INVESTIGATION OF POTENTIAL TARGETS AND THEIR INHIBITORS IN *MYCOBACTERIUM TUBERCULOSIS*

Abstract

Mycobacterium tuberculosis (MTB) poses a significant global health threat due to its rapid development of drug resistance, making it difficult to monitor, treat, and prevent tuberculosis (TB) effectively. As a result, scientists have heightened their efforts in exploring novel bioactive substances that possess a unique mechanism of action when combating drug-resistant strains of *Mycobacterium tuberculosis*. In this study, a high-throughput virtual screening of 30,417 compounds from three independent repositories against putative antitubercular targets, namely *MmpL*, *GlfT2,* and *MurB*, involved in cell-wall biosynthesis was performed. Seventy-five top-ranked inhibitors for further evaluation have been identified.

To assess the candidate compounds' ability to withstand an active site environment, a Molecular Dynamics Simulation on the top fourteen best-docked complexes of *GlfT2* and *MurB* targets, with seven complexes against each target was conducted. The complexes exhibiting the most stable and consistent interactions were selected for further *in vitro* experiments. Our findings revealed that the identified compounds demonstrated inhibitory activity against the enzymes of interest, with the most notable inhibition. Compounds ZINC000095092808 and DB12424 have showcased noteworthy efficacy in inhibiting *GlfT2*, while ZINC254071113 and DB15688 have demonstrated compelling inhibitory activity against *MurB*. Among the screened

compounds, both compounds ZINC000095092808 and ZINC254071113 emerged as the most promising, exhibiting highly effective MIC values.

An in-depth analysis, including the ligands' binding affinity through docking studies, coupled with insights from MD simulation on contact stability, and rigorous *in-vitro* validation, collectively suggest that Compounds ZINC000095092808 and DB12424 hold significant promise as novel potential targets for *GlfT2*. Similarly, Compounds ZINC254071113 and DB15688 have demonstrated potential as inhibitors of *MurB*. Notably, ZINC254071113 has displayed promising MIC values against *MurB*, solidifying its candidacy as a potential therapeutic agent.

According to our research outcomes, these newly discovered compounds show promise and may serve as potential candidates for the development of more effective drugs to combat tuberculosis. Through our study, we have introduced a series of novel compounds that exhibit the potential to act as significantly stronger inhibitors of the *GlfT2* and *MurB* enzymes, which are known targets for developing anti-tuberculosis medications. These findings hold significant clinical implications, given that TB is still a major global health burden, with 10.5 million cases reported worldwide in 2021. Our overall approach and findings provide a solid starting point for developing new MTB inhibitors that specifically target the enzymes examined in this study.

Keywords: Drug resistance, *Mycobacterium tuberculosis*, Cell wall, Potential targets, Inhibitors, Drug discovery