et al., 2018). The most important properties of metal–TSC complexes responsible for their antioxidant activity, including, in majority, free radical scavenging and inhibition of oxidative

processes are associated with the redox characteristics of the central metal ion, as well as the ability of ligands to stabilize reactive species (Ali et al. 2021). Indeed, the recycling involves the redox cycle of the copper ion between the Cu (II) and Cu(I) states and has directed the electron transfer and thus radical scavenging studies with TSC copper (II) complexes (Wang et al., 2017). Likewise, iron (III)-TSC complexes possess antioxidant effect by undergoing redox reactions which counteract oxidative stress (Prasad et al., 2016). Factors contributing to the antimicrobial activity of transition metal-TSC complexes, include the lipophilicity of the complex with regard to the penetration ability of microbial cell membranes and the nature of the metal ion that can interact with intracellular components (Kumar et al., 2019). Nickel (II) and zinc (II) complexes of some TSCs have also exhibited highly potent antibacterial activity against multiple bacterial strains (Escherichia coli and Staphylococcus aureus) and this effect is thought to be associated with complex–DNA and protein interactions resulting in inhibited essential biological processes (Chandra & Kumar, 2015). Because of their structural polymorphism, TSCs can form complexes with various geometries and coordination modes that have an impact on their biological function (Iqbal et al., 2022). This has for instance been demonstrated in square-planar copper (II) complexes with tridentate TSC ligands, which exhibited increased antimicrobial properties compared to their bidentate counterparts, a difference that was possibly related to a more planar structure in the former set allowing for intercalation with DNA (Sharma et al., 2021).

Statement of the research problem

The lack of comprehensive mechanistic understanding regarding how transition metal-thiosemicarbazone (TSC) complexes exert their biological activities has impeded their optimization and broader application in therapeutic settings, as existing studies highlight their remarkable antioxidant and antimicrobial properties driven by metal-ligand interactions and redox behaviors, with copper(II)-TSC complexes demonstrating superior radical scavenging activity due to efficient redox cycling between Cu(II) and Cu(I) states that enhances reactive oxygen species (ROS) quenching capabilities (Kumar et al., 2020), while nickel(II) complexes are shown to exhibit antimicrobial activity through DNA intercalation and membrane disruption mechanisms (Al-Majedy et al., 2018), but despite these findings, the exact pathways through which these complexes engage in biological redox reactions, interact with microbial biomolecules, and destabilize pathogenic membranes remain unclear, thereby necessitating a deeper exploration of their structure-activity relationships, particularly in light of recent advances in computational chemistry techniques like density functional theory (DFT), which have successfully elucidated electronic structures and predicted binding affinities for various metal complexes (Al-Amiery et al., 2012), yet studies often fail to correlate such theoretical insights with experimental antimicrobial assays, such as minimum inhibitory concentration (MIC) or reactive oxygen species inhibition assays, which have reported MIC values of 1-10 μg/mL for copper(II)- and zinc(II)-TSC complexes against multidrug-resistant bacteria (Devi et al., 2021), further supported by findings showing that iron(III)-TSC complexes can participate in redox reactions mitigating oxidative stress in model systems (Rani et al., 2019), highlighting a pressing need to integrate experimental results with computational predictions to better design complexes with enhanced specificity and efficacy, while challenges related to solubility, stability, and ligand modification to optimize lipophilicity and metal coordination must also be addressed as illustrated in a study on cobalt(II)-TSC complexes, which demonstrated enhanced stability and bioavailability upon ligand functionalization (Patra et al., 2018), yet inconsistencies in reported findings across various studies, such as variability in assay conditions, ligand structures, and metal coordination geometries, have hindered the development of a unified model explaining the molecular mechanisms underlying the bioactivity of TSC complexes (Sharma et al., 2021), indicating that without a holistic and interdisciplinary approach incorporating spectroscopic, electrochemical, and theoretical analyses, alongside advanced bioassays, the potential of these complexes in combating oxidative stress and multidrug-resistant infections will remain unrealized, prompting the need for future research aimed at systematically investigating their redox behavior, biomolecular interactions, and potential for synergistic activity with conventional antimicrobial agents.

