Drug Discovery Pipeline

Bench.............to.............Bedside

Some facts:

• 10% of the compounds make it to preclinical development
• 70% of these compounds make it to Phase 1
• 20% of Phase 1 compounds make it to market
• Odds are < 10% of making it to market
• 30% of new drugs return cost of development
• 6 yrs from idea to First In Human (FIH)
• 8 yrs from FIH to market approval
  • Total of ~ 12-15 yrs to get to market
• $802 MM cost of drug to market

-AlzForum.org ~2007
A Few Steps to Clinical Trials

Hit/Lead identification

Potency

Selectivity

Metabolic stability in mice and human

Hepatocyte stability in mice and human

Solubility

Pharmacokinetic profiling

CNS penetration

Formulation

In vivo toxicity (MTD)

In vivo efficacy

Clinical Trial
What is needed for ‘Hit to Lead’?

1. Identification of Hits/Lead Compounds
   a. High Throughput Screens (HTS)
   b. Rational Design / Med chemistry of literature compounds
   c. Computational Approach
   d. Structural Biology/Crystal Structure

2. Good Chemical Matter
   a. Does the compound/chemical series have drug-like properties?
   b. Can modifications be made to the compound/chemical series to make it drug-like?
   c. Is the compound or chemical series a dead-end?
Good Chemical Matter:
Guidelines, Tools and Examples

• Lead Quality Attributes
• Compound profile
• Compound Progression Pathway
• Med Chem Strategy
• Guidelines to Drug-like Properties
• Structural alerts
• Metabolic stability
• Potency, solubility, selectivity, stability
## Lead Quality Attribute Checklist

<table>
<thead>
<tr>
<th>Chemical Series Attributes</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  <strong>Potency</strong>: <em>In vitro</em> potency &lt; 100 nM</td>
<td>yes</td>
</tr>
<tr>
<td>2  <strong>SAR</strong>: Evidence of SAR with ≥ 10X change in potency</td>
<td>yes</td>
</tr>
<tr>
<td>3  <strong>Cellular activity / toxicity</strong>: Active / no toxicity</td>
<td>yes</td>
</tr>
<tr>
<td>4  <strong>Selectivity</strong></td>
<td>yes</td>
</tr>
<tr>
<td>5  <strong>Safety</strong>: No toxicophores or reactive moieties</td>
<td>yes</td>
</tr>
<tr>
<td>6  <strong>Physicochemistry</strong>: <em>In vitro</em> ADME studies of similar framework show solubility and absorption</td>
<td>yes</td>
</tr>
<tr>
<td>7  <strong>Synthesis</strong>: Synthetic chemistry flexibility is high; amenable to rapid, highly productive analog synthesis</td>
<td>yes</td>
</tr>
<tr>
<td>8  <strong>Intellectual property</strong>: Proprietary</td>
<td>yes</td>
</tr>
<tr>
<td>9  <strong>Synthesis</strong>: Amenable to parallel synthesis</td>
<td>yes</td>
</tr>
</tbody>
</table>
# Compound Profile
## Physicochemical and Pharmacokinetic Properties

<table>
<thead>
<tr>
<th>DATA</th>
<th>TARGETED VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42&lt;sub&gt;cells&lt;/sub&gt; IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>&lt; 100 nM</td>
</tr>
<tr>
<td>Selectivity (Notch Elisa&lt;sub&gt;cells&lt;/sub&gt; / Aβ42 Elisa&lt;sub&gt;cells&lt;/sub&gt;)</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>Polar Surface Area</td>
<td>&lt; 70</td>
</tr>
<tr>
<td>Solubility (µM) (In PBS at pH 7.4 (µM))</td>
<td>&gt; 10 µM</td>
</tr>
<tr>
<td>Experimental Log D</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Microsome Stability: Mouse (% remaining @ 60 min)</td>
<td>&gt; 20% (or t ½ = 1h)</td>
</tr>
<tr>
<td>Intrinsic Clearance&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;15 Low Risk; &gt;40 High Risk</td>
</tr>
<tr>
<td>Microsome Stability: Human (% remaining @ 60 min)</td>
<td>&gt; 20% (or t ½ = 1h)</td>
</tr>
<tr>
<td>Intrinsic Clearance&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;15 Low Risk; &gt;40 High Risk</td>
</tr>
<tr>
<td>Hepatocyte Stability: Mouse (% remaining @ 120 min)</td>
<td>&gt; 20% (or t ½ = 1h)</td>
</tr>
<tr>
<td>Intrinsic Clearance&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&lt;15 Low Risk; &gt;40 High Risk</td>
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<tr>
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<td>&lt;15 Low Risk; &gt;40 High Risk</td>
</tr>
</tbody>
</table>

