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Computational Docking of β-Lactamase Inhibitors to the Mycobacterium tuberculosis β-Lactamase BlaC Structure (PDB ID: 7K8F)

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ABSTRACT

Beta-lactam antibiotics are widely used, yet bacterial beta-lactamases can hydrolyze the beta-lactam ring and cause drug resistance. Beta-lactamase inhibitors (BLIs) are co-administered to protect antibiotics by occupying or inactivating the enzyme active site. Here, we used structure-based molecular docking to compare three BLIs (clavulanic acid, vaborbactam, and nacubactam) against BlaC, a class A β-lactamase from Mycobacterium tuberculosis (M. tuberculosis) that contributes to β-lactam antibiotic resistance, using a BlaC structure captured with ceftriaxone (Protein Data Bank ID: 7K8F) [1], in order to computationally screen and prioritize inhibitors most likely to bind the BlaC active site before moving to more time- and resource-intensive laboratory experiments. We validated the docking setup by re-docking ceftriaxone into the active site using GNINA docking software [2] with a 20 Angstrom cubic search box (center: 15, 2, 25). The best-scoring re-docked pose showed a root mean square deviation (RMSD) of 1.05 Angstrom from the crystallographic ligand position, supporting the use of this protocol for comparative screening. Docking scores (kcal/mol) and residue contacts within 4 Angstrom were then analyzed for each inhibitor. Nacubactam produced the most negative average score (-7.52) and engaged multiple active-site residues (ASP-241, THR-239, SER-128, LYS-236, THR-218), outperforming clavulanic acid (average -4.96) and modestly exceeding vaborbactam (average -6.71). These results suggest nacubactam is a promising candidate for strong active-site binding in this BlaC model, although enzyme inhibition assays will be required to confirm potency and mechanism.

Keywords: β-lactamase; BlaC; molecular docking; antibiotic resistance; Mycobacterium tuberculosis; GNINA

INTRODUCTION

M. tuberculosis is intrinsically resistant to many β-lactam antibiotics in part because it encodes an extended-spectrum serine β-lactamase, BlaC, which can hydrolyze diverse β-lactam classes [3]. Tuberculosis remains a major global health burden: the World Health Organization estimated 10.7 million people developed TB in 2024 and 1.23 million died that year [13]. Drug-resistant TB further increases the need for effective treatment combinations; WHO estimates about 400,000 people developed multidrug- or rifampicin-resistant TB (MDR/RR-TB) in 2023 [14]. These epidemiological realities support renewed interest in revisiting selected β-lactams in combination strategies that address BlaC-mediated hydrolysis [4]. Mechanism-based BLIs can restore activity against some serine β-lactamases, and newer inhibitor chemotypes have been developed to broaden inhibition profiles and improve clinical utility, including boronic-acid–based vaborbactam and diazabicyclooctane (DBO)–based nacubactam [5,6].

We focused on clavulanic acid, vaborbactam, and nacubactam because they represent three clinically relevant BLI chemotypes and are commonly discussed as β-lactamase–targeting partners. Clavulanate is especially relevant to TB because carbapenem–clavulanate combinations have demonstrated activity against M. tuberculosis through BlaC inhibition [15], while vaborbactam (cyclic boronate) and nacubactam (DBO) represent newer inhibitor scaffolds developed to broaden inhibition profiles [5,6].

To help prioritize inhibitors against BlaC, we used structure-based molecular docking, a computational approach that predicts how a small molecule may bind within a protein's active site by generating candidate binding poses and scoring them. Docking is useful as an early screening step because it can rapidly compare multiple candidate inhibitors and highlight which molecules are most likely to fit into the active site and form favorable interactions, guiding later laboratory validation. In this study, we performed docking using GNINA, a docking tool that integrates deep learning–based scoring to evaluate protein–ligand binding poses [2]. We docked inhibitors to the BlaC structure captured with ceftriaxone (PDB ID: 7K8F) obtained from the Protein Data Bank [1,7].