Significance of the research study

Investigating the mechanistic insights into the antioxidant and antimicrobial activities of transition metal-thiosemicarbazone (TSC) complexes is significant in order to address major global health issues such as the increasing incidence of multidrug-resistant pathogens and oxidative stress-related diseases, as well as their distinctive structural properties and redox capabilities, leading to the discovery of exceptionally effective scavengers of reactive oxygen species (ROS), with studies indicating that copper(II)-TSC complexes exhibit elevated radical scavenging activity, as a result of the redox cycling between Cu(II) and Cu(I) states, facilitating electron transfer reactions to neutralize ROS (Palanimuthu et al., 2013), while nickel(II) and zinc(II) TSC complexes exhibiting remarkable antimicrobial properties through potential mechanisms involving DNA intercalation and disruption of microbial cell membranes (Devi et al., 2022) and a better understanding of these mechanisms is crucial for the rational design of more effective therapeutic agents, as it would enhance the optimization of ligand structures and metal centers to maximize the biological activity and selectivity resulting in novel treatments for infections and oxidative stress-related conditions, and furthermore, the elucidation of the interaction pathways by these complexes with biological targets providing the information on their possible cytotoxicity and side effects, thus guiding the development of safer pharmaceutical applications, and recent advancements in computational studies, such as density functional theory (DFT) have facilitated the prediction of electronic structures and reactivity patterns of metal-TSC complexes and offering a theoretical basis for understanding the mechanism of antioxidant and antimicrobial (Rani et al., 2022), and their integration with the experimental findings leading to a comprehensive understanding of the structure-activity relationships, thus informing the synthesis of the complexes with tailored properties for specific therapeutic applications and moreover, the versatility of TSC ligands in forming stable complexes with various transition metals allowing for solid exploration of a wide range of metal-ligand combinations each with distinct biological activities, thus broadening the scope for drug development and in addition, the study of these complexes contributing to bioinorganic chemistry field by elucidating the role of metal in biological systems, particularly in processes involving oxidative stress and microbial resistance, and this knowledge can be applied to design metal-based drugs that mimic or modulate biological metal ion function and therefore the research into the mechanistic aspects of transition metal-TSC complexes holds great promise for advancing medical science and offering potential solutions to address antibiotic resistance and oxidative stress-related diseases, thus paving the way for the development of innovative therapeutic agents with enhanced efficacy and safety profiles.

Review of relevant literature related to the study

Thiosemicarbazones (TSCs) are Schiff base ligands obtained from the condensation of thiosemicarbazone with aldehydes or ketones, which are interesting materials due to their rich coordination chemistry and some important biological activities, including antioxidant and antimicrobial activities (Devi et al., 2022; Jamal et al., 2019), which have been thoroughly studied. Transition metals coordination with TSCs improves their biological activity due to the formation of stable metal complexes that can interact with different biological targets. Free radical scavenging and prevention of free radical-mediated lipid peroxidation and oxidative processes are believed to be the main mechanisms of antioxidant action of metal–Thiosemicarbazone complexes, and these activities might be related to the redox properties of the central metal ion and the ability of the ligand to stabilize free radical intermediates (Muleta et al., 2019) In this regard, copper(II) complexes of TSCs showed remarkable scavenging activity of ROS, which was related to the redox capacity of the copper ion, allowing for the redox cycle of Cu(II) and Cu(I) and the electron transfer reaction to neutralize ROS (Devi et al., 2022). Likewise, the antioxidant activity of iron (III)-TSC complexes is due to their interaction with oxidation/reduction processes, which ultimately relieve oxidative stress (Jamal et al., 2019). A number of key physicochemical characteristics, including the lipophilicity, which governs the passage through microbial cell membranes, and the property of the metal ion, whose presence may substantially influence the intracellular components, can play a decisive role in the efficacy of the organometallic antimicrobial activity of such TSC-transition metal complexes

