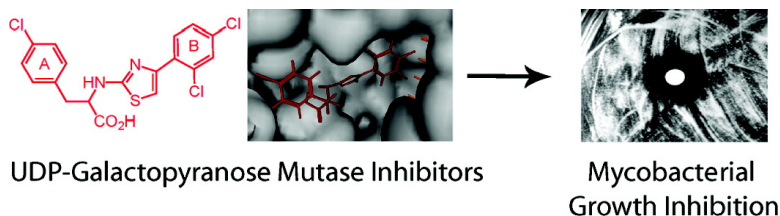Communication

Inhibitors of UDP-Galactopyranose Mutase Thwart Mycobacterial Growth

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Inhibitors of UDP-Galactopyranose Mutase Thwart Mycobacterial Growth

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Galactofuranose (Gal*f*) residues are present in many pathogens. For example, they are essential components of the arabinogalactan layer of mycobacteria. Mycobacteria cause a number of diseases, the most deadly of these is tuberculosis (TB). Each year, *Mycobacterium tuberculosis* is responsible for 8 million human infections and 2 million deaths.[1] Strains have emerged that are resistant to most or all known antibiotics.[2] Resistance can be combatted by developing an inhibitor with a new mechanism of action against a known target.[3] An alternative tack is to identify novel targets. To this end, we focused on an essential enzyme responsible for the incorporation of galactofuranose residues:[4] uridine 5′-diphosphate (UDP) galactopyranose mutase (UGM). UGM uses a unique mechanism to catalyze the isomerization of UDP-galactopyranose (UDP-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*) (Figure 1).[5,6] The gene encoding UGM is essential for mycobacterial viability;[7] the identification of UGM inhibitors could validate it as a therapeutic target. Moreover, Gal*f* residues are found in some eukaryotes; therefore, UGM inhibitors could provide insight into the role of Gal*f*-containing oligosaccharides in these organisms.[8]

Most efforts to develop UGM inhibitors have focused on UDP-sugar substrate analogues.[9–18] These approaches have not yet afforded compounds that block mycobacterial growth. Small, cell permeable compounds have been identified as inhibitors of UGM catalysis and mycobacterial growth.[19] Nevertheless, the UGM inhibition and antimycobacterial activity of these compounds were not correlated, so the utility of targeting UGM was unclear. To probe the consequences of UGM inhibition on mycobacterial viability, we sought to identify new types of UGM inhibitors. To this end, we used a high-throughput, fluorescence polarization (FP) screen.[20] We found several compounds with good IC$_{50}$ values ($\sim 10^{-6}$ M) for the UGM from *Klebsiella pneumoniae* (UGM$_{kleb}$) or *M. tuberculosis* (UGM$_{myco}$). A directed library containing a 5-arylidine-2-thioxo-4-thiazolidinone core was synthesized to identify factors influencing ligand binding.[21] This analysis revealed several thiazolidinone derivatives that serve as ligands for both homologues. The thiazolidinone scaffold, however, reacts reversibly with biologically relevant thiols in solution. Not surprisingly, inhibitors of this structural class fail to block mycobacterial growth.

Given the reactivity of the thiazolidinones, we sought an alternative scaffold that would display functionality important for UGM binding, yet be inert under physiological conditions. We reasoned that stable 2-aminothiazole derivatives would have a shape similar to that of thiazolidinones (Figure 2). In addition, compounds of this class can be assembled efficiently, as illustrated by our production of the parent 2-aminothiazole in four synthetic steps with minimal purification (Figure 3). Specifically, the methyl ester of the racemic phenylalanine analogue was converted to the thiourea using mild conditions.[22] The product was obtained using the Hantzsch thiazole synthesis, in which a thiourea generated from an aryl amino acid and an α-bromo ketone were condensed. The
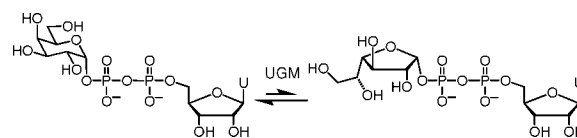
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Figure 1. UDP-galactopyranose mutase (UGM) catalyzes the conversion of UDP-galactopyranose to UDP-galactofuranose. (U ≡ uracil).
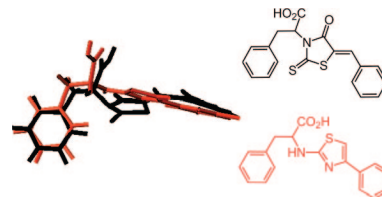

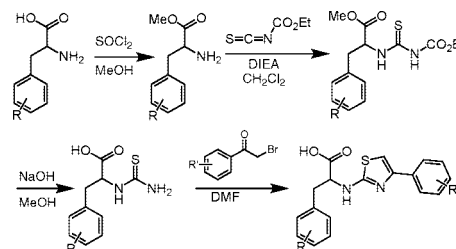**Figure 2.** Overlay of minimized thiazolidinone scaffold (black) and novel 2-aminothiazole scaffold (red).

