RESEARCH PAPER

Antibacterial Evaluation and Structure–Activity Relationship Studies of Benzothiazole-Conjugated Chalcone and Pyrimidine Derivatives

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ABSTRACT

The growing prevalence and rapid spread of antimicrobial resistance (AMR) represent a serious global health issue, significantly undermining the effectiveness of existing antibiotic therapies[1]. Antibiotic abuse and overuse have hastened the creation of bacterial strains that are resistant, increasing the risk of illness[2]. So, there is a critical need to develop new antibacterial drugs with improved potency and mechanism of action also to be improved[3].In present study, we have synthesized a set of benzothiazole-linked chalcones (CE1–CE10) and benzothiazole-based pyrimidine derivatives (PR1–PR10), which are structurally characterized, were investigated for their antibacterial properties. Using the cup plate method, the antibacterial assessment was conducted against types of bacteria that are both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli).The results demonstrated that many compounds with minimum inhibitory concentration (MIC) values between 50 and 100 µg/mL have strong antibacterial activity.Among these, compounds CE6 and PR6 has exhibited the highest level of activitydemonstrating effects similar to those of the common medication Rifampicin. Electron-withdrawing substituents on the aromatic ring are important in boosting antibacterial activity, according to structure–activity relationship (SAR) studies, and pyrimidine derivatives were more potent than their corresponding chalcone analogues.The study's overall findings demonstrate the potential of benzothiazole-conjugated hybrid molecules as promising scaffolding for the creation of new antibacterial medicines.

Keywords: Benzothiazole, Antibacterial, Sar, Mic

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INTRODUCTION

The efficacy of currently available antibiotics is seriously compromised by antibiotic[1] resistance, which has grown to be a serious threat to world health.

The rapid rise of multidrug-resistant bacterial strains has created an urgent demand for the development of novel therapeutic agents with improved antibacterial efficacy[2,3].

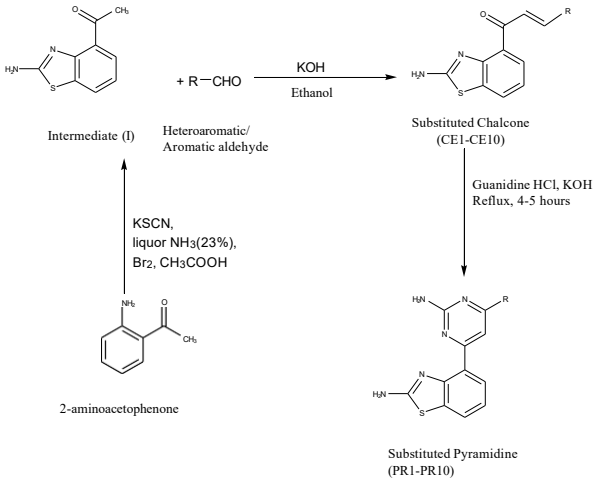
Because of their versatility and wide range of biological activity, heterocyclic molecules are essential to medical chemistry. Among them, benzothiazole derivatives have drawn a lot of interest due to their diverse pharmacological characteristics, which include anti-inflammatory, antibacterial, and anticancer actions. Chalcones[10], which have an α, β-unsaturated carbonyl moiety, are known for their varied biological activities and have been extensively researched for their antimicrobial potential. The benzothiazole framework's interaction with biological systems is improved by the presence of heteroatoms like sulfur and nitrogen, which makes it a crucial scaffold in drug discovery.Furthermore, because of their important biological activity and similarity to naturally occurring nucleic acid bases, pyrimidine derivatives constitute an important family of heterocycles that are frequently encountered in medicinal medicines.

