# A broad activity screen in support of a chemogenomic map for kinase signaling research and drug discovery

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## Abstract

**Despite the development of a number of efficacious kinase inhibitors, the strategies for rational design of these compounds have been limited by target promiscuity. In an effort to better understand the nature of structure and activity across the kinome, especially as it relates to off-target effects, we screened a well-defined collection of kinase inhibitors using "gold standard" radiometric assays for inhibitory activity toward 234 human kinases, representing all branches of the kinome tree except lipid kinases. We screened 158 small molecules initially identified in the literature as potent and specific inhibitors of kinases important from a therapeutic target as well as a signal transduction biology perspective. We performed hierarchical clustering of these benchmark kinase inhibitors based upon their kinome activity profiles and illustrate how they relate to chemical structure similarities; this provides new insights into inhibitor specificity and potential applications for probing new targets. Additionally, we developed an inhibitor accessibility score for each kinase which we believe reflects general binding site accessibility to small molecules. Knowledge of such scoring indices can play an important role in therapeutic target selection and drug discovery strategies. Using this broad set of data, we provide a framework for assessing polypharmacology. For example we provide data dependent recommendations on the use of popular JNK and Syk specific inhibitors as well as identify potential new uses for existing compounds specifically inhibiting targets such as Aurora A, fms, Ck1d, and Blk.**

## Methods

234 human recombinant kinases, a part of the Merck Millipore KinaseProfiler™ service, were assayed for activity in the absence and presence of two concentrations (1uM and 10um) of 158 different kinase inhibitors (Calbiochem® Protein Kinase Inhibitor Library I and II). The 158 kinase inhibitors were initially chosen based upon literature publications that defined their targets as important signal transduction and disease associated kinases. Importantly all assays were carried out under reaction conditions within 3 fold concentration of the measured Km for ATP for each kinase. Substrates used were specific peptides and buffers and ATP concentrations were individually optimized for each assay. Assays were calculated as % remaining activity in the absence of inhibitor or DMSO control. % inhibition was normalized based upon previously characterized standard positive control inhibitors as baseline. Kinase activity profile similarity analysis and hierarchy clustering was executed using the binary method in R statistical package. The clustering results were visualized as phylogenetic trees using the software package Mega5 (*Molecular Evolutionary Genetic Analysis*, http://www.megasoftware.net/) . Small molecule structure similarity analysis and hierarchy clustering was performed using the Pubchem Chemical Structure Clustering Tool (http://pubchem.ncbi.nlm.nih.gov/assay/?p=clustering) based on Pubchem 2D fingerprints. Inhibitor selectivity and kinase accessibility scores represent fractions of actives among subjects screened.

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## Results

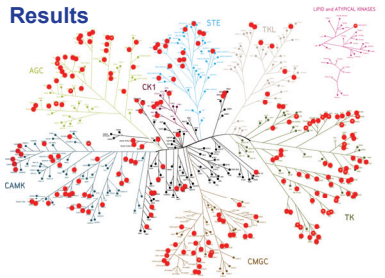


**Figure 1. Kinase Targets Profiled.** 234 human wild type kinases (Merck Millipore's KinaseProfiler™ Service) were screened in radiometric activity assay against 158 Calbiochem® inhibitors (InhibitorSelect™ Libraries I and II, 539744, 539745). Red dots represent kinases profiled and are evenly distributed across the kinome.

