

In silico evaluation of brazilin (*Caesalpinia sappan* L.) as a DprE1 *Mycobacterium tuberculosis* inhibitor

Evaluasi In Silico Brazilin (Caesalpinia sappan L.) sebagai Inhibitor DprE1 Mycobacterium tuberculosis

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ABSTRACT

Background: The increasing incidence of drug-resistant *Mycobacterium tuberculosis* (Mtb) necessitates the identification of novel molecular targets and therapeutic candidates. Decaprenylphosphoryl- β -D-ribose 2'-epimerase subunit 1 (DprE1) is a key enzyme in Mtb cell wall biosynthesis and represents a promising therapeutic target. Brazilin, a natural compound derived from *Caesalpinia sappan* L., has been reported to exhibit diverse biological activities; however, its interaction with DprE1 remains insufficiently explored, highlighting a gap for computational-based investigation.

Objective: To evaluate the binding affinity and molecular interaction profile of brazilin against DprE1 through an in silico approach.

Methods: Molecular docking was performed using the crystal structure of DprE1 (PDB ID: 5OEP) with Molegro Virtual Docker. Key parameters included an active-site grid radius of 15 Å, grid resolution of 0.30 Å, and 10 independent docking runs per ligand to ensure reproducibility. Method validation was performed via redocking of the native ligand, yielding an RMSD <2.0 Å. Binding affinity and interaction patterns of brazilin were compared with rifampicin, isoniazid, and the native ligand.

Results: Brazilin exhibited a rerank score of -71.287, indicating moderate binding affinity, stronger than isoniazid but lower than rifampicin and the native ligand. Notably, brazilin consistently occupied the catalytic binding pocket and established hydrogen bonding and hydrophobic interactions with key residues, suggesting a stable and functionally relevant binding orientation. This represents the first target-specific computational evidence linking brazilin to DprE1 inhibition.

Conclusion: Brazilin from *Caesalpinia sappan* L. shows potential as a natural DprE1 of *Mycobacterium tuberculosis* inhibitor, although further experimental validation is required to confirm its biological efficacy.

Keywords: Brazilin, DprE1, drug-resistant tuberculosis, molecular docking

ABSTRAK

Latar Belakang: Peningkatan kasus *Mycobacterium tuberculosis* (Mtb) yang resisten terhadap obat mendorong perlunya identifikasi target molekuler dan kandidat terapi baru. Decaprenylphosphoryl- β -D-ribose 2'-epimerase subunit 1 (DprE1) merupakan enzim kunci dalam biosintesis dinding sel Mtb dan target terapeutik yang menjanjikan. Brazilin dari *Caesalpinia sappan* L. diketahui memiliki berbagai aktivitas biologis, namun kajian interaksinya dengan DprE1 masih terbatas, sehingga membuka peluang penelitian berbasis komputasional.

Tujuan: Mengevaluasi afinitas ikatan dan pola interaksi molekuler brazilin terhadap DprE1 secara in silico.

Metode: *Molecular docking* dilakukan menggunakan struktur kristal DprE1 (PDB ID: 5OEP) dengan Molegro Virtual Docker. Parameter utama meliputi radius grid sisi aktif

sebesar 15 Å, resolusi grid 0,30 Å, serta 10 kali *docking run independen* untuk menjamin reproduksibilitas hasil. Validasi metode dilakukan melalui redocking ligan asli dengan nilai RMSD <2,0 Å. Afinitas ikatan dan pola interaksi brazilin dibandingkan dengan rifampisin, isoniazid, dan ligan asli.

Hasil: Brazilin menunjukkan nilai rerank score sebesar -71,287 yang mengindikasikan afinitas ikatan moderat, lebih kuat dibandingkan isoniazid namun lebih rendah dibandingkan rifampisin dan ligan asli. Secara analitik, brazilin secara konsisten menempati kantong katalitik dan membentuk ikatan hidrogen serta interaksi hidrofobik dengan residu kunci, yang mengindikasikan orientasi ikatan yang stabil dan relevan secara fungsional. Temuan ini merupakan bukti komputasional pertama yang mengaitkan brazilin secara spesifik dengan potensi inhibisi DprE1.