(Nasiri Sovari & Zobi, 2020) Nickel(II) and zinc(II) complexes of TSCs have exhibited considerable antimicrobial activity against different strains including Escherichia coli and Staphylococcus aureus, which may be due to their interaction with appropriate budding parameters followed by gross change in the DNA and proteins which may lead to inhibition of many biological processes (Muleta et al., 2019). The dissimilarity in the TSC structure enables them to pair up with different geometries and coordination modes to form complexes that can modulate their biological activity (Salah et al., 2019). For instance, tridentate square-planar copper (II) complexes of TSC ligands have demonstrated an increase in antimicrobial activity over their bidentate counterparts, possibly on account of increased molecular planarity to intercalate with DNA (Nasiri Sovari & Zobi, 2020). The ability of TSCs to form complexes with several different transition metals has motivated much research on their possible therapeutic activities, such as anti-cancer, anti-viral, and anti-fungal (Jamal et al., 2019). The above literature survey sparked interest in the synthesis of some derivatives of TSC and metallodrugs to potentiate their biological activity and selectivity (Devi et al., 2022). Heterocyclic moieties have been integrated into TSC ligands to increase antimicrobial potency (Nasiri Sovari & Zobi, 2020). In addition to simple TSC complexes, the development of mixed-ligand TSC complexes has also been explored for potential synergistic effects and thus further increase the biological activity spectrum (Muleta et al., 2019). Secondly, TSCs can be considered as versatile chelating agents that form stable transition metal complexes with transition metals in different oxidation state, which further diversifies TSC coordination chemistry (Salah et al, 2019). This characteristic has been utilized in the rational design of metal-based drugs optimized for their pharmacokinetics and pharmacodynamics (Jamal et al., 2019). Additionally, the analysis of TSC complexes has led to the understanding of the function of metal ions in biological systems and especially in enzymatic processes and in metal ion handling or homeostasis (Devi et al., 2022) Research into the therapeutic potential of TSC complexes remains an active area of interest, with further investigation into their mechanisms, structure–activity relationships, and design of new, more biologically active derivatives (Muleta et al., 2019). Computational insights through across the computational quadrant of TSCs have also allowed for greater understanding of the electronic structures and subsequent reactivity of these complexes, aiding in the rational design of new compounds to possess selected biological properties (Nasiri Sovari & Zobi, 2020). Overall, transition metal–TSC complexes represent an important and complex class of compounds with diverse biological activities, especially antioxidant and antimicrobial activities and considerable work is still going on for exploration of such metal complexes in biomedical field to develop new therapeutic agents (Salah et al., 2019).

Research Gap related to the study

Despite extensive research into transition metal-thiosemicarbazone (TSC) complexes, there remain significant gaps in understanding the precise mechanisms underlying their antioxidant and antimicrobial activities, especially in elucidating how metal ion coordination, oxidation states, and ligand structural modifications influence their biological efficacy, with studies reporting promising antioxidant activity attributed to redox cycling mechanisms, particularly in copper(II) and iron(III) complexes, yet failing to fully explain how these mechanisms translate to biological contexts such as the neutralization of reactive oxygen species (ROS) or modulation of oxidative stress pathways (Devi et al., 2022; Ivković et al., 2022), while antimicrobial activity has been linked to DNA intercalation, protein binding, and disruption of microbial membranes, but inconsistencies in experimental protocols and outcomes have prevented the establishment of generalized structure-activity relationship (SAR) models that could predict efficacy across diverse microbial strains (Khan et al., 2022; Sharma et al., 2020), and although computational methods like density functional theory (DFT) have provided valuable insights into electronic structures, charge distribution, and binding affinities of TSC complexes, these findings often lack experimental validation, leading to gaps in correlating theoretical predictions with observed biological activity, particularly for mixed-ligand and heterocyclic derivatives, which exhibit unique coordination geometries and reactivities (Rani et al., 2021; Nasiri Sovari & Zobi, 2020), and furthermore, while solubility and stability issues associated with TSC complexes have been

acknowledged as critical factors influencing their pharmacokinetics and pharmacodynamics, limited data exist on strategies for optimizing these properties to enhance bioavailability and therapeutic potential (Jamal et al., 2019; Palanimuthu et al., 2016), and the lack of comprehensive studies integrating spectroscopic, electrochemical, and computational analyses to elucidate the interplay between ligand design, metal selection, and biological outcomes represents a significant barrier to the rational development of transition metal-TSC complexes as next-generation therapeutic agents capable of addressing challenges such as multidrug-resistant infections and oxidative stress-related diseases, with recent findings highlighting the need for more systematic investigations into mixed-metal complexes, which could offer synergistic effects and broaden the spectrum of activity (Kumar et al., 2022; Al-Amiery et al., 2022), and therefore, bridging these research gaps through interdisciplinary approaches that combine advanced analytical techniques, high-throughput biological screening, and computational modeling is essential for unlocking the full therapeutic potential of transition metal-TSC complexes in modern medicinal chemistry.

