Computational Insights into Thiazole Derivatives as Dual Antifungal and Antibacterial Agents: Docking Studies on Key Cellular Targets

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Abstract-The study explores the molecular docking of thiazole derivatives to assess their potential as antifungal and antibacterial agents. Using computational methods, the researchers modeled the interaction of various thiazole derivatives with key bacterial and fungal enzymes. The results showed strong binding affinities with target microbial proteins, suggesting their efficacy as antimicrobial agents. These findings could help develop novel treatments for resistant bacterial and fungal infections by synthesizing and in vitro testing thiazole-based compounds. The ongoing threat of fungal and bacterial infections necessitates the development of novel antimicrobial agents. The methodology employed computational techniques, specifically molecular docking simulations using AutoDock Vina software. The thiazole derivative was chosen based on its structural features and preliminary antimicrobial activity data. The compound's three-dimensional structure was optimized and prepared for docking studies, using multiple crystal structures of key enzymes crucial for fungal and bacterial viability as receptor models. In silico docking experiments were conducted to evaluate the binding affinity and mode of interaction between the thiazole derivative and its respective enzyme target. The computational analysis provided insights into the structural features of the thiazole derivative that contribute to its antimicrobial efficacy, with specific functional groups and molecular motifs identified as critical for enhancing binding affinity and specificity to the target enzymes. The thiazole derivative exhibited comparable or superior binding affinities to existing antifungal and antibacterial drugs, highlighting its potential as a lead candidate for further development.

Keywords: AutoDock Vina, Antimicrobial efficacy, Computational methods, Docking simulations, Thiazole derivatives.

INTRODUCTION

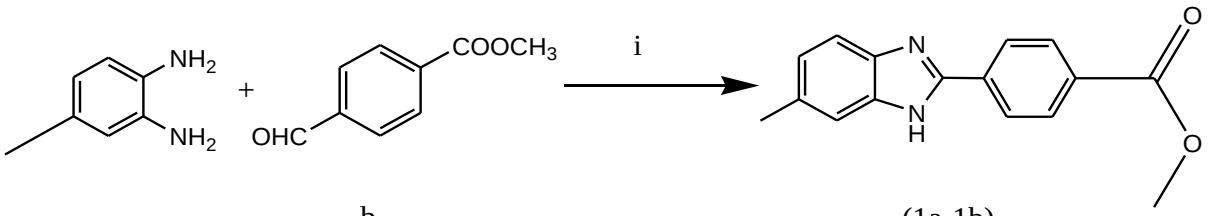
Molecular docking plays a crucial role in the rational design and optimization of pharmaceutical agents, particularly in the field of antifungal drug discovery. Thiazole derivatives have emerged as promising candidates due to their diverse biological activities, including potent antifungal properties. Understanding the molecular interactions between these derivatives and their target proteins is essential for elucidating their mechanism of action and for guiding structure-based drug design efforts. In this study, we employ molecular docking techniques to investigate the binding modes and energetics of a novel thiazole derivative against key fungal protein targets. By computationally predicting the binding conformations and affinity of the thiazole derivative within the active site of fungal enzymes or receptors, we aim to gain insights into its potential as an antifungal agent. This approach allows us to explore the structural features responsible for the observed biological activity and to optimize the chemical structure for enhanced efficacy and selectivity. (1)

The findings from this molecular docking study not only contribute to our understanding of the molecular basis of thiazole derivative interactions with fungal targets but also provide valuable information for the development of novel antifungal agents with improved therapeutic profiles. Such computational studies bridge the gap between chemical structure and biological activity, paving the way for the rational design of next-generation antifungal drugs with enhanced potency and reduced side effects. (2)

RESULT

(I)Methyl 4-(5(6)-substituted-1H-benzimidazol-2-yl) benzoate derivatives (1a–1b) were synthesized by reacting methyl 4-formylbenzoate with various o-phenylenediamines in the presence of Na2S2O5 (i). This two-step procedure involves the condensation of

o-phenylenediamines with aromatic aldehydes, forming Schiff base intermediates that are then cyclodehydrogenated oxidatively to yield the benzimidazole products. Na2S2O5 was chosen as the oxidizing agent due to its reported effectiveness in achieving high yields in similar reactions according to previous studies. (3,4)