Purpose and hypothesis. The purpose of this work was to computationally compare clavulanic acid, vaborbactam, and nacubactam for their likelihood of binding the BlaC active site, as an initial prioritization step before more time- and resource-intensive laboratory experiments. We hypothesized that these BLIs would show distinguishable predicted binding poses and docking scores in BlaC's active site, enabling a preliminary ranking of candidate inhibitors most likely to inhibit BlaC and support β-lactam/BLI combination strategies relevant to Mtb drug resistance [3–6]. While BlaC docking and virtual screening have been reported previously [8–12], our contribution is a focused, reproducible comparison of three clinically relevant BLIs using the ceftriaxone-captured BlaC structure (PDB 7K8F) with an explicitly validated re-docking protocol (RMSD) and repeated docking trials to support consistent ranking.

MATERIALS AND METHODS

Protein structure selection

M. tuberculosis β-lactamase BlaC structure (PDB ID: 7K8F) was downloaded from the Protein Data Bank [1,7]. This structure contains the cephalosporin ceftriaxone, which was used as a reference ligand to

validate our docking workflow by re-docking ceftriaxone into the BlaC active site and comparing the predicted pose to the crystallographic binding position.

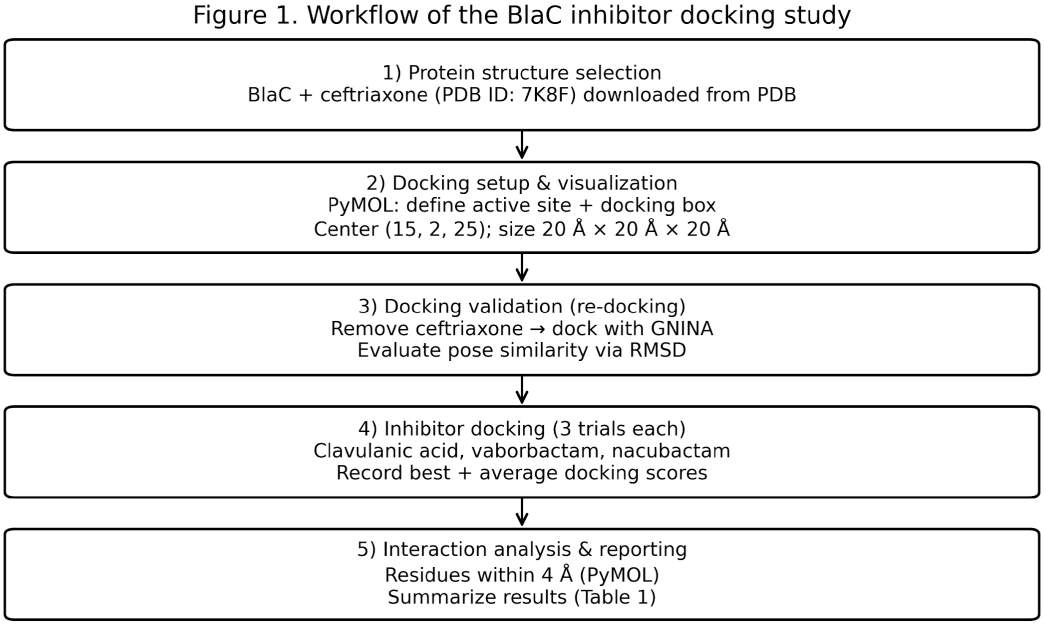

Figure 1. Workflow of the BlaC inhibitor docking study. The BlaC structure (PDB ID: 7K8F) was obtained from the Protein Data Bank, the docking region was defined in PyMOL, docking was performed using GNINA, ceftriaxone was re-docked for validation (RMSD), and three inhibitors (clavulanic acid, vaborbactam, nacubactam) were docked in three trials each. Key contacts (≤4 Å) and docking scores were summarized (Table 1).

