The Published Kinase Inhibitor Set: A resource to develop probes for the untargeted kinome

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Why the Pharmaceutical Industry is Changing

Top 25 prescribed drugs in US

Only 3 non-generics
All 3 lose patent protection in 2011/2
...but the Rate of Drug Discovery is Constant!


“Nothing that companies have done in the past 60 years has affected their rates of new-drug production”
Still Searching Under the Street Light?

- <10% of the genome has been the focus of pharmaceutical drug discovery
- We work on the same limited set of proteins in industry AND academia

Al Edwards, U. Toronto and The SGC
NR Publications (1990-1994)
NR Publications (1990-1994 and 2009)

Impact of readily available chemical probes

Nature (2011) 470 163-165
Open access chemical and clinical probes to support drug discovery

Aled M Edwards, Chas Bountra, David J Kerr & Timothy M Willson

Drug discovery resources in academia and industry are not used efficiently, to the detriment of industry and society. Duplication could be reduced, and productivity could be increased, by performing basic biology and clinical proofs of concept within open access industry-academia partnerships. Chemical biologists could play a central role in this effort.

- Chemical probes freely available to the scientific community
- Combine the innovation of academia with infrastructure of industry
- Identification of new molecular targets for drug discovery
- Precompetitive publicly-funded endeavor for the benefit of society
Protein Kinases

- 518 kinases in the human genome
- Key regulators of cellular physiology and pathology
- Successful targets for drug discovery using ATP competitive inhibitors

C-AMP-dependent protein kinase (PKA)
Chemically Connected System: Why it works

- ATP site - conserved but not optimized for ATP
- large database of structures allows for:
  - greater understanding of key pharmacophores and SAR
  - improved homology models
  - novel template design

- Exploit unique features of ATP site to achieve potency and selectivity
The (Orphan) Human Kinome

The orphan kinome: why do we keep focus on the “usual suspects”?

Reasons for this vicious circle

- Kinome size leads to “looking in the light”
- Conservative funding mechanisms and decision making
- Historical lack of methods for broad kinome activity assessment
- Lack of high quality, well-characterized chemical probes
How do we prosecute the orphan kinome? A proposal

- **Situation**: The therapeutic potential of the orphan kinome remains unrealized

- **Task**: Seed kinase research by establishing a loose collaborative network of researchers

- **Proposal**: Define and release an open access set of kinase inhibitors
  - Engage a diverse range of experts
  - ID probes or, more likely, chemical starting points for probe development
  - ID interesting phenotypic profiles and kinases for therapeutic targeting
But wait... we don’t do that!

- Why would we give away compounds!??!? 
- Mitigate risk: include only published compounds 
- Mitigate cost: include only materially available compounds 
- Stipulation of material transfer: all data deposited into public domain 
- Move from individual engines of innovation to an *innovative network of experts* 
- Open the door for future collaboration: further dispensing of compounds under the MTA is facilitated
Defining the Set of Kinase Inhibitors

- GSK has long track record with kinases
  - 2 marketed drugs
  - Numerous clinical compounds
  - >100 publications describing 1000s of compounds

- Compound selection
  - Must be published and materially available in house
  - Removed clinical compounds
  - Reduced over-representation of kinases and chemotypes
  - Maximized potential for broad kinome coverage

- End result
  - 367 compounds
  - Not a perfect set but a useful starting point
GSK Published Kinase Inhibitor Set (PKIS)

- **Set design**
  - 367 inhibitors published by GSK
  - >20 chemotypes
  - Limited annotation across <50 kinases

- **Availability**
  - Available to any academic investigator with structures and selectivity data
  - Investigators required to deposit data in the public domain ([www.sarfari.org/kinasesarfari](http://www.sarfari.org/kinasesarfari) is the suggested site)

- **Contacts**
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  - william.j.zuercher@gsk.com
Exemplars from set


Kinase
- Akt1: 6 nM
- Akt2: 200 nM
- Akt3: 22 nM

Cellular proliferation
- LNCaP: 0.3 μM
- HLF: > 30 μM

WHERE R =

- p38α IC₅₀ values range from 100 nM to 10 μM
- Cellular activity and pharmacokinetic properties described

• 10 PLK inhibitors
• variation at 3 sites
• PLK activity from 10 nM to > 1 μM
How Broad is the Kinome Coverage?

- **PKIS vs. 220 kinases**
  - ID of starting points for probes
  - a map to guide phenotypic results

- **NANOSYN Microfluidics Assay**
  - Activity-based assay
  - Ratiometric detection of product and substrate = increased precision
  - Performed at $K_m$ of ATP for each kinase
  - Dual assay at 1.0 and 0.1 µM
### Kinome Coverage (Nanosyn)

<table>
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<tr>
<th>131 non-TKs</th>
<th>89 TKs</th>
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- PKIS had activity across the TKs and non-TKs
- Potent inhibitors were found more often against the TKs
- PKIS had activity on 127/130 non-TKs

- **367 Inhibitors**
  - **>10 μM**
  - **0.1-10 μM**
  - **<0.1 μM**
Selectivity results

