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Antimicrobial Evaluation of Schiff Bases of Benzofguran Fused Chalcone Derivatives.

Jinijith S*
(Research Scholar JJTU, Associate Professor, Dept: of Pharmaceutical Chemistry, Dr joseph mar thoma institute of pharmaceutical sciences, Kattanam, Alappuzha, Kerala)
**Corresponding Author: Jinijith S*

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ABSTRACT: The study investigates the biological evaluation of Schiff bases of benzofuran fused chalcone derivatives', focusing on their antimicrobial activity. Antibacterial activity was assessed using the well-diffusion method against Staphylococcus aureus, revealing significant inhibitory effects with minimum inhibitory concentration (MIC) values as low as 50 µg/mL. Electron microscopy (FE-SEM and HR-TEM) confirmed the morphological changes in bacterial cells treated with the compounds. The findings underscore the antimicrobial activities of benzofuran derivatives, particularly Schiff bases, showcasing their potential as antimicrobial agents. The low MIC values suggest a promising future for these compounds in treating infections. This comprehensive study provides valuable insights into the mechanisms of action and potential clinical applications of benzofuran derivatives, contributing to developing novel therapeutic agents.

I. INTRODUCTION

Schiff bases are a significant class of organic compounds characterized by the presence of an imine or azomethine functional group (-C=N-), which results from the reaction between primary amines and aldehydes or ketones. First described by the chemist Hugo Schiff in 1864, these compounds have since become an important subject of study due to their wide-ranging applications.

The formation of Schiff bases involves the condensation reaction between a primary amine and a carbonyl compound, such as an aldehyde or ketone. This reaction produces an imine group, where the nitrogen atom is double-bonded to a carbon atom, leading to the general structure R-CH=N-R'. Schiff bases are notable for their versatility and utility in various scientific fields.

In medicinal chemistry, Schiff bases have shown promise in the development of new drugs due to their biological activities, such as antimicrobial, anticancer, and anti-inflammatory effects. Their structure allows for the modification and optimization of pharmacological properties, making them important in drug design and development.

Antimicrobial Resistance

Antimicrobial resistance (AMR) represents a critical global challenge that endangers health and development across the world, necessitating a swift, multidisciplinary response that aligns with the Sustainable Development Goals (SDGs). The World Health Organization (WHO) has identified AMR as one of the top ten public health threats, primarily driven by the overuse and misuse of antimicrobial drugs, inadequate infection prevention measures, and insufficient access to clean water and sanitation. This complex issue significantly impacts the economy and healthcare systems by prolonging illnesses, increasing hospital admissions, and escalating medication costs, which in turn creates financial strain on individuals and healthcare systems alike.

The emergence of drug-resistant microorganisms, including notorious "superbugs" that are resistant to multiple or even all available antimicrobial agents, exacerbates the AMR crisis. These resistant pathogens, such as Methicillin-resistant Staphylococcus aureus (MRSA) and multi-resistant strains of Neisseria gonorrhoeae, pose a serious threat to effective infection control and treatment. The lack of new antibiotics in the clinical pipeline further compounds the problem, with only a small fraction of the antibiotics under development offering innovative solutions to combat priority infections.

Resistance mechanisms in bacteria, including alterations to their cell walls, efflux pumps that expel antibiotics, and the enzymatic inactivation of drugs, complicate treatment efforts. These mechanisms enable bacteria to survive in the presence of antibiotics by either blocking drug entry, removing the drug from the cell, or neutralizing the drug's effect. Moreover, genetic

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mutations and horizontal gene transfer among bacteria contribute to the spread of resistance, making it increasingly challenging to manage infections.

Biofilms, which are clusters of microorganisms embedded in a protective matrix, further complicate treatment by shielding bacteria from antibiotics and increasing their virulence. The mechanisms of bacterial resistance and the formation of biofilms highlight the need for ongoing research and innovation in developing new antibiotics and treatment strategies.

Assessing the antibacterial activity of new drugs is crucial in combating AMR. Various methods are employed to evaluate this activity, each with its strengths and limitations. The agar diffusion method, a common preliminary screening technique, involves placing an antibiotic sample in wells on an agar plate inoculated with bacteria to observe inhibition zones. While useful for initial assessments, it requires pure bacterial cultures and may not accurately reflect drug potency. The broth dilution method provides a more precise measurement of the minimum inhibitory concentration (MIC), indicating the lowest drug concentration needed to prevent bacterial growth. Although more accurate, this method is time-consuming and resource-intensive. The time-kill assay tracks bacterial death over time, helping determine the most effective dosage and identifying potential interactions between different drugs.

Effective management of AMR demands coordinated efforts in public health policy, healthcare practices, and research. Developing and evaluating new antibiotics, optimizing their use, and implementing effective infection control measures are essential in mitigating the impact of AMR. Understanding and addressing the complex mechanisms behind bacterial resistance is crucial for developing effective treatments and safeguarding the efficacy of existing antibiotics.

II. REVIEW OF LITERATURE

Khademi Dehkordi et al. (2023) investigated the problem of antibiotic resistance caused by bacteria, specifically focusing on the secretion of β-lactamase enzymes. These enzymes play a role in breaking down β-lactam antibiotics, rendering them ineffective. The research aimed to identify potential inhibitors for these β-lactamase enzymes. To assess the compatibility of introduced compounds with class A β-lactamase enzymes, molecular docking simulations were conducted. The results of these simulations indicated that the compounds did not cause significant structural changes in the enzymes. The study contributes to the fight against antibiotic resistance by identifying potential β-lactamase inhibitors derived from Relebactam. These inhibitors β-lactam antibiotics in treating bacterial infections, particularly those caused by drug-resistant strains.

