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Design, Antimicrobial Evaluation, and Molecular Docking Studies of Benzimidazole, Benzothiazole, and Benzoxazole Derivatives Targeting DNA Gyrase B and CYP51

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A B S T R A C T

The increasing prevalence of antimicrobial resistance necessitates the development of novel therapeutic agents with improved efficacy and distinct mechanisms of action. In this study, a series of benzimidazole, benzothiazole, and benzoxazole derivatives were evaluated for their antibacterial and antifungal potential using a combined in vitro and in silico approach. The synthesized compounds were screened against Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, and fungal strains *Candida albicans* and *Aspergillus niger* using the agar well dilution method. Several derivatives exhibited significant antimicrobial activity, with benzimidazole-based compounds demonstrating superior potency compared to the other heterocyclic scaffolds. Molecular docking studies were performed to investigate the binding interactions of the synthesized compounds with DNA Gyrase B (PDB ID: 6F86) and Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z), key targets involved in bacterial DNA replication and fungal ergosterol biosynthesis, respectively. The docking results revealed favorable binding affinities and interaction pro les for the top-ranked compounds, comparable to standard reference drugs Ciprofloxacin and Fluconazole. Structure activity relationship analysis indicated that electron-donating substituents, particularly amino and methoxy groups, enhance antimicrobial efficacy through improved target interactions. Overall, the combined experimental and computational findings highlight benzimidazole derivatives as promising lead candidates for further antimicrobial drug development.

Introduction

The rapid emergence of antimicrobial resistance (AMR) has become a major global public health concern, severely compromising the effectiveness of existing antibacterial and antifungal therapies (16, 12). Pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and opportunistic fungal species including *Candida albicans* and *Aspergillus niger* have developed resistance mechanisms against commonly used drugs, leading to increased morbidity, mortality, and healthcare costs (14). Consequently, there is an urgent

need to identify and develop new antimicrobial agents with novel scaffolds and improved mechanisms of action. Heterocyclic compounds represent a cornerstone of medicinal chemistry due to their diverse pharmacological properties and structural versatility (6). Among them, benzimidazole, benzothiazole, and benzoxazole frameworks have attracted considerable attention owing to their broad spectrum of biological activities, including antibacterial, antifungal, anticancer, antiviral, and anti-inflammatory effects (15, 1). In particular, the benzimidazole nucleus is considered a privileged scaffold, as it closely resembles purine bases and readily participates in hydrogen bonding and π π interactions with biological targets (3). Several benzimidazole-based derivatives have been reported to inhibit key microbial enzymes, such as DNA Gyrase B in bacteria and Sterol 14-α-demethylase (CYP51) in fungi, both of which play essential roles in DNA replication and ergosterol biosynthesis, respectively (10, 11). DNA Gyrase B is a validated antibacterial target involved in ATP- dependent DNA supercoiling, and its inhibition results in impaired bacterial proliferation (4). Similarly, CYP51 is a well-established antifungal target, and its inhibition disrupts fungal cell membrane integrity, leading to cell death (8). The clinical success of drugs such as Ciprofloxacin and Fluconazole further validates these enzymes as attractive molecular targets.

In recent years, the integration of in vitro biological screening with in silico molecular docking approaches has become a powerful strategy for accelerating drug discovery (9). Molecular docking enables the prediction of ligand protein interactions at the atomic level, offering valuable insights into binding affinity, interaction patterns, and structure activity relationships (SAR). When combined with experimental antimicrobial evaluation, docking studies can effectively guide lead optimization and rational drug design.

In this context, the present study focuses on the synthesis and evaluation of a series of benzimidazole, benzothiazole, and benzoxazole derivatives with diverse substituents. The synthesized compounds were assessed for their in vitro antibacterial and antifungal activities against clinically relevant bacterial and fungal strains. Furthermore, molecular docking studies were performed against DNA Gyrase B (PDB ID: 6F86) and Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z) to elucidate binding modes, interaction profiles, and potential mechanisms of antimicrobial action. The combined experimental and computational approach provides a comprehensive understanding of the antimicrobial potential of these heterocyclic scaffolds and highlights promising lead compounds for further development.

Materials and Methods

Synthesized Compounds

All chemicals and solvents were of analytical grade and procured from commercial suppliers (Sigma-Aldrich, Merck) and were used without further puri cation. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel G plates, and spots were visualized under UV light. Melting points were determined using the open capillary method and are reported uncorrected. The synthesized benzimidazole, benzothiazole, and benzoxazole derivatives evaluated in this study are listed in Table 1.

