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Antibacterial Bioevaluation of Novel Chalcone, Dihydropyrazoline, and 1,2,3-Triazole Derivatives and Identification of Broad-Spectrum Antibacterial Leads

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Abstract

The rapid emergence of antimicrobial resistance has created an urgent need for new antibacterial agents capable of overcoming the limitations of existing antibiotics. In the present study, a series of structurally related aromatic aldehyde intermediates, chalcones, dihydropyrazoline derivatives, and 1,2,3-triazole analogues were evaluated for their in vitro antibacterial activity against three clinically relevant bacterial strains: Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. Antibacterial potency was expressed as IC_{50} values in µg/mL. The early aminoalkyl-substituted benzaldehyde derivatives (2a–3) were essentially inactive against all tested organisms, with IC_{50} values greater than 50 µg/mL. Transformation of these intermediates into chalcone derivatives (5a–5d) resulted in moderate antibacterial activity, particularly against S. aureus and K. pneumoniae, with compound 5b displaying the most favorable activity in this series. Cyclization of chalcones into dihydropyrazoline analogues (8a–8d) produced a substantial enhancement in antibacterial potency. Among them, compound 8c demonstrated pronounced activity against S. aureus, while compound 8d exhibited a broader spectrum of inhibition against both Gram-positive and Gram-negative organisms. The most significant improvement was observed following conversion of the propargyl-containing precursor into 1,2,3-triazole derivatives (10a and 10b), both of which showed excellent broad-spectrum activity, including strong inhibition of E. coli with IC_{50} values of approximately 1.25 µg/mL. Structure–activity relationship analysis revealed a clear stepwise evolution in antibacterial potency from inactive aromatic precursors to highly active triazole-based leads. The results indicate that incorporation of the chalcone pharmacophore, pyrazoline ring, and triazole nucleus progressively optimized physicochemical properties, target affinity, and membrane permeability. Compounds 10a and 10b emerged as the most promising antibacterial leads and warrant further mechanistic, toxicity, and in vivo investigations.

Keywords: Antibacterial activity; Antimicrobial resistance; Chalcones; Dihydropyrazolines; Pyrazolines; 1,2,3-Triazoles; Structure–activity relationship; Heterocyclic compounds.

Introduction

Antimicrobial resistance (AMR) has become one of the most serious problems in modern healthcare.[1-3] Many antibiotics that were highly effective a few decades ago are now losing their usefulness because bacteria have developed resistance to them.[4-5] This problem has increased due to the overuse and misuse of antibiotics in human medicine, veterinary practice, and agriculture. As a result, common bacterial infections are becoming harder to treat, leading to longer hospital stays, higher treatment costs, and increased mortality. The World Health Organization has identified AMR as one of the major global health threats of the present century.[6] Among the

most important bacterial pathogens are Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae.[7-8] These bacteria are responsible for a wide variety of infections, including pneumonia, urinary tract infections, wound infections, and septicemia.[9] Resistant strains such as methicillin-resistant S. aureus (MRSA), extended-spectrum β-lactamase (ESBL)-producing E. coli, and carbapenem-resistant K. pneumoniae have made treatment much more difficult.[10-11] Bacteria can resist antibiotics in several ways, such as breaking down the drug, altering the target site, pumping the drug out of the cell, or reducing drug entry through the cell membrane.