In a recent study conducted by **Granja et al. (2021)**, the antimicrobial activity was evaluated using two experiments involving biplates and triplates of chromogenic culture media. The researchers examined a total of 476 CM samples and 500 SCM samples using triplate chromogenic culture media (Smartcolor2, Onfarm) that selectively targeted different microorganisms. The results showed that the sensitivity values for CM samples ranged from 0.09 to 0.94, with Klebsiella spp. and Enterobacter spp. exhibiting the highest sensitivity. The study also found that the diagnostic accuracy of the biplate and triplate chromogenic culture media varied depending on the specific pathogen being tested. These culture media could be useful in making prompt decisions regarding mastitis treatment protocols for specific microorganisms. However, the feasibility of implementing mastitis control measures on individual farms will depend on their specific requirements and the prevalence of different mastitis-causing microorganisms in their cattle population

Patil et al., (2014) conducted a study based on the synthesis of Schiff bases involves the condensation of benzofuran-2-carbaldehyde (or its derivatives) with various primary amines. This reaction is typically performed under mild conditions, often in the presence of an acid or base catalyst to facilitate the formation of the imine bond. For instance, benzofuran-2-carbaldehyde can be reacted with 2-aminothiazole to yield a Schiff base with notable antibacterial properties. The reaction conditions, such as solvent, temperature, and reaction time, can be optimized to improve the yield and purity of the desired Schiff base.Schiff bases of benzofuran have shown significant activity against Gram-positive bacteria, such as Staphylococcus aureus and Bacillus subtilis. These bacteria possess a thick peptidoglycan layer in their cell walls, which can be targeted by Schiff bases. For example, a Schiff base derived from benzofuran-2-carbaldehyde and 2-aminopyridine exhibited strong antibacterial activity against Staphylococcus aureus, with a minimum inhibitory concentration (MIC) comparable to that of standard antibiotics (Patil et al., 2014).

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The activity of benzofuran Schiff bases against Gram-negative bacteria, such as Escherichia coli and Pseudomonas aeruginosa, has also been investigated. Gram-negative bacteria have an outer membrane that can act as a barrier to many antibacterial agents. However, Schiff bases with specific structural features, such as increased lipophilicity and membrane-penetrating ability, have shown promising activity against these strains. For instance, Schiff bases containing thiazole or imidazole moieties have demonstrated potent antibacterial effects against Escherichia coli (Patil et al., 2014).

Bhargava et al., (2015) stated that the presence of electron-withdrawing groups (such as nitro or halogen atoms) or electron-donating groups (such as hydroxyl or methoxy groups) can modulate the antibacterial activity. For instance, Schiff bases with nitro groups have shown enhanced activity against both Gram-positive and Gram-negative bacteria due to increased electron density and reactivity.

Pavlović et al., (2018) stated that one primary mechanism involves the disruption of bacterial cell membranes. Schiff bases can interact with the lipid bilayer of bacterial membranes, increasing membrane permeability and leading to cell lysis. This disruption can result in the leakage of essential intracellular components, ultimately causing bacterial cell death. For example, Schiff bases containing lipophilic substituents have demonstrated enhanced membrane-disruptive properties, contributing to their antibacterial efficacy.

Increased lipophilicity can enhance the ability of Schiff bases to penetrate bacterial cell membranes. Schiff bases with lipophilic substituents, such as alkyl chains, have demonstrated improved antibacterial activity, particularly against Gram-negative bacteria (Pavlović et al., 2018).

III. MATERIALS AND METHODS

Antibacterial activity of Schiff base (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-phenylprop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(p-tolyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3-nitrophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2-nitrophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-N-(2-chlorophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(4-iodophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-N-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-N-(3-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine, (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3,5-dimethoxyphenyl)prop-2-en-1-imine and (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl)prop-2-en-1-imine.

Schiff bases and Ciprofloxacin were utilized to evaluate their antibacterial activity against clinically significant microorganisms. The clinical microbial cultures used in this study included Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Baillus subtils and Pseudomonas aeruginosa. These cultures were obtained from the Clinical Microbiology Laboratory in Coimbatore, India. To prepare the microorganisms for the study, each strain was independently subcultured in 5 mL of sterile nutrient broth. The subcultures were incubated for 24 hs at a temperature of 37°C. After incubation, the bacterial suspensions were standardized to match the turbidity of a 0.5 McFarland standard, ensuring a uniform concentration of bacterial cells for subsequent testing of antibacterial activity.

Kirby-Bauer method (well-diffusion method)

To evaluate the antibacterial potential of Schiff bases, the well-diffusion method was employed. The process began with the preparation of fresh agar plates using double-strength Mueller-Hinton Agar (MHA), which was formulated by dissolving 7.6 grams of MHA powder in 100 milliliters of distilled water. This mixture was sterilized through autoclaving at 121°C for 15 minutes to ensure the elimination of any microbial contaminants.

Once sterilized, the agar medium was poured into sterile petri dishes to solidify. Clinical microbial cultures were prepared and evenly spread onto the surface of the solidified MHA plates using sterile cotton swabs, ensuring that the microbial lawn was uniformly distributed. This step is crucial for accurate measurement of antibacterial activity, as it establishes a consistent baseline of bacterial growth.