Methodology adopted for the purpose of study

In the study the adopted methodology encompassed the synthesis of thiosemicarbazone ligands through the condensation reaction between thiosemicarbazone derivatives and aldehydes or ketones, followed by complexation with transition metals such as Co(II), Ni(II), Cu(II), and Zn(II) to form metal-thiosemicarbazone complexes; these synthesized compounds underwent comprehensive characterization utilizing various analytical and spectroscopic techniques, including Fourier-transform infrared spectroscopy (FT-IR) to identify functional groups, nuclear magnetic resonance (NMR) spectroscopy for structural elucidation, ultraviolet-visible (UV-Vis) spectroscopy to assess electronic transitions, mass spectrometry for molecular weight determination, and elemental analysis to confirm composition, ensuring the verification of their chemical structures and purity (Devi et al., 2022; Borhade & Tryambake, 2022). The antioxidant activity of these complexes was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, a widely accepted method for measuring free radical scavenging efficacy, where the decrease in absorbance at 517 nm upon reaction with the complexes indicated their potential to neutralize free radicals; concurrently, the antimicrobial efficacy was assessed through in vitro assays against a spectrum of bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as fungal strains like *Candida albicans* and *Rhizopus oryzae*, employing the broth dilution method to determine minimum inhibitory concentrations (MICs), thereby quantifying the antimicrobial potency of each complex (Devi et al., 2022; Jamal et al., 2019). To gain deeper mechanistic insights, molecular docking studies were conducted, particularly focusing on the interaction between the most biologically active complexes and target proteins, such as the Chromosome partition protein Smc (PDB ID:5H67) from *Bacillus subtilis*, to predict binding affinities and modes, which provided a theoretical basis for understanding the observed biological activities at the molecular level; this integrative approach, combining synthetic chemistry, comprehensive physicochemical characterization, biological activity assays, and computational modeling, facilitated a thorough investigation into the structure-activity relationships governing the antioxidant and antimicrobial properties of transition metal-thiosemicarbazone complexes, thereby contributing valuable knowledge to the field of medicinal inorganic chemistry and aiding in the rational design of metal-based therapeutic agents (Borhade & Tryambake, 2022; Jamal et al., 2019).

Major objectives of the present study

1. To synthesize and characterize novel transition metal-thiosemicarbazone (TSC) complexes
2. To evaluate the antioxidant activity of the synthesized complexes
3. To assess the antimicrobial properties against pathogenic microorganisms
4. To investigate the molecular interactions between active complexes and biological targets

Synthesize and characterize novel transition metal-thiosemicarbazone (TSC) complexes related to new TSC complexes using Co (II), Ni (II), Cu (II), and Zn (II) metals and confirm their structural integrity and purity through spectroscopic and analytical techniques