Kesimpulan: Brazilin dari *Caesalpinia sappan L.* berpotensi sebagai inhibitor alami DprE1 *Mycobacterium tuberculosis*, namun masih diperlukan validasi eksperimental lebih lanjut untuk mengonfirmasi aktivitas biologisnya.

Kata kunci: Brazilin, DprE1, drug-resistant tuberculosis, molecular docking

INTRODUCTION

Tuberculosis (TB) remains a major global health challenge, largely driven by the persistence of *Mycobacterium tuberculosis* (Mtb) and the rapid emergence of drug-resistant strains [1], [2]. Despite the availability of first-line therapies such as isoniazid and rifampicin, treatment outcomes are often compromised by prolonged therapeutic regimens, adverse drug reactions, and poor patient adherence [3], [4]. These challenges are further exacerbated by the increasing prevalence of multidrug-resistant TB (MDR-TB), which significantly limits the effectiveness of existing treatment strategies. Consequently, there is a critical need to identify novel therapeutic agents that target essential bacterial pathways with improved efficacy and safety profiles [5], [6].

Among the validated molecular targets in antituberculosis drug discovery, decaprenylphosphoryl-β-D-ribose 2'-epimerase subunit 1 (DprE1) has emerged as a key enzyme involved in the biosynthesis of arabinogalactan, an essential component of the mycobacterial cell wall [7], [8]. Disruption of this pathway compromises cell wall integrity and ultimately leads to bacterial death. Recent advances in drug discovery have demonstrated that DprE1 inhibition is a highly effective strategy, with several synthetic compounds, particularly benzothiazinones and their derivatives, exhibiting potent antimycobacterial activity [9], [10]. These findings underscore the strategic importance of DprE1 as a target for structure-based drug design and highlight its relevance in the development of next-generation antituberculosis agents [11], [12].

Natural products continue to represent a valuable source of chemically diverse bioactive compounds for drug discovery. *Caesalpinia sappan L.* is a medicinal plant known for its rich phenolic content, including brazilin, which has been reported to possess antibacterial, antioxidant, and anti-inflammatory activities [13], [14]. Previous studies have primarily focused on evaluating the general antibacterial effects of brazilin or its pharmacological properties in non-target-specific contexts [15]. However, despite these promising bioactivities, its interaction with validated molecular targets in Mtb, particularly DprE1, has not been systematically investigated using structure-based computational approaches. This lack of target-specific mechanistic insight represents a significant gap, especially in the context of rational drug discovery strategies [16].

To address this gap, the present study employs a structure-based computational biotechnology approach to evaluate the binding affinity and molecular interaction profile of brazilin against DprE1 [17]. Molecular docking simulations were performed using a validated protein structure and systematically compared with established antituberculosis drugs and a co-crystallized reference ligand. By integrating quantitative

binding analysis with detailed interaction profiling, this study aims to provide mechanistic insights into the potential inhibitory activity of brazilin at the molecular level [18] [19]. The findings offer pre-experimental evidence to support the positioning of brazilin as a promising natural lead compound and contribute to the rational prioritization of candidates for subsequent in vitro and in vivo validation.

METHODS

Study design

This study was designed as a structure-based computational biotechnology investigation employing molecular docking simulations to evaluate ligand–enzyme interactions. The workflow comprised target protein selection, protein and ligand preparation, docking protocol validation, docking simulation, and comparative binding affinity analysis. Molecular docking was selected as an established in silico method for early-stage screening and prioritization of bioactive compounds, as supported by previous studies demonstrating its reliability in predicting ligand–protein binding modes and relative affinities. This study was conducted as a pre-experimental in silico screening without in vitro or in vivo validation [20]. Docking results were interpreted cautiously due to inherent scoring limitations and approximations in the scoring function. The study was carried out from April to June 2025 at Poltekkes Kemenkes Surabaya, Indonesia.