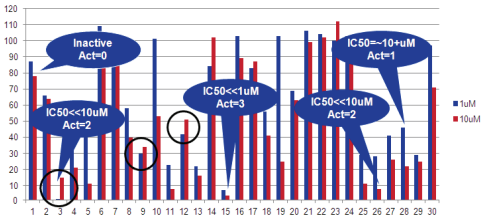


**Figure 2. Potency Assignment Strategy (Syk Inhibitor as example).** Error estimation for assay and potency assignment less than 5 % based on two duplicates, assay SD, and follow up IC50s.

| | |
|---|---|
| 3 =very active | Act% ≤ 10 at 1uM and 10uM |
| 2= active | Act% ≤ 10 at 10uM or 1uM |
| 1= weakly active | Act% < 50 at 10uM or 1uM |
| 0=inactive | Act% ≥ 50 at 1uM or 10uM |

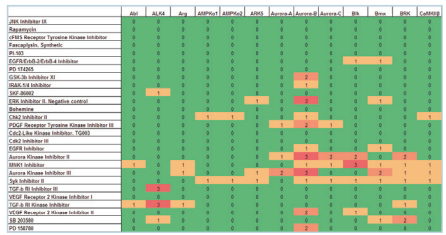

**Figure 3. Heat Map of potency assignments identifies off-target effects of tool compounds.** Snapshot of heatmap showing specificity of TGFβ inhibitors and broad sensitivity of Aurora-B kinase. Inhibitor potency was plotted vs. protein targets and conditionally formatted in Excel.

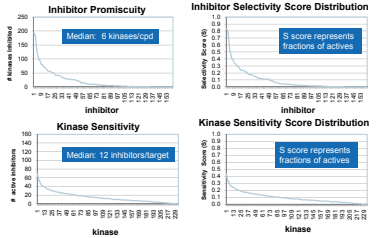


**Figure 4. Selectivity Score and Distribution of inhibitors and targets for identification and quantitation of off-target effects.** Median: ~6 kinase/cpd, 12 inhibitors/target. Inhibitor selectivity and protein target accessibility represents fraction of actives with potency level Act ≥ 2. The 20 most inhibited kinases ranked: Flt4, Aurora-B, Lyn, Flt3, TrkA, Fms, Flt1, Blk, Yes, TrkB, ACK1, KDR, MLK1, Fyn, FGFR1, Rsk3, CaMKIIγ, SIK, Hck, Fgr

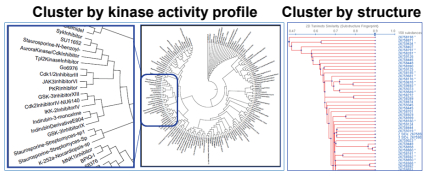


**Figure 5. Clustering of small molecules by activity fingerprint and structure allows intuitive exploration of similar compounds.** MEGA5 was used to illustrate compound neighbor and relationships by activity. Clustering method: Hierarchy clustering (method = binary). Distance matrix: Tanimoto distance (1-Ts). The distance represents the proportion of bits in which only one is on amongst those in which at least one is on. Chemistry Fingerprints: Pubchem 2D

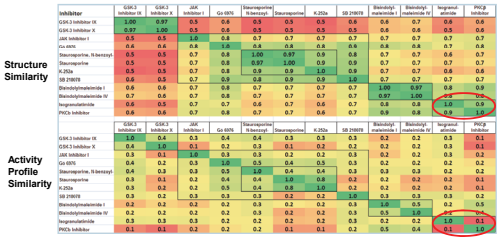


**Figure 6. Comparison of pairwise similarity matrix based on structure and activity profiles.** Similarity matrix can be used to help researchers find the closest compounds in terms of structure or activity profile for screening follow-up or explaining experimental observations Generally, similar compounds in terms of activity profiles tend to have similar structures. However similar structures do not always predict activity profiles. For example a PKCβ inhibitor and a Chk1 inhibitor have similar structural profiles but very different activity profiles.

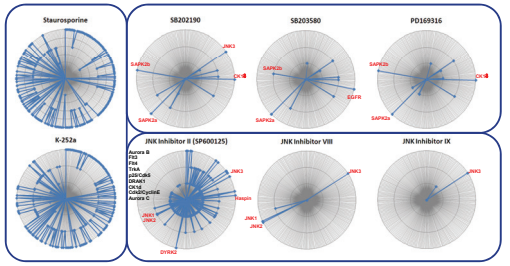


**Figure 7. Selectivity and potency (0-3) visualization for small molecule selection strategies.** p38 map kinase inhibitors are relatively selective but have potential to hit other pathways. Second generation JNK inhibitors are very specific even for easy to inhibit JNK3. Potency was graphed using a radar chart with kinase targets distributed in a clock-wise direction beginning with most inhibitable at 12 o'clock.