Antimicrobial Activity Assay

The in vitro antimicrobial activity of the synthesized compounds was evaluated using the agar well diffusion method. The test organisms included two bacterial strains,

Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923), as well as two fungal strains, *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404).

Antibacterial Assay

Nutrient agar (NA) medium was prepared and sterilized by autoclaving at 121◦C for 15 min. Bacterial suspensions equivalent to the 0.5 McFarland standard (1.5 × 108 CFU/mL) were uniformly inoculated onto the agar plates. Wells of 6 mm diameter were aseptically punched using a sterile cork borer. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 100 µg/mL, and 100 µL of each solution was introduced into the respective wells. Ciprofloxacin was used as the standard antibacterial drug. Plates were incubated at 37◦C for 24 h, and antibacterial activity was assessed by measuring the zone of inhibition (ZOI) in millimeters (mm).

Table.1 List of synthesized benzimidazole, benzothiazole, and benzoxazole derivatives used for antimicrobial and in silico studies along with their corresponding bio- logical entry IDs.

Entry Code	IUPAC Name	Biological Entry ID
Benzimidazole Series		
Benzimidazole-1	2-Phenyl-1H -benzo(d)imidazole	**270**
Benzimidazole-2	2-(4-Methoxyphenyl)-1H -benzo(d)imidazole	**269**
Benzimidazole-3	2-(4-Chlorophenyl)-1H -benzo(d)imidazole	**271**
Benzimidazole-4	4-(1H -Benzo(d)imidazol-2-yl)phenol	**272**
Benzimidazole-5	2-(4-Nitrophenyl)-1H -benzo(d)imidazole	**273**
Benzimidazole-6	4-(1H -Benzo(d)imidazol-2-yl)aniline	**274**
Benzimidazole-7	2-Phenyl benzimidazole-5-sulfonic acid	**254**
Benzothiazole Series		
Benzothiazole-1	2-Phenylbenzo(d)thiazole	**260**
Benzothiazole-2	2-(4-Methoxyphenyl)benzo(d)thiazole	**255**
Benzothiazole-3	2-(4-Chlorophenyl)benzo(d)thiazole	**259**
Benzothiazole-4	4-(Benzo(d)thiazol-2-yl)phenol	**258**
Benzothiazole-5	2-(4-Nitrophenyl)benzo(d)thiazole	**257**
Benzothiazole-6	4-(Benzo(d)thiazol-2-yl)aniline	**261**
Benzothiazole-7	2-(Benzo(d)thiazol-2-yl)phenol	**256**
Benzoxazole Series		
Benzoxazole-1	2-Phenylbenzo(d)oxazole	**265**
Benzoxazole-2	2-(4-Methoxyphenyl)benzo(d)oxazole	**262**
Benzoxazole-3	2-(4-Chlorophenyl)benzo(d)oxazole	**263**
Benzoxazole-4	4-(Benzo(d)oxazol-2-yl)phenol	**264**
Benzoxazole-5	2-(4-Nitrophenyl)benzo(d)oxazole	**267**
Benzoxazole-6	4-(Benzo(d)oxazol-2-yl)aniline	**268**
Benzoxazole-7	**2-(Benzo(d)oxazol-2-yl)phenol**	**266**

Table.2 Grid box parameters used for AutoDock Vina docking studies against DNA Gyrase B and CYP51.

Target Protein	PDB ID	Center Coordinates ( )			Grid Size ( )	
		X	Y	Z	Dimensions	Spacing
DNA Gyrase B	6F86	63.256	30.246	63.374	18.2 ×16.9 ×22.9	**1.0**
CYP51	**5V5Z**	**-38.662**	**-13.830**	**24.225**	**27.4 ×29.6 ×26.7**	**1.0**

Results and Discussion

Antibacterial and Antifungal Activity

The synthesized derivatives belonging to the benzimidazole (1 7), benzothiazole (1 7), and benzoxazole (1 7) series were evaluated for their in vitro antimicrobial activity against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus*, and two fungal strains, *Candida albicans* and *Aspergillus niger*. Antimicrobial efficacy was qualitatively assessed using the agar well diffusion method by measuring the zone of inhibition (ZOI), and the results are summarized as a heatmap in Figure 1.

