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Original Article

Pharmacophore-Guided Virtual Screening and Molecular Dynamics Validation of Candidate Inhibitors of the Vietnamese Extended-Spectrum β-Lactamase

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ABSTRACT

Vietnamese extended-spectrum β-lactamase (VEB) is a class A extended-spectrum β-lactamase (ESBL) associated with high-level ceftazidime and monobactam resistance in Enterobacterales and *Pseudomonas aeruginosa*. No VEB-specific inhibitor is in clinical use, and structure-based discovery against VEB is complicated by the absence of an experimental crystal structure. A 3D pharmacophore model built from four template inhibitors (avibactam, zidebactam, aminobenzylboronic acid, phenylboronic acid) was used to screen over 300 million molecular conformers across 11 chemical databases. Hits were docked with AutoDock Vina against a SwissModel homology model of VEB, validated by Ramachandran analysis. The four top-ranked leads were subjected to 300.0 ns molecular dynamics (MD) simulation in GROMACS and *in silico* ADMET profiling. Docking identified four VEB-directed leads (PubChem-75298942, -87448357, -25144947, -75298821) with binding energies of -9.3 to -8.9 kcal/mol. ADMET profiling of the two boronic-acid leads showed zero Lipinski violations and favourable physicochemical properties, including low LogP (as low as -0.49) and low predicted hepatotoxicity. However, 300.0 ns MD simulation revealed pronounced ligand displacement from the catalytic pocket, with a mean ligand RMSD of approximately 8.55 nm, indicating that the docked poses did not correspond to a durable, kinetically stable complex. Favourable docking scores and ADMET profiles were insufficient to predict genuine target engagement against VEB. The candidates identified here are best classified as docking-stage false positives rather than viable leads. Extended MD validation should be treated as a mandatory, not optional, filter in VEB-directed computer-aided drug design, and future campaigns will require an improved VEB structural model, longer or replicate MD sampling, and scaffold redesign before candidates can be progressed.

Keywords: Extended-spectrum β-lactamase, virtual screening, molecular dynamics, false positive, ADMET, boronic acid inhibitors.

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INTRODUCTION

Antimicrobial resistance (AMR) remains one of the most pressing threats to global public health. Bacterial AMR was associated with an estimated 4.95 million deaths in 2019, and mortality attributable to resistant infections is projected to rise substantially by 2050 without effective intervention(1). Economic modelling by the 2016 Review on Antimicrobial Resistance projected that unchecked AMR could cost the global economy up to $100 trillion in lost output by 2050(2). Among the mechanisms driving this crisis, β-lactamase production by Gram-negative bacteria is one of the most consequential, as these enzymes inactivate the β-lactam antibiotics that form the backbone of modern antibacterial therapy.

The less prevalent Vietnamese extended-spectrum β-lactamase (VEB) is a class A extended-spectrum β-lactamase (ESBL) enzyme first described in Enterobacterales and subsequently reported at high prevalence in *Pseudomonas aeruginosa*, where it mediates marked resistance to ceftazidime, other oxyimino-cephalosporins, and monobactams (3,4). Bacterial isolates producing VEB enzyme have been documented across diverse geographic settings, and recent regional surveillance continues to detect *bla*VEB alongside other ESBL genes in contemporary Enterobacterales collections, underscoring that VEB has not disappeared as a clinically relevant resistance determinant(5). In 2024, the World Health Organization reaffirmed third-generation cephalosporin-resistant Enterobacterales, the phenotype produced by ESBLs including VEB, as a Critical Priority pathogen group(6).

Clinical management of ESBL infections is constrained by a narrow inhibitor pipeline. Older inhibitors such as clavulanic acid, sulbactam, and tazobactam are increasingly ineffective, while newer non-β-lactam inhibitors (avibactam, vaborbactam, relebactam) require intravenous administration and have already encountered emerging resistance within a few years of introduction(7). Newer boronate-based inhibitors have broadened coverage across Ambler classes, but their activity against VEB is variant-dependent rather than uniform: in a recent isogenic strain panel, taniborbactam potentiated cefepime activity more than 500-fold against VEB-9, yet VEB-14 and VEB-25 substitutions substantially reduced potentiation of ceftazidime-avibactam and aztreonam-avibactam, placing VEB among the class A enzymes that most compromise these newer combinations(8). This variant sensitivity, combined with the absence of a solved VEB crystal structure, makes VEB a genuinely difficult and comparatively under-characterised structure-based drug design target relative to CTX-M or SHV.

