Targets, Tools, and Drugs: Advances in Molecular Discovery at BU

April 4, 2017
Adrian Whitty
Associate Professor
Chemistry
CAS
Drugs and probes for highly challenging protein-protein interaction (PPI) targets

Most disease modifying genes are not druggable by conventional means

- **Topology** – lack suitable sized cleft or pocket
- **Polarity** – binding site too polar (or too hydrophobic)
- **Disorder** – additional energetic barrier to ligand binding; difficult to employ structure-based approaches

OUR APPROACH: Non-canonical drug chemotypes
- High MW synthetic macrocycles
- Targeted covalent inhibitors

Team approach (with Porco, Allen, Vajda labs, Brown/BU-CMD, et al.)
EXAMPLE: Synthetic Macrocycles for PPI targets

- Computational assessment of target “druggability”
- Clone & express target, develop assay
- Design and make MC screening library
- Screen target, validate hits
- Obtain X-ray structures of bound hits
- Biological evaluation of hits
- Further optimization of compounds

OPPORTUNITY: We are keen to collaborate with BU investigators who seek to inhibit challenging PPI targets

Beyond HTS: BU-CMD Strategies for Focused Molecular Discovery

Lauren Brown

Assistant Director | Center For Molecular Discovery
Research Assistant Professor | Chemistry, CAS
Center for Molecular Discovery: A Small Molecule Resource for Biomedical Research

Faculty:
John Porco (Director), Lauren Brown (Asst. Director), Karen Allen, Aaron Beeler, Scott Schaus, Adrian Whitty, Sandor Vajda, Arturo Vegas

Staff:
Lisa Holik (Center Administrator)
Richard Trilles (Organic Synthesis Specialist)
TBD Analytical Core Manager
Postdoctoral Researchers (6-8)
Undergraduate students (3-4)

Small molecule screening collection (3K-5K compounds)

BU-CMD Library Synthesis

BU Chemistry Department
Student & Postdoc Contributions

Neuroscience

Cancers

Bacteria

Parasites

Viruses

Fungi

CLC Collaborator Network
Screens for bioactivity

Boston University Office of the Vice President and Associate Provost for Research
Strategies for Discovery Beyond High-Throughput Screening: Focused Subset Generation

**Known target** vs. **Known actives**

**Small molecule screening collection**

**Expanding virtual libraries** synthesized vs. synthesizable

**Similars to known actives or pharmacophore**

**Improving training sets** for computational similarity/overlay/pharmacophore analysis

**Improved collection quality:**
- Physiochemical properties
- Privileged substructures
- “Rule-breaking” druglike molecules
- Expanding depositors

**Molecule “kits”** e.g. “kinase inhibitor-like,” “anti-infectives”

**Computational docking hits**

**De novo designed inhibitors**

**Small fragments (FBDD)**
Carmela Abraham

Professor
Biochemistry, Medicine, and Pharmacology & Experimental Therapeutics
MED
Neuroprotection in Alzheimer’s disease (1)

Reducing the levels of the neurotoxic Amyloid beta protein (Abeta)

- The Abeta peptide is toxic to neurons and their synapses
- High throughput screen (HTS) to reduce Abeta identified compound Y
- Y analogs, such as Y10, inhibit the receptor tyrosine kinase cKit
- Inhibitors of a down stream effector also inhibit Abeta production
- Efforts are under way to optimize Abeta lowering compounds in close collaboration with Drs. Porco, Brown and Camara
Neuroprotection in Alzheimer’s disease (2)

Increasing the levels of the neuroprotective and cognition enhancer protein Klotho

- Klotho is a large protein hormone that is essential for the function of most organs, including the brain.
- Our group found that Klotho is low in the aged brain, is protective against Abeta and glutamate excitotoxicity \textit{in vitro}, and Abeta \textit{in vivo}.
- Klotho also improves remyelination in a mouse model of MS.
- Two HTS are being conducted to identify compounds that enhance Klotho expression using a novel coincidence reporter.