<sup>1</sup> (mL/min/mg/protein)
Screening Tree – Compound Selection

- Solubility
- Mouse + Human liver microsome stability
- Mouse + Human hepatocyte stability
- Log D

- PK studies
- Brain penetration
- P450 enzymes
- Selectivity panel
- Toxicity

New Lead Series

IC$_{50}$’s: Aβ40 + Notch Cellular Assays + Cellular Toxicity (MTS)

Active & Interesting Compounds

- In vivo Candidates

Mouse Studies

Efficacious cpds

Proof of Principle

- Microdialysis Studies
- Neuronal Culture Assay

SAR & Lead Development

Lead Optimization

Total compounds >1400

5/4/2018 Southern Research Drug Discovery
Medicinal Chemistry Strategy

Synthesis of Analogs

Test Potency and Selectivity → PK Parameters on Leads

- IC₅₀ > 10 µM
- Nonselective
- Poor PK

- IC₅₀ < 10 µM; < 1 µM
- (Notch) Selective
- Good PK

< 100 nM
2- to 3-fold > Avagacestat (known drug)

Test In Vivo

Terminate Series

5/4/2018 Southern Research Drug Discovery
Guidelines to Drug-Like Properties

**STEP 1 / Early Stage**
- Rule of 5
- Structural ‘alerts’
- Ease of synthesis
- Solubility (>10 µg/mL)
- Log D (2-4)
- Mouse (Rat) Micr/Hep (>20%)
- Human Micr/Hep (>20%)
- IC<sub>50</sub> (< 1 µM)

**STEP 2 / Optimization**
- % Bioavailability (30)
- t<sub>1/2</sub> (> 2h, i.e., BID)
- Clearance
- Non-target selectivity profile
- Plasma protein binding
- hERG testing
- P450 interactions (>3 µM)
- Pgp
- Mini-Ames
- Minimum effective dose
- Brain / Plasma (0.5 – 1)

**STEP 3 / Pre-Clinical Development**
- Genetic toxicology
- In vivo studies / dose response
- Side-effect profiling
- Biomarker
- Defined mechanism of action
- Differentiation vs. known drugs

POC = Proof of Concept
Structural Alerts

• Structural alerts have the potential for:
  • Intrinsic reactivity
  • DNA intercalation
  • Metal coordination or metabolic activation (e.g., to a species capable of covalent binding)

• This binding could then lead to:
  • Mutagenicity
  • CYP inhibition
  • Direct toxicity
  • Carcinogenicity
  • Idiosyncratic toxicity

• A structural alert alone is not predictive of an adverse outcome.

• Other factors to consider:
  • Clinical dose
  • Route of drug clearance
  • Presence of metabolic routes (e.g., that divert metabolism away from the structural alert)
Examples of Structural Alerts

Potential DNA Damaging Agents
- Amino triazoles
- Furocoumarins/tricyclics

Potential Metal Chelators
- Imidazoles and Triazoles
- Pyridine derivatives

Undergo Metabolic Activation.........or not....... 
- Furan
- Thiophene
- Pyrrole
- Thioamides
- Aldehyde
- Michael Acceptor
- Quinones
- (Hydroxy)anilines
- Thiocarbamates
- Thioureas
- Thiols and Disulfides
Metabolism...How to improve?

• Avoid structural alert moieties in a molecule

• Find replacements (bioisosteres) of particular groups/rings

• Block potential sites of metabolism on aryl rings

• Use calculations such as CSL (StarDrop) to prioritize analogs with predicted better metabolic stability
  • CSL (Composite Site Liability) is a measure of the efficiency of metabolism of a molecule by e.g., CYP3A4, one of the CYP isoenzymes.
  • CSL varies between 0 and 1 (lower values indicate greater metabolic stability)
Solubility...how to improve?