Computer-aided drug design (CADD) pipelines that combine pharmacophore modelling, large-scale virtual screening, molecular docking, molecular dynamics (MD) simulation, and ADMET profiling have proven effective at identifying viable leads against bacterial targets while filtering out unsuitable candidates before costly experimental validation(9,10) . Pharmacophore-based screening in particular allows the 3D chemical-feature

requirements of known active inhibitors to be used as an efficient filter ahead of computationally expensive docking(9). Extended MD simulation is a critical complementary step, static docking poses can appear energetically favourable yet fail to persist once solvent, protein flexibility, and thermal motion are modelled explicitly. Therefore, MD is widely used to distinguish genuine binders from docking-stage false positives (11,12).

This study reports a pharmacophore-guided virtual screening campaign directed specifically at VEB, in which the top docking-ranked leads were carried through 300.0 ns MD simulation and ADMET profiling to test whether favourable docking and pharmacokinetic predictions translate into durable target engagement. As detailed below, they did not, and the resulting dataset serves as a cautionary, data-grounded illustration of why kinetic validation is indispensable in VEB-directed CADD.

MATERIALS AND METHODS

A multi-stage computational workflow was used to narrow a chemical library of several hundred million conformers down to a small set of VEB-directed leads, combining pharmacophore modelling, large-scale virtual screening, molecular docking, MD simulation, and ADMET profiling.

Pharmacophore Model Generation

Four structural templates were used to derive 3D pharmacophore hypotheses: avibactam (AVI), zidebactam (ZID), aminobenzylboronic acid (ABA), and phenylboronic acid (PAB). Pharmacophore virtual screening was performed using Pharmit, a computational webtool from the National Institute of General Medical Sciences and a generous gift from Relay Therapeutics (https://pharmit.csb.pitt.edu/). Each model encoded hydrogen-bond donor/acceptor features corresponding to the catalytic pocket, hydrophobic features capturing van der Waals and π-stacking contacts 300.0 ns with residues such as tyrosine and tryptophan, and exclusion volumes to prevent steric clashes with the protein backbone.

Virtual Screening

The four pharmacophore hypotheses were used as sequential filters against 11 chemical databases (including PubChem, Mcule-ultimate, and Enamine) comprising more than 300 million molecular conformers. Conformational sampling identified 1,109 hits satisfying the geometric and chemical requirements of all four templates; these constituted the candidate pool carried forward to docking against all five ESBL families examined in the parent screening campaign, of which VEB is the focus of this paper.

VEB Structural Preparation and Validation

Due to the absence of an experimental VEB crystal structure, a three-dimensional VEB model was generated using the SwissModel homology-modelling server. Stereochemical quality was assessed by Ramachandran analysis, which confirmed that backbone dihedral angles (φ and ψ) fell within energetically favourable regions, supporting the use of this model for docking. This structural approach is a necessary limitation of VEB-directed CADD relative to targets with solved crystal structures (e.g., CTX-M, PDB 4HBT) and is revisited in the Discussion.

Molecular Docking

Docking was performed with AutoDock Vina (v1.1.2) via PyRx 0.8, selected for its empirical scoring function and iterated local-search global optimizer (13). A grid box of approximately 48 × 50 × 45 Å, centred on the predicted catalytic pocket, was used to allow full rotational and translational freedom of each ligand. The 1,109 pharmacophore hits were docked against the VEB model, with binding poses ranked by predicted affinity (kcal/mol); the top four VEB-directed leads were retained for downstream analysis.

Molecular Dynamics Simulation

Protein–ligand MD was performed using GROMACS 2025.4. Protein topology used the AMBER99SB-ILDN force field; ligands were parameterised with GAFF2 via ACPYPE, with partial charges derived from xTB quantum-mechanical calculations. Each complex was solvated in a dodecahedral box (1.0 nm buffer) with explicit water and neutralising Na+/Cl- ions. Following steepest-descent energy minimisation, systems were equilibrated for 300.0 ns under NVT and 0.1 ns under NPT conditions (V-rescale thermostat and Parrinello-Rahman barostat for NPT), with hydrogen bonds constrained by LINCS and non-bonded cutoffs of 0.1 ns. Production MD was run for 300.0 ns using the leapfrog integrator, with frames saved every 10.0 ps. Ligand and protein RMSD, RMSF, radius of gyration, SASA, and hydrogen-bond persistence were computed to assess structural and kinetic stability, and MM/PBSA binding free energies were calculated over the final 1,000 frames using MM/PBSA.

