Discovery of Selective Inhibitors for 123 Protein Kinases Utilizing Internal Kinase Panel Dataset

Akito Hata, Tatsuya Okuno, Yutaka Matsuyama, Tsutomu Henta, Satoshi Sogabe, Nobuyuki Takakura, Yoshi Nara, Takaharu Hirayama, Tomohiro Kawamoto

Axcelead Drug Discovery Partners, Inc.

Background

Protein kinases are one of the most frequently targeted classes in small molecule drug discovery research. More than 60 small molecules have been approved by the FDA and hundreds are in the clinical trial stages[1,2]. Although there have been extensive drug discovery efforts, exiting therapeutics are targeting only a small fraction of 518 human protein kinases. A challenge in protein kinase inhibitor development is optimizing selective compounds to the target of interest in order to avoid risks of off-target toxicities[2,3]. Discovering selective lead compounds is considered one of the most critical challenges in advancing protein kinase inhibitor drug development.

Summary

Here, we report several approaches that are introduced to discover selective protein kinase inhibitors for 123 kinases:

- A global kinase panel assay with over 320 kinases was developed internally[4] to test large number of compounds with feasible cost. Approximately 6,500 compounds from in-house compound library have been tested to date.
- Approximately 5,000 compounds with global kinase panel data were selected to construct a kinase-focused library. This library was evaluated against Protein kinase “X”, which had not been previously assessed in our global kinase panel, resulting in the identification of five potent and highly selective hits.
- Through AI analysis of the kinase panel database, combination of 46 kinases were identified that reproduces selectivity scores of the global kinase panel. By utilizing a cost-effective mini kinase panel, it becomes possible to gain a general understanding of selectivity across the entire kinases. This allows for the evaluations on large number of lead candidates, facilitating the early validation of selectivity profiles.

Kinase Panel Assay and Internal Dataset

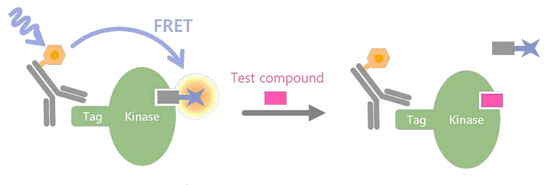

Figure 1. Assay principle

TR-FRET competitive binding assay method was used to develop kinase panel assay (Fig 1). Internally purified recombinant protein, overexpression cell lysate, or commercial recombinant protein were used as a protein source. Tb labeled anti-tag antibodies are used for TR-FRET donor. Bodipy or Cy5-labeled fluorescence probes have been synthesized internally[4]. Test compound, protein kinase, anti-tag antibody, and fluorescence probe were dispensed in 1536 well plate, incubated over 1 hr, and TR-FRET signal was measured on EnVision plate reader.

Table 1. Probe selection for Kinase X

Probe conc.	10 nM	100 nM	1000 nM
Probe 1	0.93	0.94	1.02
Probe 2	0.98	0.91	0.85
Probe 3	0.82	0.82	0.99
Probe 4	0.86	0.80	1.37
Probe 5	0.54	0.66	0.88
Probe 6	1.08	1.09	1.47
Probe 7	1.01	0.94	0.95
Probe 8	0.93	0.97	0.94
Probe 9	0.99	1.00	1.04
Probe 10	1.00	1.48	1.15
Probe 11	0.96	0.92	0.92
Probe 12	1.00	1.06	1.11
Probe 13	1.07	1.06	1.03
Probe 14	1.38	1.67	1.44
Probe 15	1.06	1.09	1.36
Probe 16	0.96	1.14	1.17
Probe 17	0.68	0.71	0.92
Probe 18	1.17	2.14	2.26
Probe 19	1.18	1.91	2.79
Probe 20	0.54	0.66	1.51
Probe 21	1.04	1.06	1.28
Probe 22	1.03	1.05	1.05
Probe 23	1.23	2.52	2.14
Probe 24	0.67	0.79	1.02
Probe 25	1.01	1.08	1.51
Probe 26	1.17	1.53	1.25
Probe 27	1.92	2.34	2.32
Probe 28	1.08	1.17	1.50
Probe 29	0.97	1.12	1.29
Probe 30	0.64	0.73	1.05