Data Source and Sampling Procedure

The three-dimensional crystal structure of *Mycobacterium tuberculosis* dpre1 in complex with an inhibitor (PDB ID: 5OEP) was retrieved from the RCSB Protein Data Bank. The selected structure had a resolution of ≤ 2.5 Å and contained a co-crystallized ligand, enabling active site identification and docking validation. The ligands analyzed included brazilin as the test compound, rifampicin and isoniazid as standard first-line anti-tuberculosis drugs, and the co-crystallized ligand 9T2_502[E] as the native reference ligand.

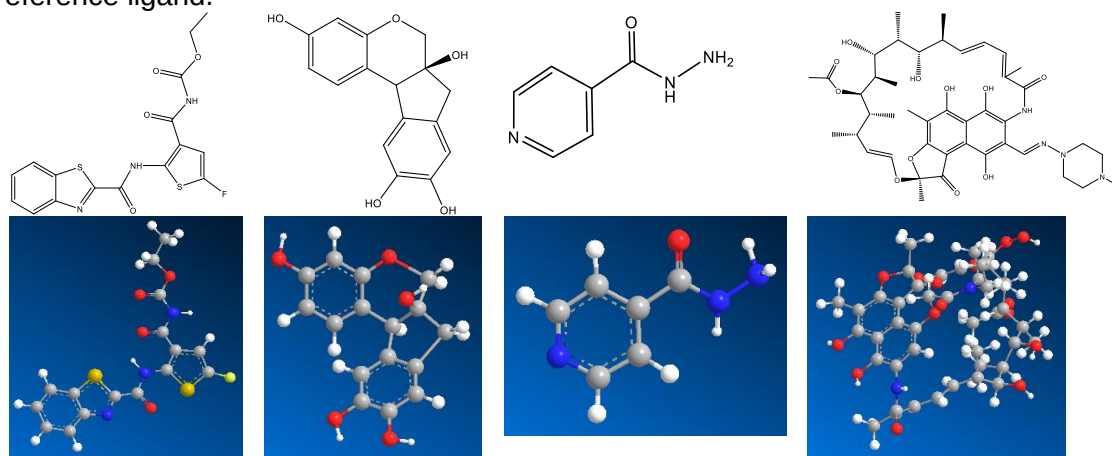


Figure 1. Two-dimensional and three-dimensional optimized structures of 9T2_502[E], brazilin, isoniazid, and rifampicin.

Variables of the Study

The independent variable was ligand type, consisting of brazilin, rifampicin, isoniazid, and 9T2_502[E]. The dependent variables were predicted binding affinity and molecular interactions with the dpre1 active site.

Data Collection

Target Protein Preparation

Protein preparation was performed using Molegro Virtual Docker (MVD) on Windows 10. Water molecules and non-essential heteroatoms were removed, while the co-crystallized ligand was retained for validation. Polar hydrogen atoms were

added, protonation states were assigned at physiological pH (~7.4), and partial charges were generated using default MVD settings.

Ligand Preparation

Brazilin was constructed in two-dimensional form using ChemDraw Professional 16.0 and converted into a three-dimensional structure using Chem3D 16.0. Energy minimization was performed using the MMFF94 force field. All ligands were prepared using the same protocol, including hydrogen addition, protonation adjustment, Gasteiger charge assignment, and optimization into SYBYL2 (*.mol2) format.

Measurement and instrument

Docking Protocol Validation

The docking protocol was validated through redocking of the co-crystallized ligand (9T2_502[E]) into the DprE1 active site. The predicted binding pose was compared with the crystallographic conformation, and the root-mean-square deviation (RMSD) was calculated. An RMSD value ≤ 2.0 Å was considered acceptable, indicating reliable reproduction of the native binding mode.

