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**Title: Antibiotic Discovery of Inhibitors of the CarD-RNA Polymerase Protein-Protein Interaction in *Mycobacterium tuberculosis.***

## Abstract

Multidrug-resistant tuberculosis (MDR-TB) is estimated to account for 3.7% of new cases of TB annually worldwide and is becoming a major threat to global public health. Rifampin is considered the most important antibiotic for killing the slowly metabolizing *Mycobacterium tuberculosis* (*Mtb*) and acts by targeting DNA-dependent RNA polymerase (RNAP) in order to inhibit transcription. Rifampin resistance is one component of all MDR-TB and extensively drug-resistant tuberculosis (XDR-TB). Resistant TB occurs in the presence of partially suppressive drug concentrations that enable replication of bacteria, the formation of mutants, and overgrowth of wild-type strains by mutants. Due to the prevalence of the MDR-TB and XDR-TB cases, there is an urgent need to improve treatment by either enhancing the application of existing agents or introducing new drugs with novel mechanisms of action. CarD is an essential global transcription regulator in *Mtb* that binds RNAP and activates transcription by stabilizing the transcription initiation complex (also called the open complex). CarD homologues are highly conserved in many eubacteria and have been found to be essential for the survival of the organism in the stringent host environment. Because new treatments for MDR-TB are urgently needed, we propose that CarD will be an effective target for therapeutic discovery for the treatment of TB. Previously, CarD has been selectively labeled with the green BODIPY FL fluorophore or the red DAOTA fluorophore via engineered Cys mutations at multiple locations. A fluorescence polarization assay which monitors the association of *Mtb* RNAP, native rRNA promoter DNA and labeled CarD has been developed, optimized and validated with a known RNAP inhibitor. From an HTS pilot campaign of 24,000 small molecules, one compound (CCG-249580) has shown activity in both CarD FP assays; however, further studies are needed in order to confirm compound binding to CarD. One of my aims is to complete the CarD FP HTS pilot study and advance the hit compound by employing solution NMR spectroscopy in order to investigate CCG-249580 binding to CarD. By using the crystal structure of CarD (PDB ID:4KBM), the molecular docking of CCG-249580 will be performed to obtain a model of the molecular interactions using Molecular Operating Environment (MOE). We also intend to perform an HTS of an additional 5,000 small molecules (a drug repurposing library generated by Prof. Sexton) by employing the CarD FP assay to identify potential inhibitors of the CarD/RNAP interaction. In addition, a virtual screening with the ASINEX database against the potential binding site identified on the CarD (PDB ID: 4KBM) crystal structure will be pursued after we obtain confirming evidence for the binding site. The highest-scoring components of these virtual libraries will be synthesized and evaluated biochemically using the CarD FP assay and microbiologically (e.g., MIC and MBC) to determine their *in vitro* potency. Overall, our objective is to identify and characterize small-molecule inhibitors that block the CarD/RNAP interaction and to understand the mechanisms by which CarD interacts with the molecules. We expect that the development of a new and improved anti-TB compound with a novel mechanism of action that overcomes the burden of resistance will lead to lower mortality and faster recovery rates, regardless of strain or stage of infection.