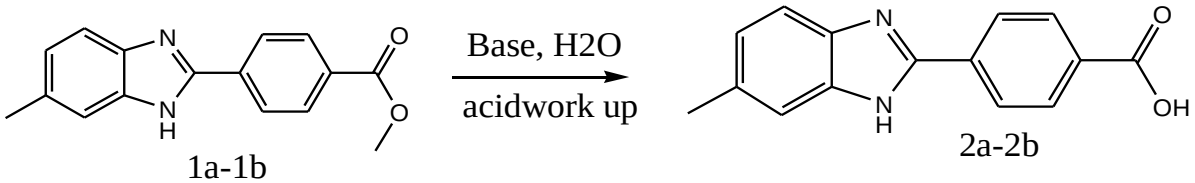


a b (1a-1b)

(II) Esters are hydrolyzed either by aqueous base or acid to yield carboxylic acids plus alcohol. In base-catalyzed hydrolysis (saponification), a hydroxide ion attacks the ester carbonyl to produce a tetrahedral alkoxide intermediate. Elimination of the alkoxide ion generates the carboxylic acid, which is immediately deprotonated by the alkoxide ion. An acid work-up restores the carboxylic acid.

Acid-catalyzed hydrolysis can occur by more than one mechanism, depending on the structure of the ester. In a reverse Fisher esterification, the carbonyl group is protonated by the acid, which activates it for nucleophilic attack by water, yielding a tetrahedral intermediate. Transfer of a proton and elimination of alcohol yields the carboxylic acid.

- Reagents: Acid or Base
- Reactant: Ester
- Product: Alcohol, Carboxylic Acid
- Type of Reaction: Nucleophilic Acyl Substitution
- Bond Formation: RCO_2H (5)



1a-1b 2a-2b

(III)The reaction where an alcohol is converted to an alkyl chloride in the presence of thionyl chloride (SOCl2) (ii) at 70°C is known as the thionyl chloride reaction. This process involves the substitution of the hydroxyl group (-OH) of the alcohol with a chlorine atom (Cl), resulting in the formation of an alkyl chloride. The overall reaction can be summarized as follows:

Mechanism:

1. Formation of Intermediate Complex*: The alcohol (R-OH) reacts with thionyl chloride (SOCl2) to form an intermediate complex where the hydroxyl group is replaced by a chlorosulfite group (ROSOCl).
2. *Chlorosulfite Intermediate*: This intermediate is relatively unstable and decomposes to form the alkyl chloride (R-Cl), releasing sulfur dioxide (SO2) and hydrogen chloride (HCl) gases as by-products.

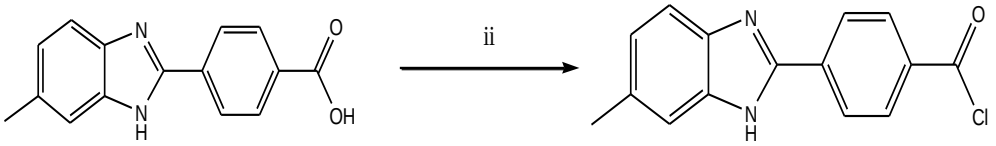
Conditions:

- *Temperature*: The reaction is typically carried out at 70°C, which provides the necessary energy for the reaction to proceed efficiently.
- *Catalyst*: Sometimes, a base such as pyridine is added to the reaction to neutralize the hydrogen chloride formed and drive the reaction to completion.

#Characteristics:

By-products: The reaction produces two gaseous by-products, SO2 and HCl, which can be removed from the reaction mixture easily.