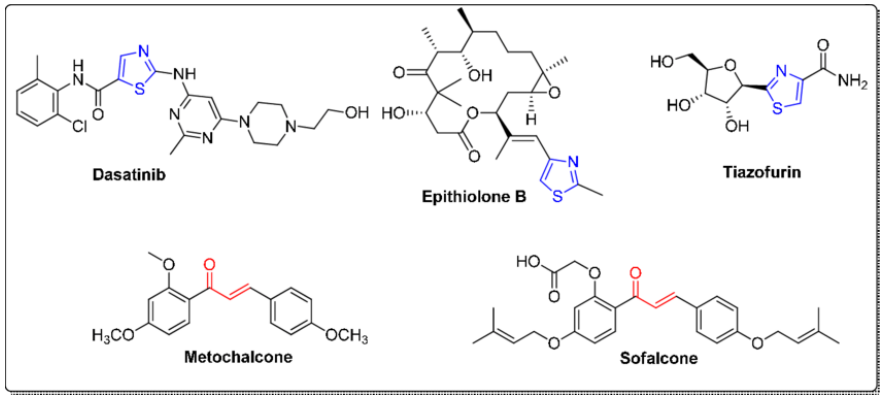


Figure 1: Structure of some clinically approved chalcone drug molecules

Most currently used antibiotics belong to well-known classes such as β-lactams, aminoglycosides, macrolides, quinolones, tetracyclines, and glycopeptides.[12] These drugs act by inhibiting cell wall synthesis, protein synthesis, or DNA replication. Although these medicines have saved millions of lives, resistance has reduced their effectiveness.[13] At the same time, the discovery of entirely new classes of antibiotics has slowed considerably. Many recently introduced drugs are only modified versions of older molecules and often face the same resistance problems.[14-15] Therefore, there is a strong need to identify new chemical scaffolds with different structural features and improved antibacterial properties. Heterocyclic compounds play a central role in medicinal chemistry because they are found in a large number of approved drugs. Their structures contain atoms such as nitrogen, oxygen, or sulfur, which help them interact effectively with biological targets. Heterocycles also allow chemists to change molecular properties such as lipophilicity, polarity, and steric bulk, all of which influence biological activity. Because of these advantages, heterocyclic scaffolds continue to be widely explored in the search for new antibacterial agents.

Chalcones are among the most studied bioactive molecules in medicinal chemistry. They consist of two aromatic rings connected by an α,β-unsaturated carbonyl system.[16-17] Chalcones are easy to synthesize and can be modified in many ways. Their antibacterial activity has been linked to membrane disruption, inhibition of fatty acid biosynthesis, and interference with important bacterial enzymes. The presence and position of substituents on the aromatic rings strongly affect their activity. Dihydropyrazolines (pyrazolines) are five-membered nitrogen-containing heterocycles usually prepared by cyclization of chalcones with hydrazine derivatives.[18] Conversion of chalcones into pyrazolines often leads to improved biological activity because the ring system provides additional sites for hydrogen bonding and gives the molecule a more rigid shape. Pyrazoline derivatives have been reported to inhibit bacterial DNA gyrase and other enzymes involved in bacterial growth and replication.[19] 1,2,3-Triazoles are another important class of heterocycles known for their high chemical stability and strong ability to interact with proteins and nucleic acids.[20] They are commonly synthesized through copper-catalyzed azide–alkyne cycloaddition, a reliable reaction often referred to as click chemistry. Triazole-containing compounds have shown a wide range of biological activities, including antibacterial effects.[21] The triazole ring can improve both target binding and pharmacokinetic properties, making it a useful structural motif in drug design.

In the present study, a series of aminoalkyl-substituted benzaldehydes, chalcones, dihydropyrazoline derivatives, and 1,2,3-triazole analogues were synthesized and evaluated for their antibacterial activity against E. coli, S. aureus, and K. pneumoniae. The purpose of this work was to examine how stepwise structural changes influence antibacterial potency and to identify promising lead compounds. Special attention was given to understanding the structure–activity relationship of these heterocyclic scaffolds. The findings of this study provide useful information for the design of new antibacterial agents with potential to address the growing problem of drug-resistant bacterial infections.

Materials and Methods

Chemistry

All compounds used in this study were synthesized in our laboratory following the procedures described in the synthetic section of the thesis. The compounds belonged to four related series: aminoalkyl-substituted benzaldehydes (2a–2c and 3), chalcones (5a–5d), dihydropyrazoline derivatives (8a–8d), and 1,2,3-triazole derivatives (10a and 10b). The general synthetic route involved initial functionalization of 4-hydroxybenzaldehyde with cyclic amines or propargyl bromide, followed by Claisen–Schmidt condensation with the appropriate acetophenone derivative to obtain chalcones. Cyclization of the chalcones with hydrazine hydrate afforded the corresponding dihydropyrazolines. The propargyl-containing intermediate was further converted into 1,2,3-triazoles by copper(I)-catalyzed azide–alkyne cycloaddition.