The biological screening revealed a clear dependence of antimicrobial activity on the nature of the heterocyclic scaffold. Overall, the benzimidazole derivatives exhibited superior antimicrobial potency compared to the corresponding benzothiazole and benzoxazole analogues. This enhanced activity may be attributed to the presence of the imidazole N H moiety, which can act as an effective hydrogen bond donor during interactions with microbial target enzymes.

In terms of antibacterial activity, Gram-negative *E. coli* was generally more susceptible to the synthesized compounds than Gram-positive *S. aureus*. Among all tested deriva- tives, Benzimidazole-6, bearing a para-amino substituent, emerged as the most potent antibacterial agent, exhibiting ZOI values of 30 mm against *E. coli* and 22 mm against S. aureus. The presence of the electron-donating amino group (−NH2) likely enhances bind- ing interactions with bacterial DNA Gyrase B, a trend further supported by molecular docking studies. In contrast, compounds containing strong electron-withdrawing substituents, such as nitro (Benzimidazole-5) and sulfonic acid (Benzimidazole-7) groups, displayed comparatively reduced antibacterial activity.

Regarding antifungal activity, several compounds demonstrated pronounced inhibitory effects, particularly against *C. albicans*. Benzimidazole-2, substituted with a para-methoxy group, exhibited the highest antifungal activity with a ZOI of 38 mm, followed by Benzimidazole-3 (para-chloro substituted), which showed a ZOI of 35 mm. Although ben- zothiazole and benzoxazole derivatives were generally less potent, their amino-substituted analogues, Benzothiazole-6 and Benzoxazole-6, displayed selective and notable activity against A. niger (30 mm), suggesting that the presence of an amino group can partially compensate for the absence of the benzimidazole core in certain fungal targets.

Figure.1 Heatmap representation of antimicrobial activity expressed as zone of inhibition (mm) for synthesized benzimidazole, benzothiazole, and benzoxazole derivatives against selected bacterial and fungal strains

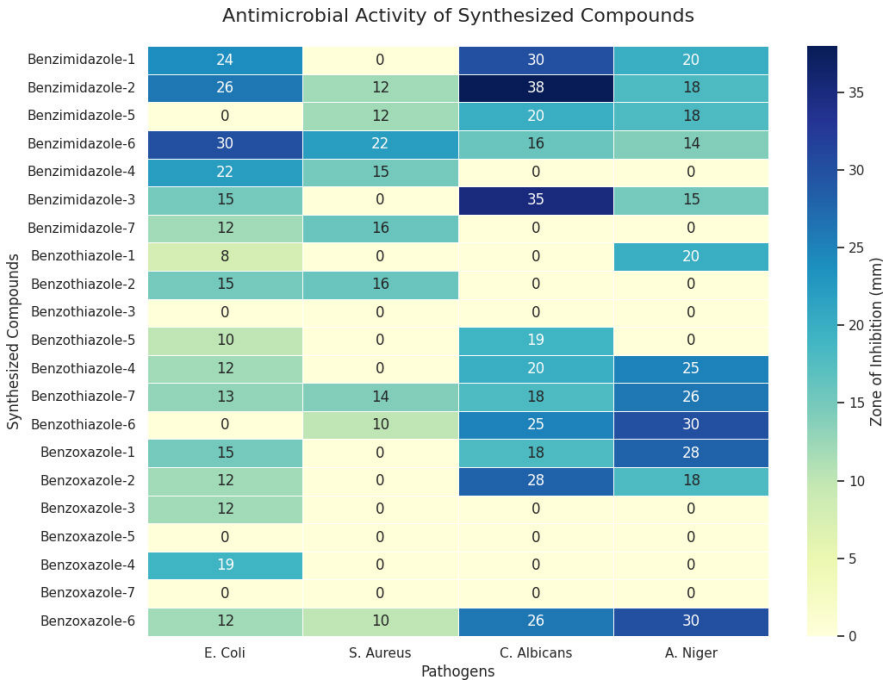


Figure.2 Representative agar well diffusion assay images illustrating the antibacterial and antifungal activity of selected benzimidazole, benzothiazole, and benzoxazole derivatives against *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, and *Staphylococcus aureus*. Compound codes correspond to biological activity numbering, while DMSO was used as a negative control.