• Add polar or solubilizing groups
  • OH, N, OR, COOH

• Include cycloalkyls containing heteroatoms

• Insert heterocycles

• Interrupt intramolecular hydrogen bonding

• Insert groups that will allow rings to rotate within a molecule....disrupting planarity
Example of Potency, Stability and Selectivity

1st Generation

- $\alpha\beta_40 \text{ IC}_{50} = 113 \text{ nM}$
- Notch IC$_{50} = 560 \text{ nM}$ (5X)

2nd Generation

- $\alpha\beta_40 \text{ IC}_{50} = 4.1 \text{ nM}$
- Notch IC$_{50} = 960 \text{ nM}$ (230X)

- BPM = 0.01 (low BBB penetration)
- RLM (19%), HLM (44%) @10 min incub

Racemate

- $\alpha\beta_40 \text{ IC}_{50} = 3400 \text{ nM}$

Resolution

BMS-708163 (Avagacestat)

- $\alpha\beta_40 \text{ IC}_{50} = 0.3 \text{ nM}$
- Notch IC$_{50} = 58 \text{ nM}$ (190X)

Potency

Selectivity

Stability

- $\alpha\beta_40 \text{ IC}_{50} = 113 \text{ nM}$
- Notch IC$_{50} = 560 \text{ nM}$ (5X)

- $\alpha\beta_40 \text{ IC}_{50} = 4.1 \text{ nM}$
- Notch IC$_{50} = 960 \text{ nM}$ (230X)

- B/P = 0.01 (low BBB penetration)
- RLM (19%), HLM (44%) @10 min incub
Example of Potency and Stability

**Hit**

- **Aβ_{40} IC_{50} = 5549 nM**
- **Selectivity: 3.7-fold**

**Lead**

- **Aβ_{40} IC_{50} = 294 nM**
- **Selectivity: 10-fold**

**Stability**

- **Aβ_{40} IC_{50} = 15 nM**
- **Selectivity: 14-fold**
  - 48 min (mice)
  - >90 min (human)

- **Aβ_{40} IC_{50} = 16 nM**
  - 24 min (mice)
  - 8 min (human)

- **Aβ_{40} IC_{50} = 25 nM**
  - 1 min (mice)
  - 8 min (human)

**Potential for Oxidation and Glucuronidation**

GSI-953
Identification of Hits/Lead Compounds

1. High Throughput Screens (HTS)

2. Rational Design / Med chemistry of literature compounds

3. Computational Approach

4. Structural Biology/Crystal Structure
Case Studies - HTS

1. TXNIP – Diabetes

2. LRRK2 – Parkinson’s disease

3. CHIKV (Chikungunya) virus – Antiviral
Compound Libraries at Southern Research

One million small molecules

- Chembridge, Enamine, Tripos
- Kinase library
  - 26,734 compounds theoretically designed to inhibit kinases
- Approved drugs and biological actives library
  - 2,500 FDA approved drugs
  - 460 unapproved drugs
- Fragment libraries

- Southern Research Proprietary library (34K)
  - Compounds from SR Research Programs
1. TXNIP (Thioredoxin Interacting Protein)

Goal

• Develop a small molecule inhibitor of TXNIP as a novel approach for diabetes therapy

Results

• 300,000-compound primary screen resulted in 3197 hits
• 3197 compounds re-screened for both primary and cytotoxic effects in INS1 cells, resulting in 1258 compounds
• 1258 compounds counter-screened for off-target activity resulted in 651 “good” compounds
• Focus on 4 series for medicinal chemistry
## Lead Compound Profile