Compound promiscuity

Kinase promiscuity
PKIS %I at 100 nM vs. original targets
Potential LOK (STK10) starting point

- LOK (STK10) associates with PLK1 and phosphorylates it in vitro
- Crystal structure 2J7T by SGC of different scaffold (Met kinase oxindole SU11274 from Sugen)
- A chemical starting point for a LOK probe?
Potential BRSK2 starting point

- BRSK2 expressed in brain and required for neuronal polarization; regulation of neurotransmitter release
- SAR between BRSK2 and PLK1 appears divergent (at least some differences)
BRSK2 Hits: SAR diverges from PLK1
Orphan Kinase Activity

- 40 orphan kinases
- Screened by thermal melt
- Stefan Knapp (SGC-Oxford)

Thermal stability (DSF)

- 41 kinases
- 17 inhibitors
- $\Delta T_m$ ($^\circ$C)
- $pK_d$ (Ambit)
- $R = 0.95$
PKIS vs SGC Orphan Kinase Panel

- Sub-μM hits for 39/40 kinases
- Multiple analogs with structure-activity
- Identification of promiscuous kinases
- Identification of selective inhibitors
- Vice versa

40 Kinases

367 Inhibitors

\[ \Delta Tm > 9 \, ^\circ C \]
\[ \Delta Tm > 5 \, ^\circ C \]

\[ \text{--promiscuous} \quad \text{--selective} \]
Orphan Kinase Inhibitors

GSK1511931
IGF-1R inhibitor

GW853606
PLK1 inhibitor

GSK312948
PLK1 inhibitor

GAK: involved in centrosome maturation
STK33A: interacts with oncogenic KRAS
TTKA: associated with breast cancer

CLK4A: CDC-like kinase 4
SLKA: STE 20-like kinase
STK10: mutated in testicular cancer
Phenotypic screening: NCI60

- 60 different cell lines spanning 9 cancer types
- **Extensively** characterized biologically and pharmacologically
- Dose response curves for PKIS obtained
- Results for cmpds with known MOA (eg, EGFR inhibitors) as expected
NCI-60 Cancer Cell Lines: high level view

Growth Inhibition

Toxicity

PKIS Compounds

Cancer Cell lines

Cancer Cell lines

$\text{XC}_{50}$ (µM)

- < 0.1
- 0.1
- 1.0
- > 10
JNK3 compounds
Crystal structure

- 2.45 Å crystal structure of GW572738X/JNK3 (PDB code 2O2U)
- Unusual hinge binding: Met149 backbone NH with ligand CN
- H-bond donation from ligand amide NH to Met146 S
- Water-mediated interaction of ligand CO with Lys93

Selective growth inhibition

Selective growth inhibition of KM12 colon cancer cell line by GW768505A

>50% I @ 100 nM: MUSK, EPHA2/3/4, EPHB2/3/4, RET, TRKA/B/C, TIE2, KDR, HIPK4, DDR2
The PKIS Collaboration Network

PKIS dispensed to over 60 laboratories across 35 institutions
Ependymoma

- **Background**
  - 3rd most common brain tumor in children
  - Survival: 24-75% at 5 years; Incurable in up to 40% of cases

- **Screening paradigm**
  - Proliferation of mEP<sub>Ephb2</sub> vs. parental NSCs

- **IGF-1R as ependymoma target?**
  - IGF-1R upregulated in mEP<sub>Ephb2</sub> NSCs relative to parental
  - PKIS screening IDed GSK2110236A as hit

GSK2110236A
IGF-1R
pIC<sub>50</sub> > 9.0

>50% @ 0.1 uM: IGF-1R, PLK1, TSSK1/2, ALK, FER, FES, FMS, IR, LTK, PYK2, ROS, LRRK2, GRK6/7

R. Gilbertson, K. Guy et al.
St. Jude Childrens Research Hospital
High-Content Neuronal Imaging

- *Real time* measurement of multiple morphologic parameters
- Previous molecular genetic studies have identified kinases and phosphatases

Vance Lemmon, John Bixby (U. Miami)
High-Content Neuronal Imaging

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Vance Lemmon, John Bixby  
The Miami Project to Cure Paralysis, University of Miami
Applications of PKIS

- ID of selective probes or chemical starting points
- Multiple learnings from SAR
- Comparison of assay types and conditions
- Annotation around cell lines (e.g., NCI60) combined with activity map may ID kinases or combinations of kinases for targeting
  - Mechanistic insight
  - Synthetic lethal/sensitization screens

Collective data will enable an improved PKIS

Unlocking the Orphan Kinome

1) Dispense inhibitor set. Screen broadly across the kinome and release all data into public domain

2) Refine PKIS by addition of more compounds from GSK and other Pharma + academics.

3) Create open network to enable optimization of new kinase chemical probes
Closing Thoughts

- The challenges of drug discovery demand new ways of doing things
- An experiment in open preclinical target validation:
  - Created PKIS, a set of 367 kinase inhibitors
  - Obtained activity map vs. 220 kinases
  - Engaged several dozen collaborators (and growing)
- Annotation of the orphan kinome creates opportunities for new drug discovery
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A growing network of collaborators!