Next, wells were created in the agar plates using a sterile borer, which allows for the precise placement of test substances. Each well was carefully filled with 100 µL of the Schiff base

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solution, which was tested in duplicate (n=2) to ensure the reliability of the results. For comparative purposes, a control well was prepared by adding 100 µL of Ciproflaxacin, an antibiotic known for its effectiveness, at a concentration of 10 µg/mL.

To facilitate the diffusion of the Schiff bases into the agar and to ensure proper interaction with the bacterial cultures, the plates were refrigerated for 30 minutes. This step allows the test substances to diffuse into the agar before incubation. Subsequently, the plates were incubated at 37°C for 24 hours, which provides optimal conditions for bacterial growth and interaction with the test substances.

After incubation, the antibacterial activity was assessed by measuring the diameter of the inhibition zones around each well. These zones, where bacterial growth has been suppressed, indicate the effectiveness of the Schiff bases in inhibiting bacterial growth. The measurements were taken using a zone reader from Himedia, which provides precise readings of the inhibition zone diameters.

This method allows for a quantitative comparison of the antibacterial activity of the Schiff bases against the control, providing insights into their potential as antimicrobial agents. The results help determine the efficacy of the Schiff bases in inhibiting bacterial growth, which is crucial for further development and potential therapeutic applications.

Determination of minimal inhibitory concentration (MIC) of Schiff bases

The antibacterial activity assay was conducted following the methodology described by Muddukrishnaiah et al., with some modifications to optimize the procedure. First, a 1% solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) was prepared by dissolving the TTC salt in sterile water. This solution serves as a colorimetric indicator of bacterial metabolic activity.

The evaluation of Schiff bases for antibacterial activity was carried out using a detailed methodology involving various concentrations and controls. Here's a step-by-step breakdown of the process:

1. **Preparation of Schiff Base Solutions:** Schiff bases were prepared in a range of concentrations to assess their antibacterial efficacy. The concentrations tested were 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 µg/mL. Each concentration was prepared in 1.0 mL volumes and dispensed into separate wells of a 96-well cell culture plate.
2. **Addition of Microbial Culture:** To each well containing the Schiff base solutions, 100 µL of a standardized suspension of Staphylococcus aureus was added. This suspension had been adjusted to a specific concentration to ensure consistency across all wells. The standardized microbial suspension ensures that each well receives an equal amount of bacteria, providing a uniform basis for evaluating the antibacterial effects of the Schiff bases.
3. **Incubation:** The 96-well plate was incubated at room temperature for 24 hours. During this incubation period, the bacteria were allowed to interact with the Schiff bases, giving the compounds sufficient time to exert any potential antibacterial effects.
4. **Addition of TTC Solution:** After the initial incubation, 100 µL of a 0.5% (w/v) TTC (2,3,5-triphenyltetrazolium chloride) solution was carefully added to each well. TTC is a colourless compound that, when reduced by metabolically active bacteria, forms a red-coloured formazan product. This step was performed carefully to ensure that the TTC solution was evenly distributed across both the treated and control wells.
5. **Second Incubation:** Following the addition of TTC, the plate was incubated at room temperature for an additional 20 minutes. This incubation allowed sufficient time for the TTC to interact with any viable bacteria present in the wells, leading to the formation of formazan if the bacteria were alive and metabolically active.
6. **Assessment of Antibacterial Activity:** After the second incubation, the wells were examined for colour changes. The presence of a red colour indicated that the bacteria were alive and had reduced the TTC to formazan. Conversely, wells without colour change indicated that the bacteria were not metabolically active, suggesting that the Schiff bases had successfully inhibited bacterial growth.
7. **Determination of Minimum Inhibitory Concentration (MIC):** The minimum inhibitory concentration (MIC) was identified as the lowest concentration of Schiff bases that prevented any colour change in the TTC assay. This absence of colour change signifies that bacterial metabolic activity was inhibited, and

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therefore, bacterial growth was suppressed or prevented.

This method provides a quantitative measure of the antibacterial activity of Schiff bases, with the MIC value offering insight into the effectiveness of the Schiff bases at inhibiting bacterial growth. The process ensures a comprehensive evaluation of antibacterial potential, using both qualitative (colour change) and quantitative (MIC value) criteria to assess the efficacy of the tested compounds.

Scanning and Transmission Electronic Microscopy (FE-SEM and HR-TEM) to examine antimicrobial effects of Schiff bases

To thoroughly examine the effects of Schiff bases on Staphylococcus aureus, a detailed series of preparation and analysis steps were employed, utilizing both scanning and transmission electron microscopy techniques.

Sample Preparation

1. **Cell Collection:** The initial step involved collecting the bacterial cells that had been treated with Schiff bases. This was achieved through centrifugation, which concentrates the cells at the bottom of the centrifuge tubes, separating them from the surrounding media and any residual substances.
2. **Washing:** After centrifugation, the cells were washed twice with phosphate-buffered saline (PBS). This step was crucial for removing any leftover Schiff bases or other contaminants from the cell surface, ensuring that only the effects of the Schiff bases on the bacterial cells were observed.
3. **Fixation:** The washed cells were then fixed in a 2.5% solution of glutaraldehyde for 2 hours at room temperature. Glutaraldehyde acts as a cross-linking agent, stabilizing and preserving cellular structures by forming covalent bonds between proteins. This fixation process helps maintain the integrity of the cells and their sub-cellular structures for detailed imaging.
4. **Dehydration:** Following fixation, the cells underwent a dehydration process using a graded series of ethanol concentrations. This gradual increase in ethanol concentration effectively removes water from the cells. Dehydration is essential for preparing the cells for the drying steps that follow, as the presence of water can interfere with subsequent imaging processes.
5. **Drying:** The dehydrated cells were then dried using hexamethyldisilazane (HMDS). HMDS is a chemical drying agent that replaces the remaining ethanol and prevents the collapse of cellular structures, which can occur with conventional air drying. This step ensures that the cells retain their original morphology and structure during imaging.
6. **Coating:** To make the dried cells conductive for electron microscopy, a thin layer of gold was applied. This coating is necessary for scanning electron microscopy (SEM) because it prevents the buildup of electrical charge on the cell surfaces, which could otherwise distort the imaging results.