- *Efficiency*: This method is highly efficient for converting primary and secondary alcohols to alkyl chlorides. However, it may be less effective for tertiary alcohols due to steric hindrance.
- *Mild Conditions*: The reaction generally proceeds under mild conditions without the need for extreme temperatures or pressures. (6)



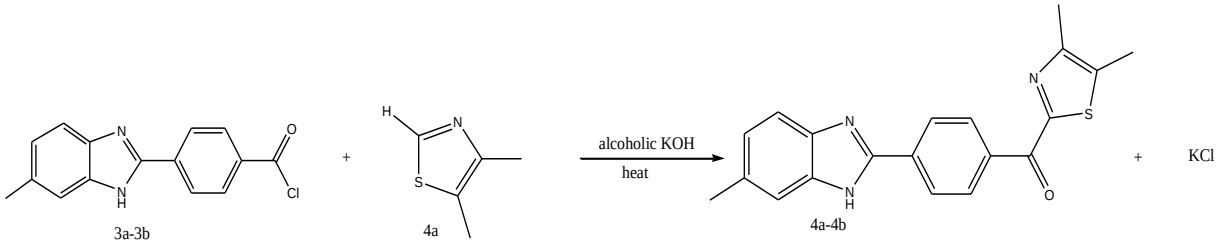
2a-2b 3a-3b

(IV)The reaction of removing a chlorine atom from an alkyl chloride in the presence of alcoholic potassium hydroxide, also known as the β-elimination reaction or dehydrohalogenation, typically results in the formation of an alkene. Here's a possible reaction:

R-Cl + KOH (alc.) -> R-H + KCl + H2O

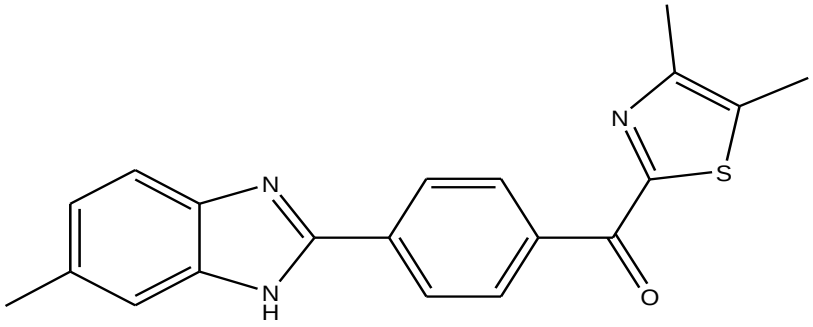
This reaction is a classic example of nucleophilic substitution. The alcoholic KOH serves as a strong base, abstracting a proton from the β-carbon (the carbon adjacent to the carbon bearing the halogen). The resulting carbanion then expels the leaving group (chloride ion) to form the alkene.

If you're looking for a reaction byproduct or a side product, it would be potassium chloride (KCl). This reaction is widely used in organic synthesis to prepare alkenes from alkyl halides.



3a-3b 4a 4a-4b

The reaction given the actual product 4a-4b which showing therapeutic effect in front of fungal and bacteria. Fomation of thiazole derivative make this structure as combinational effective. (7,8)



(4,5-dimethyl-1,3-thiazol-2-yl)[4-(6-methyl-1H-1,3-benzimidazole-2-yl) phenyl] Methanone

This the thiazole derivative showing the action as antibacterial as well as antifungal activity. Thiazole increase therapeutic index. All the structure and reaction are drawn in chemdraw 3D and chemsketch software and name detected by chemsketch.

- Thiazole ring (4,5-dimethyl-1,3-thiazol-2-yl):
- Thiazole rings are found in many biologically active compounds and can influence the electronic properties and shape of the molecule.
- Substituents on the thiazole ring (such as the methyl groups in this case) can affect the lipophilicity, electronic distribution, and potentially the interaction with biological targets.
- Benzimidazole ring (5-methyl-1H-1,3-benzimidazol-2-yl):
- Benzimidazole derivatives are known for their diverse biological activities, including antimicrobial, antiviral, and anticancer properties.
- The position and nature of substitutions on the benzimidazole ring (here, a methyl group at position 5) can significantly influence the biological activity. For instance, substitutions can

affect the hydrogen bonding capacity, lipophilicity, and receptor binding affinity.

- Phenyl group (4-(5-methyl-1H-1,3-benzimidazol-2-yl) phenyl):
- Phenyl rings are common in pharmaceuticals and can modulate the electronic properties, steric effects, and hydrophobic interactions of a compound.
- Substitutions on the phenyl ring can alter the receptor specificity, potency, and metabolic stability of the molecule.

Methanone group (attached to the thiazole ring):

- The methanone group can influence the molecular conformation and possibly participate in hydrogen bonding or electrostatic interactions with biological targets.