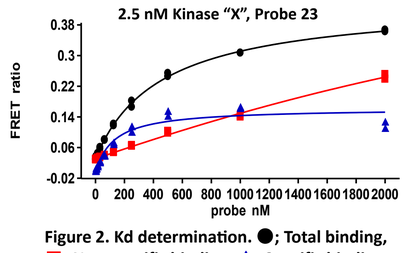

Figure 2. Kd determination. ●; Total binding, ■; Non-specific binding, ▲; Specific binding

The assay conditions for each kinase are determined based on the Kd values of fluorescent probes. First, binding assays are conducted on 30 proprietary fluorescent probes developed in-house, and probes that yield high TR-FRET signals are selected for Kd determination (Table 1) . Subsequently, probe titration assay is performed on the selected probes to measure their Kd values with different kinase concentrations (Fig 2). The probe concentrations around the Kd value are then employed for the panel assay system.

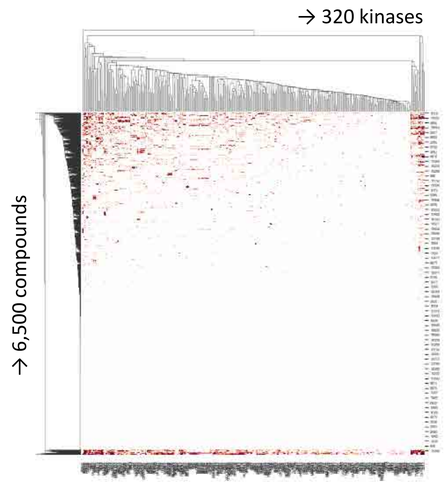

Figure 3. Heat map of internal protein kinase panel dataset. Red indicates high binding affinity and white indicates low binding affinity.

The compounds evaluated in the kinase panel were selected from the Axcelead small molecule compound library, which comprises around 1.5 million compounds.
Representative examples of the criteria for compound selection are;

- Compounds that have been tested with any protein kinase assays prior
- Synthesized compounds in kinase drug discovery project
- Similar compounds of the one that showed high selectivity or high potency

A heatmap depicting the current panel data is shown in Fig 3. It is characterized by a higher selectivity compared to public kinase inhibitor databases.

Kinase-Focused Library and Small Scale HTS

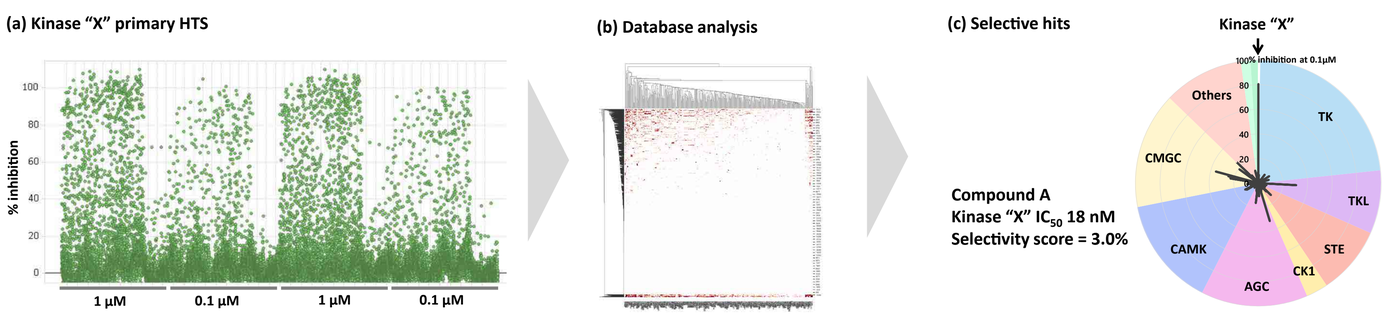

Figure 4. (a) Kinase “X” primary screen results. 4,995 kinase-focused library compounds were tested at 1 μM and 0.1 μM in duplicates. (b) Primary screen data was analyzed comparing to global kinase dataset in order to identify selective hit. (c) Selectivity profile of one of selective hit compounds identified from the kinase-focused library. IC_{50} against Kinase “X” was 18 nM and selectivity score (% of kinases IC_{50} < 1μM) was 3.0%.