<table>
<thead>
<tr>
<th>Data</th>
<th>Targeted Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TXNIP % Inhibition at 1.5 µM</td>
<td>≥ 40</td>
</tr>
<tr>
<td>TXNIP % Inhibition at 12.5 µM</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>Cytotoxicity CC&lt;sub&gt;50&lt;/sub&gt; (72h) µM</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>&lt; 500</td>
</tr>
<tr>
<td>Total Polar Surface Area (tPSA)</td>
<td>40 – 70</td>
</tr>
<tr>
<td>Solubility (µM) (In PBS @ pH7.4)</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Experimental Log D</td>
<td>2 – 4</td>
</tr>
<tr>
<td>Microsomal Stability: Mouse/Human (MLM/HLM: t&lt;sub&gt;1/2&lt;/sub&gt; in min)</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>Hepatocyte Stability: Mouse (% Remaining @120 min)</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Mouse &lt;em&gt;in vivo&lt;/em&gt; PK: IV (1mg/Kg) &amp; PO (5mg/Kg) t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Bioavailability: %F</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Plasma protein binding: Fraction unbound %</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>hERG IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>
# Examples of TXNIP inhibitors

<table>
<thead>
<tr>
<th>Target Values</th>
<th>Data</th>
<th>HTS hit</th>
<th>SRI-36222</th>
<th>SRI-36650</th>
<th>SRI-36434</th>
<th>SRI-36407</th>
<th>SRI-37330</th>
<th>SRI-38182</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 40</td>
<td>TXNIP % Inhibition [1.5 µM]</td>
<td>32</td>
<td>44</td>
<td>19</td>
<td>54</td>
<td>56</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>TXNIP % Inhibition [12.5 µM]</td>
<td>52</td>
<td>54</td>
<td>30</td>
<td>65</td>
<td>69</td>
<td>53</td>
<td>66</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>CC&lt;sub&gt;50&lt;/sub&gt; at 72h (µM)</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>Molecular weight</td>
<td>304</td>
<td>338</td>
<td>363</td>
<td>305</td>
<td>313</td>
<td>388</td>
<td>363</td>
</tr>
<tr>
<td>&lt; 70</td>
<td>tPSA</td>
<td>74</td>
<td>74</td>
<td>97</td>
<td>36</td>
<td>36</td>
<td>74</td>
<td>97</td>
</tr>
<tr>
<td>≥ 10 µM</td>
<td>Sol (µM)</td>
<td>8.4</td>
<td>85.3</td>
<td>80</td>
<td>1.4</td>
<td>29</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>2-4</td>
<td>Log D</td>
<td>4.3</td>
<td>1.5</td>
<td>1</td>
<td>3.2</td>
<td>4.7</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; &gt; 60 min</td>
<td>Mouse Liver Microsomal Stability</td>
<td>3</td>
<td>51</td>
<td>226</td>
<td>9</td>
<td>26</td>
<td>46</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Human Liver Microsomal Stability</td>
<td>27</td>
<td>285</td>
<td>172</td>
<td>36</td>
<td>56</td>
<td>116</td>
<td>300</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; &gt; 30 min</td>
<td>Mouse PK (IV, 1 mg/kg)</td>
<td>12</td>
<td>12</td>
<td>96</td>
<td>--</td>
<td>--</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>CSL (Composite Site Lability)</td>
<td></td>
<td>0.99</td>
<td>0.71</td>
<td>0.38</td>
<td>0.88</td>
<td>0.74</td>
<td>0.31</td>
<td>0.38</td>
</tr>
</tbody>
</table>

- CSL: varies between 0 and 1 (lower values imply greater metabolic stability); Formulation for PK studies: 2%DMSO/3% solutol/95% WFI
- SRI-37330 and 38182: both free bases and the corresponding HCl salts have the same biological and stability profiles

**Within target value** | **Borderline target value** | **Outside target value** | **NA: not available**

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Southern Research Drug Discovery 22
Status: Leads SRI-37330 & SRI-38182

- **In vivo** proof-of-concept efficacy mouse studies
  - SRI-37330 & SRI-38182 protect against diabetes by increasing insulin-producing beta cells of the pancreas

- **In vivo** mouse Tox studies
  - SRI-37330 & SRI-38182 tolerated at all doses tested
    - (10, 30 & 100 mg/kg; 14 days)

- Safety & Off-Targets assessment: SRI-37330 & SRI-38182
  - Negative for: Ames, L-Calcium channel, hERG, CEREP receptor & CYP panel

- Ongoing Studies
  - Metabolite Identification in Human, Monkey, Mouse and Dog Hepatocytes