MOLECULAR DOCKING STUDIES:

This study aimed to provide a good simulation of what happens during the protein ligand binding. Docking was conducted to predict the activity of tested compound on various targets. Antibacterial agents most common mechanism includes destroying cell wall and membrane integrity, inhibiting protein expression, reducing nucleic acid synthesis and affecting bacteria's energy metabolism on (pdb:6Y1M and 6E2P) homosapiens enzyme with interaction between the thaizole derivative as a ligand. The docking studies score indicate that low free energy of binding indicat strong ligand-enzyme binding, with all tested compound showing low binding energy.

Preprocess Files:

- Ensure that both the ligand (thiazole derivative) and the protein are prepared appropriately. This involves adding polar hydrogen atoms, assigning charges, and removing water molecules and any unwanted heteroatoms.

Define Binding Site:

- Identify the binding site or active site of the protein where the thiazole derivative is expected to bind. This can be done by analyzing the protein structure, ligand-binding assays, or by using computational tools to predict binding sites.

Docking Parameters Setup:

- Set up parameters such as search space dimensions, grid spacing, and docking algorithms in the docking software. These parameters may vary depending on the software being used.

Perform Docking:

Run the docking simulation using the prepared ligand and protein files and the defined parameters. This step will generate multiple binding poses (conformations) of the thiazole derivative within the binding site of the protein.

Scoring and Analysis:

- Evaluate the binding poses based on scoring functions provided by the docking software. Scoring functions assess the binding affinity and predict the most probable binding mode of the ligand.
- Analyze the interactions between the thiazole derivative and the protein residues to understand the key interactions stabilizing the complex. (9,10,11)

MODE	AFFINITY (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd v.b
1	-8.5	0.000	0.000
2	-7.9	3.921	11.510
3	-7.7	2.160	2.713
4	-7.3	2.562	3.397
5	-7.1	7.890	10.334
6	-7.1	3.883	10.691
7	-7.0	6.660	9.584
8	-7.0	2.564	2.974
9	-6.9	10.401	12.382

Table .1 Docking score for 6Y1M (PDB) enzyme.

Theoretically all the nine-mode showed very good binding energy and docking energy ranging from-8.5kJmol $^{-1}$, -7.9kJmol $^{-1}$, 7.71kJmol $^{-1}$, -7.3kJmol $^{-1}$, -7.1kJmol^{-1}, -7.0kJmol-1, -6.9kJmol^{-1} respectively.

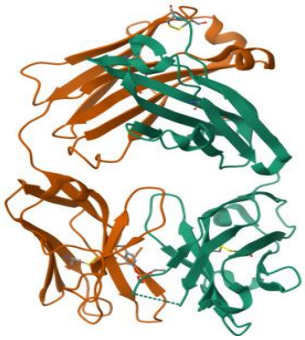


Fig.1 Crystal structure of the paraoxon-modified A.17 antibody FAB fragment - L47K mutant

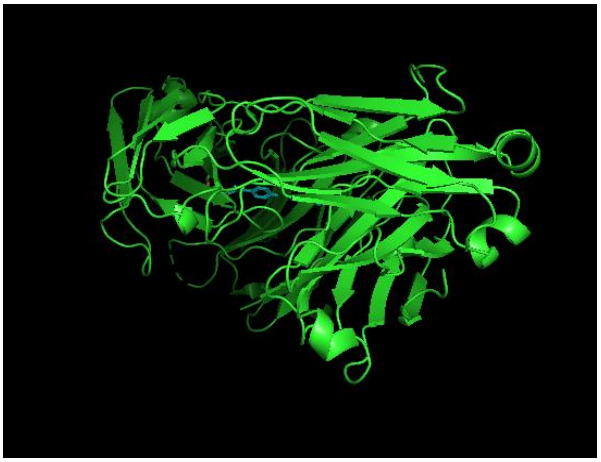


Fig.2Interaction of 4a-4b with A.17 antibody FAB fragment - L47K mutant

MODE	AFFINITY (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd v.b
1	-7.2	0.000	0.000
2	-7.1	17.720	18.822
3	-6.9	19.629	22.874
4	-6.9	18.212	19.349
5	-6.8	19.037	20.133
6	-6.6	23.961	25.555
7	-6.6	22.097	23.805
8	-6.6	19.753	22.418
9	-6.5	18.279	20.625

Table .2 Docking score for 6E2P (PDB) enzyme.

