SOUTHERN RESEARCH

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Antituberculosis High Throughput Drug Screening at Southern Research Institute

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Abstract

The global burden of tuberculosis (TB) remains enormous. An estimated 3 million people worldwide die of tuberculosis each year and another 1.7 billion are infected with *Mycobacterium tuberculosis* (*Mtb*). An increase in the incidence of multi-drug-resistant TB (MDR TB) and the recent emergence of extensively drug-resistant TB (XDR TB), especially in developing countries, underscores the current need for new drugs to treat these infections. The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established in 1994 by the National Institute of Allergy and Infectious Diseases to facilitate the discovery of new antitubercular drugs. The program, managed by Southern Research Institute, assists commercial, academic and government laboratories worldwide in identifying new chemical classes of compounds for evaluation in *in vitro* and *in vivo* models. Through the direction and funds of NIAID, Southern Research Institute added high-throughput screening (HTS) services to the program in the fall of 2001 (NIH-NIAID *Antimicrobial Drug Screening Contract*; N01-A1-15449). The HTS component allows for screening of large compound libraries against specific validated targets and expanding the primary *in vitro* screening efforts to a high throughput format. The efforts through this program have resulted in the identification of several promising compounds that have entered advanced evaluations in animal models. The promises and challenges of the HTS component of this NIH program will be presented.

Introduction

Mycobacterium tuberculosis (*Mtb*) is a notorious pathogen whose increasing resistance to antibiotics and heightened lethality in combination with AIDS makes it a major health concern worldwide. The World Health Organization estimates that one-third of the world's population is infected with *Mtb*; eight million people worldwide develop tuberculosis annually while nearly two million die. *Mtb* causes more deaths than any other infectious agent in the world. Immunocompromised individuals, particularly those infected with human immunodeficiency virus (HIV), are at great risk for infection with *Mtb*. WHO estimates that 11.4 million people worldwide are infected with both *Mtb* and HIV. Although infections with drug sensitive strains can be effectively cured with a 6 to 9 month regimen of multiple antibiotics, the inability to deliver and complete appropriate courses of therapy on a global level has led to the selection of resistant strains over the past 50 years. Especially alarming is the upsurge in cases of multidrug-resistant tuberculosis. The selection and spread of multiple drug resistant *M. tuberculosis* continued for decades leading to selection and spread of two operationally distinct forms, multiple drug resistant (MDR-TB resistant to isoniazid and rifampicin) and extensively drug resistant (XDR-TB resistant to isoniazid, rifampicin, a fluoroquinolone and at least one of the injectable second-line agents). The estimate for global MDR-TB and XDR-TB cases for 2005 were 424,000 and 27,000 respectively, and the situation is worse in areas with high incidences of HIV infection. XDR-TB has been found in 41 countries. The recent outbreak of XDR-TB in a rural area in KwaZulu Natal, South Africa brought XDR-TB to the attention of global health organizations. During this outbreak 52 of the 53 patients with XDR tuberculosis died with a median survival from the time of specimen collection to death of 16 days (range 2–210 days). New XDR-TB strains continue to arise and the global situation is very dire for the future.

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established in 1994 by the National Institute of Allergy and Infectious Diseases to facilitate the discovery of new antitubercular drugs (www.taacf.org). The program, managed by Southern Research Institute, assists commercial, academic and government laboratories worldwide (fig.1) in identifying new chemical classes of compounds (fig. 2) for evaluation in *in vitro* and *in vivo* models. These screening activities have resulted in several promising agents that have reached advanced stages of testing. Compounds are initially evaluated to determine their maximum tolerated dose (MTD) in mice and their oral bioavailability. Antituberculosis activity is determined *in vivo* first in an interferon gamma knockout (GKO) mouse model followed by murine aerosolized *Mtb* mouse model (standard model).

Introduction

In 2001, the program was augmented by the inclusion of a high throughput screening contract "Microbiological Drug Screening." The statement of work for this contract includes 1) implementing biochemical, target-specific drug screening assays and 2) implementing *Mtb* drug screening in HTS format. In the early years of this contract a number of biological targets were validated as automated assays and offered to the public through the TAACF. In 2007 when the H37Rv HTS assay came on line >200,000 compounds were screened. Several chemical scaffolds were identified from this screening effort that were different from known anti-TB drugs.

