ABSTRACT

Multi-drug resistant tuberculosis (MDR-TB) has emerged as a major challenge for global health that compels an everlasting endeavor for newer molecular targets and treatment strategies. In this context, L-asparaginase of *Mycobacterium tuberculosis* (MtA), an amido-hydrolase is proposed as a novel metabolic target. MtA has been implicated in nitrogen assimilation and neutralization of acidic micro-environment inside human alveolar macrophages. To investigate whether this enzyme could serve as a crucial metabolic target, structural details of MtA was obtained using small angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations. Multiple sequence alignment demonstrated high conservation among catalytic residues of asparaginases from other intracellular pathogens. Interestingly, MtA showed a critical Tyr to Val replacement in its catalytic triad I alongside a substrate induced structural reorganization of the active site β-hairpin loop. We utilized a differential active site-based screening protocol using natural products (TCM) and FDA-approved drug library and ZINC database to identify five potential inhibitors against MtA. Three MtA-specific inhibitors, M3, M26 and M29 showed promising results on Mycobacterium cultures, with inhibitory concentrations (IC_{50}) of 431 μM, 100 μM and 56 μM respectively. Extensive morphological evaluation of compound-treated Mycobacteria using atomic force microscopy (AFM) showed major distortion in rod-shaped bacilli architecture along with deposited cell debris. Overall, in lieu of its major structural differences with human asparaginase, MtA is presented as a new, druggable metabolic target that could be harnessed to improve treatment outcomes against Multi-drug resistant TB.