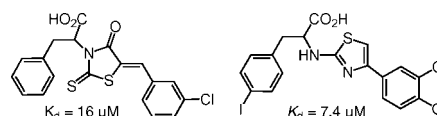**Figure 3.** Synthetic route to substituted 2-aminothiazoles.

Figure 4. 2-Aminothiazoles bind to UGM$_{myco}$ with affinities similar to those of thiazolidinones.

desired products were obtained after a single purification step (silica gel chromatography) in good overall yields (50–70%). Using this approach, we employed 18 commercially available phenylalanine analogues and 23 α-bromo ketones to generate 62 aminothiazoles.[23]

Members of the resulting focused library were screened against UGM$_{kleb}$ (see Supporting Information) and UGM$_{myco}$ using a previously described fluorescence polarization assay.[20,21] Twenty-five 2-aminothiazoles were identified as UGM ligands ($K_d < 60$ μM). Compared to the thiazolidinone ligands identified previously, the most effective 2-aminothiazoles had comparable K_d values (Figure 4). We assessed their ability to inhibit UGM$_{myco}$[21] and found a correlation between their binding affinity and their inhibitory activity (see Supporting Information). Kinetic assays revealed that the active 2-aminothiazoles function as competitive inhibitors with respect to UDP-Gal*f* (see Supporting Information).

With access to a new class of UGM$_{myco}$ inhibitors, we explored which structural features contribute to binding. The 2-aminothia-
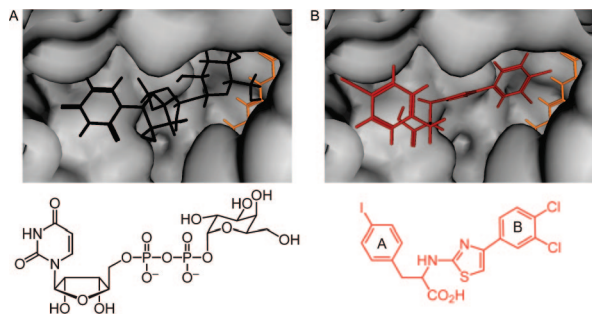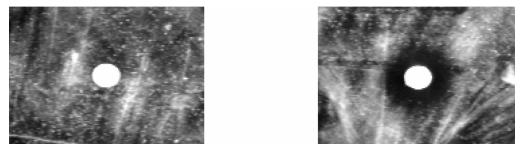
Figure 5. Model of UGM_myco[24] complexed to (A) UDP-galactopyranose (black) (the uracil group is on the left and the sugar on the right) or with (B) inhibitor (red) docked (ring A is on left, ring B on right). A portion of the flavin cofactor (orange) is visible in the binding pocket.
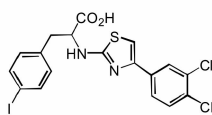

Figure 6. Mycobacterial growth inhibition by a 2-aminothiazole. Discs applied with DMSO (left) or sample in DMSO (right) were placed on agar medium innoculated with *M. smegmatis*. The zone of inhibition persisted throughout the 4 days of the assay.

zoles and the thiazolidinone inhibitors possess aryl substituents in similar relative orientations. We tested the consequences of perturbing this orientation. Specifically, when a phenylglycine rather than phenylalanine building block was used, the resulting 2-aminothiazole was less potent (2−3-fold). Compounds with halogen substituents on either aryl ring or both had increased activity. In contrast, compounds bearing electron-rich rings were less effective inhibitors. A greater variety of substituents could be appended to the B than the A ring (Figure 5), a finding that indicates that the B ring occupies a region of the binding site with fewer steric constraints. This observation is consistent with the effects of aryl substituents on thiazolidinone derivative activity.[21] Together, the results suggest that ring A occupies the uracil binding pocket and aryl group B resides in the sugar binding pocket (Figure 5).

We used a disk susceptibility test to evaluate several active (11 compounds) as well as inactive (3 compounds) 2-aminothiazoles for growth inhibition of *M. smegmatis*. Only the UGM inhibitors block mycobacterial growth (Figure 6). To test for off-target effects, *Escherichia coli* (BL21(DE3)), which lacks the gene encoding UGM,[8,26] was exposed to several mycobacterial growth inhibitors. None inhibited *E. coli* growth. To further characterize the observed antimycobacterial activity, minimum inhibitory concentrations (MICs) were determined for five 2-aminothiazoles with different UGM inhibitory activities. The MIC for the most potent UGM inhibitor was 50 μM, a value in the same range as the clinically used antimycobacterial agents, ethambutol and rifampicin.[25] A direct relationship was observed between UGM inhibitor potency and the MIC (see Supporting Information). This finding suggests

that the ability of the compounds to block mycobacterial growth is related to their ability to inhibit UGM.

Our results support the validity of UGM as a target for antimycobacterial agents. Our findings also highlight the utility of the 2-aminothiazole scaffold for targeting the UDP-sugar binding site of UGM. The similarity between the 2-aminothiazole and compounds found to inhibit other enzymes that act on nucleotide−sugar substrates[27] suggests that this scaffold could yield inhibitors of other UDP-sugar utilizing enzymes.

Supporting Information Available: Experimental procedures, compound characterization data, and sample binding curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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