

Abstract

A goal of the NIH Roadmap Initiative is to empower the scientific community with small molecule probes as tools to study complex biological processes involved in health and disease. To accomplish this goal, the mission of the SRMLSC is to provide HTS and chemistry resources to identify probes for academic investigators. Since 2005, the SRMLSC has conducted over 30 HTS campaigns and has uploaded over 2.5 million data points to PubChem. Biochemical, cell-based and phenotypic assays have been run to support a broad range of biological research. Many of the assays are cell-based and involve genetically-engineered yeast, bacteria, or mammalian cells. For example, a number of yeast-based screens have been run to identify probes that affect lifespan, post-Golgi transport, mitochondrial division or other cellular processes. We have also run various potency-directed assays. For example, a screen was run to identify inhibitors of the enzyme, pantothrenate synthase, implicated in tuberculosis, which yielded a novel high potency probe. A variety of phenotypic-based screens have also been conducted. For example, a series of probes were identified that reverse the multidrug resistant phenotype. Other assays were conducted that required BSL-3 containment, for example, to identify probes that inhibit cytopathic effects of various viral pathogens. We also perform image-based screenings using high speed confocal or laser scanning microscopy. Given that most assays conducted by the MLCSC involve non-conventional drug targets, this program is anticipated to result in a rich and unique database of compounds for investigators with interests in drug discovery. The presentation will highlight specific assays and our process for probe identification and optimization to encourage a discussion of differences with big pharma. In addition, a major challenge for the network relating to the need for assessing probe specificity and the use of cell-based assays to profile hits will be discussed.

Introduction

For more than sixty-five years, Southern Research Institute has been a leader in life sciences research specifically in the fields of drug discovery and drug development. The primary goal of this pharmacological research is to identify novel, small molecule compounds that lead to progress through the drug discovery to FDA acceptance. SRI leads the anti-cancer agent market by discovering six FDA-approved compounds with an additional six drug leads currently in clinical trials. Additional therapeutic discovery work is being done in infectious diseases including bio-defense targets and in neuroscience. Three major groups in the company's drug discovery division have led the way to such successes: medicinal chemistry, biochemistry and molecular biology, and high-throughput screening. The medicinal chemistry department's role not only involves developing new compounds with drug-like characteristics but also redesigning hit compounds to improve such characteristics through computational chemistry, protein crystallography, and combinatorial and parallel synthesis. The function of the biochemistry and molecular biology department is to develop new biological and biochemical assays for compound screening, analyze and explain new pathway targets, and determine drugs' mechanisms of actions. The high-throughput screening center is a multi-million dollar facility designed to run target and cell-based assays on robotics platforms screening large compound libraries to find lead compounds for optimization and advancement.

As a not-for-profit entity, SRI's drug discovery research is often funded by funding from the National Institute of Health. Even as the government funding is granted to a particular department or group, the three mentioned above have all had a hand in the achievements of Southern Research's drug discovery efforts in the following contracts or projects: the Southeast Regional Center of Excellence for Biodefense (SERCEB), the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), NIAID's Antimicrobial Acquisition and Coordinating Facility (AACF), the NINDS Drug Screening Facility for Neurodegenerative Diseases, the *Mycobacterium Tuberculosis* and bio-defense targets screening contract, the SARS and influenza *in vitro* antiviral screening contract, and the HIV targets screening contract. After such great success in screening hundreds of thousands of compounds through these NIH-funded programs, SRI was again recognized for its outstanding drug discovery accomplishments and chosen to be a screening center as part of the Molecular Libraries Screening Centers Network (MLSCN), a major campaign of the NIH Roadmap initiative. In June of 2005 the Southern Research Molecular Libraries Screening Center (SRMLSC) was created. The goals and achievements of the SRMLSC and its future will be presented here.