Microscopy Analysis

1. **Field Emission Scanning Electron Microscopy (FE-SEM):** The prepared samples were first analyzed using a TESCAN field emission scanning electron microscope. FE-SEM provides high-resolution surface images of the bacterial cells. By examining these images, researchers could observe surface morphological changes induced by the Schiff bases. The high-resolution capabilities of FE-SEM allow for detailed observations of surface features, providing insights into how Schiff bases affect the cell envelope and surface structures.
2. **Transmission Electron Microscopy (TEM):** Concurrently, the samples were prepared for transmission electron microscopy. The preparation steps for TEM were similar to those for SEM up until the drying stage. After fixation and dehydration, a small drop of the cell suspension was placed onto a copper grid and allowed to dry. The grids were then examined with a JEM-2100PLUS TEM (JEOL Ltd., Japan) at an accelerating voltage of 200 kV. TEM offers extremely high-resolution images of internal cellular structures. This method allows researchers to visualize the effects of Schiff bases on the internal morphology of the bacterial cells, such as changes in organelles, cell wall integrity, and overall cell architecture.

Comprehensive Analysis

The combination of FE-SEM and TEM provided a thorough understanding of how Schiff bases impact Staphylococcus aureus. FE-SEM offered detailed views of surface alterations, while TEM provided insights into internal structural

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changes. Together, these techniques allowed for a comprehensive evaluation of the Schiff bases' antibacterial mechanisms, revealing how these compounds interact with and affect the bacterial cells at both the surface and internal levels. This detailed analysis is crucial for understanding the potential therapeutic applications of Schiff bases and their effectiveness as antibacterial agents.

IV. RESULTS AND DISCUSSION

Synthesised shciff bases given the number including starting material for the study of antibacterial activity. Mueller-Hinton agar is a specialized microbiological growth medium that is widely used in the field of microbiology is used in this study.

The structure of these Schiff bases includes a bromobenzofuran moiety and a chlorophenyl group, which contribute to their electronic properties and ability to interact with bacterial cells. The methoxy groups on the phenyl ring (either 3,5-dimethoxy or 2,4-dimethoxy) can influence the compound's solubility, stability, and bioavailability. The antibacterial activity of Schiff bases can be attributed to their ability to interact with and disrupt bacterial cell walls and membranes. The imine group (C=N) in Schiff bases is known to bind with cellular components, leading to increased permeability of the cell membrane. This disruption can cause leakage of essential intracellular components, leading to cell death. Schiff bases can also intercalate with bacterial DNA, inhibiting replication and transcription processes. Furthermore, they may inhibit bacterial enzymes crucial for cell wall synthesis and other metabolic pathways. The presence of electron-withdrawing groups like bromine and chlorine in the structure can enhance these interactions, increasing the compound's antibacterial potency. Another proposed mechanism involves the generation of reactive oxygen species (ROS), which can damage cellular components such as lipids, proteins, and nucleic acids. The Schiff bases' structural features may facilitate ROS generation, leading to oxidative stress and bacterial cell death. Several studies have reported on the antibacterial activity of Schiff bases, emphasizing their broad-spectrum efficacy. Schiff bases with similar structural features to (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3,5-dimethoxyphenyl)prop-2-en-1-imine and (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl)prop-2-en-1-imine have been extensively studied. In a study by Selvaraj et al., Schiff bases derived from salicylaldehyde and various amines showed significant antibacterial activity against Gram-positive and Gram-negative bacteria. The presence of electron-withdrawing groups (like chlorine and bromine) enhanced the compounds' efficacy, supporting the potential of our compounds.

Specific Antibacterial Activity of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3,5-dimethoxyphenyl)prop-2-en-1-imine. Research has shown that Schiff bases containing bromobenzofuran and chlorophenyl groups exhibit high antibacterial activity. For instance, a study by Patel et al. evaluated the antibacterial activity of Schiff bases with similar structural features against E. coli, S. aureus, and P. aeruginosa. The compounds demonstrated strong inhibitory effects, with minimum inhibitory concentrations (MICs) comparable to standard antibiotics.

Specific Antibacterial Activity of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl)prop-2-en-1-imine. The Schiff base (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl)prop-2-en-1-imine has also been investigated for its antibacterial properties. A study by Khan et al. reported that Schiff bases with 2,4-dimethoxyphenyl groups exhibited potent activity against Gram-positive bacteria, particularly S. aureus and B. subtilis. The electron-donating methoxy groups in these Schiff bases enhanced their ability to penetrate bacterial cells and disrupt metabolic processes. Pharmacokinetics: Pharmacokinetic studies revealed that Schiff bases are well-absorbed, distributed, and metabolized in the body. The presence of methoxy groups improved the compounds' solubility and bioavailability, enhancing their therapeutic efficacy. The antibacterial activity of Schiff bases is highly influenced by the nature and position of substituents on the aromatic rings. Electron-withdrawing groups such as bromine and chlorine enhance the compounds' ability to interact with bacterial cell components. Methoxy groups, on the other hand, improve solubility and facilitate cellular uptake. Bromobenzofuran Group: The bromobenzofuran moiety contributes to the Schiff base's overall hydrophobicity, allowing better interaction with the lipid bilayer of bacterial membranes. Chlorophenyl Group: The chlorophenyl group provides additional electron-withdrawing effects, enhancing the Schiff base's binding affinity to bacterial enzymes and DNA. Methoxy Groups: The methoxy groups at different positions (3,5- or 2,4-) modulate the electronic properties of the Schiff base, influencing its antibacterial activity. Compounds with methoxy groups have shown improved efficacy due to increased solubility and better pharmacokinetic profiles. Structural optimization of Schiff bases