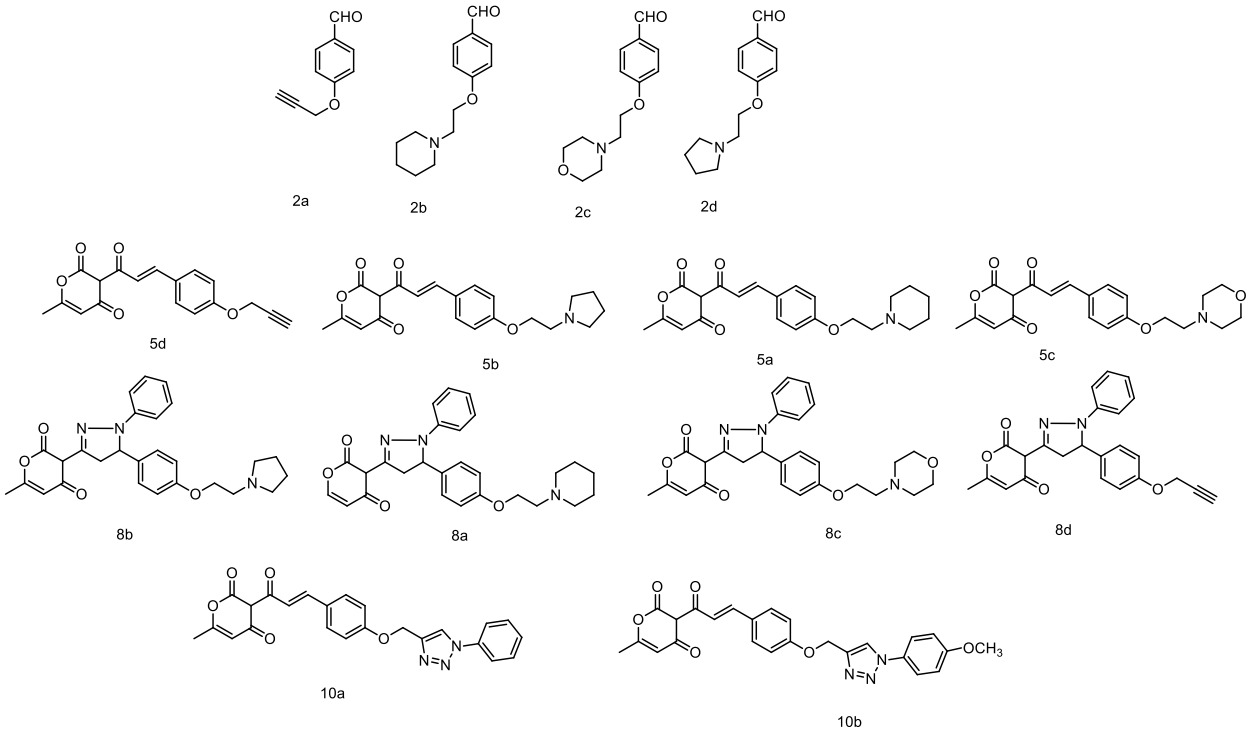


Figure 2: The library of compounds synthesized for antibacterial evaluation

All reagents and solvents were obtained from commercial suppliers and were used without further purification unless otherwise stated. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel plates. Melting points were determined in open capillary tubes and are uncorrected. The structures of the synthesized compounds were confirmed by infrared (IR) spectroscopy, proton nuclear magnetic resonance (1H NMR), ^{13}C nuclear magnetic resonance (13C NMR), and mass spectrometry. The spectral data were in good agreement with the proposed structures.

Test Microorganisms

The antibacterial activity of the synthesized compounds was evaluated against three common pathogenic bacteria: Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. These organisms were selected because they are frequently associated with hospital- and community-acquired infections and are widely used in antibacterial screening studies.

Preparation of Test Solutions

Each synthesized compound was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. Further dilutions were made using sterile nutrient broth to obtain the required concentrations for biological testing. The final concentration of DMSO in the assay was kept low enough to avoid any effect on bacterial growth. A control experiment containing the same amount of DMSO without test compound was included in each assay.

Antibacterial Assay

The antibacterial activity was determined using a broth dilution method. Fresh bacterial cultures were grown overnight and adjusted to match the turbidity of a standard inoculum. Aliquots of the bacterial suspension were added to tubes or microplate wells containing different concentrations of the test compounds. The inoculated samples were incubated at 37 °C for 18–24 h. After incubation, bacterial growth was assessed by visual observation of turbidity. The percentage inhibition at each concentration was calculated by comparing the growth in treated samples with that of the untreated control.

Determination of IC_{50} Values

The inhibitory activity of each compound was expressed as IC_{50}, defined as the concentration required to inhibit 50% of bacterial growth. IC_{50} values were estimated from the dose–response data obtained from at least three independent experiments. Results are reported in µg/mL. Compounds showing lower IC_{50} values were considered more potent antibacterial agents.

Data Analysis

The antibacterial data were analyzed to compare the activity of compounds belonging to different structural classes. Particular attention was given to the effect of converting the initial benzaldehyde intermediates into chalcones, dihydropyrazolines, and 1,2,3-triazoles. The influence of substituents such as piperidine, pyrrolidine, morpholine, and propargyl groups was also examined.

