Machine learning and DFT-based discovery of natural inhibitors against β-lactam-resistant PBP2x mutants in *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae continues to show increasing resistance to β-lactam antibiotics, a trend largely driven by mutations in penicillin-binding protein 2x (PBP2x), one of the key enzymes involved in cell wall synthesis. Mutations within conserved catalytic motifs such as STMK and KSG are frequently reported in clinically important serotypes including 19A, 19F, and 23F. These alterations weaken antibiotic binding, disrupt enzymatic function, and contribute directly to the emergence of multidrug-resistant pneumococcal infections such as pneumonia, meningitis, and septicaemia. In this study, we focused on five clinically relevant PBP2x mutations (T338A, T338G, T338P, K547G, and K547T) located within the active site and investigated natural compounds as potential alternatives to conventional β-lactam therapy. A curated library of phytochemicals was first screened using a machine learning classifier trained to identify antibacterial candidates. Promising hits were refined through ADMET-based filtering and evaluated for electronic stability using HOMO–LUMO energy gaps and electrostatic potential mapping via density functional theory. Among the shortlisted compounds, Glucozaluzanin C, derived from *Elephantopus scaber*, consistently demonstrated favorable chemical reactivity and stability. Molecular docking revealed high-affinity interactions with all selected PBP2x mutants, which were further validated through 100-ns

molecular dynamics simulations. RMSD, RMSF, and hydrogen-bond analyses confirmed stable binding and minimal structural deviation across the simulation period. Overall, the study highlights Glucozaluzanin C as a promising plant-derived inhibitor with potential activity against β-lactam-resistant PBP2x variants and presents an integrated machine learning and DFT-based workflow for accelerated antimicrobial lead discovery in drug-resistant S. pneumoniae.

Keywords: PBP2x mutations, Machine learning virtual screening, Density functional theory, Phytochemical inhibitors