Development and Discovery of Novel Inhibitors of *Mycobacterium tuberculosis* RNA Polymerase for the Treatment of Tuberculosis

Shireen R. Ashkar

Tuberculosis (TB) is an infectious pulmonary disease caused by the pathogen *Mycobacterium tuberculosis* (MTB). It is the leading cause of death from a single bacterial pathogen and second, only to COVID-19, as the deadliest infectious disease overall. In 2020 alone, approximately 10 million new cases of TB infection and 1.5 million TB-associated deaths were reported. Additionally, it's estimated that one third of the world's population carries latent TB, an asymptomatic, dormant form of the infection capable of evading the human immune system.

Bacterial RNA polymerase (RNAP) is an enzyme responsible for transcribing DNA to RNA in the process of gene expression. Essential to survival and highly conserved among bacteria but not between prokaryotes and eukaryotes, bacterial RNAP is an attractive, well-studied target which has been validated for the development of potent and selective antitubercular drugs. The rifamycins are a class of macrolide antibiotics which inhibit bacterial RNAP by binding to a pocket on one of its subunits within close proximity of the transcription active site, sterically blocking the elongation of RNA transcripts and effectively inhibiting the enzyme.

Rifampin is the cornerstone of current TB treatment. It is highly potent against MTB RNAP and MTB with the capability of permeating its thick cell envelope. It has also displayed sterilizing effects against low-metabolizing and slow-replicating MTB in vitro which mimic latent infection, a feature which few antitubercular drugs have. Despite such favorable qualities however, the rifamycins suffer from serious drawbacks. Especially problematic, is the emergence of rifamycin-resistant MTB with mutations in or near the rifamycin-binding pocket of MTB RNAP, weakening binding and diminishing potency, resulting in significantly less successful treatment. In addition to this, the rifamycins are potent off-target agonists of the human pregnane xenobiotic receptor (hPXR), leading to the upregulation of metabolic CYP450 enzymes and resulting in drug-drug interactions. Severely impacted by these complications is the treatment of TB-HIV coinfection, as the majority of front-line HIV therapeutics are substrates for the CYP enzymes upregulated and the two infections are highly prevalent in the same regions.

In the work described here, three simultaneous approaches were utilized with the goal of developing novel, potent, inhibitors of MTB RNAP. A structure-based approach was used to design novel benzoxazinorifamycin analogs with improved potency for wild-type and rifamycin-resistant MTB RNAP and MTB, while maintaining minimal activation of hPXR. Initial investigation determined the minimal substitutions necessary on the benzoxazino moiety to avoid hPXR activation, while also probing larger substitutions with minimal hPXR activation and improved MTB RNAP inhibition. This set the basis for more elaborate optimization resulting in a lead molecule with excellent potency for MTB, wild-type and rifamycin-resistant MTB RNAP, negligible hPXR activation, good mouse pharmacokinetics, and excellent activity with no adverse effects in an acute TB mouse model.

A library of microbial natural product extracts was also explored in search of novel inhibitory scaffolds for MTB RNAP. Using bioactivity-guided fractionation and purification paired with high-resolution mass spectrometry, some interesting molecules have been identified in active fractions and are being pursued with follow-up studies to confirm activity. Virtual screening was also used to identify novel inhibitors of a relatively new binding site that does not exhibit cross-resistance with rifampin. A ligand-based screen and an artificial intelligence-based screen have identified novel scaffolds with moderate inhibition which are being studied with computational models to design potentially optimized analogs.