Docking setup and visualization

PyMOL (The PyMOL Molecular Graphics System; maintained and distributed by Schrödinger, LLC) was used to visualize the BlaC structure, identify the binding pocket, define the docking region, and inspect predicted protein–ligand contacts. Docking was performed with GNINA (an open-source molecular docking program that integrates deep learning–based scoring; developed and maintained by an academic research group and released as described by McNutt et al. [2]). The docking search box was centered at (15, 2, 25) with dimensions 20 Å × 20 Å × 20 Å, selected to encompass the ceftriaxone binding site.

Figure 2 shows the docking search box positioned relative to the full BlaC structure and the ceftriaxone binding region.

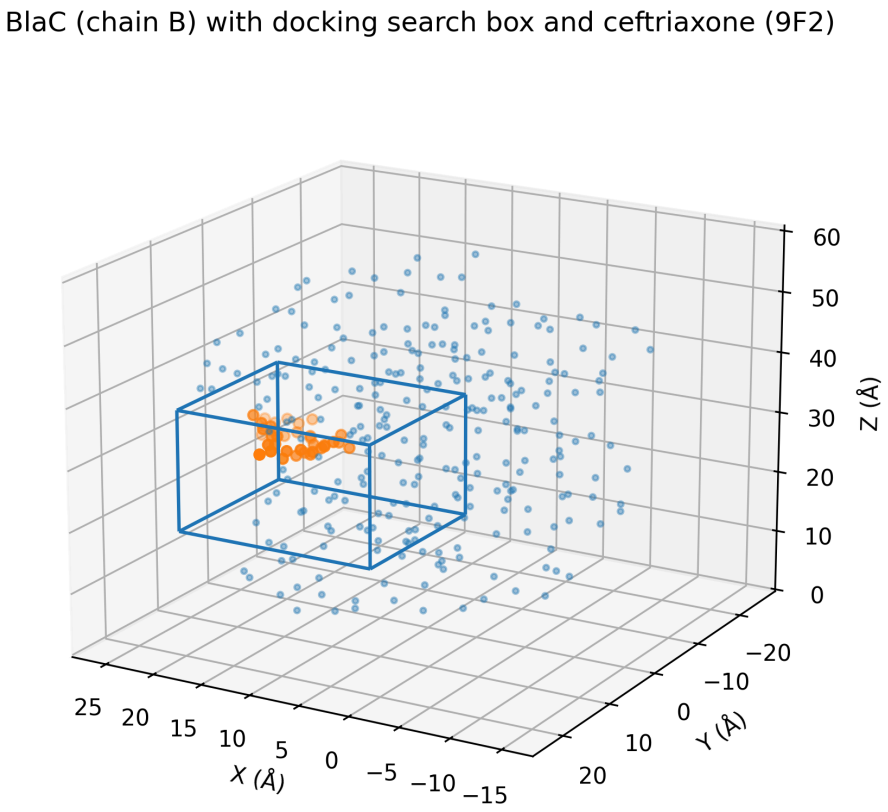

Figure 2. Docking region in the context of the full BlaC enzyme (PDB ID: 7K8F). BlaC (one chain shown) is displayed with the cubic docking search box centered at (15, 2, 25) with dimensions 20 Å × 20 Å × 20 Å. Ceftriaxone (crystal ligand) is shown to indicate the binding region used for docking setup and validation.

Docking validation by re-docking

Ceftriaxone was removed from the structure and re-docked using GNINA to assess whether the workflow could reproduce the crystallographic pose. Pose similarity was quantified by RMSD between crystallographic and re-docked ligand coordinates.

Inhibitor docking and interaction analysis

Three BLIs were evaluated: clavulanic acid, vaborbactam, and nacubactam [5,6]. For each ligand, GNINA docking was repeated across three trials. Docking scores (kcal/mol) were recorded and summarized as the best score and across-trial average. Residues within 4 Angstrom of the ligand were defined as nearby contacts and annotated in PyMOL using distance measurements.