ADMET Profiling

Physicochemical and pharmacokinetic properties (molecular weight, LogP, LogS, TPSA, Lipinski rule compliance, Caco-2 permeability, P-glycoprotein and blood-brain-barrier interaction probability, plasma protein binding, CYP450 inhibition, plasma clearance, half-life, hERG blockade, Ames mutagenicity, and DILI risk) were predicted *in silico* for the VEB leads to assess their translational potential independent of binding kinetics. Admetlab 3.0 webtool was used for this screening (https://admetlab3.scbdd.com/).

RESULTS

Molecular Docking Against VEB

Docking of the 1,109 pharmacophore-filtered hits against the VEB homology model identified four leads with binding energies between -9.3 and -8.9 kcal/mol (Table 1). All four leads share a boronic-acid pharmacophore, consistent with the boronate-inhibitor templates used to build the screening pharmacophore. On docking score alone, these compounds appeared broadly comparable to leads obtained for the other ESBL families examined in the parent screening campaign, providing no indication from static docking of the instability subsequently revealed by MD (Section 3.3).

Table 1. Molecular Docking Results for VEB

S/N	Lead Compound ID	Compound IUPAC Name	Docking Score (kcal/mol)
1	PubChem-75298942	3-[2-borono-2-[[2-[4-[(4-methylmorpholin-4-ium-4-yl)methyl]phenyl]acetyl]amino]ethyl]-2-hydroxybenzoic acid	-9.3
2	PubChem-87448357	3-[2-borono-2-[[2-[4-[(4-methylmorpholin-4-ium-4-yl)methyl]phenyl]acetyl]amino]ethyl]-2-hydroxybenzoic acid formate	-9.1
3	PubChem-25144947	(4R,6S)-3-[(3S,5S)-5-[4-carboxyfuran-2-yl)methylcarbamoyl]pyrrolidin-3-yl]sulfanyl-6-[(1S)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid	-9.0
4	PubChem-75298821	3-[2-borono-2-[[2-[4-(methylaminomethyl)phenyl]acetyl]amino]ethyl]-2-hydroxybenzoic acid	-8.9

Interaction mapping showed that the VEB leads engaged the catalytic pocket predominantly through a combination of π-alkyl, π-stacking, and carbon-hydrogen bond interactions, rather than the more extensive hydrogen-bonding networks observed for leads docked against CTX-M in the parent campaign. This more limited interaction profile is illustrated in Figure 1.