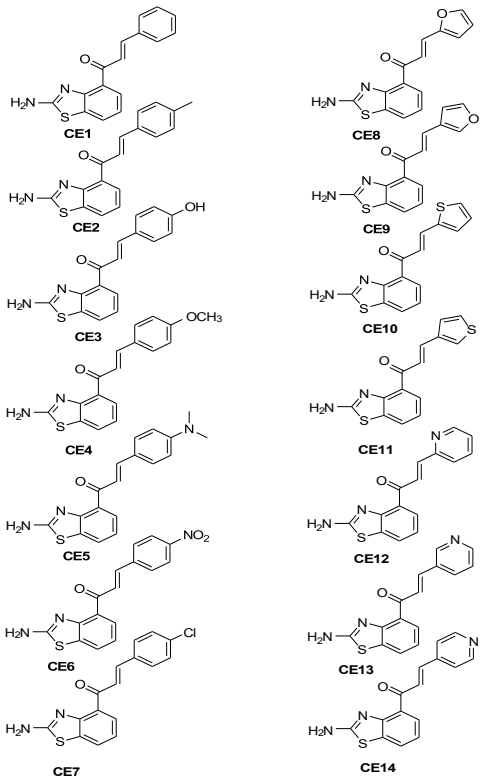
The strategy of pharmacophore hybridization has gained prominence in recent years as an effective tool in drug design, enabling the combination of multiple biologically active units within a single molecular framework to enhance efficacy and selectivity[8]. Despite the established importance of benzothiazole, chalcone, and pyrimidine scaffolds individually, systematic investigations focusing on their combined antibacterial activity and detailed structure–activity relationships are still limited[9].

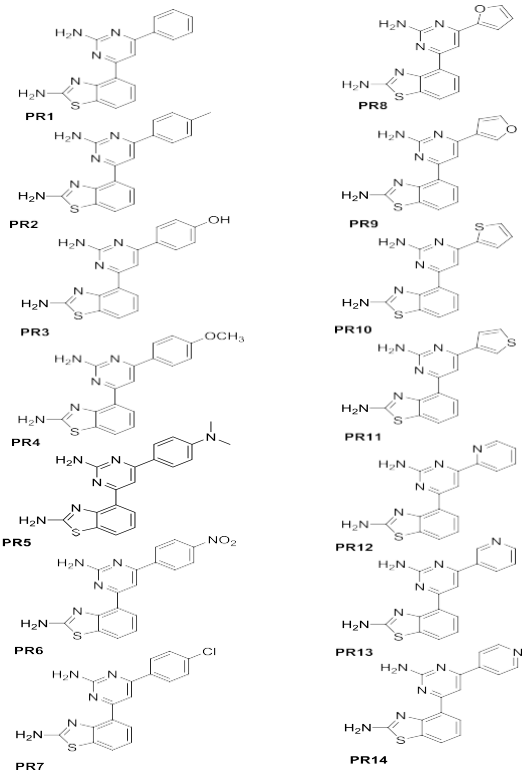
In our previous work, a series of benzothiazole-conjugated chalcones (CE1–CE10) and pyrimidines (PR1–PR10) were synthesized and structurally characterized[11-14]. Building on this, the present study aims to evaluate their antibacterial activity and establish structure–activity relationships to identify potential lead compounds.

MATERIALS AND METHODS

The benzothiazole-conjugated chalcones (CE1–CE10) and pyrimidines (PR1–PR10) were synthesized from the scheme and characterized.







Benzthiazole conjugated chalcones and benzthiazole conjugated pyrimidines were tested for their antibacterial efficacy against harmful bacteria, namely

- Gram positive bacteria, such as Staphylococcus aureus
- Gram-negative bacteria, such as Escherichia coli

1. *Staphylococcus aureus*: These are 1 micron-diameter spherical cocci that are organized in grape-like structures. They can also be seen in rings, pairs, and short chains. They are gram +ve, non-motile, non-sporulating, and non-capsulated. After 24 hours of incubation, they grow easily on nutrient agar media at the ideal temperature of 370C and pH of 7.4 to 7.6.
2. *Escherchia coli*: It is gram-positive, non-capsulated, short, plump bacilli with a diameter of 2 to 4 μ × 0.4 to 0.7 μ, motile, and non-sporulating. It is a facultative and aerobic anaerobe that grows best at 370C on basic media.

Nutrient agar media preparation: Escherchia coli and Staphylococcus aureus use this medium as their basal (solid) medium.

MEDIA COMPOSITION:

- 20 grams of bacteriological peptone
- 5 grams of bacteriological beef extract
- 15 grams of bacteriological sodium chloride
- 20 grams of agar (bacteriological)
- Up to 1 liters of distilled water

Agar is added and dissolved by heating on a water bath after the aforementioned ingredients have been gently warmed in distilled water. Distilled water is used to increase the volume to 1000 ml, and the medium's pH is set to 7.2. The media is then autoclaved under 15 pounds of pressure for 20 minutes at 1210 degrees Celsius to disinfect it.