Theoretically all the nine-mode showed very good binding energy and docking energy ranging from- 7.2kJmol $^{-1}$, -7.1kJmol $^{-1}$, 7.71kJmol $^{-1}$, -6.9kJmol $^{-1}$, -6.8kJmol^{-1}, 6.6kJmol-1, 6.5kJmol^{-1} respectively

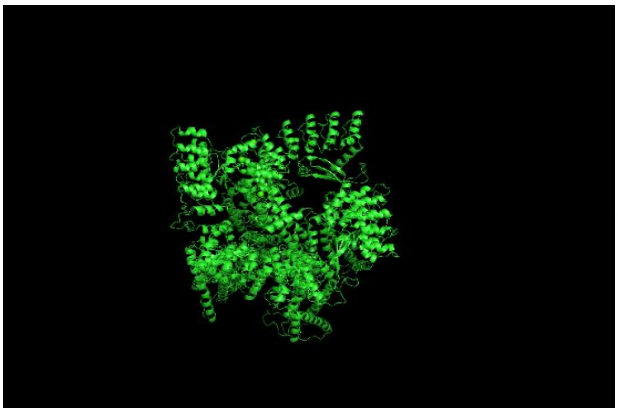


Fig.3Interaction of 4a-4b with JAK2 FERM/SH2

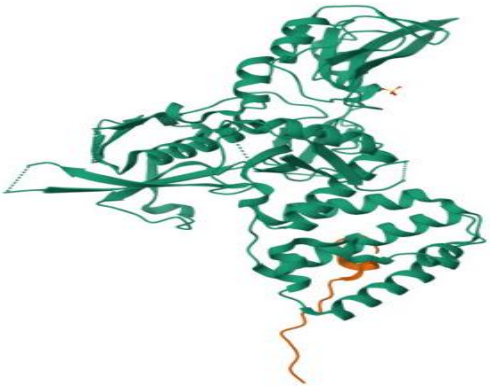


Fig.4 Structure of human JAK2 FERM/SH2 in complex with Leptin Receptor

MODE	AFFINITY (kcal/mol)	Distance from best mode rmsd l.b	Distance from best mode rmsd v.b
1	-7.6	0.000	0.000
2	-6.6	27.036	28.686
3	-6.6	2.431	9.485
4	-6.5	25.072	26.270
5	-6.4	7.456	12.844
6	-6.3	2.839	9.340

7	-6.2	7.605	8.968
8	-6.1	2.050	3.230
9	-6.1	17.321	20.131

Table .3 Docking score for 1JXA (PDB) enzyme.

Theoretically all the nine-mode showed very good binding energy and docking energy ranging from-7.6kJmol $^{-1}$, -6.6kJmol $^{-1}$, -6.4kJmol $^{-1}$, -6.3kJmol $^{-1}$, -6.2kJmol^{-1}, 6.1kJmol-1 respectively

In vitro antimicrobial results suggest the need for in silico studies to confirm the in vitro activity. Automated docking was used to determine inhibitors' orientation in the active site of GlcN-6-P synthase (PDB:1jxa), using a Lamarckian genetic algorithm method in Auto Dock 3.0. The docking of ligand molecules 4a-4b revealed all inhibitor compounds are effective. (12)

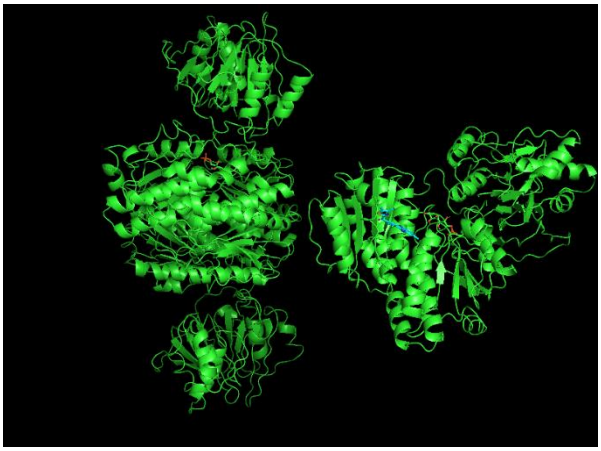


Fig.5Interaction of 4a-4b with GlcN-6-P.