like FT-IR, NMR, UV-Vis, mass spectrometry, and elemental analysis

In the study the researchers synthesized novel thiosemicarbazone (TSC) ligands by condensing thiosemicarbazide derivatives with aldehydes or ketones, followed by complexation with transition metals such as Co(II), Ni(II), Cu(II), and Zn(II) to form metal-TSC complexes, and these synthesized compounds underwent comprehensive characterization utilizing various analytical and spectroscopic techniques, including Fourier-transform infrared spectroscopy (FT-IR) to identify functional groups, nuclear magnetic resonance (NMR) spectroscopy for structural elucidation, ultraviolet-visible (UV-Vis) spectroscopy to assess electronic transitions, mass spectrometry for molecular weight determination, and elemental analysis to confirm composition, ensuring the verification of their chemical structures and purity (Devi et al., 2022; Jamal et al., 2019), while the antioxidant activity of these complexes was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, a widely accepted method for measuring free radical scavenging efficacy, where the decrease in absorbance at 517 nm upon reaction with the complexes indicated their potential to neutralize free radicals, and concurrently, the antimicrobial efficacy was assessed through in vitro assays against a spectrum of bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as fungal strains like *Candida albicans* and *Rhizopus oryzae*, employing the broth dilution method to determine minimum inhibitory concentrations (MICs), thereby quantifying the antimicrobial potency of each complex (Borhade & Tryambake, 2022; Devi et al., 2022), and to gain deeper mechanistic insights, molecular docking studies were conducted, particularly focusing on the interaction between the most biologically active complexes and target proteins, such as the Chromosome partition protein Smc (PDB ID:5H67) from *Bacillus subtilis*, to predict binding affinities and modes, which provided a theoretical basis for understanding the observed biological activities at the molecular level, and this integrative approach, combining synthetic chemistry, comprehensive physicochemical characterization, biological activity assays, and computational modeling, facilitated a thorough investigation into the structure-activity relationships governing the antioxidant and antimicrobial properties of transition metal-thiosemicarbazone complexes, thereby contributing valuable knowledge to the field of medicinal inorganic chemistry and aiding in the rational design of metal-based therapeutic agents (Borhade & Tryambake, 2022; Jamal et al., 2019).

The antioxidant activity of the synthesized complexes the minimum inhibitory concentrations (MICs) of the complexes against bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, etc.) and fungal strains (*Candida albicans*, *Rhizopus oryzae*, etc.) to evaluate their spectrum of antimicrobial efficacy

The various thiosemicarbazone (TSC) ligands identified in the study were synthesized via condensation of thiosemicarbazide derivatives with appropriate aldehydes or ketones followed by complexation with a selection of transition metal such as Co(II), Ni(II), Cu(II) and Zn(II) and characterization of the resultant metal–TSC complexes employing a comprehensive suite of analytical techniques (Al-Amiery et al., 2012; Nasiri Sovari & Zobi, 2020; Salah et al., 2019), which included Fourier-transform infrared spectroscopy (FT-IR) for the identification of functional groups, nuclear magnetic resonance (NMR) spectroscopy for structural elucidation, ultraviolet-visible (UV-Vis) spectroscopy to study electronic transitions, mass spectrometry for molecular weight confirmation, and elemental analysis to ensure the purity of the complexes which validated the expected chemical structure of the synthesized compounds, that ultimately enabled the physicochemical characterization of the synthesized compounds, while subsequent physicochemical manipulations were performed to assess the antioxidant activities based on the DPPH radical scavenging assay, in which the potential of the complexes to mitigate oxidative stress was observed via decreasing absorbance values at 517nm upon interaction with the complexes (Jamal et al., 2019; Muleta et al., 2019); so that the antioxidant activity of the compounds would appear to correlate favourably with metal cation charge, with strong findings also observed for the antimicrobial activities, which were also studied in vitro for a diverse range of microbial strains, including strains of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa bacterium and the fungal

strains, Cytophaga johnsonae, Candida albicans and Rhizopus oryzae using the classical broth dilution method to yield minimum inhibitory concentrations (MICs) for comparison of their spectrum of efficacy (Jamal et al., 2019; Muleta et al., 2019); molecular docking studies into the mechanisms of interaction of the most active complexes versus the biological target Chromosome partition protein Smc (PDB ID:5H67) from Bacillus subtilis were also performed to predict binding affinities and binding modes as a theoretical basis for understanding the observed biological activities at the molecular level (Jamal et al., 2019) and to describe structure-activity relationships governing the antioxidant and antimicrobial properties of transition metal–thiosemicarbazone (TSC) complexes in addition to the progress of the concepts of medicinal inorganic chemistry pertaining to the rational design of next-generation therapeutic agents aimed at combating oxidative stress and microbial infections (Nasiri Sovari & Zobi, 2020; Salah et al., 2019; Jamal et al., 2019).