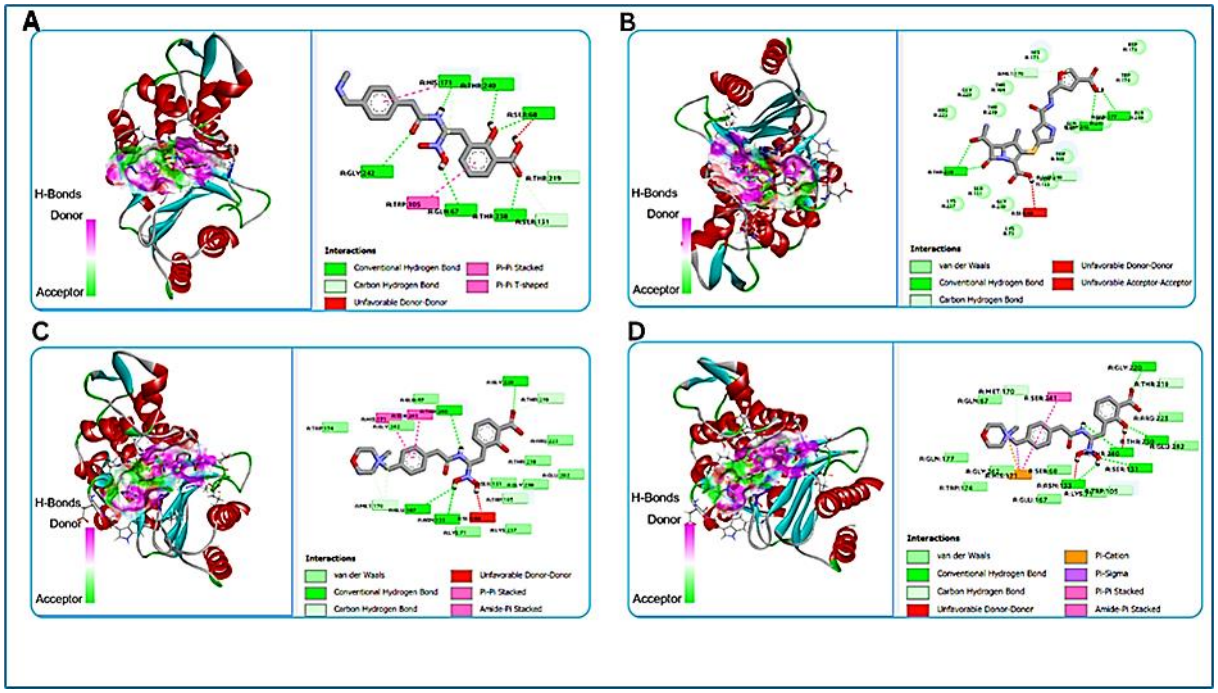

Figure 1. Molecular Interaction Profiles for VEB.

Panels A–D depict the predicted binding orientations and two-dimensional protein–ligand interaction maps of PubChem-75298942, PubChem-87448357, PubChem-25144947, and PubChem-75298821, respectively, highlighting hydrogen-bonding,

hydrophobic, π-alkyl, and π-stacking interactions within the binding site.

ADMET Profiling of VEB Leads

ADMET profiling was performed for the two most extensively characterised VEB leads, PubChem-75298942 and PubChem-75298821. Both compounds satisfied all Lipinski criteria (zero rule violations) and showed low lipophilicity (LogP -0.20 and -0.49, respectively), favourable aqueous solubility (LogS -2.35 and -2.25), and negligible predicted blood-brain-barrier penetration and P-glycoprotein inhibition. PubChem-75298942 showed low predicted CYP1A2/CYP3A4 inhibition (≤0.01) and low Ames mutagenicity (0.39), while PubChem-75298821 showed comparably low mutagenicity (0.30) but a higher predicted DILI risk (0.91) than its analogue (0.14). Neither compound showed meaningful hERG liability (0.13 and 0.36, respectively). Taken in isolation, these predictions describe orally tractable, low-toxicity boronic-acid scaffolds which is an encouraging pharmacokinetic profile that, as shown in Section 3.3, was not matched by kinetic stability at the target.

Molecular Dynamics Simulation

In contrast to the favourable docking scores and ADMET predictions, 300.0 ns MD simulation of the VEB complexes revealed pronounced instability. The ligand RMSD trajectory showed early and progressive departure from the docked pose, culminating in a mean ligand RMSD of approximately 8.55 nm, an order of magnitude greater than the sub-nanometre RMSD values considered consistent with stable active-site retention. This magnitude of displacement indicates that the ligand did not remain associated with the catalytic pocket over the simulated timescale but instead dissociated and diffused into the surrounding solvent. Protein backbone RMSD and radius of gyration for the VEB target itself remained comparatively stable, indicating that the instability was specific to the ligand-binding event rather than global unfolding of the modelled protein. Under the same simulation protocol applied elsewhere in the parent screening campaign, at least one other ESBL complex (targeting SHV) retained a stable, sub-nanometre ligand RMSD throughout an equivalent 300.0 ns run, indicating that the instability observed here reflects a property of the VEB complexes specifically rather than an artefact of the MD protocol.

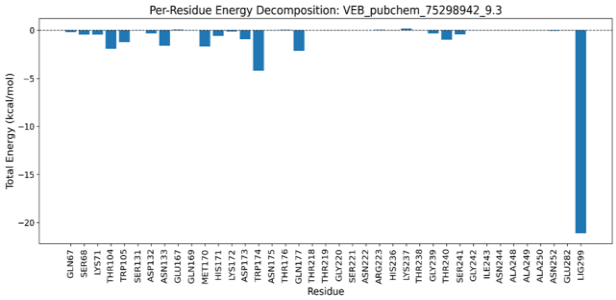

Figure 2. Per-Residue Binding Energy Decomposition for VEB.