TAACF Program

TAACF-Acquired Compound Libraries

Libraries Procured

- 97,920 TAACF Suppliers (Figures 1 &2)
- 102,000 compounds in conjunction with NINDS
- 101,000 compounds in NIAID's ChemBridge diversity library
- 50,000 compounds in Dr. Jim Sacchettini's ChemDiv (Tripos) diversity library
- 1120 compounds in the TAACF's Prestwick library
- 26,734 kinase inhibitor-like compounds from Live Chemicals

Figure 1 TAACF Compounds Received Distribution Summary

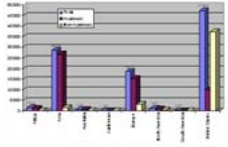
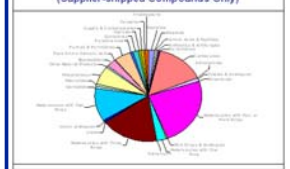


Figure 2 Breakdown of Compound Classifications (Supplier-shipped Compounds Only)



Status of *In Vivo* Candidates

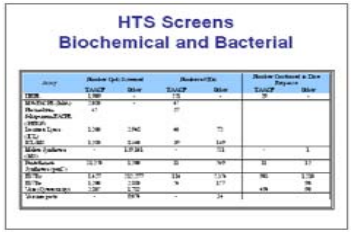
Samples with in vivo data (includes TB drug analogs)

173 Total
126 MTD
133 GKO
33 Standard Model
79 pK BioAssay

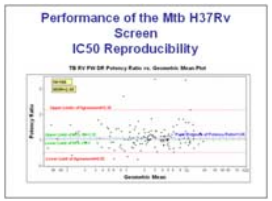
In Vivo Testing Results
48 Actives in GKO
12 Actives in Standard Model
4 Actives in both GKO and Standard

Microbiological Drug Screening Program

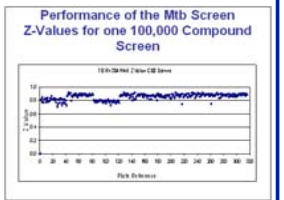
HTS Screens Biochemical and Bacterial



Performance of the Mtb H37Rv Screen IC50 Reproducibility



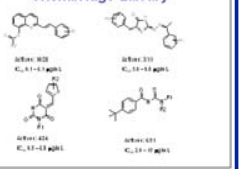
Performance of the Mtb Screen Z-Values for one 100,000 Compound Screen



High Throughput Screening of Chembridge Library of Compounds

Number of compounds Screened in Primary Assay: 100,680
Number of compounds with > 90% Inhibition at 10 μg/ml = 1782
Number of compounds with IC_{50} < 10 μg/mL = 1104
Number of selected active compounds: 1064
(M. tb IC_{50} < 10 μg/mL and VERO cell CC_{50} > 10 μg/mL)
Number of chemical clusters: 44 (covers ~ 250 active compounds)
Active nonclustered: ~ 800 compounds

Active Scaffolds from Chembridge Library



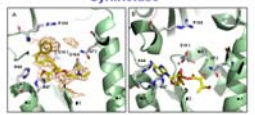
Microbiological Drug Screening Program

Biochemical Targets - panC

- Biology:
- The enzyme catalyzes the formation of pantothenate, an essential precursor in the biosynthesis of coenzyme A (CoA) and acyl carrier protein (ACP).
- Biosynthesis of pantothenate is essential for Mycobacterium tuberculosis
- Important factor in virulence
- Pantothenate Synthetase (EC 6.3.2.1)
- Inhibitors - none
- Collaborators:

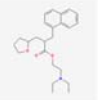
Celia Goulding and David Eisenberg, Howard Hughes Medical Institute, UCLA

Pantoyl Adenylate Bound in the Active Site of Pantothenate Synthetase



Biochemical Targets - panC

- Screened against Prestwick and Microsource Compound Libraries
- Naltrexyl oxalate – a vasodilator used in the treatment of vascular and cerebral disorders
- K_i= 75 μM
- Soaked into PS crystal – electron density in both active sites of the dimer
- White et al. JBS:12:100, 2007.



Acknowledgments

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