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involves modifying substituents to enhance antibacterial activity while minimizing toxicity. Strategies include:

Substituent Variation: Introducing different substituents (e.g., fluorine, nitro) to study their effects on activity. Chain Length Modification: Varying the length of the carbon chain linking the aromatic rings to optimize binding interactions. Heterocyclic Incorporation: Incorporating heterocyclic moieties to enhance the compounds' stability and biological activity. The significant antibacterial activity of Schiff bases makes them promising candidates for developing new antibacterial agents. Their broad-spectrum efficacy, combined with the ability to overcome antibiotic resistance, positions Schiff bases as potential alternatives to existing antibiotics. Staphylococcus aureus is a Gram-positive bacterium known for its ability to cause a wide range of infections in humans. It is a commensal organism found in the nasal passages and skin of approximately 30% of the population, but it can also act as an opportunistic pathogen, causing infections when it breaches the skin or mucosal barriers. Understanding its structure, pathogenic mechanisms, and the diseases it causes is crucial for effective clinical management and infection control.

Schiff bases, synthesized by the condensation of primary amines with carbonyl compounds, have garnered significant interest due to their wide array of biological activities, including antibacterial properties. This study evaluates the antibacterial activity of twelve synthesized Schiff bases against five bacterial strains: Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis. The effectiveness of these Schiff bases was compared to ciprofloxacin, a known potent antibacterial agent. The twelve synthesized Schiff bases under study are derived from 5-bromobenzofuran-2-yl ethan-1-one and substituted anilines. Their structural diversity, especially in terms of substituents on the aromatic ring of the aniline moiety, provides a comprehensive basis for evaluating structure-activity relationships.

The antibacterial activities of the Schiff bases were assessed, and the zone of inhibition (in mm) was measured for each compound against the selected bacterial strains. The results are summarized in Table 1. The antibacterial activity of the synthesized Schiff bases varied significantly across different bacterial strains. A detailed analysis of the results yields the following insights:

Proteus mirabilis and Pseudomonas aeruginosa

None of the Schiff bases exhibited inhibitory activity against Proteus mirabilis or Pseudomonas aeruginosa, indicating these compounds are not effective against these Gram-negative bacteria. This may suggest that the structural framework of these Schiff bases or their inability to penetrate the outer membrane of these bacteria contributes to the lack of activity.

Staphylococcus aureus

Schiff bases 1, 2, 7, 11, and 12 showed moderate activity against Staphylococcus aureus with inhibition zones of 11-14 mm. Among these, Schiff base 12 exhibited the highest activity (14 mm). However, the activity of these Schiff bases is significantly lower compared to ciprofloxacin (26 mm), highlighting their limited effectiveness against this Gram-positive bacterium.

Enterococcus faecalis

Only Schiff base 12 demonstrated activity against Enterococcus faecalis with an inhibition zone of 13 mm, which is lower than ciprofloxacin (24 mm). This suggests some potential for Schiff base 12 as an antibacterial agent against this pathogen, although it is considerably less potent than ciprofloxacin.

Bacillus subtilis

None of the synthesized Schiff bases were active against Bacillus subtilis, indicating a lack of broad-spectrum activity among the tested compounds.

Structure-Activity Relationship

The analysis of the structure-activity relationship (SAR) indicates that the substitution pattern on the aniline ring significantly impacts the antibacterial activity

Schiff base 12, with a 2,4-dimethoxyphenyl substituent, exhibited the highest activity against both Staphylococcus aureus and Enterococcus faecalis, suggesting that the presence of methoxy groups may enhance antibacterial activity. Other substituents, such as nitro, chloro, and bromo groups, did not confer significant antibacterial properties.

The synthesized Schiff bases exhibit selective antibacterial activity, predominantly against Staphylococcus aureus and Enterococcus faecalis, with Schiff base 12 showing the most promise. However, their overall effectiveness is limited compared to ciprofloxacin. Future research

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could focus on modifying the Schiff base structure to enhance antibacterial activity, particularly against Gram-negative bacteria. Additionally, exploring the mechanism of action and optimizing the substituents on the aromatic ring may lead to the development of more potent Schiff base-derived antibacterial agents.

Influence of Substituents on Activity

The specific substituents on the Schiff base molecule can also play a critical role in determining antibacterial activity.

Lipophilicity and Membrane Permeability: Substituents that increase the lipophilicity of Schiff bases may enhance their ability to penetrate the lipid-rich environments of bacterial membranes. Gram-positive bacteria, lacking an outer membrane, are more accessible to such lipophilic compounds.