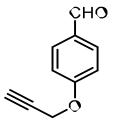
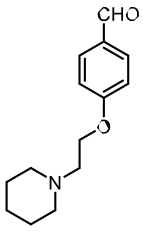
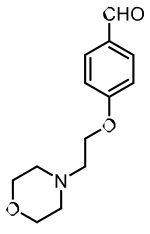
Structure–Activity Relationship Study

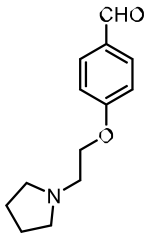
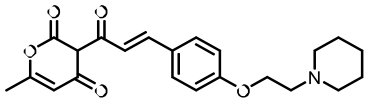
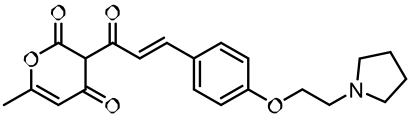
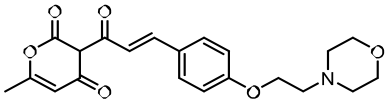
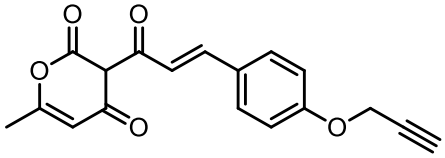
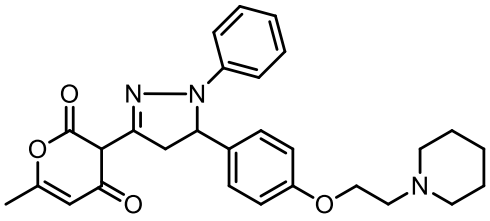
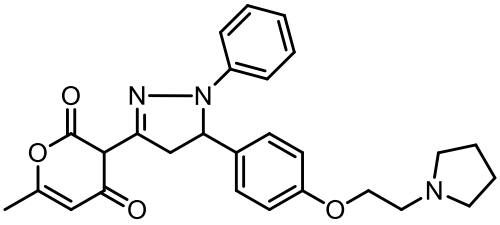
A structure–activity relationship (SAR) analysis was carried out by correlating the observed IC_{50} values with the structural features of the synthesized compounds. Changes in antibacterial potency were interpreted in terms of electronic effects, lipophilicity, ring formation, and the introduction of additional heterocyclic systems. This analysis helped identify the structural elements that contributed most strongly to antibacterial activity and guided the selection of the most promising lead compounds for further study.

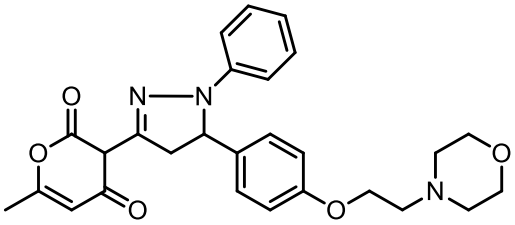
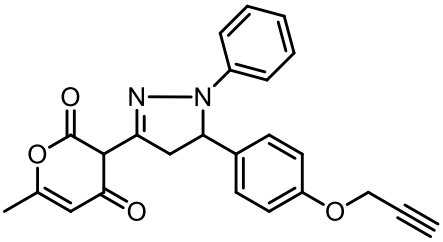
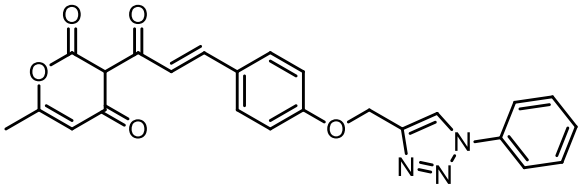
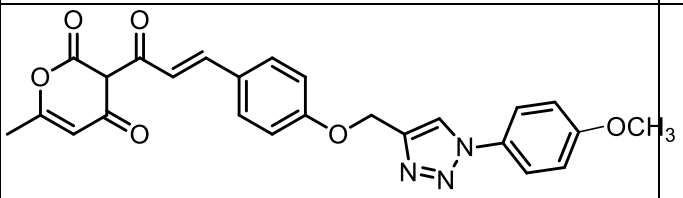
Results and Discussion

The synthesized compounds were evaluated for their antibacterial activity against Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. The activity was expressed as IC_{50} values in µg/mL, and the results revealed a clear improvement in antibacterial potency as the molecular framework was systematically modified from simple benzaldehyde intermediates to chalcones, dihydropyrazolines, and finally 1,2,3-triazole derivatives. the study showed a consistent stepwise increase in activity, indicating that each structural transformation contributed positively to antibacterial behavior.

