Whole cell target engagement identifies novel inhibitors of *Mycobacterium tuberculosis* decaprenylphosphoryl-β-D-ribose oxidase

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We have targeted the *Mycobacterium tuberculosis* decaprenylphosphoryl-β-D-ribose oxidase (Mt-DprE1) for tuberculosis. Using a whole cell target engagement strategy that overexpressed Mt-DprE1 in *M. bovis* BCG, we profiled the anti-mycobacterial set of compounds publically released by GlaxoSmithKline and identified one compound (GSK-D1) that showed an 8-fold higher minimum inhibitory concentration relative to the control strain. Analogues of GSK-D1 show a clear relationship between whole cell potency and *in vitro* activity using an enzymatic assay employing recombinant Mt-DprE1, with binding affinity measured by fluorescence quenching of the flavin cofactor of the enzyme. *M. bovis* BCG spontaneous resistant mutants to GSK-D1 and a closely related analogue were isolated and sequencing of ten such mutants revealed a single point mutation at two sites, E221Q or G248S within DprE1, providing further evidence that DprE1 is the main target of these compounds. Finally, time-lapse microscopy experiments showed that exposure of *M. tuberculosis* to a compound of this series arrests bacterial growth rapidly followed by a slower cytolysis phase.