Antifungal activity:

The study screened antifungal activity of newly prepared compounds using a serial plate dilution method. Sabourands agar media was prepared by dissolving peptone, D-glucose, and agar in distilled water and adjusting the pH to 5.7. A suspension of spores from fungal strains was prepared, and a 20 ml agar media was added to each petridish. Excess suspension was decanted and dried in an incubator at 37 C for 1 hour. Agar punch wells were made and 10 mg/ml of the tested compounds in DMSO were added to each well. The activity of each compound was compared with Amphotericin B as a standard. (13)

- Ergosterol Biosynthesis: Fungi rely on ergosterol for membrane stability. Inhibiting enzymes like lanosterol 14-alpha demethylase (target of azole antifungals) disrupts ergosterol synthesis, weakening fungal membranes.
- Chitin Synthesis: Fungi produce chitin for cell walls. Inhibiting chitin synthase enzymes prevents wall formation, impeding fungal growth.
- Cell Membrane Ion Channels: Targeting specific ion channels (e.g., calcium and potassium channels) disrupts cellular homeostasis, leading to fungal cell death. (14)

Antibacterial activity:

The screening of newly synthesized compounds for antibacterial activity against several bacterial strains. Among these compounds, 3a-3b displayed significant activity against all tested microorganisms, with 4c showing moderate activity and 4a and 4b exhibiting none. The presence of thiazole moieties in compounds 4a and 4b is suggested to contribute to their enhanced antibacterial activity, potentially comparable to known antimicrobial drugs like sulfthaizole and 2-aminothiazole acid. However, the passage cautions against premature conclusions about the structure-activity relationship of these compounds, emphasizing the need for further evaluation before considering them for clinical use. Overall, compounds 4a and 4b hold promise as antibacterial agents, but additional research is required to fully understand their potential and optimize their efficacy. (15,16)

- Cell Wall Synthesis: Inhibiting enzymes like penicillin-binding proteins (PBPs) disrupts bacterial cell wall formation, leading to cell lysis.
- DNA Gyrase and Topoisomerases: Targeting enzymes involved in DNA replication and repair (e.g., DNA gyrase, topoisomerases) disrupts bacterial DNA metabolism.
- Metabolic Pathways: Blocking key enzymes in metabolic pathways (e.g., dihydrofolate reductase, ribosomal proteins) inhibits bacterial growth and reproduction. (17)

ADME DETECTION:

Physicochemical Properties	
Formula	C20H17N3OS
Molecular weight	347.43 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	20
Fraction Csp3	0.15
Num. rotatable bonds	3
Num. H-bond acceptors	3
Num. H-bond donors	1
Molar Refractivity	101.97
TPSA	86.88 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.24
Log $P_{o/w}$ (XLOGP3)	5.02
Log $P_{o/w}$ (WLOGP)	4.84
Log $P_{o/w}$ (MLOGP)	2.53
Log $P_{o/w}$ (SILICOS-IT)	6.56
Consensus Log $P_{o/w}$	4.44

Water Solubility	
Log *S* (ESOL)	-5.55
Solubility	9.78e-04 mg/ml; 2.81e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-6.58
Solubility	9.04e-05 mg/ml; 2.60e-07 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-7.91
Solubility	4.24e-06 mg/ml; 1.22e-08 mol/l
Class	Poorly soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes

CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-4.86 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	2.95

CONCLUSION

This study highlights the importance of molecular docking in drug discovery and development. It explains the antimicrobial activity of a thiazole derivative by examining its interactions with target enzymes. Future research will validate these findings through in vitro and in vivo experiments, assessing the compound's efficacy, safety profile, and clinical application. The study highlights the thiazole derivative's potential as a dual-action antifungal and antibacterial agent, highlighting the need for further research to address multidrug-resistant pathogens in clinical settings. The thiazole derivative's competitive potential in antimicrobial drug discovery is evident through its dual-action capability against fungi and bacteria, making it a promising candidate for tackling multidrug-resistant pathogens, as demonstrated by docking results.

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