Table 1: Antibacterial Potential of synthesized compounds

			Antibacterial Activity (IC_{50}) µg/ml		
S. No.	**Comp. No.**	**Structure**	**E. Coli**	**S. *aureus***	**K. pneumoniae**
1	2a		>50	>50	>50
2	2b		>50	>50	>50
3	2c		>50	>50	>50

4	3		>50	>50	>50
5	5a		>50	>25	>25
6	5b		>50	**>12.5**	**>12.5**
7	5c		>50	>25	>25
8	5d		>50	>25	>50
9	8a		**>12.5**	>25	>50
10	8b		>25	>25	>25

11	8c		>25	**>1.25**	**>12.5**
12	8d		**>12.5**	**>6.25**	**>6.25**
13	10a		**>1.25**	**>6.25**	**>6.25**
14	10b		**>1.25**	**>6.25**	**>6.25**

Antibacterial Activity of Aminoalkyl Benzaldehyde Derivatives (2a–2c and 3)

The first group of compounds, 2a, 2b, 2c, and 3, served as starting intermediates in the synthetic sequence. These molecules contained aminoalkyl or propargyl substituents attached to the 4-hydroxybenzaldehyde nucleus. When tested against the selected bacterial strains, all four compounds were essentially inactive. Their IC_{50} values were greater than 50 µg/mL against E. coli, S. aureus, and K. pneumoniae. The lack of activity suggests that the substituted benzaldehyde scaffold alone does not possess the structural features needed for meaningful antibacterial action. Although the compounds contain polar functional groups, they do not provide a suitable arrangement for strong interaction with bacterial enzymes or other vital cellular targets. In addition, their physicochemical properties may not favor penetration through the bacterial cell envelope. The inactivity against both Gram-positive and Gram-negative organisms indicates that these compounds are poor antibacterial templates by themselves. Nevertheless, they provided a useful starting point for further structural modifications.

Antibacterial Activity of Chalcone Derivatives (5a–5d)

Conversion of the inactive aldehyde intermediates into chalcone derivatives resulted in the first noticeable improvement in antibacterial activity. The chalcones 5a–5d contained piperidine, pyrrolidine,

morpholine, and propargyl substituents and were tested against the same three bacterial strains. Most compounds in this series showed moderate activity against S. aureus and K. pneumoniae, while they remained weak against E. coli. Compound 5b, which bears a pyrrolidine moiety, was the most active member of the series and displayed IC_{50} values of approximately 12.5 µg/mL against S. aureus and K. pneumoniae. Compounds 5a and 5c showed weaker inhibition, whereas 5d was less effective against K. pneumoniae and inactive against E. coli.

The improved activity of this series can be attributed to the presence of the α,β-unsaturated carbonyl system of the chalcone framework. This conjugated enone system is known to act as a Michael acceptor and can react with nucleophilic residues present in bacterial proteins. The aromatic rings on either side of the enone unit may also enhance hydrophobic interactions with biological targets. However, the continued poor activity against E. coli indicates that the chalcone scaffold alone was not sufficient to overcome the permeability barrier of this Gram-negative bacterium.

Antibacterial Activity of Dihydropyrazoline Derivatives (8a–8d)

Cyclization of the chalcones with hydrazine hydrate produced the corresponding dihydropyrazoline derivatives 8a–8d. This structural change led to a marked increase in antibacterial potency and a broader spectrum of activity. Compound 8c, containing a morpholine substituent, was particularly effective against S. aureus and showed an IC_{50} value of about 1.25 µg/mL, making it one of the most potent compounds in the study. It also exhibited moderate activity against K. pneumoniae. Compound 8d, which carries a propargyl group, displayed consistent activity against all three bacterial strains, with IC_{50} values ranging from 6.25 to 12.5 µg/mL. Compounds 8a and 8b also demonstrated measurable inhibition, although their potency was somewhat lower.

The enhanced activity of the pyrazoline derivatives highlights the importance of the five-membered nitrogen-containing ring. The pyrazoline nucleus introduces additional hydrogen-bonding sites and increases conformational rigidity, which may improve binding to bacterial enzymes such as DNA gyrase and topoisomerase IV. The better performance against Gram-negative bacteria suggests that these compounds possess a more favorable balance between polarity and lipophilicity, allowing improved penetration through the outer membrane.

