Synthesis and Antimicrobial Activity of 3-Acetyl Coumarin-Based Metal Complexes

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Abstract- ***Synthesis and antimicrobial study of 3-acetyl coumarin based metal complexes have been a field of interest over the last two decades as they exert therapeutic effect against a broad spectrum of microbes even drug resistant strains, possibly due to the transition metals such as copper, zinc, nickel, iron which play a versatile role in governing the biological property of coumarin ligand, which was prepared by ligand-metal coordination reaction where the 3-acetyl coumarin act as a bidentate ligand, coordinated to metals through the oxygen atom of carbonyl portion and hydroxyl group of chromene ring to stable complexes with unique geometry and spectroscopic properties (UV-visible, FT-IR, NMR, and X-ray diffraction have been employed to establish the coordination environment and confirm the metal-ligand interaction); different investigations revealed that the nature of the metal ion, the steric and electronics aspects of the ligand, as well as coordination geometry have a remarkable influence on the antimicrobial activity of the metal complexes, as some complexes have been found to exhibit significant activity against both Gram-positive and Gram-negative bacteria including, multidrug-resistant strains(of Staphylococcus aureus and Escherichia coli, fungi such as Candida albicans, the copper(II) complexes have been identified as being the most practical, probably due to their ability to disrupt microbial cell membranes and interfere with intracellular processes, antimicrobial assays exhibited minimum inhibitory concentrations (MICs) in the micromolar range, values are comparable or superior properties to traditional antimicrobial agents; Furthermore, it was demonstrated that the chelation of metal ion with 3-acetyl coumarin also has the benefit of consequent enhanced solubility and bioavailability of the ligands which facilitates better penetration of cell walls of microbes, while lowering damage associated with free metals, and a double gain in activity and practical safety; Also, computational studies and molecular docking simulations also provided significant information about the pharmacokinetic profiles of these complexes with microbial targets such as bacterial enzymes and cell wall contributors; giving valuable understanding of fundamental modes of mechanisms of antimicrobial action involving metal mediated generation of reactive oxygen species (ROS), invasive DNA replication, and inhibition of vital enzymatic processes; the synthesized metal complexes and their free ligands are a potential advance class of antimicrobial agents, requiring further investigation of the clinical application of the 3-acetyl coumarin bioactive family members.***

Indexed Terms- ***3-Acetyl Coumarin, Metal Complexes, Antimicrobial Activity, Copper (II) Complexes, Minimum Inhibitory Concentrations (MICs), Molecular Docking Simulations***

I. INTRODUCTION

Synthesis and Antimicrobial Activity of Metal Complexes of 3-Acetyl Coumarin Coumarins are a class of compounds of plant origin that have diverse biological activities, including anti-inflammatory, anticancer, and lore and action as potential antimicrobial agents are widely used in the field of drug discovery as many drug-resistant pathogens have appeared in the past few years, thus enhancing the demand for novel and effective antimicrobial agents in the medicinal chemistry field for the treatment of infectious diseases where several studies been devoted to the synthesis of these types of complexes, where we used transition metals such as copper (Cu2+), zinc (Zn2+), iron (Fe2+), nickel (Ni2+), and silver (Ag1+), which have many profound coordination properties leading to an increase in bioactivity (Kumar et al.,

2013; Dios et al.; Sahu et al 2020; Yadav et al., 2017; Bamidele et al., 2020; Hassan et al., 2019; Singh et al., 2020; Jadhav et al., 2021); it is well established that the coordination of metals with 3-acetyl coumarin can greatly improve its resistance and linking through a chelation-like formation of a ring-like structure between the metal and the ligand, which reduces the undetectable toxicity of free metal ions (Bamidele et al., 2020); the copper-free complex of a copper (Cu) or zinc (Zn) complex of 3-acetyl coumarin has been shown to have a high antimicrobial potency by generating reactive oxygen species (ROS) affecting the binding site and the physical properties of the coupling ligand of course (Kumar et al., 2019; Kong et al., 2018; Pinto et al., 2018).

Statement of the research problem

The escalating global health crisis posed by antimicrobial resistance necessitates the development of novel therapeutic agents capable of combating resistant microbial strains; in this context, 3-acetyl coumarin-based metal complexes have emerged as promising candidates due to their potential enhanced bioactivity compared to uncomplexed ligands (Balewski et al., 2021); despite the known antimicrobial properties of coumarin derivatives, the specific interactions and mechanisms by which 3-acetyl coumarin coordinates with various metal ions to form complexes that exhibit superior antimicrobial efficacy remain inadequately explored (Rohman et al., 2023); furthermore, the influence of different metal ions on the structural, electronic, and biological properties of these complexes is not fully understood, hindering the rational design of more effective antimicrobial agents (Kruger & Tolba, 2022); therefore, this research aims to synthesize a series of 3-acetyl coumarin-based metal complexes, systematically investigate their structural and electronic characteristics, and evaluate their antimicrobial activities against a spectrum of pathogenic microorganisms, thereby elucidating the structure-activity relationships that govern their efficacy and providing a foundation for the development of new antimicrobial therapeutics (Nongpiur et al., 2022).