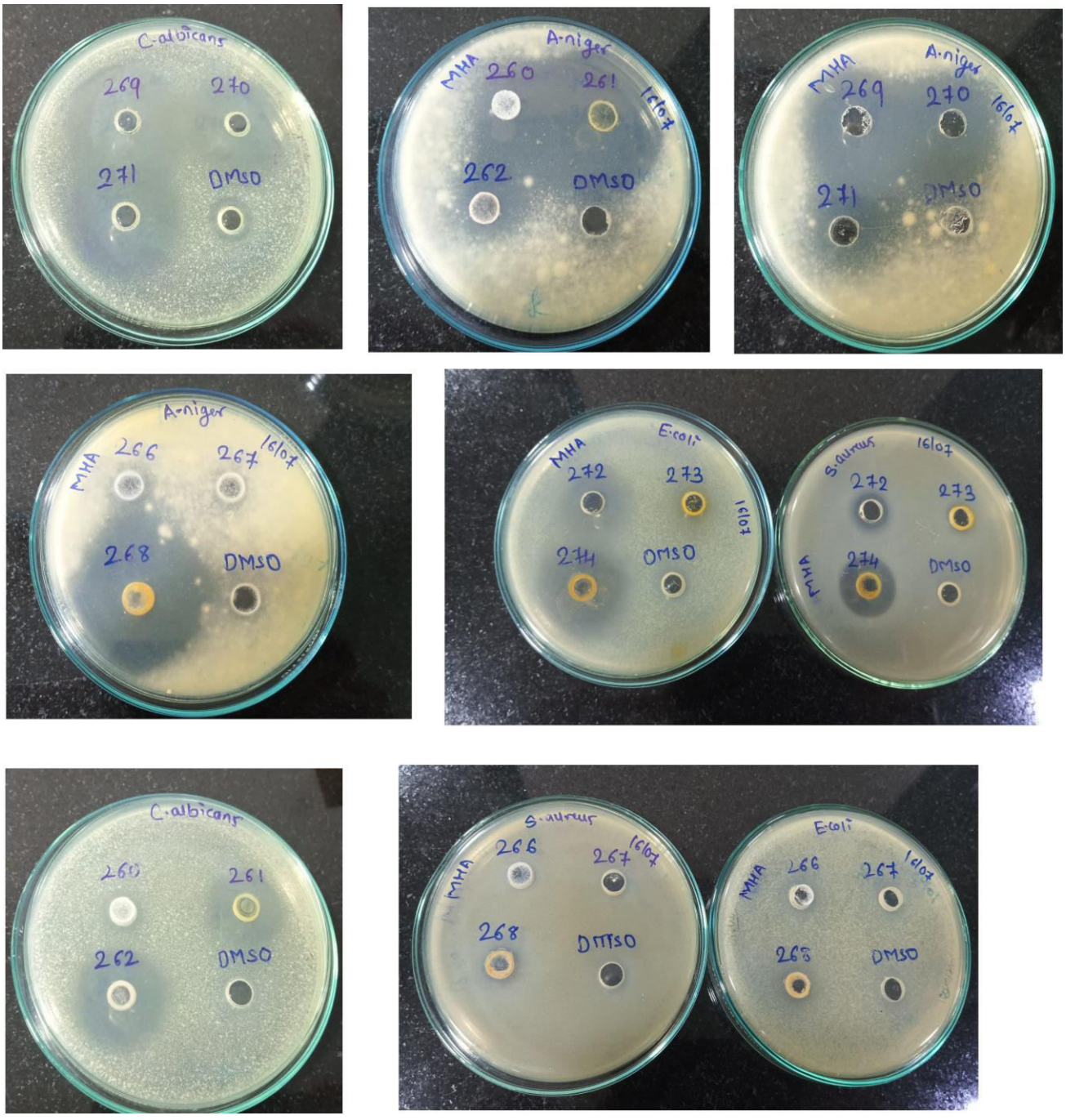


Figure.3 Quantitative analysis of predicted binding pocket properties for the antifungal target CYP51 (5V5Z, top row) and the antibacterial target DNA Gyrase B (6F86, bottom row). Pocket 1 (Rank 1) exhibits the highest pocket score, druggability score, and cavity volume, supporting its selection as the primary docking site. The corresponding positive control using two-dimensional interaction maps and three-dimensional binding conformations.