RESULTS

Docking validation with ceftriaxone

The best-scoring re-docked ceftriaxone pose overlapped closely with the crystallographic ligand (RMSD 1.05 Angstrom). The crystallographic pose displayed polar contacts with THR-239 (two contacts), THR-237, and GLY-240, while the re-docked pose retained contacts to THR-239 and introduced an interaction with ASN-172. Figure 3 shows the active-site close-up with ceftriaxone in the binding pocket and the key nearby residues used for comparison.

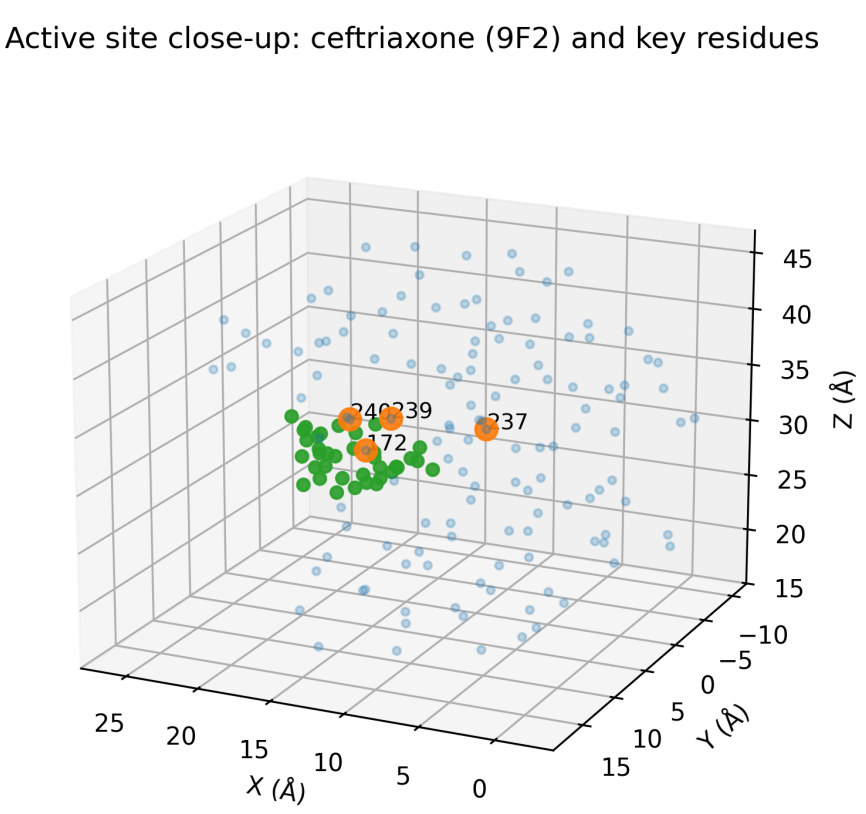

Figure 3. Active-site close-up showing ceftriaxone and key residues. Close-up of the 7K8F binding pocket showing ceftriaxone (9F2) and nearby residues referenced in docking validation and contact analysis.

Comparative docking of inhibitors

Clavulanic acid, used as a control BLI, interacted with SER-70, SER-128, and THR-237 (within 4 Angstrom) and produced an average docking score of -4.96 kcal/mol (best -5.47). Vaborbactam interacted with THR-237, THR-239, and SER-128 and produced an average score of -6.71 kcal/mol (best -7.47). Nacubactam showed the most negative average score (-7.52 kcal/mol; best -7.53) and contacted multiple residues (ASP-241, THR-239, SER-128, LYS-236, THR-218) in the active-site region. Table 1 summarizes docking scores and key interacting residues. Several contacts involve residues near the active-site region (e.g., SER-128 and THR-239), suggesting these positions may contribute to stabilizing inhibitor binding across multiple ligands. Nacubactam's additional contacts (e.g., ASP-241 and LYS-236) may provide extra stabilization and could explain its stronger predicted score in this docking framework.