\textit{Klotho overexpression improves cognitive deficits and ameliorates synaptic hippocampal dysfunction in the J20 model without affecting Abeta levels.}

A new company is born; Klogene

\textit{Klogene is a startup company developing novel \textit{neuroprotective therapeutics} for neurodegenerative diseases.}
Collaborative Research in the Beeler Lab

Aaron Beeler
Assistant Professor
Chemistry
CAS
The Beeler Research Group is truly multidisciplinary, combining organic chemistry, engineering, and biology to solve problems in medicinal chemistry. All of these elements are combined and directed toward significant problems in human health. The Beeler Group is addressing focused disease areas (e.g., schizophrenia, Parkinson’s, cystic fibrosis), as well as project areas with broader impact potential (e.g., new methods for discovery of small molecules with anti-cancer properties).
Collaboration with David Harris @ BUMC Department of Biochemistry

A. Zeocin or G418
384-well plates pretreated with compounds

B. Rescue by drug

C. Chemical clustering: 12 lead compounds

D. 70,000 compounds screened with DBCA
68 compounds inhibitory in DBCA (>99%)
61 compounds purchased
24 compounds reduce PrPSc in RML-infected N2a cells

LSF-targeted small molecules as hepatocellular carcinoma chemotherapeutics

Scott E Schaus
Department of Chemistry

Ulla Hansen
Department of Biology
**Clinical relevance of LSF in hepatocellular carcinoma**

- **Stage 1**: normal liver
- **Stage 2**: poorly differentiated
- **Stage 3**: moderately differentiated

Late SV40 Factor (LSF)

In vivo Tumor Reduction

xenograft model

Tumor Volume (mm$^3$)

- FQI1  + FQI1

2 mg/kg I.P.
2 weeks, treat on 3rd day
followed by 2 weeks no treatment

orthotopic

Before treatment

After treatment

-FQI1

+FQI1

2012, 109, 4503.
Structure-Aided Inhibitor Design

Karen N. Allen

Professor
Chemistry
CAS
Assay Development and Screening

SAR by SAXS

Structure Aided Design
Targeting a Novel Signaling Interface in Metastasis

Mikel Garcia-Marcos

Assistant Professor
Biochemistry
MED
GPCR

G protein

Outside

“Oncogenic”

receptors

Inside

CANCER CELLS

CANCER METASTASIS

“anything could happen”

NORMAL CELLS

Overexpressed in
most carcinomas

“GIV”

Non-GPCR

G protein

activators

“Oncogenic”

receptors

Overexpressed in
most carcinomas

“GIV”

Non-GPCR

G protein

activators
High-throughput screen for “good” inhibitor molecules:  
*From 1,000 to 200,000 compounds*

FILTER 1  
Confirm in other assay formats

FILTER 2  
Medicinal chemistry quality control and assessment

FILTER 3  
Toxicity and tumor cell migration assays

ICCB-L high-throughput screening facility, Harvard School of Medicine

- Experimental
- Vehicle (DMSO)
- Positive Inhibitor (AIF4-)

*Switch II*
Hui Feng, MD, PhD

Assistant Professor of Pharmacology and Medicine
Department of Pharmacology and Experimental Therapeutics

Can Novel Cancer Therapeutics be Identified through Combined Genetic and Chemical Efforts?
Our Strategies

**zebrafish genetic studies**

- **A:** T GFP (Control)
- **B:** GFP; Myc (Leukemia)
- **C:** GFP; Myc; X+/- (Lymphoma)
- **D:** GFP; Myc; X+/- (Tumor free)

**Collaboration with Chemists**

**Human cell culture studies**

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DLST inactivation impairs T-cell leukemogenesis

**rag2-GFP**

<table>
<thead>
<tr>
<th>Leukemia</th>
<th>early stage of tumor</th>
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<tbody>
<tr>
<td>Myc;dlst +/-</td>
<td>Myc;dlst +/-</td>
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![Graph showing cell growth and growth rate](image)

**Boston University** Office of the Vice President and Associate Provost for Research
Searching for DLST inhibitors

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Collaboration with Dr. John Porco’s group
Searching for DLST inhibitors

Collaboration with Dr. John Porco’s group
Searching for DLST inhibitors

![Graph showing relative cell viability (%)](image)

**PBMC-1**
**PBMC-2**
**PEER**
**JURKAT**

*** $P<0.0001$

Collaboration with Dr. John Porco’s group
Tsuneya Ikezu

Professor
Neurology and Pharmacology & Experimental Therapeutics
MED

Neurology Director | Laboratory of Molecular NeuroTherapeutics
Member | BU Alzheimer’s Disease Center

Boston University Office of the Vice President and Associate Provost for Research
Variant of TREM2 Associated with the Risk of Alzheimer’s Disease