- Current Status
  - Pre-clinical development

- Intellectual Property
  - Patent application filed on Nov. 30, 2017. No: 62579594
Next Studies

• Dog *in vivo* PK (IV/PO) Studies

• Metabolite Identification in Human, Monkey, Mouse and Dog Hepatocytes

• Maximum Tolerated Dose (MTD) in CD1 Mice and Liver Histology Study studies
2. LRRK2 (Leucine-Rich Repeat Kinase 2) Inhibitors

- Developing a small molecule inhibitor of LRRK-2 is a novel approach for Parkinson’s Disease therapy
- Initial 28,000 HTS of computationally derived kinase library
- Results:
  - Triazolopyridazine series identified
  - Tool compound: SRI-29132
  - Kinase IC$_{50}$ = 260 nM (WT); IC$_{50}$ = 352 nM (Mutant)
  - CNS target engagement in mice and rats
  - No inhibition > 20% in a panel > 320 kinases
  - High clearance compound *in vivo*
  - Series abandoned: labile functionality required for activity
  - Approximately 300 compounds synthesized

- Need for New chemical matter
**LRRK2 2\(^{nd}\) HTS**

184K Compound HTS: Novel autophosphorylation assay

2566 Hits → Hit Rate: 1.4%
Several diverse hit series identified

Hit-to-Lead: Modeling & multi-parameter optimization

Compounds with IC\(_{50}\) < 100 nM from 4 hit series identified

Focus on most drug-like series: Pyridopyrrolidines
LRRK2: New Chemical Series

- New LRRK-2 screening campaign resulted in pyrrolopyrimidine hits for medicinal chemistry follow-up
- Identified key moieties (e.g., 4-chiral amines & 2-aminotriazole) of pyrrolopyrimidine scaffold for improving LRRK-2 inhibition and kinase selectivity

• SRI-32708
  - IC_{50} = 50 nM ((LRRK-2, mutant, photo.); IC_{50} = 3.3 nM (LRRK-2, mutant, peptide)
  - Sol. = 7 µM; Mouse PK: t ½ = 204 min (IV), t ½ = 324 min(PO); F% =47
  - Free concentration [brain, 4h] = 50 nM (15-fold IC_{50} (LRRK-2, mutant, peptide)

• SRI-33974
  - IC_{50} = 72 nM ((LRRK-2, mutant, photo.); IC_{50} = 6.7 nM (LRRK-2, mutant, peptide)
  - Sol. = 7 µM; Mouse PK: t ½ = 138 min (IV), t ½ = 200 min(PO); F% =40
  - Free concentration [brain, 4h] = 76 nM (11-fold IC_{50} (LRRK-2, mutant, peptide)
Status: Lead SRI-32708

- *In vivo* Rat Tox studies
  - SRI-32708 was tolerated at 10 & 30mg/kg (14 days)
- Drug Safety & Off-Target assessment
  - Negative for: Ames; hERG, CEREP receptor & CYP panel
- Planned Studies
  - Target engagement
- Current Status
  - Preclinical development
- Intellectual Property
3. Chikungunya Virus (CHIKV): Lead SRI-36498

• Hit to lead efforts by structure activity relationship (SAR) studies

• Identified key moieties (e.g., t-butyl and amide) of benzoannulene scaffold for activity
  – Activity profile: EC\textsubscript{50} = 0.4 µM; VTR = 3.2 logs, MLM t\textsubscript{1/2} = 57 min, Sol. = 0.5 µM

• \textit{In vivo} PK proof-of-concept mouse studies
  – Subcutaneous (SC) route provided best PK profile at 20 mg/kg dose
  – Partial efficacy observed

• Pitfall – Not soluble at > 20 mg/kg dose

• Ongoing chemistry to increase solubility
CHIKV Virus: Target Identification of Lead SRI-36498

- Target identified as Non-Structural Protein-3 (NSP3) by resistant and mutation studies
  - Resistant virus obtained; binding interaction/binding pocket determined

- Protein expression, purification and crystallization
  - Protein crystallized and structure determined at 1.46Å
  - Co-crystallization efforts failed due to poor solubility of lead compound

