Acceleration of TB drug discovery by AI-based virtual screening against underexploited targets

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ABSTRACT

The global impact of Tuberculosis (TB), which causes ~10 million new infections and ~1 million deaths annually, is exacerbated by the lack of effective therapeutics and the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis* (Mtb). Historically, clinically used TB drugs and late-stage candidate compounds have been discovered by whole-cell phenotypic screening campaigns, which account for the formidable permeability barrier presented by the mycobacterial cell wall. While this approach has proven successful, it is logistically demanding to execute an HTS of large compound libraries in a Biosafety Level 3 facility. Subsequent identification of the target and mechanism of action of hits, which is important for structure-based hit-to-lead optimization, presents a second daunting challenge. As a result, there remains an urgent need for new anti-TB drugs and many genetically validated drug targets in Mtb remain to be exploited. In this project, we sought to harness the power of AtomNet® technology, the world's first deep convolutional neural network for structure-based drug discovery developed by Atomwise Inc., to accelerate the discovery of novel scaffolds and lead compounds for TB drug development. We conducted virtual screens of 2.5 million commercially available compounds for predicted inhibitors of FusA1, an essential ribosomal accessory factor involved in ribosome translocation, and LdtMt2, an L,D-transpeptidase responsible for unusual 3'-3'cross-linking of the peptidoglycan layer of the cell wall. Primary screening of 94 compounds for each target against a bioluminescent reporter strain of Mtb revealed 10 novel scaffolds (5 per target) with dose-dependent whole-cell antimycobacterial activity. In addition to establishing structure-activity-relationship (SAR) profiles for prioritized scaffolds by assaying of analogs, demonstration of target-specific activity is also underway using both microbiological and biochemical strategies. The discovery of first-in-class inhibitors of underexploited targets with potent bactericidal activity against Mtb would validate this AI-driven strategy for the rapid, more cost-effective development of novel TB therapeutics.

BACKGROUND

LdtMt2

- Dominant L,D-transpeptidase (Ldt) in Mtb responsible for forging 3→3 crosslinking of peptidoglycan (PG) stem peptides. Cysteine active site. No mammalian homologs.
- ~80% of PG is 3→3 linked in stationary phase Mtb
- Most bacteria have 4→3 crosslinked PG made by D,D-transpeptidases (aka penicillin binding proteins, PBPs). Serine active sites
- Most classes of beta-lactam antibiotics can inhibit PBPs but NOT Ldts
- Loss of LdtMt2 → altered morphology, slow in vitro growth, attenuated in chronic phase of infection in mouse model
- GOAL: ID of first-in-class, non-covalent, tight binding, β-lactamase-proof inhibitors of LdtMt2

FusA1

- Auxiliary translation factor with dual role – Ribosome translocation + ribosome recycling.
- Essential in vitro. Efficacy of natural product fusidic acid proves druggability of this target.
- Fusidic acid is difficult to synthesize, analogs have shown no improvement in activity
- GOAL: ID of novel inhibitors with no cross-resistance with current TB drugs, selectivity for bacterial FusA1

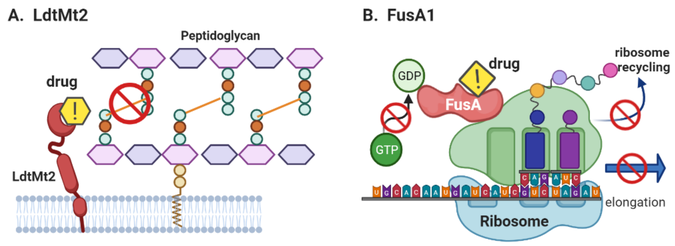
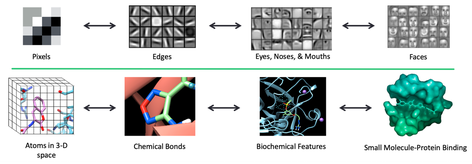


Figure 1: Mechanism of action of proposed inhibitors.

METHODS

AtomNet® Virtual Screening



AtomNet® model – Deep Convolutional Neural Networks applied to drug discovery

To identify novel inhibitors of two Mtb targets - FusA1 and Ldt2, AtomNet® model was utilized to score a library of 2.5 millions commercially available molecules. A set of 94 compounds, optimized for the combination of binding affinity, drug-likeliness, and cell permeability, were selected and made available for experimental validations for each target.

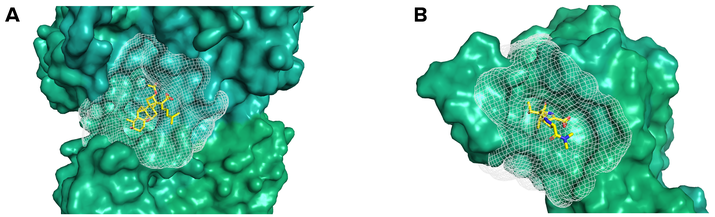


Figure 2: Models of known inhibitors bound to target site utilized in defining binding pockets for virtual screening. (A) Fusidic acid- bound pocket of T. thermophilus FusA, the template used to build homology model for Mtb FusA1. (B) Meropenem-bound pocket of Mtb LdtMT2.