Table 1. Summary of docking outcomes for ceftriaxone and BLIs in 7K8F

Ligand	Role	Avg docking score (kcal/mol)	Best docking score (kcal/mol)	Key residues within 4 Angstrom
Ceftriaxone	Antibiotic reference (crystal ligand)	NA	-9.65	THR-239; THR-237; GLY-240
Ceftriaxone (re-docked)	Docking validation	NA	NA	THR-239; ASN-172
Clavulanic acid	Control inhibitor	-4.96	-5.47	SER-70; SER-128; THR-237
Vaborbactam	Novel inhibitor	-6.71	-7.47	THR-237; THR-239; SER-128
Nacubactam	Novel inhibitor	-7.52	-7.53	ASP-241; THR-239; SER-128; LYS-236; THR-218

To help interpret Table 1, Figures 4A–4C visualize the active-site region and highlight the predicted residue contacts (≤4 Å) associated with each inhibitor.

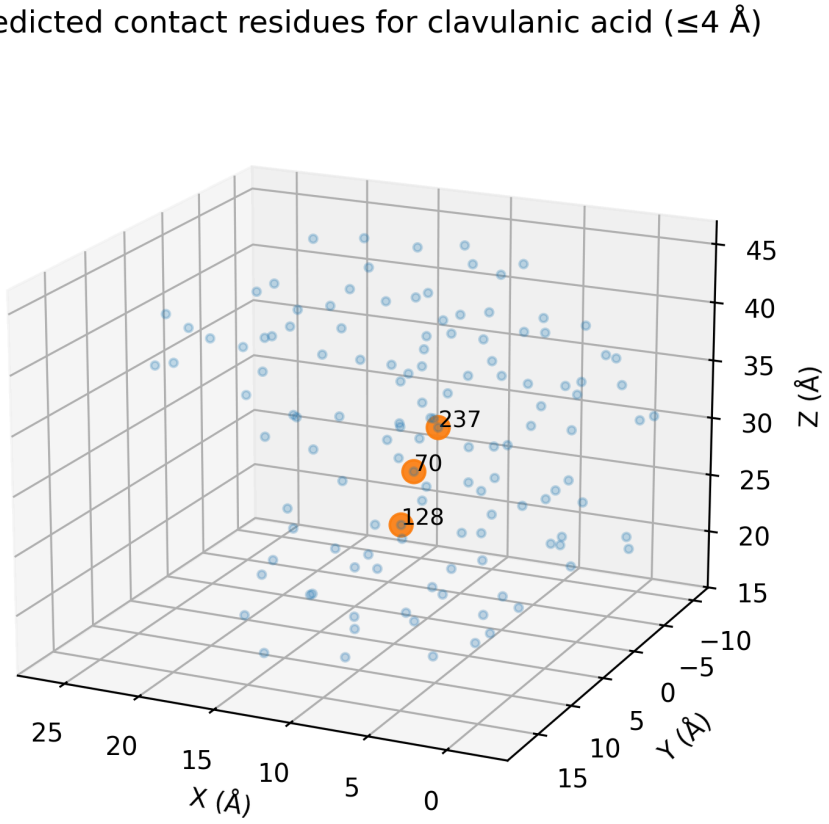

Figure 4A. Predicted contact residues for clavulanic acid (≤4 Å). Residues identified as within 4 Å of the docked ligand pose are highlighted in the BlaC active-site region (contact residues derived from docking-based proximity analysis).

