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Tamoxifen inhibits histidine kinases of *M. tuberculosis* two-component signaling systems

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Two-component signaling systems (TCSSs) serve as primary signal transduction pathways in bacteria, regulating essential processes including growth, physiology, and virulence, making them attractive drug targets. In *Mycobacterium tuberculosis* (*M. tb*), the PhoPR system plays a critical role in pathogenesis, with the PhoR histidine kinase (HK) functioning at the intersection of cognate and noncognate signaling networks. Using PhoR as a prototypical HK, we hypothesized that targeting this system would compromise *M. tb's* adaptive capacity. We developed and optimized a high-throughput screen of pharmacologically active small-molecule libraries to identify PhoR autophosphorylation inhibitors. Selected compounds were evaluated using kinase assays, protein interaction analyses, molecular docking studies, and growth inhibition using *Mycobacterium bovis* BCG. Screening identified 11 potential inhibitors, with tamoxifen (TAM) demonstrating the most potent activity. TAM inhibited PhoR autophosphorylation at micromolar concentrations both *in vitro* and *in vivo*. Mechanistic studies revealed that TAM competitively binds to the ATP-binding pocket of PhoR with a dissociation constant (K_d) of 108.5 ± 44 nM. Although maximum inhibition was found with PhoR, additional screening of HKs revealed MtrB as another low-affinity target of TAM (K_d = 412 ± 83 nM). Treatment with TAM significantly inhibited *M. bovis* BCG growth in culture and suppressed PhoPR-regulated acid-responsive gene expression. Our findings establish PhoR HK as a promising antimycobacterial drug target and demonstrate the potential for repurposing the clinically approved anticancer drug TAM as an anti-tuberculosis therapeutic with TCS-targeting effects. This work provides proof of concept for targeting bacterial TCSSs and supports further development of TAM derivatives for tuberculosis treatment.

IMPORTANCE Two-component signaling systems are essential for bacterial growth, metabolism, and survival, making them ideal candidates for selective antimicrobial therapy. Tamoxifen (TAM), a well-known anticancer drug, has recently been shown to exhibit antimicrobial activity and is emerging as a potential anti-tuberculosis (TB) agent. In this study, we report for the first time that TAM inhibits *Mycobacterium tuberculosis* histidine kinases, PhoR and MtrB, implicated in virulence. Using a combination of biochemical and computational biology techniques, we demonstrate that TAM competes with ATP for PhoR binding and impairs its autophosphorylation activity, thereby disrupting downstream regulation of gene expression. Dissociation kinetics revealed that in comparison to PhoR, TAM bound MtrB with a lower affinity. These findings establish PhoR as a novel drug target, highlight a plausible mechanism of TAM's antimycobacterial action, and, more importantly, support its repurposing as a promising therapeutic candidate against TB.

KEYWORDS *M. tuberculosis*, two-component signaling system, histidine kinase, tamoxifen, PhoR and MtrB, small-molecule inhibitors, anti-TB drug target

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