The plot shows the energetic contribution of individual VEB residues to ligand binding, with negative values representing favorable contributions and larger negative values indicating stronger predicted stabilization of the complex

Because the ligand did not maintain a defined binding pose, the MM/PBSA binding free energies computed over the final 1,000 frames are not interpretable as estimates of specific target affinity; any favourable energetic terms most likely reflect weak, non-selective, or transient solvent-exposed interactions rather than a genuine bound state.

DISCUSSION

The central finding of this study is a clear discordance between static docking/ADMET predictions and dynamic kinetic behaviour for VEB-directed leads. All four candidates scored competitively by docking, and the two profiled in ADMET showed orally favourable, low-toxicity pharmacokinetic predictions, including zero Lipinski violations and low predicted mutagenicity. Yet none of the corresponding complexes survived 300.0 ns of explicit-solvent MD simulation without substantial ligand dissociation. On the criteria that matter for progressing a candidate toward synthesis and testing, durable target engagement, these leads must be regarded as false positives rather than viable inhibitors.

Several factors plausibly contribute to this outcome. First, VEB lacks a solved crystal structure; the model used here was a Ramachandran-validated SwissModel homology structure, which can capture correct backbone geometry while still misrepresenting side-chain packing and loop flexibility around the catalytic pocket, particularly for the more mobile omega-loop region characteristic of class A β-lactamases. Docking against such a model can identify a geometrically plausible pose that does not correspond to the true induced-fit binding mode, a discrepancy that only becomes apparent once the system is allowed to relax and explore conformational space dynamically (11,12). Secondly, the boronic-acid warhead common to all four leads is designed to engage the active-site serine through a reversible, quasi-covalent interaction; rigid-body docking scoring functions are not well suited to modelling this chemistry, and may overestimate the stability of a pose that depends on a specific transition-state-like geometry rarely sampled in a single static docking run(13) . Thirdly, VEB itself is a known problem enzyme for even well-optimised boronate inhibitors. In a recent large isogenic panel, taniborbactam potentiated cefepime activity against VEB-9 by more than 500-fold, yet loss-of-function against ceftazidime-avibactam and aztreonam-avibactam was specifically associated with VEB-14 and VEB-25 substitutions, placing VEB among the class A enzymes most likely to compromise next-generation inhibitor combinations (8). This variant-dependent behaviour suggests that VEB's active-site environment is unusually sensitive to small structural perturbations, a property that a single homology model and a single 300.0 ns trajectory are unlikely to capture in full.

This discordance between docking and MD is not unique to the present dataset. MD-based re-evaluation of docking poses has repeatedly been shown to expose poses that appear favourable by scoring function alone but drift or dissociate once solvated and thermally equilibrated, particularly for flexible or homology-modelled targets (11). Recent reviews of hybrid docking-MD pipelines in other drug discovery contexts similarly emphasise that MD validation, rather than docking score, should be the deciding filter before a compound is prioritised for synthesis or experimental testing (14). The present results reinforce that recommendation specifically for VEB: a favourable docking score and an attractive ADMET profile were, on their own, actively misleading.

These findings should be interpreted with several limitations in mind. The VEB structure used was a single homology model rather than an experimentally solved structure, so some of the observed

instability may reflect model inaccuracy rather than a genuine property of the biological target. Only a single 300.0 ns trajectory was run per complex, without replicate seeds, so the extent to which the observed dissociation is deterministic versus a single unlucky trajectory cannot be fully distinguished without repeat simulations. MM/PBSA rescoring was performed on a trajectory that had already lost the bound pose, limiting the interpretability of the reported energetics. Finally, ADMET predictions are *in silico* estimates and were not benchmarked against experimental pharmacokinetic data for these specific compounds.

Taken together, these limitations point toward concrete next steps for a genuine VEB-directed discovery campaign: (i) pursuing an experimentally solved VEB structure, or at minimum a higher-confidence, induced-fit-refined model, before committing to structure-based design; (ii) running replicate and/or extended MD (or enhanced-sampling methods such as metadynamics) to distinguish genuine binders from single-trajectory artefacts; (iii) redesigning the boronic-acid scaffold with explicit attention to VEB's apparently unusual sensitivity to active-site perturbation, potentially guided by structure-activity relationships already established for taniborbactam and related cyclic boronates; and (iv) re-running pharmacophore-guided screening with a VEB-specific (rather than shared) pharmacophore hypothesis, since the four templates used here were derived from inhibitors not specifically optimised against VEB.

