Background: DNA is critical for bacterial initiation of replication and has no known inhibitors. This enzyme binds at the origin of replication, holo-enzymes, and regulates the double helix, allowing helicase to load. DNA is an ideal antibacterial target due to its multiple functions in a vital pathway, its high level of conservation across many bacterial species, and the absence of homologs in humans. To date, inhibitors of DNA, we altered an E. coli nsp3 gain-of-growth cell-based screen (5), applied to a compound library using a high throughput screening (HTS) campaign and characterized the hits.

Methods: Strain SF3 (ΔtolC Δrnh) was rendered efflux deficient by deletion of tolC. Compounds were screened for rescue of growth (E. coli ΔtolC Δrnh), which exhibits a cold-sensitive phenotype due to toxic overproduction of DNA replication at 20°C unless DNA is inhibited. Because DNA is required for replication, toxicity inhibition would also be lethal. To counter this, SF3ΔtolCΔrnh also contains a deletion of Fossum H, allowing for a less efficient alternative mechanism of replication. Positive control strain SF8 maintains a plasmid-encoded non-catalytic DnaA, which inhibits cellular DNA via competitive binding of the origin.

Results: The HTS had an average signal to noise ratio of 0.4 and Z’ factor of 0.65. Screening 162,600 compounds yielded a primary hit rate of 0.8%. A confirmatory screen, performed in quadruplicate, yielded a confirmed hit rate of 0.05%. In dose-dependent studies, these compounds showed minimum growth inhibiting concentrations ranging from 4.2-2548 µM. Eight hits have been shown to inhibit growth of SF3ΔtolCΔrnh at permissive temperature with minimum inhibitory concentrations ranging from 10-80 µg/mL. Of these hits, compounds also inhibited growth of E. coli 701, which carries wild type DnaA with MICs of 40-320 µg/mL. Mammalian cytotoxicity (CC50) ranged from 20-40 µg/mL.

Conclusion: The DNA-specific assay was successfully transferred to HTS to detect inhibitors of DNA, an excellent target for a new class of antibiotics.

- Infections by multi-drug resistant bacteria such as Acinetobacter baumannii, extended spectrum β-lactamase-producing Xanthomonas campestris and Klebsiella species. Pseudomonas aeruginosa, vancomycin-resistant Enterococcus faecalis, Mycobacterium tuberculosis, and methicillin-resistant Staphylococcus aureus (MRSA) are becoming more abundant and effective treatments are becoming limited.

- These bacteria have become resistant to many antibiotic classes, which share many of the same targets and mechanisms. There is an immense need for innovative antibiotic classes that are directed at novel targets. Microbiotix has approached this need by developing inhibitors of an unexplored target in an validated pathway of DNA replication: the enzyme DNAA.

- DNA is a multifunctional protein that is essential to bacterial DNA replication, is highly conserved across a broad range of bacteria, and has no homolog in humans. DNAA is an ideal target for an antibiotic.

- Figure 2. Structure of most promising hits.

- Figure 3. Optical Densities within Four Screening Plates.

- Figure 4. Configuration of 96-well plates in DnaA inhibitor screen.

- Table 1. DnaA Inhibitor Screen Metrics.

- Table 2. Summary of screening results.

- Table 3. Screening results for final hits.

- Table 4. Minimum Inhibitory Concentrations (MICs) and Cytotoxicity for final hits.

- Table 5. Compound ID Number.

- Table 6. Compound ID Number.

- Table 7. Compound ID Number.

- Table 8. Compound ID Number.