Significance of the research study

The aim of this investigation is to find 3-acetyl coumarin-based metal complexes as promising additions to the class of antimicrobial agents used to combat the growing global health crisis resulting from antimicrobial resistance (Balewski et al., 2021); malaria providing well-documented due to their antidepressant, anti-inflammatory, and antioxidant effects; the coordination of these metals to the bioactive parent ligand has been shown to significantly improve the desired activity (e.g. increased solubility, stability, enhanced permeability and cellular binding) of organometallic compounds in place of metal ion complexes with several pathogens, including Staphylococcus aureus and Escherichia coli (Rohman et al., 2023), where utilization with copper and zinc has led to high efficacy against free limiting pathogens, however the mechanism of action related to unique metallic activities (e.g. reactive oxygen species (ROS) production or DNA binding) remain to be defined, necessitating a systematic investigation to evaluate these interactions and contributions towards antimicrobial potency (Kruger & Tolba, 2022); furthermore, the apparent dual benefit presented by metal complexes includes potentially decreasing the free metal ion toxicity while enhancing the action of the ligand (Nongpiur et al., 2022); the diversity of 3-acetyl coumarin to form coordinated complexes with copper, zinc, iron and nickel expands its scope and it is possible to modulate the metal coordination to finely modify its antimicrobial action through this research (Saikia et al., 2021); the application of these coordinated complexes extends not only to enhanced antimicrobial potency but includes synergies effector drug delivery systems where they have the potential to prolong or even reduce increasing antibiotic effects of resistance (Rajan et al., 2022), highlighting the significant impact this study could have in relation to addressing expansive health issues (Sahu et al., 2020).

Review of relevant literature related to the study

Now a days many studies have been studied on 3-acetyl coumarin (AC)-based metal complexes owing to their promising potential as an antimicrobial (Avdovic et al., 2019); coumarins being a large class of benzopyrone derivatives well investigated for wide range of biological properties such as antimicrobial, anticoagulant, and anticancer activities (Abdel-Kader & El-Ghamry, 2021); introduction of the metal ion has also shown to further increase the bioactivities of the coumarin derivatives leading to a series of the metal complexes with improved efficacy (Abdel-Kader &

El-Ghamry, 2021); there are already a number of reports related to the synthesis and characterization as well as biological evaluation of various coumarin-metal complexes in which a number of important transition metals (such as copper, cobalt, nickel, and zinc) were used (Linert & Kabil, 2018); for example, palladium(II) complexes with bis(Coumarin-4-yl) thiomethylphosphine,synthesized by a conventional method, exhibited moderate/low antimicrobial activity with selective action against Aspergillus flavus and Bacillus subtilis (Avdovic et al., 2019); Similarly, hydrazone Schiff base ligands prepared from 8-acetyl-7-hydroxy-4-methylcoumarin and 3-amino-1,2,4-triazole and their silver and copper(II) complexes demonstrated remarkably enhanced antibacterial activity against both Gram-positive and Gram-negative bacteria, wherein the metal-assisted coordination play a key role in their enhanced antimicrobial efficacy (Abdel-Kader & El-Ghamry, 2021); to the best of our knowledge, literature surveys reveal abundant structural characterization studies using various modalities including X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and electronic spectroscopy helping in understanding the coordination geometry and electronic transitions (Linert & Kabil, 2018); among the ligands, hydrazone Schiff base ligands from 3-formyl-4-hydroxycoumarin have been shown to exhibit varied coordination modes thereby indicating the importance of the ligand on the antimicrobial activity of the metal complexes (Patil & Bugarin, 2022); biological evaluations have indicated that metal in the metal complexes essentially acts to enhance the antimicrobial potency of the coumarins via lipophilicity and stability (Todorov & Kostova, 2024) thus leading to enhanced interaction of the metal complex to the microbial cell membrane and/or leading to disruption of its function (Ravikumar & Bindu, 2024) and many of these metal complexes demonstrated antioxidant effects as well providing (Saad, 2024)) studies show that metal ions enhances radical scavenging ability of the compounds, thus adding further to their therapeutic potential (Saad, 2024); despite the above promising findings, there continues highest challenges on a structural modification point of view in solubility, bioavailability as well as side effects and potential toxicity (Patil & Bugarin, 2022); As such, in our future work, we will prioritize the optimization of ligand structures and examine a variety of metal ions to form complexes that can increase selectivity while minimizing side effects (Todorov & Kostova, 2024); potential mechanistic research must be done to clarify the pathways through which the AC derivatives and/or metal-aided are exerting their effects, laying the groundwork for the rational design of new antimicrobial agents (Todorov & Kostova, 2024).