- Molecular model prepared for computational molecular dynamic studies
Case Studies - Rational Design

1. Gamma-Secretase Inhibitors – Alzheimer’s

2. TGF-β – Multiple Myeloma (MM)

3. Vif dimerization – HIV-1
Evolution of Chemical Matter

Gleevec

Inhibitor 2

WJ Netzer, P Greengard, et. al.  
PNAS, 100 (2003) 12444

Tocris Library  
(50 cpds)

Rational Design

Structure Modification

Pyridopyrimidines

Aryl Amides

γ-Amino Alcohols

Structural Alerts

PC Fraering, et. al.  
JBC, 280 (2005) 41987

1366  
IC$_{50}$ = 60 µM

(Reactive)

1367  
IC$_{50}$ = 20 µM

(Unstable)
Conversion of: Aryl Amides to Aryl Sulfonamides

1. Common replacement for amide
2. Sulfonamide conformation may offer more binding orientations
3. Naphthyl to aryl and alkylbenzyl amine to more diversified amines

Results

• Increased in vitro and cellular potency
• Improved selectivity
2. TGF-β (Transforming Growth Factor-β)

• Blockade of TGF-β signaling has been shown to reduce MM progression.

• TSP1 (Thrombospondin 1) has been shown to activate latent TGF-β; It is expressed by human MM cell lines.

• Developing a small molecule inhibitor for TSP-1 mediated TGF-β activation is a novel approach to inhibit disease specific TGF-β.
TGF-β: Evolution of chemical series

**Tripeptide series**

- **Approach:** Modification at stereo center and amino acid sequence
- **Innovation:** US Patent No. 9,353,149 issued May 31, 2016
- **Results:** $IC_{50} = 1 \text{ nM to } 30 \text{ nM}$
- **Issues:** Low bioavailability ($%F < 13$): Low permeability, Log D < 0

**Dipeptide series**

- **Approach:** Finding minimum active sequence (MAS), 2D NMR of active tripeptide SRI-31277
- **Innovation:** Provisional patent filed in 2017
- **Results:** $IC_{50} = 1 \text{ nM to } 50 \text{ nM}$
- **Issues:** $%F < 5$; Low permeability, Log D = 1; but IV $t_{1/2} = 1.9 \text{ h}$

**Terminated Peptidomimetic series**

- **Approach:** Synthesis of Peptoids and Oligoureas
- **Results:** Biological activity in $\mu$M range
TGF-β: Bioisosteric series/small molecule

• **Approach:** Replace amide with Oxadiazoles and Thiadiazoles
• **Innovation:** Provisional patent filed in 2017
• **Results:** $IC_{50} = 1$ nM to 30 nM
• **Issues:** %F < 10; Low permeability, Log D < 0
• **Approach:** Designed compounds with improved ADME profile
  - Nova Idea Generation, P450/permeability model (StarDrop)
  - 3D Model Generation and Docking (Schrodinger Maestro)
• **Results:** Lead SRI-40000: $IC_{50} = 28$ nM, Log D = 1.1; %F = 40
• **Studies:** Mini-Ames, hERG, CEREP receptor panel (at 10 µM), plasma protein binding, CYP panel, 2 wk Mini-Tox (10, 30 mg/kg)
• **Status:** *In vivo* study planned for SRI-40000
  Synthesis ongoing; Improve Log D, permeability, bioavailability
3. HIV-1 Vif Dimerization

**Lead BRD-25**
- $EC_{50} = 5.4 \mu M$ (anti-HIV-1 Vif) activity; good PMBC anti-viral activity, non-cytotoxic, good therapeutic index

**Issues**
- Poor solubility and metabolic stability

**Goal**
- Improve potency and ADME profile for *in vivo* animal studies

**Status**
- SRI-38445, an aminotriazolophthalazine, new lead
- Rationally designed, novel, structurally distinct from BRD-25
- $EC_{50} = 0.1 \mu M$; Improved PBMC anti-viral activity; Good solubility

**Issue**
- Microsomal stability
- Ongoing synthesis of analogs that should improve metabolic stability and maintain anti-viral activity
Case Study - Computational Approach