We created a kinase-focused library from compounds with available global kinase panel data in order to identify highly selective hit compounds through small-scale screening for kinases we do not possess a kinase panel dataset. This library is divided into three layers: approximately 1,200 representative compounds from those with a selectivity score of 20% or less, approximately 2,600 compounds with inhibitory activity against at least one kinase, and another 1,200 compounds, totaling 5,000 compounds.
We conducted a small-scale screening of the kinase-focused library against protein kinase X, which had no prior testing history in the global kinase panel, using two concentration points, 1uM and 0.1uM in duplicates (Fig 4a). By analyzing these results with the global kinase panel dataset (Fig 4b), we successfully identified five compounds that exhibited both high activity and selectivity. The selectivity profile of a representative compound is presented in Fig 4c.

Selective Inhibitors for 123 Kinases

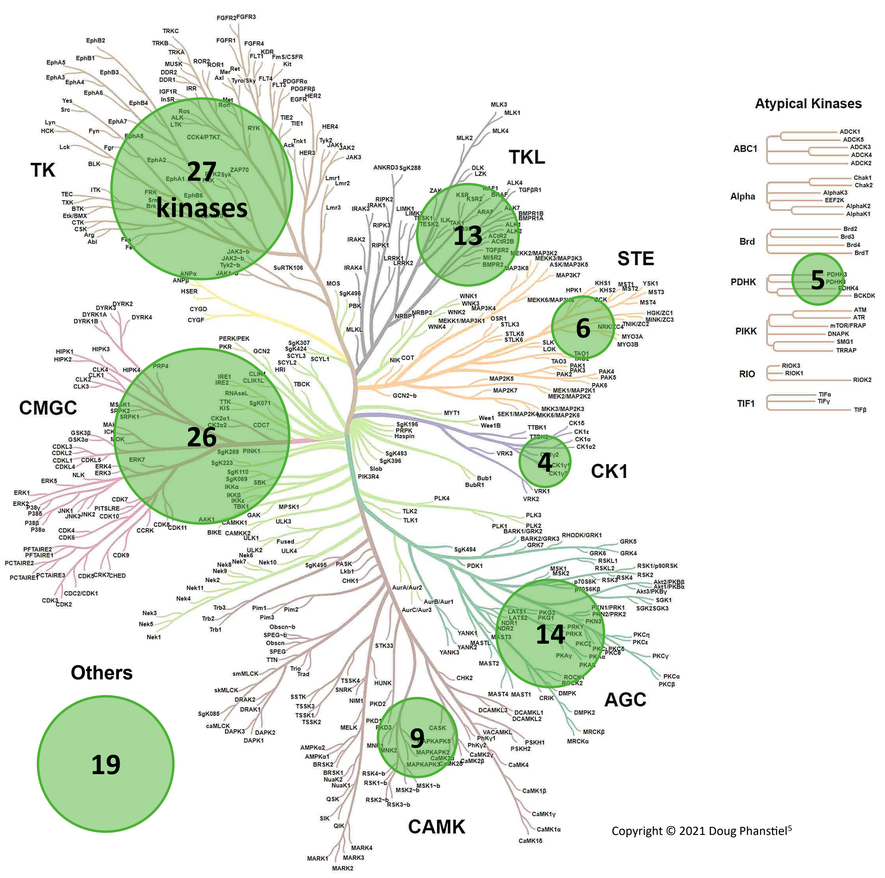

Figure 6. The distribution of 123 protein kinases on kinome map. The numbers represent the count of kinases within each kinase family for which selective kinase inhibitors have been obtained.

