

Investigating the potential of BTD7 Compound as a Novel Anti-Mycobacterial tuberculosis Agent: An In Vitro Study

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Background: Tuberculosis remains a global health concern, with the increasing prevalence of multidrug-resistant and extensively drug-resistant TB undermining the efficacy of current anti-TB drugs. In light of the stagnant progress in novel anti-TB drug development over the past five decades, there is an urgent need for innovative therapeutic strategies. **Objective:** This research aimed to characterize the efficacy of a novel compound, BTD7, against mycobacteria and to elucidate its mechanism of action. **Method:** The *M. tuberculosis* was subjected to the selection of spontaneous resistant mutants with different concentrations of BTD7. After three rounds of evolution, BTD7-resistant mutants were isolated and subjected to whole-genome sequencing (WGS). The results were confirmed through Sanger sequencing. **Results:** The novel compound BTD7 exhibited potent, selective activity against *M. tuberculosis* autoluminescent strains (MIC = 0.0078125 µg/mL in broth) and inhibited intracellular growth in RAW264.7 macrophages (intracellular MIC = ~0.5-1 µg/mL). The compound showed no measurable activity against a panel of common Gram-positive and Gram-negative bacteria or pathogenic fungal strains (MICs > 128 µg/mL), indicating narrow-spectrum mycobacterial specificity. WGS of resistant isolates identified a recurrent amino-acid substitution in the essential two-component histidine kinase PrrB (Arg266Leu), and overexpression of PrrB increased the BTD7 MIC, consistent with on-target activity. Molecular docking and short molecular dynamics simulations place BTD7 in the ATP-binding cleft of PrrB and predict that the Arg266Leu substitution disrupts ligand interactions, providing a structural rationale for resistance. **Conclusion:** Conclusively, BTD7 is a promising lead compound that selectively targets the PrrB histidine kinase in *M. tuberculosis*, shows potent in vitro and intracellular activity, and displays a relatively high genetic barrier to resistance. These properties warrant further preclinical development, including biochemical target validation, high-resolution structural studies, ADME/Toxic profiling, and in vivo efficacy testing.

Biography

Haftay Abraha Tadesse is a researcher and lecturer at Mekelle University, Tigray, Ethiopia, with expertise in medical microbiology, molecular biology, and infectious diseases. He holds a BSc in Animal Health and an MSc in Medical Microbiology from Mekelle University. His research focuses on antimicrobial resistance, tuberculosis, foodborne pathogens, molecular diagnostics, and drug discovery. He has authored several peer-reviewed publications and is actively involved in microbiology and molecular biology research aimed at improving infectious disease diagnosis, treatment, and public health.