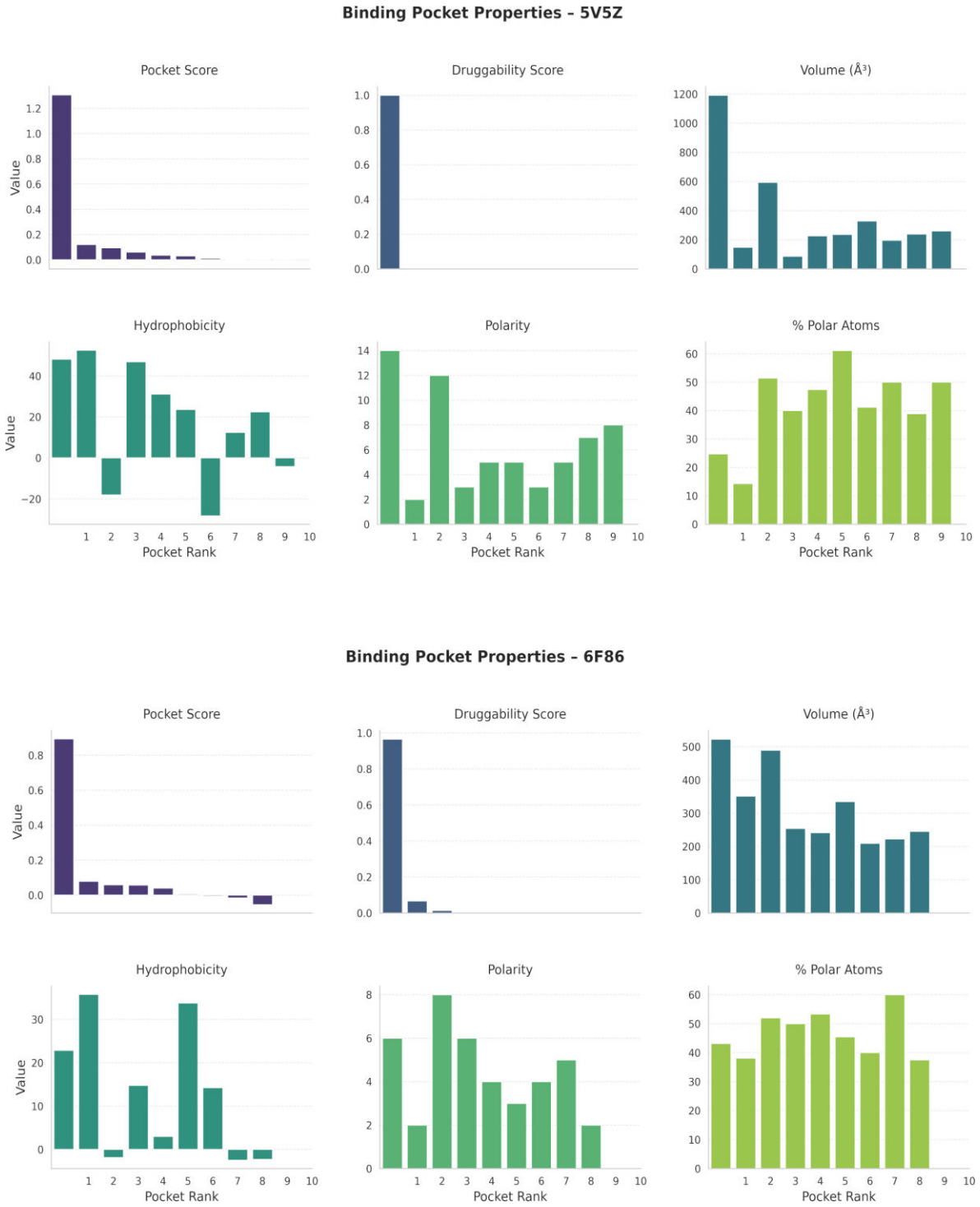