Research Gap related to the study

Despite a considerable amount of research having been performed on 3-acetyl coumarin-based metal complexes, there is still an unmet focus on design for bioactivity, specifically on selective (or differential) toxicity to pathogenic microorganisms and the combination of activity by ligand–metal interactions. Although the antimicrobial effects of coumarin and its metal complexes have been studied extensively, no systematic investigations have been conducted to study their interactions with a wider range of pathogenic microorganisms, especially multi-drug-resistant strains, and the mechanisms of action. Although many previous works have discussed the anticancer, anti-inflammatory, and antioxidant activities of coumarin-metal complexes (Avdovic et al., 2019; Abdel-Kader et al., 2021), few studies are focused on their activities as potential effective antimicrobial agents against both Gram-positive and Gram-negative bacteria beside fungi. Furthermore, although some studies such as those of Luan et al. (2020) and Di Pietro et al. The antimicrobial activity of coumarin-metal complexes, although reported and with coumarin-metal complexes obtaining promising results as fluorescent probes (Luan et al., 2020; Di Pietro et al., 2021), still need to be studded against more resistant strains of pathogens. In addition, even though a number of metal ions (copper (Cu), zinc (Zn), nickel (Ni) and palladium (Pd)) have been evaluated with 3-acetyl coumarin, their specific control of some microbial activity and selectivity needs more of detailed, systematic and mechanistic studies (Karcz et al., 2021; Avdović et al., 2019). Another issue that I see as not addressed relates to the SAR (structure-activity relationship) of these complexes: the impact of the size, charge, and geometry of the metal center on the antimicrobial activity and bioavailability is still very poorly defined (Todorov et al., 2023). Other studies assessing the stability of these complexes under physiological conditions as well as their

bioaccumulation profiles are still scarce, despite being key in determining their potential suitability as therapeutic agents of clinical relevance (Di Pietro et al., 2021). For example, the literature inadequately discusses the impact of environmental factors (e.g., pH, temperature, presence of biological ligands) on the stability and reactivity of these complexes. Additionally, although computational studies have been carried out for predicting biological activities for some of the metal complexes, data are still missing in terms of more detailed molecular modeling and in silico studies which could help to clarify potential interaction mechanisms at molecular level between metal complexes and their microbial targets (Abdel-Kader et al., 2021; Avdovic et al., 2019). As such, this computational data gap prevents a fuller mechanistic understanding of the antimicrobial action but prohibit a more rational design of a more potent and specific coumarin-metal complex antimicrobial agent. Filling these research gaps will not only broaden the antimicrobial profile of 3-acetyl coumarin-based metal complexes but will also allow the generation of novel Drug Attack Targets (DATs) for multidrug-resistant infections that are one of the major problems of present medicine (Todorov et al., 2023; Abdel-Kader et al., 2021).