Results

Figure 1. Distribution of SRMLSC assays by type

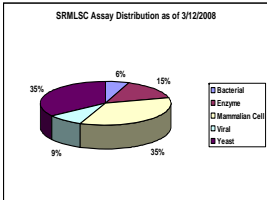


Figure 2. Total number of compounds screened by the SRMLSC

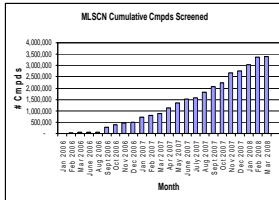


Table 2. A Selection of Scaffolds Entering Probe Optimization Chemistry

ASSAY - STATUS - COMPOUND TYPE	SCAFFOLD	ASSAY - STATUS - COMPOUND TYPE	SCAFFOLD
Pantothrenate Synthase Inhibitors Probe identified, chemistry completed	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Dimethylamino pyrimidine Nucleoside Kinase Inhibitors Probe identified, chemistry completed	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
<i>S. pneumoniae</i> Mevalonate (SCAFFOLD 2)	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Dual <i>S. pneumoniae</i> Inhibitors Probe identified, chemistry completed	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
SAR work on probe with increased potency compared to parent compound	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	IPEN tumor suppressor (isogenic cell line screen)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
<i>S. pneumoniae</i> Disubstituted Probe identified, chemistry completed	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	β -catenin oncogenic protein (isogenic cell line screen)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
40 SAR work on probe with increased potency compared to parent compound	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Adenovirus - inhibitor of viral-induced apoptosis	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
41 Mitochondrial Drug Resistant Reversal (SCAFFOLD 1)	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Mitochondrial fusion inhibitors (yeast)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
42 RAM network inhibitors - yeast morphology regulated pathway	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Identification of NF- κ B activators for neurodegenerative disease	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
HANCE-Specific Antagonism (SCAFFOLD 1)	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	HMG-CoA reductase inhibitors as novel antimicrobials	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
43 Probe optimization complete	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Inhibitors of intracellular A β aggregation for Alzheimer's disease	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
44 Yeast Post-Golgi Protein Transport (SCAFFOLD 1)	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	A cell based assay to identify inhibitors of HSV infectious disease	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
45 Probe optimization complete	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Inhibitors of Plasmodium falciparum amphoterpines M1 for malaria	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
46 Yeast lifespan extension (SCAFFOLD 1)	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Compounds that augment coxsyphilic viruses for prostate cancer	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
47 Probe optimization complete	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Mycobacterial GlnM (Glucosamine-1-phosphate acetyl transferase)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
48 H2O2 Effluxion Inhibitors Probe identified, chemistry completed	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Inhibitors of Mycobacterium tuberculosis strain 37Rv (BSL3)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
49 Probe identified, chemistry completed	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Cell viability - HTS assay (M59 lung tumor cells)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>
50 Probe identified, chemistry completed	<chem>Cc1ccc(cc1)C(=O)Nc2ccc(cc2)C(=O)N</chem>	Cell health - HCS assay (HT29 colon tumor cells)	<chem>Cc1ccc(cc1)Nc2ncnc3c2n(C)cn3</chem>

*Excluding original ML-SMR compounds tested in HTS

Results

Table 1. Assays assigned to Southern Research Molecular Libraries Screening Center.

Assay Description	Date Assigned	Compounds Screened	Assay Submitter
Pantothrenate synthase inhibitors for tuberculosis	Jan-06	10011	White, SRI
Pantothrenate synthase inhibitors	Jan-06	10011	White, SRI
He22 AlphaScreen for breast cancer	Jan-06	135281	Chicago, Sloan-Kettering
Probes that extend yeast lifespan	Jan-06	134293	Goldfarb, Univ. Rochester
Probes that suppress yeast lifespan	Jan-06	159372	Goldfarb, Univ. Rochester
Inhibitors of MRP8 multidrug resistance protein (cell based)	May-06	190098	Piazza, SRI
Post-Golgi transport (yeast)	May-06	118095	Hansay, Univ of Kansas
Counter screen for post-Golgi transport assay (yeast)	May-06	97552	Hansay, Univ of Kansas
Antimicrobial pro-drugs (U-95963 strain)	May-06	119727	Lewis, Northeastern Univ.
Antimicrobial pro-drugs (E. coli ToC mutant strain)	May-06	65385	Lewis, Northeastern Univ.
Bacterial mevalonate kinase (kinetic enzyme assay)	Jul-06	65643	Leyh, Albert Einstein
Phosphoenolpyruvate kinase (kinetic enzyme assay)	Jul-06	65737	Leyh, Albert Einstein
Diphosphoenolpyruvate decarboxylase (fluorescence enzyme assay)	Jul-06	65730	Leyh, Albert Einstein
MDPI1 activates as probe for pseudomonas discolorum	Sep-06	149073	Piazza, SRI with P&E International
Endothelial cell proliferation (angiogenesis)	Nov-06	96560	Qi, SRI
Fibroblast (IL-6) proliferation - counter screen for endothelial cells	Apr-07	85227	Qi, SRI
Highly pathogenic avian influenza	Apr-07	95825	Sevenson, SRI
IPEN tumor suppressor (isogenic cell line screen)	Apr-07	148445	Waldman, Georgetown
β -catenin oncogenic protein (isogenic cell line screen)	Apr-07	148445	Waldman, Georgetown
Adenovirus - inhibitor of viral-induced apoptosis	Apr-07	214017	LL, SRI
Mitochondrial fusion inhibitors (yeast)	May-07	Pending	Nunnari, Univ. CA, Davis
Mitochondrial fusion inhibitors (yeast)	May-07	388470	Nunnari, Univ. CA, Davis
RAM network inhibitors - yeast morphology regulated pathway	May-07	204175	Weiss, Northwestern Univ.
Identification of NF- κ B activators for neurodegenerative disease	Sept-07	193257	Grimaldi, SRI
HMG-CoA reductase inhibitors as novel antimicrobials	Oct-07	194425	Staufferich, Purdue
Inhibitors of intracellular A β aggregation for Alzheimer's disease	Oct-07	Pending	DeLisa, Cornell
A cell based assay to identify inhibitors of HSV infectious disease	Dec-07	Pending	Sevenson, SRI
Inhibitors of Plasmodium falciparum amphoterpines M1 for malaria	Dec-07	Pending	Gardiner, Queensland Institute of Medical Research, Australia
Compounds that augment coxsyphilic viruses for prostate cancer	Dec-07	Pending	Passer, Mass General Hosp
Mycobacterial GlnM (Glucosamine-1-phosphate acetyl transferase)	Jan-08	Pending	Mitchell, Colorado State
Inhibitors of Mycobacterium tuberculosis strain 37Rv (BSL3)	Mar-08	Pending	White, SRI
Cell viability - HTS assay (M59 lung tumor cells)	NA	3317	QC assay for liquid handling equipment
Cell health - HCS assay (HT29 colon tumor cells)	NA	9950	QC assay for HCS instrumentation