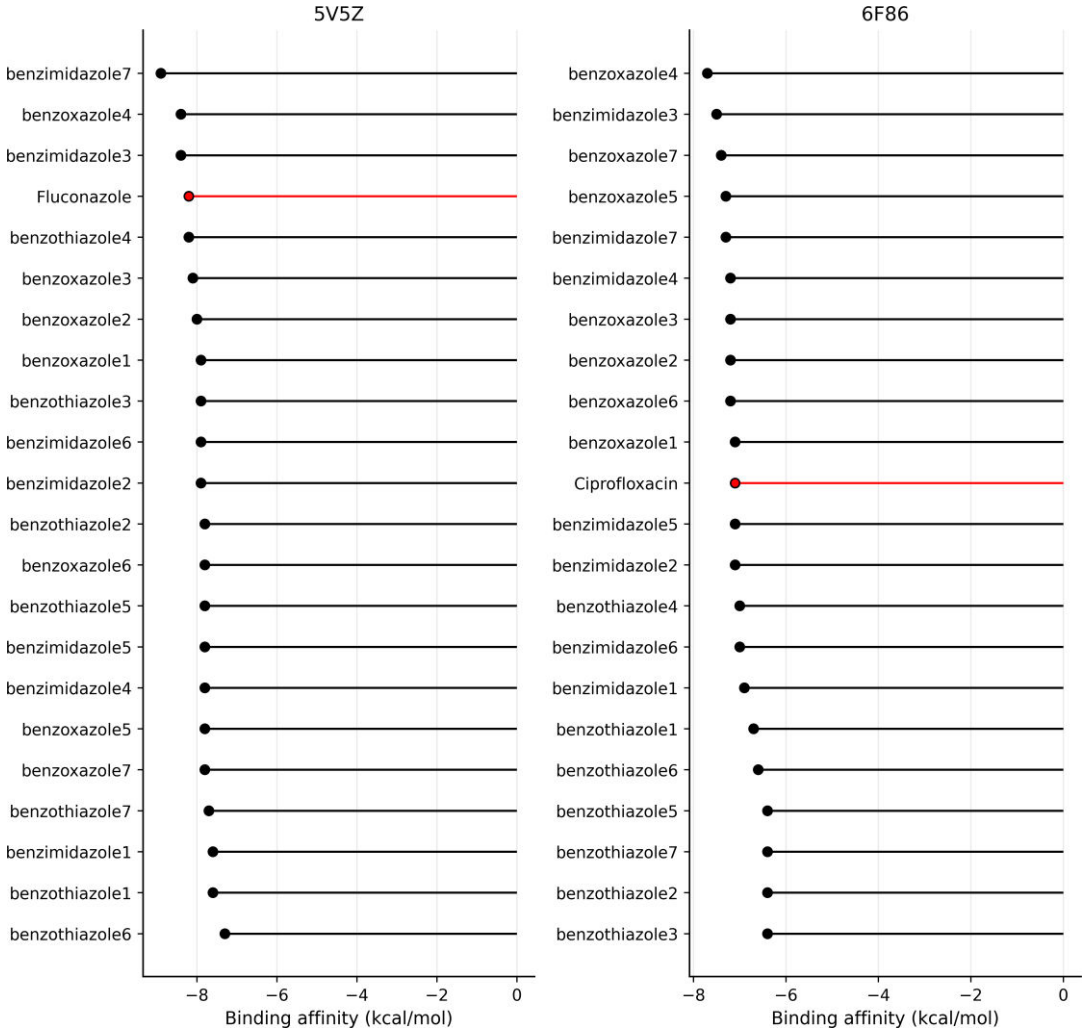