We have identified selective tool inhibitors against 123 kinases (Fig 6) through analysis of kinase panel dataset, testing structurally similar compounds in Axcelead compound library, or small scale HTS using kinase-focused library. The criteria are; the IC_{50} against the kinase of interest being 30nM or below, a Selectivity score of 5% or less, and the number of kinases with IC_{50} of less than 30nM being limited to four or fewer.
Currently, we are continuing our efforts to expand kinase coverage, while conducting cell-based assay, ADME profiling, and PK profiling. These selective compounds have the potential to be promising lead candidates in kinase inhibitor drug discovery efforts as well as the possibility of being useful tools for POC validation using compounds.

Mini Kinase Panel Development by AI Analysis

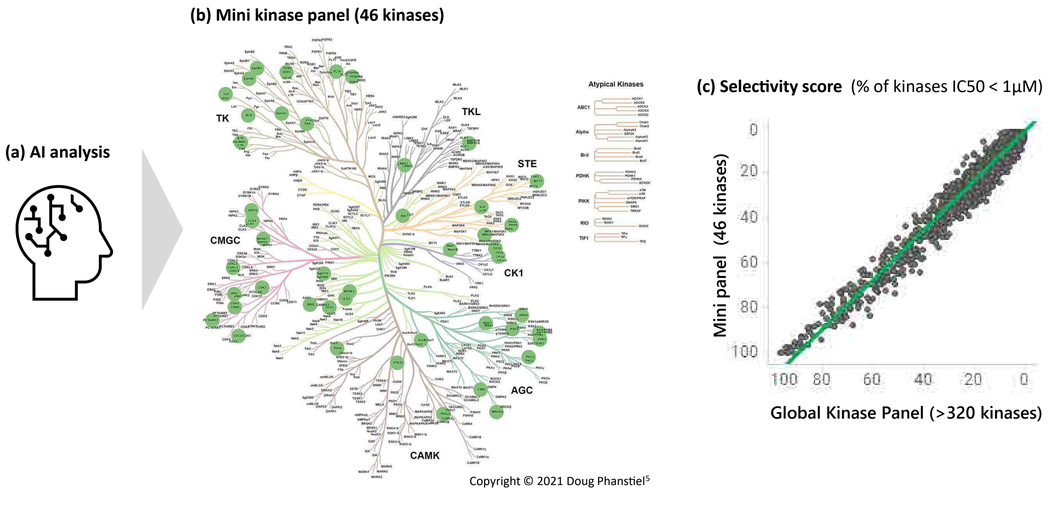

Figure 5. (a) The kinase panel data set was analyzed using AI to identify the combination of kinases that reproduces selectivity score of the global kinase panel. (b) The distribution of selected 46 kinases on kinome map. (c) Comparison of selectivity scores obtained from the selected 46 kinases and the global kinase panel with 320 kinases (R^2 = 0.96).

A focused mini kinase panel was established in order to estimate selectivity across the entire kinases at the early stage of lead generation in cost-effective way. The selection of the mini kinase panel was based on the selectivity score, an indicator of the number of kinases showing inhibitory activity relative to the total number of kinases tested. Using AI analysis on the kinase panel dataset, we identified combinations of kinases that could replicate the selectivity score with the global kinase panel results. Consequently, we selected a mini-panel comprising 46 kinases (Fig 5a, b). The selectivity scores obtained from the global kinase panel and the selected mini-panel exhibited a good correlation, with an R^2 value of 0.96 (Fig 5c).

Reference

(1) R. Roskoski Jr., *Pharmacological Research,* 2020, 152, 104609-
(2) M. Zhang et.al., *J. Chem. Theory Comput.* 2023, 19, 1615-
(3) A. Lin and C. J. Giuliano et. al., *Sci Transl Med.* 2019, 11, 509
(4) Y. Hirozane et. al., *Bioorganic & Medicinal Chemistry Letters,* 2019, 29, 126641-
(5) Kathleen Metz et. al., *Cell Systems*, 2018, 7, 347-, Released under the MIT license; https://opensource.org/license/mit/