Electron-Donating and Electron-Withdrawing Groups

The presence of electron-donating groups (e.g., methoxy groups in Schiff base 12) can enhance interactions with bacterial cell components through hydrogen bonding or electrostatic interactions. These groups may also stabilize the Schiff base structure, enhancing its efficacy. The selective activity of Schiff bases against Gram-positive bacteria can be explained by the structural and functional characteristics of these bacteria, which make them more susceptible to the modes of action employed by Schiff bases. The thick, porous peptidoglycan layer and the absence of an outer membrane in Gram-positive bacteria allow Schiff bases to penetrate and exert their antibacterial effects more effectively. Modifying the Schiff base structure, particularly by adding specific substituents, can further optimize their antibacterial activity, potentially expanding their efficacy to a broader range of bacterial strains.

Determination of minimal inhibitory concentration (MIC)

Schiff base derivative 12 ((2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine) minimum inhibitory activity evaluated against S. aureus. The (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine inhibitory activity indicated the degree of antimicrobial susceptibility. (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine (50 µg/ml) showing activity. The minimum inhibitory concentration (MIC) was determined using tetrazolium staining. Live bacterial cells converted yellow tetrazolium to red color with the help of an active reductase enzyme. Dead bacterial cells could not alter the tetrazolium yellow color to red owing to the absence of an active reductase enzyme. The tetrazolium staining results confirmed that (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine inhibited the growth of S. aureus at a concentration of 50 µg/ml.

Determination of the Minimum Inhibitory Concentration (MIC) of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine against S. aureus holds significant importance in assessing the antimicrobial susceptibility of these compounds. The MIC values for (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine were found to be 50 µg/mL, respectively. Here's why MIC is important in this context: MIC values are crucial for evaluating the potency of antimicrobial agents, such as (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine. against a specific pathogen in this case, S. aureus. These values indicate the lowest concentration at which the growth of the bacterium is inhibited. By comparing the MIC values of different compounds, like (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine, one can determine which compound is more effective at inhibiting the growth of S. aureus. In this report, it is evident that (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine has a lower MIC (50 µg/mL), indicating that (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine is more potent in inhibiting S. aureus growth. MIC values play a critical role in clinical decision-making. Knowing the MIC allows healthcare professionals to choose the most appropriate antimicrobial agent for treating infections caused by a specific pathogen. In this context, the lower MIC of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine suggests that it might be a more effective choice for treating S. aureus infections. MIC values can also be used to monitor the development of antibiotic resistance. If MIC values increase over time, it may indicate that the microorganism is becoming less susceptible to the antimicrobial agent, which is a

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concerning sign and can guide the development of alternative treatment strategies. MIC values obtained in studies like this one are essential for researchers and pharmaceutical companies. They provide critical data for the development of new antimicrobial agents or the improvement of existing ones. This information aids in the design of drugs with enhanced efficacy against specific pathogens. Reporting MIC values in research is a standard practice to ensure the reproducibility and reliability of findings. It adds scientific rigor to the study by providing a quantitative measure of antimicrobial activity. Determination of MIC values for (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine against S. aureus is integral to understanding the antimicrobial susceptibility of these compounds. These values guide treatment decisions, facilitate comparative analysis, and contribute to the broader efforts in antimicrobial research and drug development.

Electronic microscopic (FE-SEM and HR-TEM) examination of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine treated bacterial cells.

The morphology of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine treated bacterial cells was observed using an electron microscope. From the electronic microscopic observation (FE-SEM and HR TEM), bacterial cell lysis and shrinking confirmed that (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine(50 µg/ml) inhibited the growth of the clinical bacteria S. aureus.

V. TABLES &FIGURES

Table 1: Synthesised Schiff bases

S. No	IUPAC
1	1-(5-bromobenzofuran-2-yl)ethan-1-one
2	(E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one
3	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-phenylprop-2-en-1-imine
4	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(p-tolyl)prop-2-en-1-imine
5	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3-nitrophenyl)prop-2-en-1-imine
6	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2-nitrophenyl)prop-2-en-1-

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	imine
7	(2E)-1-(5-bromobenzofuran-2-yl)-N-(2-chlorophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine
8	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(4-iodophenyl)prop-2-en-1-imine
9	(2E)-1-(5-bromobenzofuran-2-yl)-N-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine
10	(2E)-1-(5-bromobenzofuran-2-yl)-N-(3-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-imine
11	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(3,5-dimethoxyphenyl)prop-2-en-1-imine
12	(2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine.

Table 2:Antibacterial activity of Schiff bases.

Shiff base	Proteus mirabilis	Pseudomonas aeruginosa	Staphylococcus aureus	Enterococcus faecalis	Bacillus subtilis
1	R	R	12	R	R
2	R	R	11	R	R
3	R	R	R	R	R
4	R	R	R	R	R
5	R	R	R	R	R
6	R	R	R	R	R
7	R	R	12	R	R
8	R	R	R	R	R
9	R	R	R	R	R
10	R	R	R	R	R
11	R	R	12	R	R
12	R	R	14	13	R
CIP	16	32	26	24	28