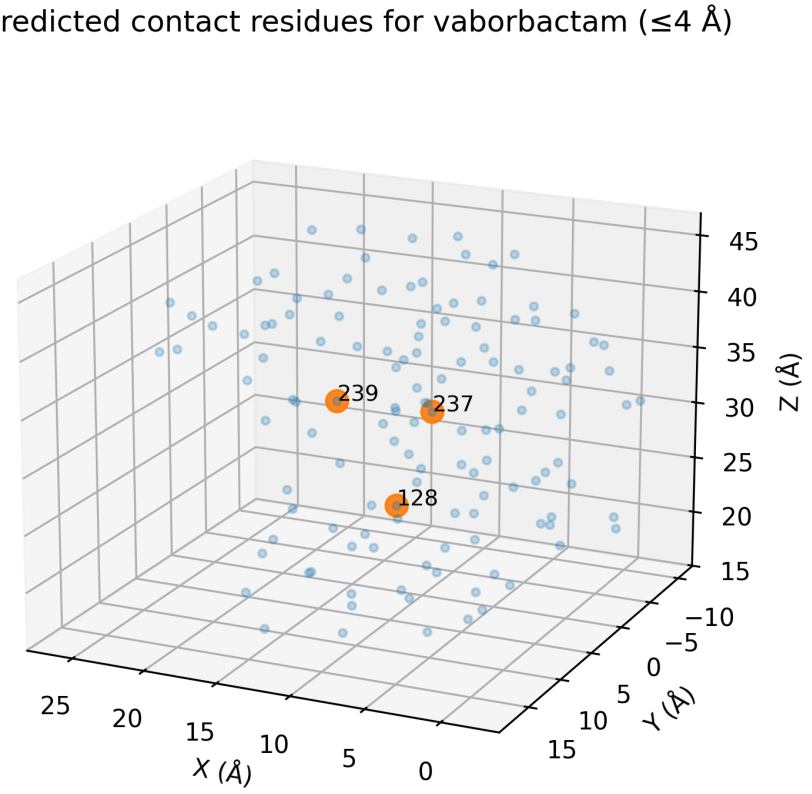

Figure 4B. Predicted contact residues for vaborbactam (≤4 Å). Active-site close-up highlighting residues predicted within 4 Å of vaborbactam.

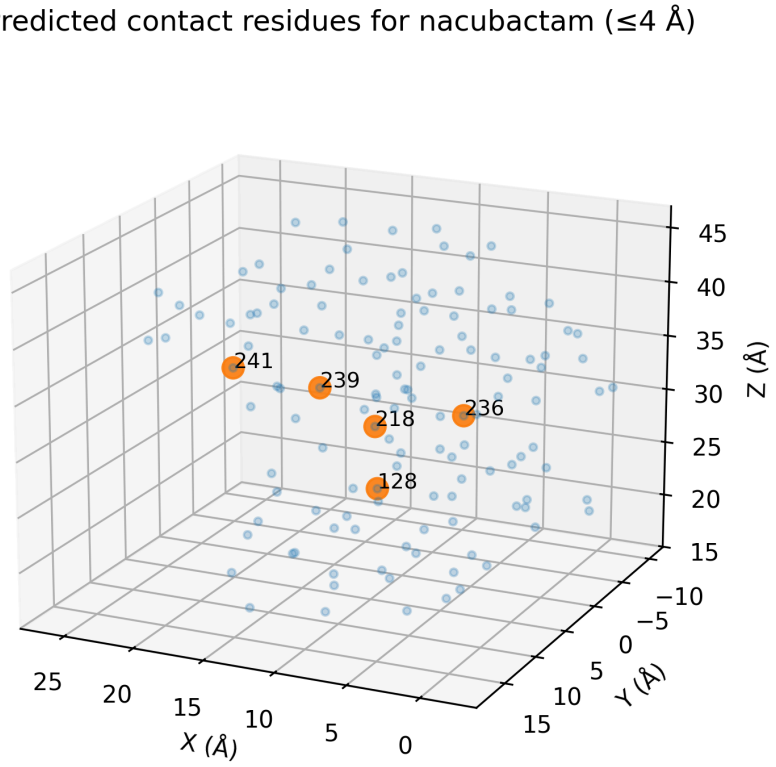

Figure 4C. Predicted contact residues for nacubactam (≤4 Å). Active-site close-up highlighting residues predicted within 4 Å of nacubactam.

