A Multi-Tiered Computational Framework for Functional and Structural Characterization of Hypothetical Proteins in *Nocardia asteroides* FDAARGOS1485: An Integrated Structure-Based Virtual Screening and Molecular Dynamics Validation of Marine-Derived Inhibitors

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Abstract

Nocardiosis is a global life-threatening disease the predominantly affects immunodeficient populations. This disease was caused by the nosocomial pathogen *Nocardia asteroides,* a gram-positive bacterium. Existing therapeutics offer limitations and challenges, highlighting the need for novel drug targets. The pathogenic strain FDAARGOS1485 contains 6,521 proteins, with a higher completeness level of 98% as determined by CheckM analysis (v1.2.2). Among these proteins, 1246 (19.11%) were identified as hypothetical proteins (HPs), and upon eliminating pseudogenes, 863 (13.20%) HPs were designated as targets. The functional annotation pipeline revealed 30 (4.40%) HPs classified to 24 enzymes (80.02%), 2 transporters (6.66%), 2 binding proteins (6.66%), and 2 cell regulatory process proteins (6.66%). Metabolic pathway analysis elucidated the functionally annotated 3-oxoacyl-synthase 3 protein (WP_223513777.1) as a promising target. The structure-based virtual screening program identified three potential MNPs (CMNPD22130, CMNPD23347, and SWMDRR052) with favourable docking scores of –8.23 to –10.05 kcal/mol and post-docking scores of –62.42 to –72.91 kcal/mol, along with the key interacting residues of Ile49, Phe50, Gly89, Ala90, Val91, Thr119, Pro121, Pro125, Val127, Ala128, Tyr134, Ser135, Cys137, Hie255, and Ala285. Remarkably, none of the candidates involved in the Ro5 violation exhibited satisfactory pharmacokinetic profiles and favourable frontier molecular orbital scores. The molecular dynamics (MD), essential dynamics (ED) and post-dynamics confirmed the stable binding of the MNPs within the active site of the 3-oxoacyl-synthase 3

protein. Moreover, the computational study warrants *in vitro* validations to explore the therapeutic potential of the identified MNPs.

Keywords: *Nocardia asteroides*, Hypothetical Proteins, Functional Annotation, Structure-based Virtual Screening, Molecular Dynamics, Essential Dynamics.

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