- CHIKV (Chikungunya) virus – Antiviral
CHIKV Virus: Virtual screen

• NSP3 binding site studied by molecular modeling and dynamics studies
  • Docked commercial fragment libraries in binding pocket
  • Binding efficacy determined by computational analysis
  • 40 small molecule fragments selected based on binding energy

• Co-crystallization by soaking compounds with protein
• Co-crystallized structure of SRI-40582 identified
• SRI-40582 fragment found in SRI-40507 ($EC_{90} = 0.5 \mu M$), a confirmed CHIKV hit from a phenotypic screen
  • Design, synthesis and biological testing ongoing
Case Studies –
Structural Biology/Crystal Structure

1. Nef – HIV-1

2. PD-1/PD-L1 – Cancer
1. HIV-1 Nef: Structural Biology

- HIV-1 Nef core domain expressed and purified from *E.coli*.
- Nef crystal structure in presence of 0.1% β-octyl-glucoside (βOG) was determined at 3.2Å.
- Novel homodimeric interface of Nef was formed by helix A, helix B and the extra N-terminal helix with 1 βOG molecule inserted in the hydrophobic interface of the Nef homodimer.
- Identification of small molecules that occupy the βOG-binding pocket could restrict Nef dynamics and act as global inhibitors of the multiple functions of this critical HIV-1 virulence factor.
Crystal structure: Dimeric Nef with βOG

(a) The homodimer of Nef core domain viewed from the top; (b) Side view of a Nef homodimer; (c) Close-up view of the contribution of helix B from each Nef monomer to the dimer interface.
2. PD-1/PD-L1 Inhibitors

Objective

• Develop a peptide inhibitor for PD-1/PD-L1 interaction as an alternative to existing antibody-based immunotherapy for cancers

Approach

• Crystal structure of PD-1 crystal structure indicates highly conserved single PDL-1 binding site across species
• Competitive inhibitor could be designed to fit into the PD-L1 binding site
  • PD-1 interacting residues: Met64 to Lys78
  • PDL-1 interacting residues: Gly120 to Asp131 PD-1
• SRI-40269: A peptide mimetic of PDL-1 – PL120131 (12-mer) designed to act as a competitive inhibitor

David Yin-wei Lin et al. PNAS 2008;105:3011-3016
PD-1/PD-L1 Inhibitors: Results-to-date

• Biolayer interferometry (BLI) experiments
  – PL120131 acts as a competitive inhibitor of PD-L1 which binds by associating with the binding groove in PD-1

• Luciferase reporter PD-1/PD-L1 blockade assay
  – indicates PL120131 acts as a checkpoint inhibitor

• In an attempt to develop small molecule inhibitors, 12-mer PL120131 was truncated into small peptide units that show similar inhibitory effect

• New lead, SRI-39240, a tetra-peptide, was recently identified
The Right Balance......

- Solubility
- Molecular weight
- Metabolic stability

- Potency
- Selectivity
- Toxicity

- Brain penetration
- Bioavailability
- Half-life

'BALANCED' OPTIMIZED Candidates

In vivo Efficacy in Animal Models

POC
Southern Research Chemistry Department
Capabilities for Medicinal Chemistry and Drug Discovery

Iterative Medicinal Chemistry

Computational Chemistry

Informatics
• Electronic Notebooks
• Dotmatics

US Patent x,xxx,xxx

Intellectual Property

Parallel Synthesis

In Vitro Pharmacokinetics

• In Vivo Pharmacokinetics
• Toxicity Studies
• Animal Efficacy Studies

“Making Drugs that Matter!”

Lead Development & Optimization

Proof of Concept

Animal Model

In Vivo Pharmacokinetics

• Toxicity Studies
• Animal Efficacy Studies

Mass Directed Purification

Nuclear Magnetic Resonance
• Exact Mass

Structural Biology
• Protein Crystallography

Compounds that meet Activity/Efficacy Criteria
THANK YOU...

Corinne E. Augelli-Szafran, Ph.D.
Director, Chemistry Department
Drug Discovery Division
Southern Research
2000 Ninth Avenue South
Birmingham, AL 35205
205-581-2305 telephone
caugelli-szafran@southernresearch.org
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