In addition to RMSD validation, the docking protocol was supported by literature evidence demonstrating that MVD provides consistent and accurate docking predictions for enzyme–ligand systems, reinforcing the robustness of the applied methodology [20]

Molecular Docking Procedure

Docking simulations were performed using Molegro Virtual Docker version 6.0. The binding cavity was defined based on the co-crystallized ligand position, with a grid resolution of 0.30 Å, a radius of 15 Å, and coordinates centered on the native binding site.

Docking parameters included: maximum iterations = 1500, population size = 50, number of runs = 10 per ligand, and maximum poses returned = 5. A fixed random seed was applied to ensure reproducibility. Each ligand was docked independently under identical conditions.

Ethical Considerations

This study did not involve human participants, animals, or biological samples. Therefore, ethical approval was not required. All analyses were conducted using publicly available structural data.

Data analysis

Binding Affinity and Interaction Analysis

Binding affinity was evaluated using the rerank score generated by MVD, where more negative values indicate stronger predicted binding. The binding affinity of brazilin was compared with reference ligands and the native ligand as an internal control. A ligand was considered to have good binding potential if its rerank score approached that of the native ligand.

Molecular interaction analysis was conducted using Discovery Studio Visualizer and PyMOL to identify hydrogen bonds, hydrophobic interactions, and steric contacts between ligands and key amino acid residues within the DprE1 active site. The docking pose of each ligand was also visually compared with the native ligand to assess spatial consistency.

Data Interpretation, Reproducibility, and Limitations

Docking results were interpreted as preliminary molecular evidence of ligand–target interaction. Reproducibility was assessed by evaluating the consistency of binding scores and poses across 10 independent runs, with low variation indicating stable convergence.

However, this study acknowledges several limitations inherent to molecular docking, including simplified scoring functions, limited treatment of protein flexibility, and the absence of solvent dynamics. Therefore, the results should be considered predictive rather than definitive. Further validation through molecular dynamics simulations, binding free energy calculations, and experimental assays (*in vitro* and *in vivo*) is recommended to confirm the biological relevance of the findings.

RESULTS

Ligand–DprE1 Docking Outcomes

Molecular docking simulations were successfully performed for brazilin and all reference ligands using the DprE1 crystal structure (PDB ID: 5OEP). The docking protocol generated reproducible binding poses across three independent runs for each ligand, indicating methodological stability.

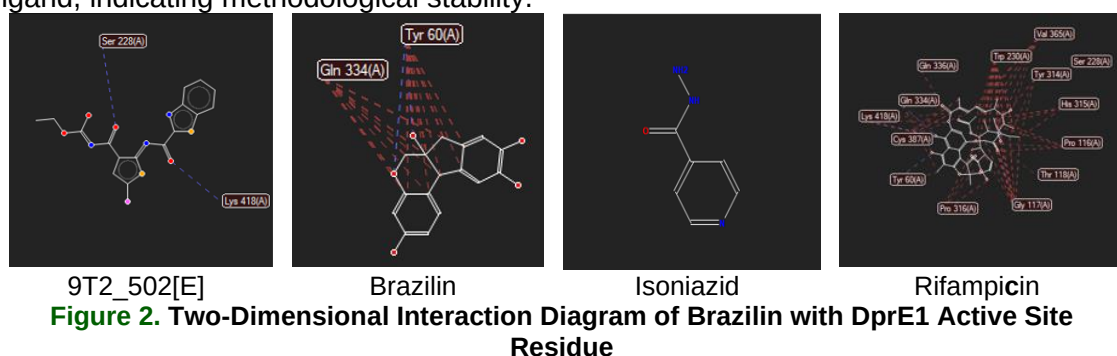


Table 1. Ligand Interactions with Amino Acid Residues of DprE1 from Docking Simulation.