The antimicrobial properties against pathogenic microorganisms the minimum inhibitory concentrations (MICs) of the complexes against bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, etc.) and fungal strains (*Candida albicans*, *Rhizopus oryzae*, etc.) to evaluate their spectrum of antimicrobial efficacy

Researchers in the study synthesized a series of transition metal-thiosemicarbazone (TSC) complexes by complexing TSC ligands with various metals including Co(II), Ni(II), Cu(II) and Zn(II), and evaluated twenty-four complexes for antimicrobial properties against pathogenic microorganisms, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Rhizopus oryzae, using broth dilution methods that quantify the minimum inhibitory concentrations (MICs) to provide the lowest concentration of each complex capable of inhibiting visible growth, with results indicating that the complexes exhibited significant antimicrobial activity in many instances, with Ni(II) TSC complexes highlighted for specific bacterial strains as possessing MIC values as low as 0.8 μg/mL, showing their potential as potent antimicrobial agents (Netalkar et al., 2014; Kizilcikli et al., 2007), furthermore, variations in activity were noted based on the metal center as well as the ligand structure where Zn(II)-based TSC complexes presented improved selectivity against particular pathogens such as Staphylococcus aureus and Candida albicans that even exhibited MICs rivalling or outperforming standard antimicrobial drugs in specific assays (Kizilcikli et al., 2007), which underscores the critical influence that metal-ligand interactions as well as the properties of the coordination environment have in determining the chemical activity, and to gain further insight into the molecular basis of this activity, docking studies were carried out, describing excellent binding interactions between the microbial target protein Chromosome partition protein Smc, from Bacillus subtilis, with active complexes (PDB ID:5H67), allowing to elucidate the potential mechanisms of action involving DNA intercalation, protein binding, or disruption of microbial membranes that operate at the molecular level, therefore providing a theoretical framework that complements the experimental data and affirms the observed antimicrobial effects (Netalkar et al., 2014), in addition, this integrative methodology which incorporates synthetic chemistry, antimicrobial assays and computational studies represents a significant step forward in the development of effective metal-based therapeutics for combating pathogenic microorganisms, where current treatments are becoming increasingly ineffective (Kizilcikli et al., 2007; Netalkar et al., 2014).

Molecular interactions between active complexes and biological targets employ molecular docking studies to explore binding affinities and interactions with target proteins, such as the Chromosome partition protein Smc, to elucidate the molecular mechanisms underlying their antioxidant and antimicrobial activities

Using molecular docking studies, the authors have identified the binding ranges and interactions of synthesized transition metal-thiosemicarbazone (TSC) complexes (i.e., Co(II), Ni(II), Cu(II), and Zn(II)) with the Chromosome partition protein Smc from Bacillus subtilis (PDB ID: 5H67) and other biological targets in order to explain their antioxidant and antimicrobial action at the molecular level, where docking simulations showed relevant interactions at the binding sites of the protein (such as hydrogen bonding, hydrophobic contacts, coordination bonds) contributing to significant binding affinities

(calculated binding energy were -8.88 kcal/mol against microbial target protein for Co(II) complexes, while -8.92 kcal/mol for Zn(II) complexes) supporting their strong inhibitory effect in the respective sequence of experiments, in which other metals (such as Cu(II) that showed superior antioxidant activity) are expected to interfere with proteins modulating the reactive oxygen species (ROS) effect, whilst the study highlighted that, why and how differences in metal centers and ligand structure influenced binding, hence also biological effect of the complexes, with the finding suggested that both metal-ligand coordination clearly affected specificity and potency with results indicating that these very metal-TSC complexes disrupt microbial processes via critical enzymes and proteins that challenge the survival of both bacterial and fungal species, such as they effectively disrupt DNA replication and protein level synthesis mechanisms of bacteria (e.g., Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa), and fungal strains (like Candida albicans and Rhizopus oryzae), so minimum inhibitory concentrations (MICs) were in agreement with the findings based on docking predictions of binding strengths indicating that there is the complementarity of computational and in vitro findings, which reflects the potential of transition metal-TSC complexes as effective antimicrobial and antioxidant agents and openness of the molecular docking to understand structure-activity relationships and supple rational designs of therapeutic agents with optimized biological properties to the right (Devi et al., 2022; Borhade & Tryambake, 2022).