Methodology adopted for the purpose of study

The synthesis and antimicrobial screening of the 3-acetyl coumarin- based metal complexes is performed through a specific protocol which includes: preparation of the metal complexes via coordination reaction of 3-acetyl coumarin (as the active bioactive ligand) with selected metal salt such as Copper(II), Nickel(II), Zinc(II) and Iron(III) (or its corresponding carbonate) (the metal salt is selected based on the target shell activity, biocompatibility, role as a redox catalyst, etc.), in different solvent, pH and temperature (optimized to prevent excessive formation of free ligand in solution) (Ardila et al., 2021; Jadhav et al., 2019) Aprotic solvents, such as dimethyl sulfoxide (DMSO) or ethanol under reflux conditions, are used for these types of reactions, where the coordination sphere of the metal ion is formed by O donor atoms from the coupled 3-acetyl coumarin molecule that coordinates to the metal ion through its carbonyl group and often leads to the formation of a chelate which was testified by other techniques (UV-Vis, FTIR, NMR, and X-ray diffraction for structural elucidation) (Nabavi et al., 2019). Microwave-assisted synthesis can be utilized to facilitate reaction rates and improve the yield of metal-ligand complexes (Anwar et al., 2020) using common metal salts such as Cu(II) and Zn(II), resulting in about a 90% reduction in reaction time with equivalent or higher yields compared to traditional heating methods (Mitra et al., 2021). Metal complexes are synthesized and then screened for their antimicrobial activity in a broad spectrum, which includes gram-positive and gram-negative bacteria and fungi, by qualitative and quantitative methods viz disc diffusion and reduced inhibitory concentration (MIC) methods (Tiwari et al., 2020). In this case, the disc diffusion method is used to see the zone of inhibition around the disc that has the metal complex, while antibiotics such as amoxicillin or ciprofloxacin used as positive controls (Pandey et al., 2022). MIC was determined by serial dilution of the complexes in nutrient broth and incubating the bacteria at 37°C after the addition of the complexes, identifying the lowest complex concentration that visibly inhibits the growth (Farhan et al., 2020). Time-kill assay: Time-kill assays are commonly used to determine the mechanism of antimicrobial action, in which a bacterial culture is treated with the complex at the determined MIC and the culture is periodically sampled to assess the decline in microbial growth as a function of time (Vargas et al., 2021) In recent years, molecular docking studies have often been included in these studies to understand the interactions between the metal complexes and the microbial targets at the molecular level, giving insights into binding affinities and possible mechanisms of how these complexes impart their antimicrobial effects (Patel et al., 2020). Stability and solubility tests have also been performed to evaluate the suitability of the complexes for potential pharmaceutical applications, investigating effects of environmental factors (e.g., pH and temperature) on the stability of metal-ligand complex (Banerjee et al., 2021). Complementing the theoretical aspects of this methodology is computational studies on metal - ligand binding interactions analysis, involving the electronic properties of the metal centers and their influence on overall antimicrobial activity, as well as the use of molecular dynamics simulation to predict the behavior of these complexes in a biological environment (Sadeghi et al., 2021). Overall, this work offers an eclectic development process via experimental and computational methods that can be

applied to the design, synthesis, and testing of 3-acetyl coumarin-derived metal complexes to enhance their suitability for clinical applications as antimicrobial agents (Rahman et al., 2020; Kumar et al., 2022).

Major objectives of the present study

1. To Synthesize and Characterize Metal Complexes of 3-Acetyl Coumarin
2. To Evaluate the Antimicrobial Activity of Synthesized Complexes
3. To Investigate the Structure-Activity Relationships (SAR)
4. To Explore the Mechanism of Antimicrobial Action

Synthesize and Characterize Metal Complexes of 3-Acetyl Coumarin besides develop and optimize a reliable synthesis methodology for creating stable coordination complexes between 3-acetyl coumarin and various metal ions (e.g., Cu^{2+}, Zn^{2+}, Ni^{2+}), followed by their detailed structural characterization using techniques such as UV-Vis, FTIR, NMR, and X-ray diffraction

The synthesis and characterization of metal complexes derived from 3-acetyl coumarin involve a stepwise approach beginning with the preparation of the ligand, which is synthesized using methodologies such as the Pechmann or Knoevenagel condensation reactions to yield a pure compound suitable for metal complexation, followed by the coordination of the ligand with metal ions such as copper (Cu^{2+}), zinc (Zn^{2+}), and nickel (Ni^{2+}) in the presence of a suitable base like sodium acetate under controlled reaction conditions, including reflux or microwave-assisted synthesis to ensure the formation of stable coordination complexes, where the reaction typically involves the interaction of the carbonyl group in 3-acetyl coumarin with the metal center to form chelates stabilized by the delocalized π-electron system of the coumarin ring, which is confirmed by analytical techniques like elemental analysis and mass spectrometry to validate the stoichiometry of the complexes (Jadhav et al., 2019; Ardila et al., 2021); structural characterization of the resulting complexes is performed using spectroscopic methods such as UV-Vis spectroscopy to analyze electronic transitions and confirm the coordination environment, Fourier-transform infrared (FTIR) spectroscopy to identify functional group shifts indicative of metal-ligand bonding (e.g., shifts in the carbonyl stretching frequency), and nuclear magnetic resonance (NMR) spectroscopy for elucidating the electronic environment of the ligand before and after coordination, along with single-crystal X-ray diffraction for detailed three-dimensional structural analysis, enabling the determination of parameters such as bond lengths, angles, and the overall geometry of the complex, whether square planar, tetrahedral, or octahedral, with additional confirmation provided by thermal analysis techniques like thermogravimetric analysis (TGA) to assess the thermal stability and composition of the complexes (Anwar et al., 2020; Mitra et al., 2021); as an example, the copper(II) complex of 3-acetyl coumarin often exhibits a characteristic blue or green color attributed to d-d electronic transitions, while the zinc complex is typically white or pale yellow due to its diamagnetic nature, and their molecular structures reveal that the carbonyl oxygen and hydroxyl group on the coumarin ring act as bidentate ligands, forming five- or six-membered chelate rings around the metal center, as evidenced by shifts in the IR absorption bands corresponding to the C=O and C-OH groups upon complexation (Rahman et al., 2020; Banerjee et al., 2021); this comprehensive characterization not only validates the successful synthesis of stable coordination complexes but also provides critical insights into their electronic and steric properties, which are foundational for understanding their reactivity and biological activity, and such robust methodologies lay the groundwork for further exploration of their antimicrobial, antioxidant, and potential therapeutic properties (Pandey et al., 2022).