Discussion

The major goals of the Molecular Libraries Screening Center Network were very much aligned with those achievements aimed at reaching by Southern Research Institute's drug discovery research group. The broadest aim of the MLCSCN was to screen large numbers of compounds to identify and subsequently optimize small molecules that selectively interact with specific biological targets so that they may be used as probes to understand the function of newly characterized proteins, and to analyze physiological processes, biological pathways, and disease mechanisms. (Piazza, RA-RL-08-005) Because of the experience and expertise of SRI's drug discovery staff, the creation of SRMLSC and its assimilation into the network as a whole proved to be a smooth process. After its inception, the SRMLSC worked diligently to meet all goals and milestones established by the NIH in order to be a successful screening center.

The SRMLSC has had a total of thirty-four assays assigned to it through this program as shown in Table 1. Only those assays commissioned to SRI since December of 2007 are currently in the validation stage awaiting primary and secondary screening. A further break-down of the assays by type is highlighted in Figure 1 demonstrating the SRMLSC's ability to fulfill one aim of the MLCSCN: to carry out multifarious assay projects. The assignments - cell-based assays including not only mammalian cells but also yeast, bacteria and viruses and enzyme-based biochemical assays - originated from an assortment of academic organizations with varying fields of expertise. The staff's depth of knowledge was tested and proven during the implementation of each of these projects and demonstrated again with the assays they themselves submitted to the network thus meeting another objective of the program: the development of innovative assays for compound screening. The quality of data produced by these screening endeavors has led to the medicinal chemistry group's work on probe development projects on nine chemical scaffolds identifying approximately seventy compounds thus complying with another one of the MLCSCN's goals (see Table 2).

The construction and assembly of the high-throughput screening center at Southern Research several years before the establishment of the SRMLSC was an advantage to the program. Removing the lag time for equipment purchase, employee training, and proper operation, the group moved the assigned assays quickly from validation phase to screening on the robotics platform. As the center had already shifted most of its screening campaigns to 384-well format, increased production as a required goal of the MLCSCN to screen compounds in a higher density 1536-well format was easily achieved in several different types of assays. To aid in the efficiency of this process one piece of equipment, an acoustic-based liquid handling dispenser, was integrated into the existing HTS operations. As a means of monitoring the quality of the data produced by the SRMLSC, a Laboratory Information Management System (LIMS) was also incorporated into the screening set-up to capture information on all constituents that could affect the outcome of a screen. Every part of the screening process, from reagents to consumables to instrumentation including QC measurements, is stored in this database for querying. Additionally the SRMLSC Informatics & Cheminformatics team used ActivityBase for collecting compound library samples' molecular structures and storing each sample's associated screening data. They have designed ABase data analysis protocols for each assay implemented by the SRMLSC and accommodated its increased workflow as well by integrating the 1536-well screening formats. In addition to the capabilities mentioned above, there is another specialized service that the SRMLSC contributed to the network - BSL-2/3 level screening. The BSL-2 and BSL-3 facilities were used in several screening projects already established by SRI's drug discovery group. The group easily adapted incoming MLCSCN assays requiring BSL-2 or BSL-3 level containment to the existing screening system. The BSL-2 and BSL-3 laboratories contain HTS equipment that is run by personnel trained both in HTS methods and biological safety techniques.

The obvious success of the SRMLSC as part of the NIH's Molecular Libraries Screening Center Network can be attributed to Southern Research's proven track record in the field of drug discovery along with the dedication and depth of expertise found in the personnel. Figure 2 shows not only the overall number of compounds screened by Southern Research over the course of the project (3,402,999) but also exactly how the production and efficiency of the SRMLSC continues to improve on a monthly basis. And to the credit of the SRMLSC, the SRI-CMC persists as they plan to complete the screening of all the assays currently assigned to them and are pursuing additional funding through the MLCSCN phase of the NIH Roadmap initiative.

References & Acknowledgments

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