Discussion related to the study

In "Mechanistic Insights into the Antioxidant and Antimicrobial Activities of Transition Metal-Thiosemicarbazone Complexes," a variety of thiosemicarbazone (TSC) ligands were synthesized by condensing thiosemicarbazide derivatives with different aldehydes and ketones, then complexed with transition metals (Co(II), Ni(II), Cu(II), Zn(II)) to give the metal-TSC complexes that were characterized by several techniques such as Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, mass spectrometry and elemental analysis to confirm their structure and purity; the antioxidant activities of these complexes were measured using the DPPH radical scavenging assay that showed the Cell (II) complex showed the highest scavenging activity, perhaps due to the redox potential and free radical stabilization, and the antimicrobial activity was performed on bacteria (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) and fungi (Candida albicans, Rhizopus oryzae) with minimum inhibitory concentrations (MIC) determined through broth dilution methods that suggested the Co(II) and Zn(II) complexes have potent antimicrobial activity at MIC with similar values to standard antibiotics, signifying their potential for a therapeutic role; to elucidate molecular insights, molecular docking studies between the top active complexes and targets Chromosome partition protein Smc from Bacillus subtilis (PDB ID: 5H67) were performed showing predicted strong binding affinities and stable interactions which could interfere with vital biological functions in microorganisms, mediating the molecular basis to the antimicrobial activity; this research underlines the significance of metal ions in the biological functions of TSC complexes, as the metal center acted to influence both antioxidant and antimicrobial behaviors with the metal center as a notable factor; as is in line with earlier research the TSC complexes having enhanced antioxidant activity with Cu(II), likely due to redox cycling while Co(II) and Zn(II) complexes have efficient antimicrobial action due to hypothesized interactions with microbial enzymes or DNA; together these findings also highlights the flexibility of thiosemicarbazones as strong ligands that allow stable transition metal complexes to form leading to varied and orthogonal biological activity set consistent with prior studies implicating metal coordination as evidence for better pharmacological activity of thiosemicarbazones, proposing them as drug development candidates (Jamal et al., 2019; Devi et al., 2022); moreover, the effective use of molecular docking offers a means to predict and rationalize the bioactivity of metal complexes thereby facilitating the advancement of more efficacious therapeutic agents which broadens this study's scope of the synthesis, characterization and biological evaluation of transition metal-thiosemicarbazone complexes that adds to the ever-expanding body of medicinal inorganic chemistry knowledge building the pathological basis for the future development of these complexes as multi-target

agents against oxidative stress and microbial infections.

Chemical implications related to the study

In this study, the researchers synthesized a series of thiosemicarbazone (TSC) ligands by the condensation of thiosemicarbazide derivatives with different aldehydes and ketones, and transition metal (Co(II), Ni(II), Cu(II), and Zn(II)) metal-TSC complexes were synthesized and characterized by Fourier-transform infrared (FT-IR), nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible/visible (UV-Vis), mass spectrometry and elements analyses to confirm their structure and purities; their antioxidant activities were evaluated using DPPH radical scavenging assay that indicates the superior scavenging activity of the Cu(II) complex, which could attribute to its redox potential and stabilization of free radicals; antibacterial effects were evaluated against bacterial strains including Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, and fungal strains such as Candida albicans and Rhizopus oryzae, and minimum inhibitory concentrations (MICs) were determined by broth dilution methods; the Co(II) and Zn(II) complexes showed promising detrimental antimicrobial activity with the MIC values comparable to the standard antibiotics, suggesting the motivation of their usage as therapeutic agents; and mechanistic insight with their interaction with target proteins including the Chromosome partition protein Smc from Bacillus subtilis (PDB ID: 5H67), and the molecular docking studies predicted strong binding affinities and stable interactions that could disrupt essential biological processes in microorganisms, which elucidates the molecular basis of the observed antimicrobial effects; which highlight the significance of metal ions almost certainly influencing the biological actions of the TSC complexes since the choice of metal center determined the antioxidant and antimicrobial actions, such as the Cu(II) complexes affected practically via the enhanced antioxidant activity possibly through the participation in the redox cycling and the Co(II) and Zn(II) complexes mediate potent antimicrobial effects probably by means of interactions with microbial enzymes or DNA; and thiosemicarbazones can act ligands to form stable complexes with various transition metals, leading to compounds with diverse and tunable biological activities consistent with previous research focused on metal coordination that could improve the pharmacological properties of thiosemicarbazones for drug development (Jamal et al., 2019; Nasiri Sovari & Zobi, 2020); and for the molecular docking, it provides a weapon to predict and rationalize bioactivity of metal complexes, thereby facilitating their design into more effective therapeutic agents; and lastly, this accumulation study into the synthesis, characterization and biological evaluation of transition metal-thiosemicarbazone complexes are indispensable in more and more knowledge research grounds of manufacture medicinal inorganic chemistry in terms of their multifunctional agents against both oxidative stress and microbial infections.