Antimicrobial Activity of Synthesized Complexes to assess the antimicrobial efficacy of the synthesized metal complexes against a broad spectrum of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi, using standardized methods such as the disc diffusion assay and minimal inhibitory concentration (MIC) determination

The antimicrobial activity of synthesized 3-acetyl coumarin-based metal complexes is evaluated by well-established microbiological methods against a wide array of pathogenic microorganisms including Gram-

positive bacteria (Staphylococcus aureus and Bacillus subtilis), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), and fungal species (Candida albicans and Aspergillus Niger), which is carried out using a disc diffusion assay, whereby sterile discs are impregnated with defined concentrations of the metal complexes and placed on agar plates seeded with test microorganisms followed by incubation under optimal growth conditions, during which the zone of inhibition around each disc is measured giving indication on antimicrobial potency, while standard antibiotics (i.e., ciprofloxacin or nystatin) serve as positive controls for comparison (Ardila et al., 2021; Banerjee et al., 2021); the minimal inhibitory concentration (MIC), a more quantitative measure, is determined by preparing serial dilutions of the cascades in a liquid growth medium, inoculating with standardized microbial suspensions, and incubating to identify the lowest concentration of the cascade that inhibits visible growth of the organism being tested, providing details on the potency and likely dosage requirements of the compounds with many metal complexes giving MIC values of 2–25 µg/mL against bacterial strains and 5–30 µg/mL against fungal species that are comparable or better than conventional antimicrobial agents (Jadhav et al., 2019; Mitra et al., 2021); further, mechanism-driven insights into the antimicrobial effects are obtained through time-kill studies, monitoring the reduction in microbial viability over time upon exposure to the metal complexes, revealing whether their mode of action is bacteriostatic or bactericidal, along with reactive oxygen species (ROS) generation assays that elucidate the oxidative stress-inducing capabilities of the complexes attributable to the redox-active nature of metal ions such as Cu^{2+} that disrupt cellular functions by damaging DNA, proteins, and membranes (Anwar et al., 2020; Rahman et al., 2020); the coordination of 3-acetyl coumarin with metals increases their lipophilicity enabling better penetration through microbial cell walls and interaction with intracellular targets such as enzymes, and nucleic acids supported by molecular docking studies showing strong binding affinities of these complexes to microbial proteins and DNA indicating strong theoretical support for their antimicrobial efficacy (Pandey et al., 2022; Tiwari et al., 2020); furthermore, structure-activity relationship (SAR) studies indicate that the metal ion type, coordination geometry, and nature of substituents on the coumarin ring significantly influence the antimicrobial activity, exemplified by superior efficacy of copper(II) complexes owing to their enhanced oxidative properties and moderate but broad-spectrum activity from zinc(II) complexes attributed to higher stability and biocompatibility, further affirming the role of metal-ligand coordination in modulating biological activity and suggesting their potential as candidates for developing novel antimicrobial therapeutics (Nabavi et al., 2019; Vargas et al., 2021).

Structure-Activity Relationships (SAR) how variations in the metal ion, ligand coordination geometry, and electronic properties of the complexes influence their biological activity, thereby elucidating the factors contributing to their antimicrobial potency and selectivity

In-depth studies to establish Structure-Activity Relationship (SAR) pattern of 3-acetyl coumarin-derived metal complexes revealed that the variation in type of metal ion, coordination geometry and electronic characteristic of the complexes greatly affects biological activity as observed by the fact that the coordination of metal ion alters the electronic distribution of the ligand modifying their lipophilicity enabling the more favourable interaction with microbial membranes and with intracellular targets where it is well established that copper(II) complexes frequently show higher antimicrobial activity compared to other metal complexes due to redox potential and production of reactive oxygen species (ROS) resulting in oxidative damage of microbial DNA, proteins and membranes, while zinc(II) complexes have been recognized for their broad-spectrum activity and biocompatibility that are attributed to the stable structure of metal complexes in physiological environments and predominant tetrahedral coordination geometry which consequently strongly binds with bacterial enzymes such as metalloproteases and provides an effective treatment strategy(Banerjee et al., 2021; Rahman et al., 2020); on contrary Nickel(II) complexes showed moderate activity due to planar coordination geometry which limits dimethylformamide and dimethyl sulfoxide penetration into the membrane that favors DNA intercalation and site-specific inhibition of bacterial reproduction (Pandey et al., 2022; Vargas et al., 2021);

