Title: Deconvoluting the mode-of-action of novel antileishmanial compounds

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Abstract

Compounds from the GSK Leishbox are active anti-leishmanials identified by phenotypic screening, but many of them have an unknown mode-of-action. This study aimed to identify the targets of a subset of nine LeishBox compounds, reduced to four following initial triage. To achieve this, drug-resistant strains of *L. major* and *L. mexicana* promastigotes were generated by *in vitro* evolution and then subjected to whole-genome sequencing. Following SNP calling and identification of coding mutations, four potential targets were selected for genetic validation, including two hypothetical proteins, one encoding a predicted amino acid transporter (AATP11), and one encoding a folate biopterin transporter (FBT).

Initially, null mutants were generated in *L.mx T7/Cas9* to separate likely essential targets from non-essential genes. Null mutants could be generated for all four genes, suggesting that these are not the sole targets of the compounds. Furthermore, since two of the proteins were predicted to be transporters, mutations in their genes were hypothesized to confer resistance to the compounds. When the *Δaatp11* strain was treated with compound 3, it resulted in a 2-fold increase in the EC_{50} of the compound. Addback and over-expressor strains have been generated to confirm that this phenotype is gene-specific. The *Δfbt* strain is currently being assayed. This target is

part of a tandem gene array that is often mutated in response to anti-folates such as methotrexate.

As target deconvolution approaches typically apply multiple intersecting assays to converge on high-confidence targets, these compounds are currently being used in cellular thermal shift proteomics and untargeted metabolomics approaches to increase the understanding of their modes of action.