Figure.4 Binding affinity distribution of synthesized compounds against the antifungal target CYP51 (5V5Z) and the antibacterial target DNA Gyrase B (6F86). Red markers indicate the binding energies of the respective positive controls. site, suggesting a competitive binding mechanism

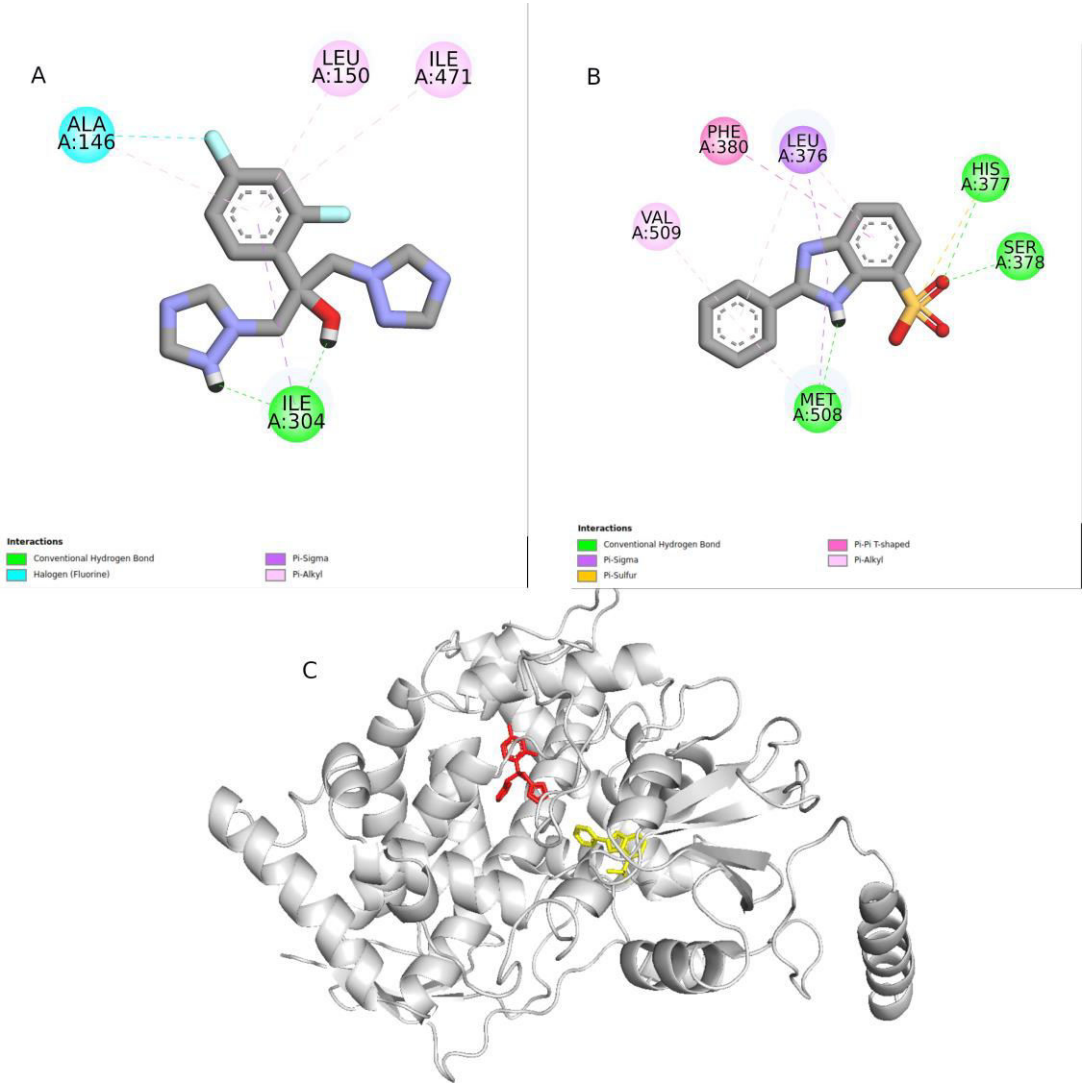


Overall, the structure activity relationship (SAR) analysis indicates that electron- donating substituents, particularly −OCH3 and −NH2, at the para-position of the phenyl ring significantly enhance antimicrobial activity. Among the investigated scaffolds, the benzimidazole core emerged as the most promising pharmacophore for further structural optimization and development of potent antimicrobial agents.

Active Site Prediction and Pocket Selection

The fpocket topology-based cavity detection algorithm was employed to identify potential drug-binding sites on the surface of DNA Gyrase B (PDB ID: 6F86) and Sterol 14- α-demethylase (CYP51, PDB ID: 5V5Z). The detected pockets were ranked based on key physicochemical descriptors, including pocket volume, hydrophobicity, polarity, and overall druggability score. As shown in Figure 3, Pocket 1 emerged as the top-ranked cavity for both target proteins, exhibiting the highest druggability scores (approaching 1.0) and favorable geometric features for small-molecule binding. In the case of CYP51 (5V5Z), Pocket 1 displayed a large cavity volume (> 10003) and pronounced hydrophobic character, consistent with the structural requirements of the heme-cofactor binding region. Likewise, for DNA Gyrase B (6F86), Pocket 1 corresponded to the known catalytic site and demonstrated superior druggability compared to secondary and auxiliary pockets.

Figure.5 Molecular docking interaction analysis with Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z). (A) 2D interaction map of Fluconazole (positive control), (B) 2D interaction map of the top-ranked compound Benzimidazole-7, and (C) superimposed 3D binding poses of Fluconazole (red) and Benzimidazole-7 (yellow) within the active site for the investigated heterocyclic scaffolds.



