

## **Kinase Selectivity Profiling as a Tool for Drug Discovery**

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Kinase inhibitors are an important new class of drugs. Imatinib, the first small molecule kinase inhibitor to be approved for use in humans, has dramatically changed the prognosis for patients with chronic myeloid leukemia (CML) and has demonstrated that targeting kinases can be a very effective therapeutic approach. The more recent success and approval of gefitinib and erlotinib for treatment of non-small cell lung cancer, sorafenib for renal cell carcinoma, sunitinib for gastrointestinal stromal tumors and renal cell carcinoma, and dasatinib for CML has further established kinase inhibitors as a promising class of compounds.

One potential liability of kinase inhibitors is their propensity for off-target activity. The vast majority of compounds target the kinase ATP site, and because all protein kinases have an ATP site there is great potential for cross-reactivity. Selectivity, therefore, has always been an important issue, and there is ongoing debate about whether it is desirable to develop compounds that are selective for a target of interest, or whether multi-targeted or even promiscuous inhibitors are more likely to have therapeutic benefit. Is promiscuity a predictor of efficacy? Is it a predictor of toxicity? Is the propensity of ATP site kinase inhibitors to interact with multiple kinases a problem, an opportunity, or a little of both? The relationship between selectivity, efficacy and safety is poorly understood and a prerequisite for answering these questions is to gain an understanding of the true inhibitory profiles of compounds across the human kinome.

Determining which kinases are inhibited by a compound is not easily accomplished with conventional technology. Compounds must be tested experimentally against many kinases to assess selectivity and to identify off-target interactions. However, building and routinely running enzymatic activity assays for large numbers of kinases is difficult, time consuming and expensive. To address this problem we have developed a novel competition binding assay that directly and quantitatively measures binding of small molecules to the ATP site of kinases. In the assay, compounds are tested for their ability to compete with the interaction between a kinase and a known ATP site binding ligand that has been immobilized on a solid support. Using this approach we have built assays for more than 250 kinases, including a number of disease-relevant mutant variants. The entire panel of assays is run in a single experiment, and hundreds of compounds may be screened against the full panel in a single day.

To gain a broad and systematic understanding of the selectivity of clinical kinase inhibitors we have profiled a diversity of compounds that are approved drugs or are in clinical or preclinical development against our panel of kinase binding assays. The resulting small molecule-kinase interaction maps provide an overview of specificity across different classes of kinase inhibitors, and reveal a wide range of selectivity among the compounds

tested. Selectivity is not dictated by the intended target of a compound, or its chemical scaffold. The study further provides a foundation from which to explore the structural basis for the observed binding profiles and for relating these profiles to biological activity.

A large number of kinases have been suggested as potential drug targets due to their central role in signal transduction and other cellular processes linked to disease. To exploit this potential it will be necessary to find potent and selective inhibitors as tool compounds for target validation and as leads for drug development. New approaches are therefore required that will enable the efficient and rapid discovery of kinase inhibitors for multiple targets in parallel.

Traditionally, a library of compounds is screened against preselected individual targets to identify hits, which are then optimized for potency and pharmacological properties. Only late in the development process are lead compounds typically screened against a larger panel of kinases to assess selectivity. While this has been an effective strategy for inhibitor discovery, it only addresses one target at a time. We propose a more parallel and efficient approach, in which a compound library is screened against an entire, large panel of kinases upfront to immediately reveal which targets may be accessed with compounds represented in the library, as well as the selectivity of compound series and structural classes. This gene-family strategy allows the rapid annotation of a chemical library against kinase space, and enables decisions about which targets to pursue to be made based not only on target biology, but also on chemistry.

We have used the approach outlined above to guide the discovery, optimization, and development of a highly potent and selective inhibitor of class III receptor tyrosine kinases, including FLT3. Activating mutations in FLT3 are present in a significant fraction of patients with acute myeloid leukemia (AML), and inhibition of FLT3 is a promising strategy for the treatment of AML. The lead compound has subnanomolar activity in vitro and in cell-based assays, is significantly more selective than other known FLT3 inhibitors, has outstanding pharmaceutical properties and is highly active in xenograft models at low doses when given orally once a day. This program demonstrates how kinase profiling technology enables a more efficient approach to the discovery of novel kinase inhibitors.