Whole Cell-based Phenotypic Screening Approach

Selective antimycobacterial activity of Atomwise compounds were evaluated using 2 assays:

- **Antimicrobial activity against replicating Mtb**
  - Primary screen: All virtual hits at single concentration (20μM)
  - Minimum Inhibitory Concentration (MIC_{99}): Dose-response curves of select hits
- **Cytotoxicity for mammalian cells**
  - IC_{50}: Concentration at which 50% loss of viability of macrophages occurs
  - Selectivity Index (SI): Measure of therapeutic window

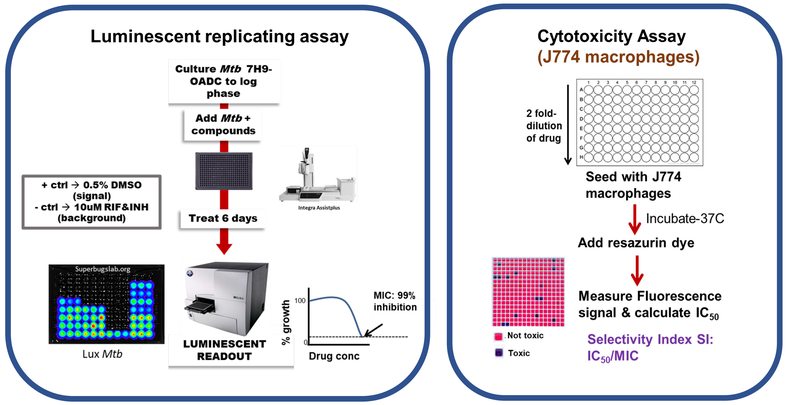
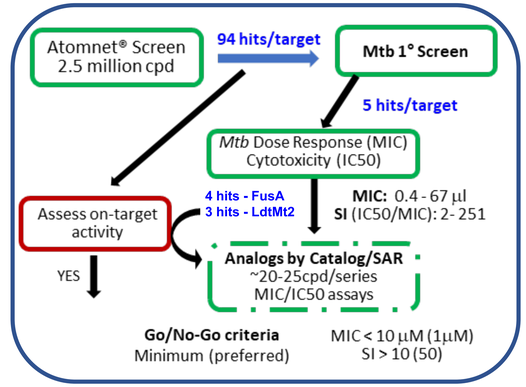


Figure 3: Whole Cell Phenotypic Assays used to evaluate Atomwise's predicted compounds

RESULTS

Primary screening against Mtb



- AI-based virtual screening identified 94 top predicted high-affinity binders per target
- 1° screen at a single concentration (20μM) identified 5 hits with >50% Mtb inhibition for each target.

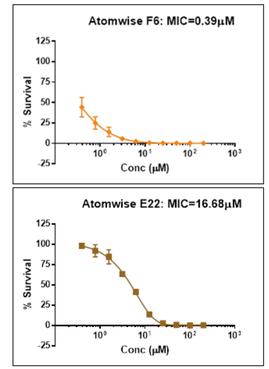
Figure 4: Screening Steps for anti-TB activity of AI–hits. **Green:** Completed, **Dotted Green:** in progress, **Red:** Future work

Mtb Dose Response and Cytotoxicity

A

Cpd	MIC-TB (μM)	IC_{50}-TB (μM)	IC_{50}-J774 (μM)	SI
F6	0.4	0.3	98.1	251
A12	32.9	9.5	592.5	18
G1	13.3	2.8	78.1	5.9
D1	21.3	4.7	73.7	3.5
E22	16.8	4.8	83	4.9
C20	32.8	10.2	123	3.8
F21	67.9	22.1	197	2.9

B



Anti-TB activity of hits: (A) Table showing MIC and SI of hits for each of the target. **Blue**: FusA1 inhibitors, **Green**: LdtMt2 inhibitors. Values represent average of 3 replicates. (B) Dose-response curves for the best compounds with respect to MIC and SI for each target.

CONCLUSION & FUTURE WORK

In vitro anti-TB activity of virtual hits identified by AtomNet® technology for FusA and LdtMt2 demonstrates the potential of AI-powered structure-based discovery of novel anti-TB drugs.

Future studies focusing on the whole-cell active and selective hits will include:

- Evaluation of target specific activity (FusA and Ldt2) through biochemical assays
- Evaluating analogs of each hit/target for anti-TB activity
- Establishing structure-activity relationship (SAR) and Hit to Lead Optimization
- Prioritizing compounds based on potency, selectivity and ADME properties