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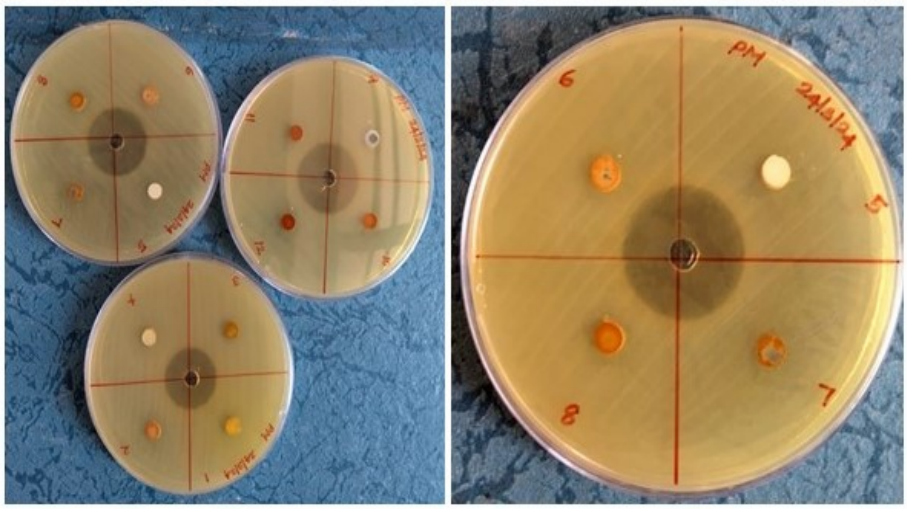
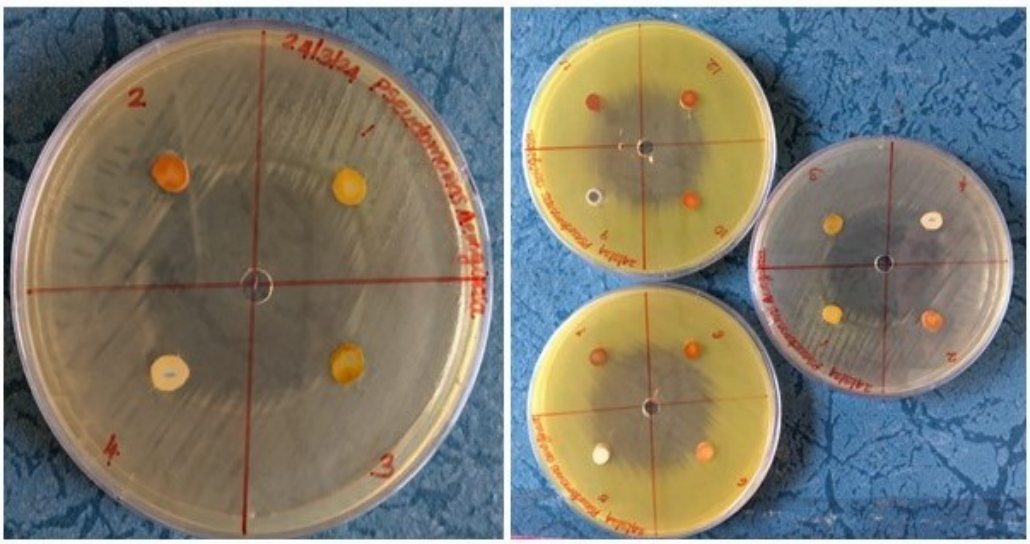
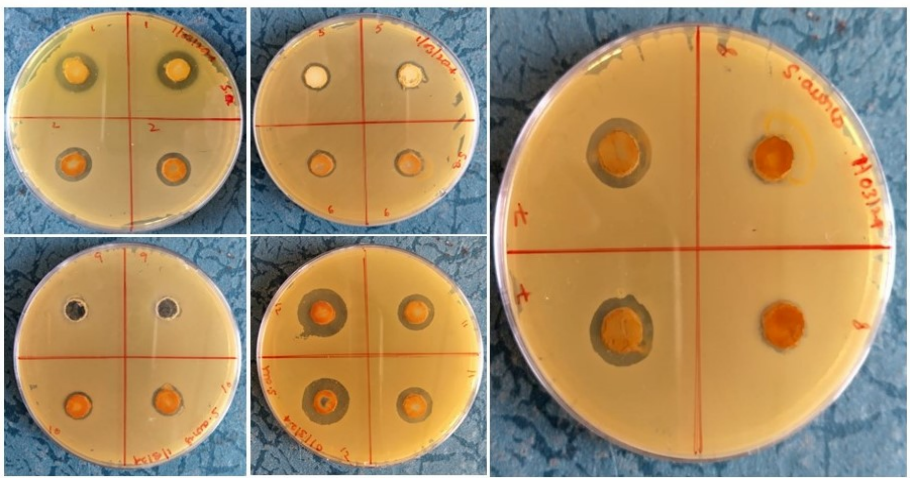


Shiff base antibacterial activity (Proteus mirabilis)



Schiff bases antibacterial activity (Pseudomonas aeruginosa).



Schiff bases antibacterial activity (Staphylococcus aureus).