Based on these findings, the geometric centroid of Pocket 1 was selected as the reference point for molecular docking simulations. The corresponding grid box center co- ordinates (x, y, z) and dimensions used for docking were defined accordingly and are summarized in Table 2.

Molecular Docking Analysis and Interaction Pro ling

Molecular docking simulations were carried out to investigate the binding affinity and interaction patterns of the synthesized compounds against Sterol 14-α-demethylase (CYP51, PDB ID: 5V5Z) and DNA Gyrase B (PDB ID: 6F86). Binding energies (kcal/mol) were calculated for all ligands and compared with the respective positive controls, Fluconazole and Ciprofloxacin. The distribution of docking scores for both targets is illustrated in Figure 4.

Several synthesized derivatives exhibited binding energies comparable to or better than the reference

drugs, indicating favorable interactions within the active sites of both proteins. For each target, the top-ranked ligand was selected based on the lowest binding energy and a stable interaction pro le and was further analyzed

Against CYP51 (5V5Z), the positive control Fluconazole (Figure 5A) demonstrated stable binding through a key conventional hydrogen bond with ILE A:304, supported by multiple π alkyl and π sigma interactions with hydrophobic residues including ALA A:146, LEU A:150, and ILE A:471. The top-ranked synthesized compound, Benzimidazole- 7 (Figure 5B), exhibited a comparable binding orientation within the same catalytic pocket. It formed strong hydrogen bond interactions with HIS A:377 and SER A:378, along with π π T-shaped, π alkyl, and π sulfur interactions involving PHE A:380, LEU A:376, VAL A:509, and MET A:508, respectively. Superposition of the docked poses (Figure 5C) confirmed that both ligands occupy overlapping regions of the CYP51 active.

Similarly, docking analysis against DNA Gyrase B (6F86) revealed that Ciprofloxacin (Figure 6A) binds effectively within the ATP-binding pocket through multiple hydrogen bonds and hydrophobic interactions. The top-ranked synthesized compound (Figure 6B) adopted a comparable binding orientation and was stabilized by conventional hydrogen bonds and π alkyl interactions with key active-site residues. The superimposed 3D bind- ing poses (Figure 6C) demonstrated close spatial overlap between the lead compound and Ciprofloxacin, supporting efficient accommodation within the catalytic cavity.

Overall, the docking results and interaction pro ling indicate that the top-ranked synthesized compounds bind efficiently within the same active-site regions as their respective positive controls. These findings correlate well with the observed in vitro antimicrobial activity and further validate CYP51 and DNA Gyrase B as relevant molecular targets.

In Conclusion, the present study, a series of benzimidazole, benzothiazole, and benzoxazole derivatives were successfully evaluated for their antimicrobial potential through a combined in vitro and in silico approach. The biological screening demonstrated that the benzimidazole scaffold exhibited superior antibacterial and antifungal activity compared to the benzothiazole and benzoxazole analogues, with specific derivatives showing notable potency against *Escherichia coli* and *Candida albicans*. Structure activity relationship (SAR) analysis highlighted the critical role of electron-donating substituents, particularly amino and methoxy groups, in enhancing antimicrobial efficacy.

Molecular docking studies against DNA Gyrase B and Sterol 14-α-demethylase (CYP51) provided mechanistic insights into ligand receptor interactions and corroborated the experimental findings. The top-ranked compounds displayed favorable binding affinities and interaction pro les comparable to standard reference drugs, occupying the same catalytic regions of the target proteins. These results validate the selected heterocyclic frameworks as promising pharmacophores for antimicrobial drug development.

Despite the encouraging outcomes, further investigations are warranted to strengthen the therapeutic potential of the identified lead compounds. Advanced computational studies, including molecular dynamics simulations and binding free energy calculations, are necessary to assess the stability of ligand protein complexes under physiological conditions. Additionally, receptor-based biochemical assays and enzyme inhibition studies are essential to experimentally con rm target engagement and elucidate the precise mechanisms of action. Collectively, these future studies will facilitate rational lead optimization and support the development of potent and selective antimicrobial agents based on these heterocyclic scaffolds.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

Dhiraj Sawant: Investigation, analysis, writing original draft, Pranali Sonawane: Methodology, investigation, Ratnamala P. Sonawane: Conceptualization, methodology, writing, Investigation, analysis,

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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