Antibacterial Activity of 1,2,3-Triazole Derivatives (10a and 10b)

The most promising results were obtained with the 1,2,3-triazole derivatives 10a and 10b. These compounds were synthesized from the propargyl precursor using click chemistry and contained aryl-substituted triazole rings. Both compounds displayed strong and broad-spectrum antibacterial activity. They inhibited E. coli with IC_{50} values of approximately 1.25 µg/mL and showed good activity against S. aureus and K. pneumoniae, with IC_{50} values around 6.25 µg/mL. These values represent the best overall performance among all synthesized compounds.

The outstanding activity of the triazole derivatives can be explained by several factors. The triazole ring is chemically stable and highly polar, enabling strong hydrogen-bonding and dipole interactions with bacterial proteins. At the same time, the attached aromatic groups contribute hydrophobic character that supports membrane penetration and target binding. The triazole unit may also improve metabolic stability and reduce susceptibility to bacterial efflux pumps. Taken together, these properties likely account for the excellent antibacterial profile of compounds 10a and 10b.

Structure–Activity Relationship (SAR)

A clear structure–activity relationship emerged from the study. The simple aminoalkyl benzaldehydes were inactive, indicating that the starting scaffold lacked essential pharmacophoric elements. Introduction of the chalcone moiety produced moderate activity, especially against S. aureus and K. pneumoniae, showing that the α,β-unsaturated ketone system contributes meaningfully to antibacterial action. Conversion to dihydropyrazoline derivatives led to a substantial increase in potency and improved activity against Gram-negative bacteria. Finally, incorporation of the 1,2,3-triazole ring produced the most active compounds, with broad-spectrum inhibition and low IC_{50} values. The nature of the substituent also influenced activity. Pyrrolidine substitution was beneficial in the chalcone series, morpholine improved the activity of the pyrazoline series, and aryl-substituted triazoles gave the best overall results. These observations suggest that both the core heterocyclic scaffold and the peripheral substituents work together to determine antibacterial potency.

The present study demonstrates how gradual and rational structural changes can transform inactive intermediates into potent antibacterial agents. Each stage of the synthetic sequence introduced new features that improved target binding, membrane permeability, and overall biological performance. The chalcone scaffold provided the first signs of activity, the pyrazoline ring significantly increased potency, and the triazole nucleus delivered broad-spectrum antibacterial effects. Among all the tested compounds, 10a and 10b were the most promising and may be considered lead molecules for further development. Their strong activity against both Gram-positive and Gram-negative bacteria, particularly the difficult-to-treat E. coli, makes them attractive candidates for future mechanistic studies, molecular docking, toxicity evaluation, and in vivo antibacterial testing. The findings of this work confirm that chalcone-derived heterocyclic systems continue to offer valuable opportunities for the development of new antibacterial agents to address the growing challenge of antimicrobial resistance.

Conclusion

The present study examined the antibacterial activity of a series of aminoalkyl-substituted benzaldehydes, chalcones, dihydropyrazoline derivatives, and 1,2,3-triazole analogues against Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae. The results clearly showed that antibacterial activity improved step by step as the molecular framework was modified from simple aromatic intermediates to more complex heterocyclic systems. The initial benzaldehyde derivatives (2a–2c and 3) were inactive against all tested bacteria, with IC_{50} values above 50 µg/mL. Introduction of the chalcone moiety produced moderate activity, particularly against S. aureus and K. pneumoniae, indicating that the α,β-unsaturated carbonyl system contributes to antibacterial action. Further cyclization to dihydropyrazoline derivatives led to a significant increase in potency and a broader spectrum of activity. Among these compounds, 8c showed excellent activity against S. aureus, while 8d exhibited consistent inhibition against all three bacterial strains.

The most promising results were obtained with the 1,2,3-triazole derivatives 10a and 10b. Both compounds displayed strong broad-spectrum antibacterial activity, including potent inhibition of E. coli, which had remained largely resistant to the earlier series. Their low IC_{50} values suggest that incorporation of the triazole ring produced an optimal combination of structural and physicochemical properties required for effective antibacterial action. The structure–activity relationship established in this work demonstrates that each synthetic transformation made a meaningful contribution to biological activity. The chalcone scaffold introduced initial antibacterial potential, the pyrazoline ring enhanced potency, and the triazole nucleus provided the highest and most consistent activity. The nature of the attached substituents also played an important role in modulating antibacterial effects.

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