CONCLUSION

The study concludes that the synthesis and characterization of Co(II), Ni(II), Cu(II), and Zn(II) thiosemicarbazone complexes, along with their comprehensive evaluation for antioxidant and antimicrobial activities, demonstrate the potential of these metal-ligand systems as multifunctional agents, where the Cu(II) complexes showed superior antioxidant activity, likely attributed to their redox cycling ability and effective free radical stabilization, as observed through DPPH radical scavenging assays, while Co(II) and Zn(II) complexes exhibited notable antimicrobial efficacy, with minimum inhibitory concentrations (MICs) against bacterial strains like *Staphylococcus aureus* and *Escherichia coli* and fungal strains like *Candida albicans* and *Rhizopus oryzae*, comparable to or exceeding the activity of standard antibiotics, and molecular docking studies further elucidated the binding mechanisms of these complexes with biological targets such as the Chromosome partition protein Smc, revealing significant binding affinities and stable interactions, which support the hypothesis that these complexes interfere with essential microbial processes, including DNA replication and protein synthesis, and additionally, the findings highlight the critical role of metal coordination in modulating the biological properties of thiosemicarbazone ligands, where the electronic and structural characteristics of the metal center significantly influenced both antioxidant and antimicrobial outcomes, suggesting that the rational design of TSC complexes with optimized metal-ligand combinations could lead to the development of

targeted therapeutic agents, particularly for combating oxidative stress and drug-resistant microbial infections, and therefore, this study contributes valuable insights into the field of medicinal inorganic chemistry by demonstrating how transition metal-thiosemicarbazone complexes can serve as a foundation for creating novel therapeutic strategies, while also emphasizing the need for further research into enhancing their solubility, stability, and bioavailability to maximize their clinical potential.

Scope for further research and limitations of the study
The limitations of the present study flagged opportunities for future research that include the lack of in vivo studies in biological systems to validate the efficiency and safety of these complexes as therapeutic agents; the absence of thorough pharmacokinetic and pharmacodynamic evaluations to investigate the fate and behavior of the complexes in physiological surroundings, including their stability, metabolism, and bioavailability; the need for further experimental proof of the molecular docking predictions on binding affinities and modes (and the exact binding mechanisms) shown with some microbial proteins like the Chromosome partition protein Smc, which requires solid proof through experimental techniques such as X-ray crystallography or nuclear magnetic resonance; a narrow range of microbial strains limiting the findings to a greater spectrum of pathogens, and thus, the antimicrobial assays should be expanded in future studies to include multidrug-resistant strains and emerging pathogens to gain a deeper insight into the compounds potential in realistic clinical conditions; the solubility and stability of the complexes were not tested extensively in water and biological media to assess their real applicability; the influence of the ligand on the biological activity and selectivity between involved complexes was not studied (for example, when introducing strong electron-donating groups in the ligand into the structures to change their biological potential); and thus, the present study demonstrated the biological potential of these metal-TSC complexes (But did not cover the toxicity profiles and side effects as involved in many such compounds to the advance of the compounds to clinical use), hence cytotoxicity studies on normal cell lines and animals should be performed in future research; while this work lays a solid foundation that addresses the gap for the use of transition metal-thiosemicarbazone complexes as potential therapeutic agents, it opens avenues for precise interdisciplinary collaborations that involve synthetic chemistry, computational modeling, advanced spectroscopy, and high-throughput screening technologies that will be needed to better update the many limitations highlighted in this study and to pave the way towards safer and more effective metal-based drugs in the clinic.

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