Ligand	Hydrogen bonds	Van der Waals interactions
9T2_502[E]	Ser 228(A) Lys 418(A)	Ser 228(A) Lys 418(A)
Brazilin	Tyr 60(A)	Tyr 60(A) Gln 334(A)
Isoniazid	No significant interactions detected	No significant interactions detected
Rifampicin	Tyr 335(A) Gln 334(A) Gly 17(A) Lys 418(A)	Tyr 335(A) Leu 333(A) Val 388(A) Gln 334(A) Gln 336(A) Asp 389(A) Cys 387(A) Trp 16(A) Gly 15(A) Tyr 60(A) Lys 418(A) Gly 61(A) Gly 17(A) Arg 58(A)

Note: Some residues participate in both hydrogen bonding and van der Waals interactions

Brazilin was consistently positioned within the active site cavity of DprE1, overlapping with the binding region of the co-crystallized reference ligand. A two-dimensional interaction diagram is recommended to complement this visualization and clearly depict residue-level interactions (Figure 2). Analysis of ligand–protein interactions revealed that brazilin formed one hydrogen bond with Tyr60(A) and van der Waals interactions with

Tyr60(A) and Gln334(A). Residue Tyr60 appears to be a critical interaction hotspot, as it is involved in both hydrogen bonding and steric stabilization, suggesting its important role in ligand anchoring within the catalytic pocket. These residues are located within or adjacent to the active site, supporting the structural plausibility of brazilin binding.

In comparison, the co-crystallized ligand (9T2_502[E]) formed stabilizing hydrogen bonds and van der Waals interactions with Ser228(A) and Lys418(A), consistent with its inhibitory role. Rifampicin exhibited the most extensive interaction network, forming multiple hydrogen bonds and hydrophobic contacts with residues such as Tyr335(A), Gln334(A), Lys418(A), and Gly17(A). In contrast, isoniazid did not form significant interactions within the DprE1 active site, consistent with its known mechanism targeting a different enzyme (Table 1).

Binding Affinity Analysis

The binding affinity was predicted using rerank scores generated by Molegro Virtual Docker. Brazilin demonstrated a rerank score of -71.287 , indicating moderate binding affinity toward DprE1. This value was more favorable than isoniazid (-48.295) but less favorable than rifampicin (-93.643) and the co-crystallized ligand (-96.682) (Table 2).

Beyond absolute scoring, the relative ranking suggests that brazilin possesses a binding profile closer to active-site inhibitors than to non-interacting ligands, reinforcing its potential as a target-relevant compound. The consistency of rerank scores across repeated docking runs supports the robustness of the predicted interaction. These findings suggest that brazilin is capable of indicating potential stable binding to DprE1, although with lower predicted affinity than established DprE1-interacting ligands.

Table 2. Rerank Scores and Docking Validation Results Against DprE1

Ligand	Rerank Score	RMSD (Å)
9T2_502[E]	-96.682	1.42
Brazilin	-71.287	-
Isoniazid	-48.295	-
Rifampicin	-93.643	-

The docking protocol was validated through a redocking procedure of the co-crystallized ligand, resulting in an RMSD value of 1.42 Å. This value is below the acceptable threshold of 2.0 Å, indicating that the docking method is reliable in reproducing the experimental binding pose.

DISCUSSION

This computational biotechnology study aimed to evaluate the potential interaction of brazilin with the essential *Mycobacterium tuberculosis* enzyme DprE1 using a structure-based in silico screening approach. The results demonstrate that brazilin can occupy the DprE1 active site and form stabilizing molecular interactions, providing pre-experimental evidence of its relevance as a potential lead compound[21]. These findings provide structure-based, pre-experimental evidence supporting the potential of brazilin as a candidate lead compound.

The moderate binding affinity of brazilin is structurally rational when considering its limited number of hydrogen bond donors/acceptors and relatively rigid phenolic scaffold. Compared to larger and more flexible ligands such as rifampicin, which possesses multiple functional groups enabling extensive hydrogen bonding and hydrophobic interactions, brazilin exhibits a more constrained interaction network, which likely contributes to its lower binding affinity. Compared to known DprE1 inhibitors such as benzothiazinone derivatives, which form covalent or highly optimized interactions within the catalytic pocket, brazilin lacks electrophilic warheads or specialized binding moieties, explaining its weaker interaction profile.