DISCUSSION

In structure-based docking, more negative docking scores typically indicate more favorable predicted binding within a given docking program. However, docking scores are model-based estimates, and their absolute values depend on the scoring function—the mathematical model used to approximate binding interactions. Different scoring functions weigh interactions (e.g., hydrogen bonding, hydrophobic contacts, sterics) differently, so the same ligand can receive different scores across software; therefore, our conclusions are based on relative comparisons within the same GNINA workflow [2].

Our approach is consistent with prior computational BlaC inhibitor–discovery studies that use structure-based docking/virtual screening to prioritize compounds predicted to bind the BlaC active site before experimental testing. For example, Sagar et al. reported an in silico screen of a large compound library against *M. tuberculosis* BlaC to identify candidate non-β-lactam inhibitors predicted to bind in the active site, illustrating how docking can efficiently triage compounds for follow-up validation [8]. Similarly, other studies have combined docking with additional computational refinement (e.g., molecular

dynamics) to shortlist potential BlaC binders, supporting the general usefulness of docking as an early step when laboratory resources are limited [9].

Among the inhibitors examined here, nacubactam had the most negative mean docking score and the broadest predicted set of active-site contacts. Shared contacts to residues such as SER-128 and THR-239 (also observed for other ligands) suggest engagement of a core binding pocket region, while additional contacts (e.g., ASP-241 and LYS-236) may stabilize nacubactam's pose. This result is consistent with the broader literature describing how newer diazabicyclooctane (DBO)-class inhibitors are designed to interact effectively with serine β-lactamase active sites and protect partner β-lactam antibiotics [10]. Vaborbactam also docked favorably in our analysis, which aligns with published biochemical/structural work showing that cyclic boronate inhibitors (including vaborbactam) can strongly inhibit class A β-lactamases through tight active-site engagement and characteristic binding kinetics [11]. In contrast, clavulanic acid scored weaker in this noncovalent docking framework, which may partly reflect that its clinically relevant inhibition involves mechanism-based, covalent, time-dependent chemistry that is not fully captured by standard docking scores [12]. Based on our GNINA results, nacubactam is the top computational candidate because it had the most favorable mean score and broad active-site contacts. However, docking does not capture time-dependent covalent inhibition well, so clavulanate remains clinically relevant for TB combinations despite weaker noncovalent docking scores [15]. This is an important limitation: docking predicts plausible binding poses and relative scoring trends, but it does not directly measure enzymatic inhibition or antibacterial effect.

Therefore, while our results provide a computational prioritization of candidate BLIs for BlaC, future work should strengthen the conclusions with (i) covalent-aware docking or kinetic modeling for mechanism-based inhibitors (particularly clavulanic acid), and (ii) experimental validation such as enzyme inhibition assays (e.g., IC_{50} or K_i) and bacterial growth assays testing β-lactam/BLI combinations against mycobacteria. Together, these follow-up steps would determine whether the predicted binding differences translate into measurable BlaC inhibition and improved antibiotic activity.

Although our docking results are specific to BlaC from M. tuberculosis, the workflow (structure selection, docking validation by re-docking, and comparative inhibitor docking) is transferable to other bacterial β-lactamases and could be applied to other pathogens where β-lactamase–mediated resistance is important [12]. In those cases, the same approach could prioritize candidate inhibitors for laboratory testing, but results would need to be re-evaluated for each target enzyme. The novelty of this work is not the discovery of a new inhibitor, but the validated and reproducible comparison of three widely discussed BLI scaffolds under a single standardized GNINA protocol, providing a clear starting point for future experimental validation.

CONCLUSION

Using a validated GNINA docking workflow on the Mtb beta-lactamase BlaC structure 7K8F, we compared three BLIs and found that nacubactam had the strongest predicted binding (average -7.52 kcal/mol) and the most extensive set of active-site contacts among the inhibitors tested. These computational results support nacubactam as a promising inhibitor candidate for further experimental study in the context of beta-lactam resistance in Mtb.

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