Thorlakur Jonsson, Ph.D., Hreinn Stefansson, Ph.D., Stacy Steinberg Ph.D., Ingileif Jonsdottir, Ph.D., Palmi V. Jonsson, M.D., Jon Snaedal, M.D., Sigurbjorn Bjornsson, M.D., Johanna Huttenlocher, B.S., Allan I. Levey, M.D., Ph.D., James J. Lah, M.D., Ph.D., Dan Rujescu, M.D., Harald Hampel, M.D., Ina Giegling, Ph.D., Ole A. Andreassen, M.D., Ph.D., Knut Engedal, M.D., Ph.D., Ingun Ulstein, M.D., Ph.D., Srdjan Djurovic, Ph.D., Carla Ibrahim-Verbaas, M.D., Albert Hofman, M.D., Ph.D., M. Arfan Ikram, M.D., Ph.D., Corneilia M van Duijn, Ph.D., Unnar Thorsteinsdottir, Ph.D., Augustine Kong, Ph.D., and Kari Stefansson, M.D., Ph.D.

Funded by Massachusetts Neuroscience Consortium, BrightFocus Foundation
“M.tuberculosis has been studying us longer than we have been studying it”

Kyu Rhee

Igor Kramnik
Associate Professor
Medicine | MED
National Emerging Infectious Diseases Laboratory
Genetic and Pharmacological Control of the Inflammatory Damage Caused by Tuberculosis and Other Infections

The sst1/Ipr1 pathway controls stress response in macrophages. Unresolved stress leads to tissue necrosis in SUSCEPTIBLE hosts.

Goals: to identify compounds that boost macrophage stress resilience to increase bacterial killing and mitigate the inflammatory damage;

Methods: novel assays based on gene expression patterns in relevant primary cells (macrophages) from susceptible individuals (mouse and humans)

Progress: in collaboration with the Porco and Beeler labs identified a novel rocaaglate that acts in synergy with low doses of IFN-gamma to activate autophagy and suppress inflammation, but does not compromise host resistance to intracellular bacteria in vitro and in vivo;

Plans: to continue the development of assays for compounds that
1. synergize with IFN-gamma;
2. correct hyperinflammatory phenotype in SUSCEPTIBLE hosts;
3. Identify inflammatory diseases that benefit from those compounds.
James S. Panek

Samour Family Professor in Organic Chemistry
CAS
**Reaction Development: Heteroatom Directed Reductive Coupling**

*Bin Cai (BU), Professor Jie Wu (NUS) and Ryan Evans (Princeton)*

**Alkyne-alkyne reductive coupling with acetylenic esters**

1. ClTi(OiPr)_3
   cC_3H_9MgCl
   -50 °C, 4 h

2.  \( \equiv \) \text{CO}_2R
   -50 °C, 12 h

minimization of \( A^{1,3} \) strain
Favored TS

10 examples
r.r. up to >20:1:1

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**Target-Oriented Synthesis**

**Alkyne-Alkyne coupling**

NFAT-68 an Immunosuppressant
Inhibits NFAT-dependent transcription factor

*Organic Letters, 2016, 18, 4304*

**Alkyne-Alkene coupling**

(-)-Q-1047H-A-A
Antioxidant

(-)-Q-1047H-R-A
Antioxidant
Convergent Synthesis of Novel Muramyl Dipeptide Analogs

The effects of MDP are biphasic: at 10 μg/ml (MDP-low), MDP activates the inflammatory process, while a dose of 100 μg/ml or higher (MDP-high) dampens the process by inhibiting the NFκB-mediated cytokine response. Analogs of MDP were prepared through a convergent strategy involving the synthesis of two unique carbohydrate fragments, using the peptide coupling reagents, EDCI and HOAt. Analogs improved MDP function and P.g-induced activities. A new signaling pathway is proposed for TNF-α induction activated after exposing macrophages to both P.g and high concentrations of MDP.

Evidence highlighting a high dose MDP-dependent signaling pathway which activates JNKs, induces AP1, up-regulates A20 expression, restricts NOD2, inhibits NFκB, and consequently, reduces P.g-induced TNF-α production in mouse macrophages (inflammation).

John Connor

Associate Professor
Microbiology | MED
Investigator | NEIDL
Small Molecule Probes of Virus Function

We Have Found Molecules That Keep Viruses From Making Copies of Themselves

Tests To Find Molecules That Stop Viruses From Working

With Snyder, Beeler, Porco

With Brown, Schaus, Porco

Range of Microcephaly Severity

Baby with Typical Head Size
Baby with Microcephaly
Baby with Severe Microcephaly