A.

| Cat ID | Inhibitors | Act≥2 | Act3 | Act2 | Act1 | Act0 | JNK1α1 | JNK2α2 | JNK3 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| 420119 | JNK Inhibitor II (SP600125) | 17.5% | 13 | 28 | 91 | 102 | 2 | 2 | 3 | non-selective, potently inhibits 12 other kinases |
| 420136 | JNK Inhibitor IX | 0.4% | 1 | 0 | 1 | 232 | 0 | 0 | 3 | most selective and potent JNK3 inhibitor |
| 420129 | JNK Inhibitor V | 6.4% | 3 | 12 | 73 | 146 | 0 | 1 | 2 | more potent for GSK3α, GSK3β, CDK9/CyclinT1 |
| 420135 | JNK Inhibitor VIII | 1.7% | 3 | 1 | 0 | 230 | 3 | 3 | 3 | potent, selective pan-JNK inhibitor |
| 420123 | JNK Inhibitor. Negative Control | 3.0% | 0 | 7 | 26 | 201 | 0 | 0 | 2 | weak inhibition of JNK3 |

B.

| Cat ID | Inhibitors | Act≥2 | Act3 | Act2 | Act1 | Act0 | Syk | | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 574711 | Syk Inhibitor | 22% | 22 | 29 | 65 | 118 | 1 | | potently inhibits 22 kinases, but weak Syk inhibitor |
| 574712 | Syk Inhibitor II | 9.8% | 1 | 22 | 78 | 133 | 3 | | most potent and selective inhibitor for Syk |
| 574713 | Syk Inhibitor III | 0.0% | 0 | 0 | 1 | 233 | 0 | | weak inhibitor of CaMKIV (86% inhibition @ 10uM) |

C.

| Cat ID | Inhibitors | Act≥2 | Act3 | Act2 | Act1 | Act0 | Published Target | Target | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 407601 | Irak-1/4 inhibitor | 0.4% | 1 | 0 | 12 | 220 | Irak-1/4 | Fms | selective fms inhibitor weaker IRAK1/4 inhibitor |
| 567305 | SKF-86002 | 1.7% | 1 | 3 | 12 | 218 | p38 | CK1δ | selective CK1δ |
| 328008 | ERK Inhibitor II, Negative Control | 0.9% | 1 | 1 | 15 | 217 | NA | Aurora B | new selective Aurora B inhibitor |
| 454861 | MNK1 Inhibitor | 3.8% | 1 | 8 | 56 | 169 | MNK1 | Blk | Selective Blk inhibitor, MNK1 not screened |

**Figure 8. Some recommendations for inhibitor use and potential new application.** The data clearly support use of JNK Inhibitor VIII for pan JNK inhibition and JNK Inhibitor IX, for JNK3 specific inhibition (A). For Syk specific inhibition the data point to Syk Inhibitor II as the most potent and specific inhibitor tested (B). Examples of potential new applications for existing molecules and inhibition of multiple pathways by single molecules (C).

## Summary

*We provide a framework for assessing polypharmacology using in-vitro inhibition profiles of 158 inhibitors vs. 234 human kinases. For example it is clear from our selectivity and potency profiles of JNK inhibitors that results using 1st generation inhibitors such as SP600125 may be difficult to interpret considering the numerous cell cycle regulators and growth factor receptors that are hit. Furthermore it is clear that some kinases are more susceptible to inhibition and that routine off target profiling of these kinases may be warranted. Finally we describe potential off-label selective inhibition of Blk, Aurora B, CK1δ, and Fms for current tool inhibitors. These represent a fraction of the useful knowledge generated from such profiling data.*

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