besides, the choice of ligands and types of substituents on coumarin ring also play a vital functions in determining overall activity of the complex since electron withdrawing groups such as nitro or halogen substituents increase the electrophilicity of the metal center facilitating stronger interaction with microbial enzymes while electron donating groups of complex like methoxy improve stability and solubility favoring bioavailability, as reported in literature (Jadhav et al., 2019; Mitra et al., 2021); moreover, the geometry of the complex (tetrahedral, square planar, or octahedral) affect its capacity to penetrate bacterial cell wall and influence the interaction with intracellular targets, where square planar copper complexes were observed to show by far higher antibacterial activity attributed to their ability to efficiently intercalate with bacterial DNA, as confirmed by theoretical docking studies reporting higher binding affinities to DNA and bacterial gyrases (Anwar et al., 2020; Tiwari et al., 2020); the importance of theoretical studies was further explained regarding density functional theory (DFT) that the electronic structure was highlighted by frontier molecular orbitals where the lower HOMO-LUMO energy gap is correlated with a higher reactivity and antimicrobial potency, where this observation was recorded across several computational models of 3-acetyl coumarin-derived Nickel and Zinc complexes (Sadeghi et al., 2021; Nabavi et al., 2019); such literature not only implies key factors that potentially influence biological activity of these complexes but also supplies an invaluable rational basis for design of next-generational antimicrobial agents by optimization of choice of metal ions, ligands substitutions and coordination geometries that potentially lead to potent and selective therapeutic agents for drug-resistant microbial infections (Patel et al., 2020; Ardila et al., 2021).

Mechanism of Antimicrobial Action utilizing computational studies (e.g., molecular docking and dynamics) and experimental approaches (e.g., time-kill assays and reactive oxygen species generation analysis) to understand the interaction mechanisms between the metal complexes and microbial targets at the molecular level, paving the way for rational design of more potent antimicrobial agents

Here, the mechanism of action of 3-acetyl coumarin-based metal complexes consisting of an integrated approach of computational studies (such as molecular docking and molecular dynamics molecular dynamics simulations) with experimental methodologies (such as time-kill ability and reactive oxygen species (ROS) generation) are elaborated, where it was shown in the molecular docking simulations that the strong binding affinities of the metal complexes with the key microbial targets like DNA, RNA polymerases, and vital enzymes such as bacterial gyrase and dihydrofolate reductase, indicated that the metal complexes have the ability to inhibit essential metabolic pathways by intercalating in between nucleic acid base pairs or by forming stable complexes in the enzyme active sites, for instance, copper(II) complexes having a binding energy of −8.5 kcal/mol for DNA gyrase active sites (Patel et al., 2020; Banerjee et al., 2021) whereas molecular dynamics simulation further confirmed the high stability of the metal complex— DNA interactions under physiological conditions by simulating the interactions, where the results suggest that sustained hydrogen bonding and π-π stacking interactions occurred over an extended time frame and, correlate with their potent bactericidal effects (Sadeghi et al., 2021); from the experimental studies, time-kill assays showed the rapid bactericidal activity, complete eradication of microbial populations were observed within 6–12 hours at concentrations equal to their minimal inhibitory concentration (MIC) indicating that the mechanism of action disrupted microbial cellular functions rather than inhibiting microbial growth by showing the generation of reactive oxygen species (ROS) indicative of oxidative stress induced by redox-active metal ions such as Cu^{2+} and Fe^{3+} (Rahman et al., 2020; Vargas et al., 2021) confirming oxidative damage causing damage to bacterial DNA, proteins and lipids leading to cellular apoptosis (Rahman et al., 2020; Vargas et al., 2021) while the additional experiments demonstrated that fluorescence microscopy and flow cytometry confirmed the increased membrane permeability in bacterial cells, while spectroscopic studies indicated significant DNA binding observed as (hypochromic shift and changes in DNA melting temperature of the DNA-bound form) further supported the intercalative mode of action (Pandey et al., 2022) whereas computational quantum chemical studied performed by using density

functional theory (DFT) elucidated the concept of electronic parameters such as the HOMO-LUMO energy gap, correlating with electron transfer capabilities of the complexes particularly in ROS generation, where the copper and nickel complexes showed the highest electron densities localized on the coumarin also enhancing their reactivity making them effectively antimicrobial (Jadhav et al., 2019; Anwar et al., 2020) and highlight not only elucidation of effectual mechanisms of action but modulating metal ion selection, ligand coordination geometry and redox properties to design and optimize the next-generation coumarin.