METHOD EMPLOYED: CUP-PLATE method

Principle: This method's antibiotic potency test is based on the diameter of zones of inhibition surrounding cylinders (cups) containing different dilutions of standard and test compounds that are poured into cups of nutrient agar medium that have previously been inoculated with a culture of an appropriate test organism. The inhibition caused by a standard at a known concentration is compared to the inhibition caused by the test medication.

Standard solution preparation: The standard drug used in this test is rifampicin. Five milliliters of sterile water were used to dissolve five milligrams of rifampicin, resulting in a 1000μg/ml solution. The necessary concentration was then reached by diluting this stock solution.

Test solution preparation: A stock solution of 1000μg/ml was created for the test solution by dissolving one milligram of the drug in one milliliter of DMSO. To achieve the required concentration, this stock solution was further diluted.

Testing procedure: The nutrient agar medium mentioned above is cooled to 450C while being gently shaken to ensure even cooling. This was injected aseptically with 0.5–0.6 ml of an 18–24 hour culture, thoroughly mixed, and gently shaken. The Petri dishes were filled with this material, which was then left to solidify.

After that, cups were created by using a sterile cork borer to punch into the set agar and then scooping off the region that was penetrated.Each cup is eight

millimeters in diameter. Two cups of standard compound and DMSO (control) are added to each Petri plate; the remaining cups are filled with medicinal solution. It was left to diffuse at room temperature for about 45 minutes after the pharmaceutical solution was added. After that, the plates were incubated at 370C for the entire day. The inhibitory zone's diameter was measured in millimeters following a 24-hour period.

Figure: Gram-positive bacterial activity S.a., or Staphylococcus aureus

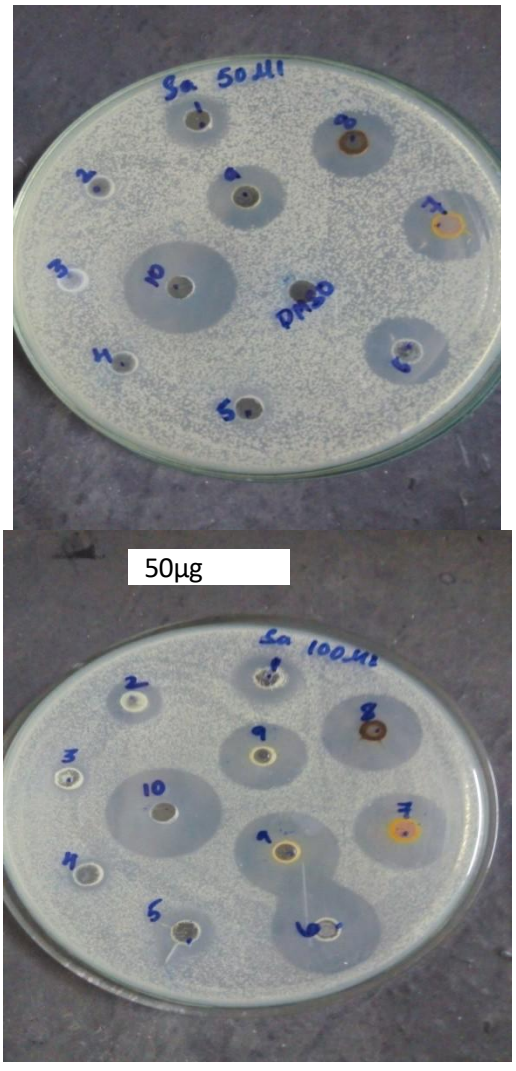
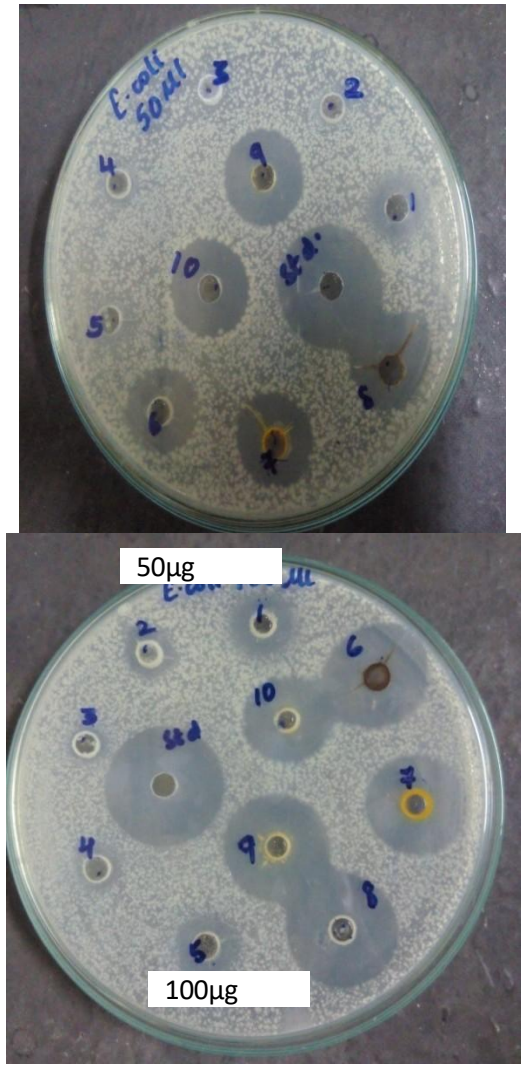


