Title: Discovery of multitargeting inhibitors for Mycobacterium tuberculosis Pkn kinases

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Abstract

Infections by the pathogen *Mycobacterium tuberculosis* (MTb) are a major cause of illness and mortality worldwide. Current treatment regimens involve the administration of multiple antibiotics over several months. The increasing prevalence of drug-resistant MTb strains, combined with the slow pace of novel drug discovery, underscores the critical need for new antibacterial agents specifically targeting MTb.

Our approach to developing new therapeutics for MTb focuses on multitargeting three Pkn kinases, which are essential for mycobacterial survival and pathogenesis. By simultaneously inhibiting two or more Pkn targets with a single agent, we can attempt to bypass the development of drug resistance. These kinases share high structural similarity among themselves but only 20-30% with human kinases facilitating the development of selective antibacterial kinase inhibitors.

Our inhibitor development pipeline relies on activity-based compound screening and validation *in vitro*, orthogonal validation using biophysical methods, cell-based assays for assessing drug effects and X-ray crystallography to visualize the binding of the compound to the kinase.

Screening of in-house small-molecule compound libraries yielded four hits targeting all three kinases *in vitro* with (sub)micromolar affinities. Target binding was validated with isothermal titration calorimetry (ITC) and X-ray crystallography. Three hits out of four prevented the growth of TB cultures in micromolar ranges.

We discovered four compounds that target three different Pkn kinases with good potencies, which were also shown to elicit an effect in cells. Going forward, computational drug development methods will be used to optimize the hits for enhanced physicochemical properties, cell permeability and potency.