The research on the synthesis and evaluation of antimicrobial activity of 3-acetyl coumarin-based metal complexes as a new generation of therapeutic agents emphasizes the important role of metal-ligand interaction in biological activity where the copper(II) and zinc(II) complexes show a promising antimicrobial activity against both Gram-positive (e.g., Staphylococcus aureus) and Gram-negative (e.g., Escherichia coli) bacteria which could be attributed to the ability of these metals to produce reactive oxygen species (ROS), serve as membrane disruptors, and possess DNA and enzyme binding properties as was able to be rationalized by molecular docking and time-kill assay (Pandey et al., 2022; Jadhav et al., 2019); the deduced molecular structures of these complexes demonstrate that the coumarin ligand can chelate to the metal center with its carbonyl and hydroxyl functionalities to yield stable coordination with fixed geometries (e.g., square planar for copper and tetrahedral for zinc), imparting substantial influence on the lipophilicity of the coordination complex and allowing penetration of biological membranes which has been supported by spectral shifts of the FTIR as well as UV-Vis data revealing significant metal-ligand interactions (Anwar et al., 2020; Mitra et al., 2021); the study continues by demonstrating that the biological activity is sharply dependent on the electronic properties of the complexes, whereby density functional theory (DFT) calculations indicate that complexes with low HOMO-LUMO band gaps are more reactive which also explains the strong biological action of the copper complexes due to their potential for catalyzing redox processes giving rise to oxidative damage in microbial cells (Rahman et al., 2020; Sadeghi et al., 2021); the possible generation of ROS is further confirmed experimentally by an increase in ROS production correlating with microbial macromolecule damage with time-kill experiments confirming that these complexes achieve total microbial inhibition at sub-MIC concentrations of 6–12 hours, suggesting rapid bactericidal efficacy (Vargas et al., 2021; Tiwari et al., 2020); besides, the proposed mechanism of action also describes a structure-activity relationship (SAR) illustrative of the influence of substituents on the coumarin ring which suggest that electron-donating groups increase the solubility and bioavailability of the complexes, while electron-withdrawing groups augment metal coordination and overall reactivity, offering a guide for determining how best to optimize these complex designs for given microbial targets (Banerjee et al., 2021; Nabavi et al., 2019); however, despite these promising results, the limitations of cytotoxicity and sub-optimal stability in physiological conditions is discussed indicating the need for in vivo testing to substantiate the therapeutic index and pharmacokinetic profile of the complexes, supported by computational simulations to evaluate the long-term behavior and interactions of these metal complexes within biological systems, paving the way toward better adaptation of next-generation antimicrobial agents to treat challenging multidrug-resistant pathogen threats (Ardila et al., 2021; Patel et al., 2020).

Chemical reaction-oriented implications related to the study

In these works, mechanistic aspects of the reactions that yield the metal complexes are often noted as they define the coordination processes between the heterocyclic ligand (acting as a chelator) and metal ions such as Cu^{2+}, Zn^{2+}, and Ni^{2+} in different stoichiometries often at more than 1:1 ratio (Banerjee et al., 2021; Jadhav et al., 2019); the reactivity features of coumarin derivatives and their metal complexes are typically studied by reacting the metal ions with the coumarin in protic or polar aprotic solvents (using mild bases like sodium acetate to deprotonate the coumarin ligand before coordination), leading to the formation of stable chelate rings that stabilize the ligand effectively by increasing the electron density of the vacant orbital of the ligand (Rahman et al., 2020; Vargas et al., 2021); depending on the strong or weak nature of the ligand, it can behave as a bidentate or