Figure: Activity on gram negative bacteria *Escherichia coli (E.c)*



RESULTS AND DISCUSSION:

S.No	Compounds	Inhibition zone in millimeters			
		Bacterial strains			
		S. aureus (Gram positive)		*E. coli (Gram negative)*	
		100 µg	**50 µg**	**100 µg**	**50 µg**
	CE 1	11	9	10	7
	CE 2	11	5	9	4
	CE 3	14	10	12	9
	CE 4	13	10	10	5
	CE 5	11	8	8	5

	CE 6	20	18	17	13
	CE 7	19	14	15	11
	CE 8	13	11	15	10
	CE 9	13	9	13	9
	CE 10	14	7	13	8
	Rifampacin	20	-	20	-
	Control	-	-	-	-

S.No	**Compounds**	**Inhibition zone in millimeters**			
		Bacterial strains			
		S. aureus (Gram positive)		***E.coli (Gram negative)***	
		100 µg	**50 µg**	**100 µg**	**50 µg**
	PR 1	9	7	10	6
	PR 2	10	6	10	8
	PR 3	18	12	19	15
	PR 4	12	8	10	8
	PR 5	12	8	11	9
	PR 6	20	18	20	17
	PR 7	18	15	18	14
	PR 8	14	9	10	8
	PR 9	15	8	13	9
	PR 10	17	14	18	13
	Rifampacin	20	-	20	-
	Control	-	-	-	-

The in vitro antibacterial activity of benzothiazole-based chalcone (CE1–CE10) and pyrimidine derivatives (PR1–PR10) was evaluated against common strains of Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria. The evaluation, which was based on minimum inhibitory concentration (MIC) and zone of inhibition (ZOI), demonstrated that the compounds' antibacterial activity was significantly influenced by the substituents on the aromatic or heteroaromatic framework.

Within the chalcone class, compounds CE6, CE7, CE8, and CE10 shown comparatively higher antibacterial activity. CE6, which possesses a 4-nitrophenyl group, was the most effective of all, as seen by its lower MIC values and larger zones of inhibition. The activity trend was followed by CE7 (4-chlorophenyl derivative), indicating the advantageous effect of electron-removing substituents. Additionally, heterocyclic derivatives such as CE8 (furan-2-yl) and CE10 (thiophen-2-yl) had moderate to noteworthy activity, suggesting that heteroaromatic substitution promotes favorable interactions with bacterial targets. Conversely, compounds like CE5, CE2, and CE1 that have unsubstituted phenyl rings or electron-donating substituents showed relatively less antibacterial activity.