CONCLUSION

This pharmacophore-guided virtual screening campaign against VEB extended-spectrum β-lactamase identified four docking-prioritised boronic-acid leads with competitive binding scores and, for the two profiled, favourable ADMET predictions. However, 300.0 ns molecular dynamics simulation showed that none of the corresponding complexes retained a stable binding pose, with ligand RMSD values indicating dissociation from the catalytic pocket. We conclude that these candidates are docking-stage false positives rather than genuine VEB inhibitors, and that favourable docking and ADMET profiles alone are insufficient evidence of target engagement for this enzyme. Extended MD validation should be treated as a mandatory, non-negotiable step in VEB-directed computer-aided drug design. Future work should prioritise an improved VEB structural model, replicate or enhanced-sampling MD, and VEB-specific scaffold optimisation before any candidate is progressed toward *in vitro* testing.

REFERENCES

1. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet. 2024;404(10459):1199-1226. doi: 10.1016/S0140-6736(24)01867-1

2. O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance; 2016. Available from: https://amr-review.org/sites/default/files/160

525_Final paper_with cover.pdf

3. Bush K, Bradford PA. Epidemiology of β-Lactamase-Producing Pathogens. Clin Microbiol Rev . 2020;33(2):e00047-19. doi: 10.1128/CMR.00047-19.

4. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. AC Antimicrob Resist. 2021;3(3):dlab092. doi:10.1093/jacamr/dlab092.

5. Brauncajs M, Bielec F, Macieja A, Machnicki P, Pastuszak-Lewandoska D. Antimicrobial Susceptibility and Genetic Epidemiology of Extended-Spectrum β-Lactamase-Positive Enterobacterales Clinical Isolates in Central Poland. Int J Mol Sci. 2024;25(15):8371. doi:10.3390/ijms25158371.

6. Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E, et al. The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. Lancet Infect Dis. 2025;25(9):1033-1043. doi:10.1016/S1473-3099(25)00118-5

7. Karaiskos I, Galani I, Daikos GL, Giamarellou H. Breaking Through Resistance: A Comparative Review of New Beta-Lactamase Inhibitors (Avibactam, Vaborbactam, Relebactam) Against Multidrug-Resistant Superbugs. Antibiotics. 2025;14(5):528. doi:10.3390/antibiotics14050528.

8. Uehara T, Chatwin CL, Miller B, Edwards M, Stevenson A, Colombo J, et al. Spectrum of cefepime-taniborbactam coverage against 190 β-lactamases defined in engineered isogenic Escherichia coli strains. Antimicrob Agents Chemother. 2025;69(5):e0169924. doi:10.1128/aac.01699-24.

9. Hamrick JC, Docquier JD, Uehara T, Myers CL, Six DA, Chatwin CL, et al. VNRX-5133 (Taniborbactam), a Broad-Spectrum Inhibitor of Serine- and Metallo-β-Lactamases, Restores Activity of Cefepime in Enterobacterales and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2020;64(3):e01963-19. doi:10.1128/AAC.01963-19

10. Schaller D, Šribar D, Noonan T, Deng L, Nguyen TN, Pach S, et al. Next generation 3D pharmacophore modeling. WIREs Comput Mol Sci. 2020;10(4):e1468. doi:10.1002/wcms.1468.

11. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461. doi:10.1002/jcc.21334.

12. Sakano T, Mahamood MI, Yamashita T, Fujitani H. Molecular dynamics analysis to evaluate docking pose prediction. Biophys Physicobiol. 2016;13:181-194. doi:10.2142/biophysico.13.0_181.

13. Shakil S, Rizvi SMD, Greig NH. High Throughput Virtual Screening and

Molecular Dynamics Simulation for Identifying a Putative Inhibitor of Bacterial CTX-M-15. Antibiotics (Basel). 2021;10(5):474. doi:10.3390/antibiotics10050474

14. Hasan GM, Mohammad T, Zaidi S, Shamsi A, Hassan MI. Molecular docking and dynamics in protein serine/threonine kinase drug discovery: advances, challenges, and future perspectives. Front Pharmacol. 2025;16:1696204. doi:10.3389/fphar.2025.1696204.