Importantly, brazilin exhibited stronger predicted interaction with DprE1 than isoniazid, which targets the InhA enzymatic pathway rather than DprE1, thereby supporting target specificity and internal consistency of the docking model [22], [23]. This is also reflected in its docking score (-71.287), which is more favorable than isoniazid (-48.295), consistent with the different target pathway of isoniazid rather than DprE1. This comparison further confirms that the observed binding is not random but structurally guided by active-site complementarity. Rifampicin and the co-crystallized ligand showed substantially stronger binding affinities and more extensive interaction networks, consistent with their optimized pharmacophore structures and established target specificity [24]. Rifampicin (-93.643) and the co-crystallized ligand (-96.682) exhibited stronger binding affinities and formed more extensive interactions, including multiple hydrogen bonds and hydrophobic contacts with residues surrounding the DprE1 active site.

From a structure–activity relationship (SAR) perspective, these differences highlight that increased molecular size, conformational flexibility, and functional group diversity correlate with enhanced binding coverage of the DprE1 active site. In contrast, brazilin's simpler polyphenolic structure limits its interaction footprint but still allows stable anchoring through key residues such as Tyr60 and Gln334.

When compared with previously reported DprE1 inhibitors in the literature, including benzothiazinone-based compounds and other small-molecule scaffolds, brazilin shows a lower binding affinity but remains within a comparable early-hit range typical of natural product scaffolds identified through virtual screening studies.

These findings reinforce that brazilin represents a moderate-affinity natural scaffold rather than a fully optimized inhibitor. The study objective was not to demonstrate clinical efficacy but to generate mechanistic hypotheses to guide further optimization [25].

Several limitations inherent to molecular docking must be acknowledged, including the use of a rigid protein model and simplified scoring functions that do not account for solvent effects, protein dynamics, or cellular context. Therefore, further validation using molecular dynamics simulations, binding free energy calculations, and *in vitro* enzymatic assays is required. Nevertheless, this study contributes to structure-guided natural product screening by integrating SAR interpretation with comparative inhibitor benchmarking, thereby supporting the rational prioritization of brazilin as a lead compound for future antituberculosis drug development [26].

CONCLUSION

This study employed a structure-based computational biotechnology approach to evaluate the interaction of brazilin, a natural compound from *Caesalpinia sappan* L., with the essential *Mycobacterium tuberculosis* enzyme DprE1. Molecular docking analysis demonstrated that brazilin can occupy the DprE1 active site and form stable interactions, exhibiting a moderate binding affinity that was stronger than isoniazid but lower than rifampicin and the co-crystallized reference ligand.

These findings provide molecular evidence supporting brazilin as a promising natural scaffold for early-stage antituberculosis drug development, particularly as a starting point for lead optimization targeting DprE1. From a translational perspective, brazilin may serve as a structural template for the design of improved derivatives with enhanced binding affinity and target specificity, thereby contributing to the expansion of natural product-based antituberculosis libraries.

In terms of future research direction, the next logical step is to perform molecular dynamics (MD) simulations to evaluate the stability and conformational behavior of the brazilin–DprE1 complex under physiological conditions. In addition, binding free energy calculations (e.g., MM-PBSA/MM-GBSA) are recommended to obtain more accurate

thermodynamic insights beyond docking scores. Experimental validation through in vitro enzymatic inhibition assays, including IC₅₀ determination against DprE1, is essential to confirm the computational predictions. Subsequent in vivo studies may further clarify the pharmacological relevance and bioavailability profile of brazilin derivatives.

Overall, this study bridges computational prediction and experimental prioritization by positioning brazilin as a viable starting point for rational DprE1 inhibitor development, while providing a clear roadmap for progressive validation toward preclinical antituberculosis drug discovery.

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