Novel HFIAP-1 Mutant Antimicrobial Peptide Identified as a Potential Inhibitor of Extended-Spectrum β-Lactamases in *Escherichia coli*: An In-silico Study

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Abstract

Extended-spectrum β-lactamases (ESβLs) produced by Escherichia coli represent a critical threat to global antimicrobial therapy, significantly undermining the effectiveness of conventional β-lactam–β-lactamase inhibitor combinations. In the present study, we employed a computational drug discovery approach to identify novel peptide-based β-lactamase inhibitors from a curated library of antimicrobial peptide (AMP) mutants exhibiting enhanced antibacterial potency (7–16%) compared to their parent sequences. Five selected peptides and their mutants were systematically analyzed for their physicochemical, pharmacokinetic, and immunogenic properties. Molecular docking identified the mutant HFIAP-1_M5 (L33K–W7C–N34C) as a promising inhibitor, predicted to interact with approximately 82% of the analyzed ESβLs spanning classes A–D. HFIAP-1_M5 demonstrated superior binding affinities (0.2–12% improvement) relative to its parent peptide, forming stable hydrogen bonds, van der Waals interactions, and salt bridges with key catalytic residues within the β-lactamase active sites. All-atom molecular dynamics simulations, along with principal component and free energy landscape analyses, confirmed the conformational stability of the ESβL–HFIAP-1_M5 complexes with minimal residue-

level fluctuations. Binding free energy and per-residue decomposition analyses further revealed critical interactions contributing to complex stabilization. Overall, this study highlights HFIAP-1_M5 as a potent peptide-based β-lactamase inhibitor with strong potential for use in combination therapy to restore the efficacy of β-lactam antibiotics against multidrug-resistant *E. coli*, upon experimental validation.

Keywords: Antimicrobial resistance, Antimicrobial peptides, *Escherichia coli,* Extended-spectrum β-lactamases