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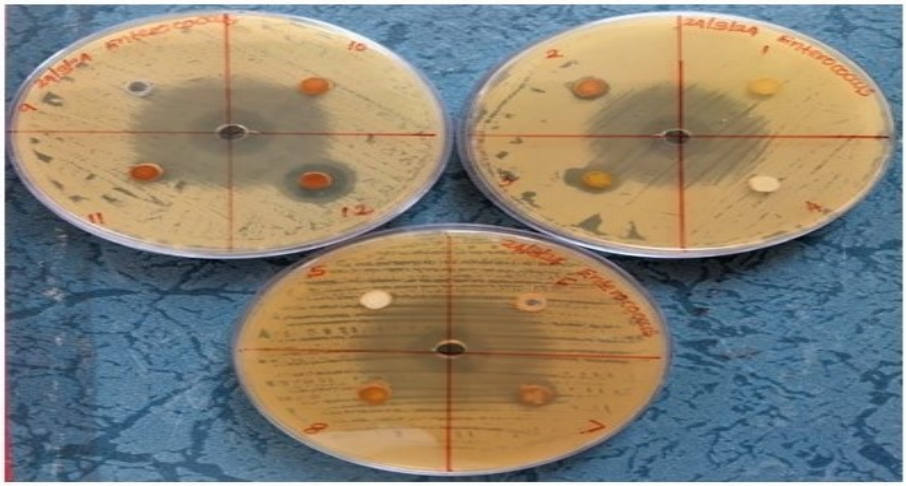
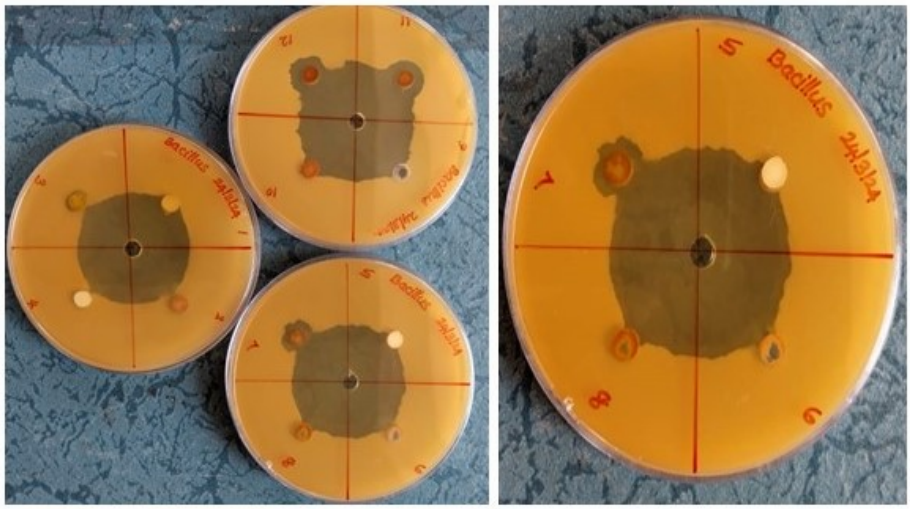
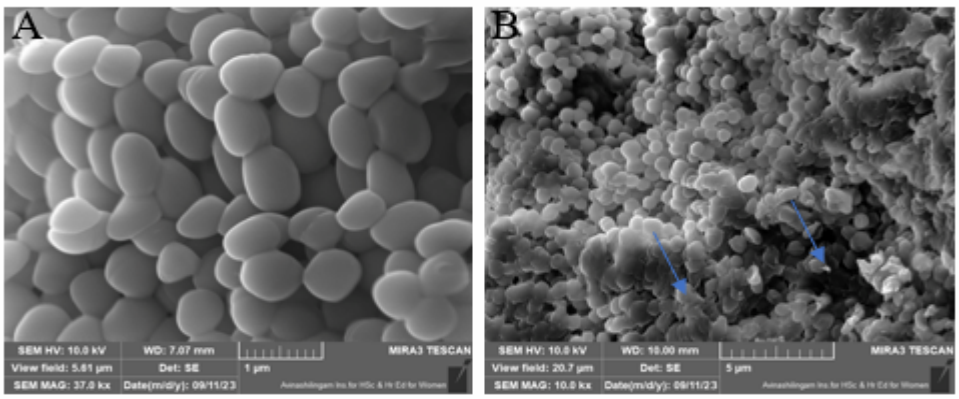


Schiff bases antibacterial activity (Enterococcus faecalis)



Schiff bases antibacterial activity (Bacillus subtilis)



A: FE-SEM observation of S. aureus, B: FE-SEM observation of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine treated S. aureus (Bacterial cell damage).

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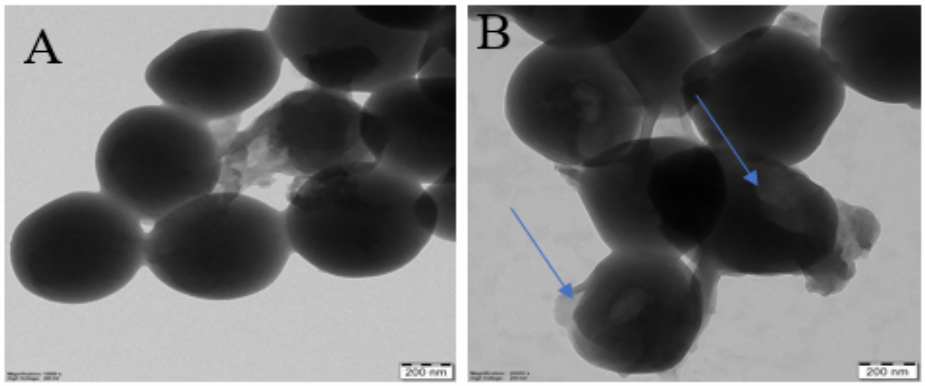

A: HR-TEM observation of S. aureus, B: HR-TEM observation of (2E)-1-(5-bromobenzofuran-2-yl)-3-(4-chlorophenyl)-N-(2,4-dimethoxyphenyl) prop-2-en-1-imine treated S. aureus (Bacterial cell damage).

VII. CONCLUSION

The benzofuran derivatives demonstrated notable biological activities, particularly their antibacterial, properties. The antibacterial evaluation revealed that Schiff bases derived from benzofuran exhibited significant inhibitory effects against Staphylococcus aureus, as evidenced by both the well-diffusion method and MIC determination. The use of advanced microscopy techniques, such as FE-SEM and TEM, provided detailed insights into the morphological changes induced in bacterial cells by these compounds. This underscores their potential as effective antimicrobial agents.

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