When compared to the chalcone analogues, the pyrimidine derivatives showed a significant increase in antibacterial activity.The most active compounds in this series were identified as PR3, PR6, PR7, PR8, and PR10. Interestingly, PR3—which contained an extra benzothiazole unit—showed the best activity, underscoring the importance of integrating several pharmacophores into a single molecular scaffold. The substantial antibacterial actions of PR6 (4-nitrophenyl) and PR7 (4-chlorophenyl) derivatives further support the idea that electron-withdrawing groups increase activity. Compounds like PR5, PR2, and PR1 only showed moderate benefits, but heterocyclic derivatives like PR8 (furan-2-yl) and PR10 (thiophen-2-yl) also demonstrated good efficacy. Because of their poor spatial orientation and decreased interaction with the biological target, PR9 and other derivatives with 3-position heterocycles were the least active molecules.

Research on the structure–activity relationship (SAR) has shown that adding electron-withdrawing substituents, such nitro and chloro groups, significantly boosts antibacterial activity.Compounds CE6 and PR6 (–NO_2) and CE7 and PR7 (–Cl), which showed increased potency, showed this tendency the most. Stronger interactions with bacterial

biomolecules may be made possible by the enhanced electrophilic nature.

On the other hand, reduced antibacterial activity was caused by the presence of electron-donating groups, such as methoxy and dimethylamino substituents (CE4, CE5, PR4, PR5). Reduced electrophilicity and reduced binding affinity are probably the causes of this drop. The action of unsubstituted phenyl compounds (CE1, PR1) was likewise only mild.

The impact of heterocyclic substitution on antibacterial effectiveness was also clearly seen. In comparison to their corresponding 3-position analogues (e.g., CE9, PR9), compounds with 2-position heterocycles (e.g., CE8, CE10, PR8, and PR10) showed superior activity, indicating that substitution at the 2-position provides a more advantageous orientation for interaction with the target site.

Moreover, a significant increase in antibacterial activity resulted from the conversion of chalcone frameworks into pyrimidine derivatives. This improvement could be explained by the pyrimidine ring's greater planarity and structural rigidity, which promote better binding affinity and stronger molecular interactions.

Pyrimidine series: PR3 (4-OHC_6H_4)> (4-$NO_2C_6H_4$)> PR7 (4-ClC_6H_4)> PR 10 (Thiophen-2-yl)> PR8(Furan-2-yl) > PR4 (4-$OMeC_6H_4$)>PR5 (4-$NMe_2C_6H_4$)> PR2 (4-MeC_6H_4)>PR1 (C_6H_5)> PR9 (Furan-3-yl).

Chalcone series: CE6 (4-$NO_2C_6H_4$) > CE7 (4-ClC_6H_4) > CE10(Thiophen-2-yl) > CE8(Furan-2-yl) > CE3(4-OHC_6H_4) > CE4 (4-$OMeC_6H_4$) > CE9 (Furan-3-yl) > CE2 (4-MeC_6H_4) > CE1 (C_6H_5)> CE5(4-$NMe_2C_6H_4$)

CONCLUSION:

This study investigated the antibacterial activity of many pyrimidine derivatives (PR1–PR10) and benzothiazole-linked chalcones (CE1–CE10) against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains. The results demonstrated that some compounds with minimum inhibitory concentration (MIC) values between 50 and 100 µg/mL have moderate to significant antibacterial activity.

The greatest antibacterial activity was shown by CE6 and PR6, two of the produced compounds having electron-withdrawing nitro substituents, highlighting the significance of substituent characteristics in influencing biological response. Additionally, comparative investigation revealed that pyrimidine derivatives typically have higher antibacterial efficacy than their chalcone counterparts, indicating that chalcones' activity is increased when they are cyclized into pyrimidine scaffolds.

The structure-based activity relationship (SAR) research revealed that while electron-donating groups often decrease activity, electron-withdrawing groups—especially nitro and chloro substituents—significantly increase antibacterial efficacy. Furthermore, it was discovered that the placement of substituents and the inclusion of heterocyclic moieties were crucial elements controlling the compounds' overall biological performance.

In conclusion, the study identifies hybrid structures based on benzothiazoles as viable options for the creation of novel antibacterial drugs. To confirm their potential and improve their pharmacological characteristics, more research is necessary, including mechanistic studies, molecular docking analysis, and in vivo assessments.

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