tridentate chelator, and coordination generally proceeds under reflux in ethanol and/or DMSO (Jadhav et al., 2019); the ligand structural and electronic properties (i.e., the type of donating and accepting group, presence of functional groups, etc.) and the identity of the dressing metals perturb the fact that many select coordination reactions such as C=O and C-OH undergo a red shift in UV-Vis absorption maximum, and exhibit reduce stretching frequencies in FTIR for vigorous rank bond formation for the metal-ligand (ML) bond (Banerjee et al., 2021; Jadhav et al., 2019); during these coordination reactions, notable predictors of the resulting oxidation states of the metals are used: when metals have such compounds as very high oxidation states and a minor ionic radii (such as Cu^{2+}), they imprison the ligand with elevated electrostatic attraction and orbital overlap, causing a greater redox potential, increase excitability, and yielding much more generation of reactive oxygen species (ROS) in biological systems with larger phenomenon of reactivity stressing the mechanism of activities (Farhane et al., 2022a, b; Rahman et al., 2020)—a critical mechanism underlying the antimicrobial activity of such coumarin-microbe complexes also recording to their high activities (Rahman et al., 2020; Vargas et al., 2021) for the strong reactivity properties of both proliferating electrostatic and electronic character, and substituents, which can increase these properties leading to overall improved reactivity through interaction of the coumarin framework with the metal ion (Ahmad et al., 2018; Pandey et al., 2022; Anwar et al., 2020), and even lower steric hindrance (where such groups are electron donating), forming more stable complexes through one or both process expressing on the bond formation to the electrons into the reaction; mechanistically, recent studies on oxidizing reactions of organics with coordination complexes in mild conditions demonstrate the involvement of the coupling of such UV-Vis and FTIR spectroscopic features via particularly, utilizing copper(II)-3-acetyl coumarin catalysts for oxidation reactions as such carriers from coumarin-yl radicals (Tiwari et al., 2020), and noting novel interaction pathways via dynamic pathway-sharpness interplay with the MAC for target discovery feature (Mitra et al., 2021; Sadeghi et al., 2021).

CONCLUSION

Novel antimicrobial activity e.g. 3-acetyl coumarin-based metal complexes: Highlights the vast potential of metal complexes themselves as new antimicrobial agents, both in the design of new chemical entities with enhanced biological properties due to coordinated metal-ligand synergy (e.g. greater lipophilicity, stability and reactivity than the parent coumarin ligand) as tested here by substantial activity against Gram-positive and Gram-negative bacteria and fungus via membrane disruptions, essential enzyme inhibition, and the generation of reactive oxygen species (ROS) to damage microbial macromolecules – by the individual complexes, and confirmed by structures derived from myriad techniques (UV-Vis, FTIR, and X-ray diffraction) and new optimal ratios of the most stable chelate complexes (with metal leaving the complex with different geometries (tetrahedral, square planar and octahedral depending on the metal ion) – but emerging copper(II) complexes can be quite potent, due to lower redox levels and the property of catalyzing ROS, suggested through different computational studies which strengthens binding interactions with microbes' DNA and enzymes (e.g. Molecular docking studies), to time-kill studies and the most effective bactericidal action at a minimal concentration per 24 h until balance MIC (end) – Structure-activity (SAR) evolutionary parameters define the effect of substituents on the coumarin ring and the electronic properties of the transition metals as major components for their changing their distinct antimicrobial efficacy- however still struggling with pharmacokinetics & fine-tuning of their bioavailability and toxicity patterns (requires future studies on in vivo application for their rational design: determining their therapeutic index versus target biological infections or comparing antimicrobial properties), catalytic role – example the driven dual antimicrobial agency (a chemical agent) towards the use- as either successful antimicrobial, while allowing further development of other multifunctional agents & materials.

Scope for further research and limitations of the study
The research reported here will pave the way for extensive future work to be done into the synthesis, characterization, pharmacokinetics, bioavailability, toxicity, and therapeutic efficacy of 3-acetyl

coumarin-based metal complexes, where in vivo work is necessary to provide sufficient data supporting the legitimacy for clinical application, combined with scalability data to ensure appropriate yields are produced at cost-effective rates alongside sustainable synthesis conditions continued to be explored (solvent-free, green chemistry processes), and further computational modeling (including molecular dynamics simulations mapped against biological environments) to predict complex interactions with biomolecules to aid in future design approaches that utilize both specificity and selectivity through metal-ligand electronic property tuning to create new targeted-complexes with antimicrobial activity against the rising number of multidrug-resistant strains, here we additionally incorporated the benefit of conjugated nanoparticles to potentially improve most delivery mechanisms for enhanced permeation through biological barriers, but the current limitations of the study lies in an, albeit fascinating, focused approach only assessment of microbial biological activity through in vitro methods which do not parallel the complex dynamics of in vivo work, coupled with a fundamental cytotoxicity assessment to determine non-target human cell killing alongside a restrained investigation between only a few selected metal ions which would benefit from assaying their other metal ion transition and rare earth behaving counterparts that likely might display unique coordination's and biological pathways, and though stable and appropriate characterization techniques are prominent to examine this interaction nature further with comprised cryo-electron microscopy or atomic force microscopy-based imaging necessary to accurately visualize the dynamics of cellular based membrane rupture followed by accumulation or pathogenic killing mechanisms alongside the need for investigating the translatability of such complexes into physiologically safe and stable work characteristically complex to ensure preserved antimicrobial efficacy against the emerging resistance sustainability challenge.

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