

Comprehensive analysis of kinase inhibitor selectivity

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We tested the interaction of 72 kinase inhibitors with 442 kinases covering >80% of the human catalytic protein kinome. Our data show that, as a class, type II inhibitors are more selective than type I inhibitors, but that there are important exceptions to this trend. The data further illustrate that selective inhibitors have been developed against the majority of kinases targeted by the compounds tested. Analysis of the interaction patterns reveals a class of 'group-selective' inhibitors broadly active against a single subfamily of kinases, but selective outside that subfamily. The data set suggests compounds to use as tools to study kinases for which no dedicated inhibitors exist. It also provides a foundation for further exploring kinase inhibitor biology and toxicity, as well as for studying the structural basis of the observed interaction patterns. Our findings will help to realize the direct enabling potential of genomics for drug development and basic research about cellular signaling.

The vast majority of small-molecule kinase inhibitors interact with multiple members of the protein kinase family. The extent of cross-reactivity for this class of compounds only became apparent once large panels of kinase assays and other approaches to interrogate the kinome with small molecules became available¹⁻⁶. Systematic kinase profiling of known inhibitors, including compounds that have been or are currently in clinical trials, has revealed diverse interaction patterns across the kinome and has provided a common resource to further study these compounds.

We previously described the interaction patterns of a set of 38 known kinase inhibitors against a panel of 317 kinase assays representing >50% of human protein kinase domains, and introduced the concept of a 'selectivity score' to facilitate an objective analysis of kinase profiling data and to quantify selectivity⁷. We now update and extend the data set to encompass a total of 72 known inhibitors, including 11 currently approved small-molecule kinase inhibitor drugs, tested against a panel of 442 kinase assays representing >80% of catalytically active, nonatypical human protein kinase domains.

The compounds tested here represent mature inhibitors that have been optimized against specific targets of interest. The data therefore provide insight into the interaction patterns and selectivity characteristics that can be achieved with optimized compounds, and complement information from screening large libraries comprising unoptimized compounds⁸⁻¹⁰. We show that most type II inhibitors, which contact a binding pocket adjacent to the ATP site and prefer a 'DFG-out', inactive kinase conformation¹¹, are indeed relatively selective as expected. In contrast, type I inhibitors, which do not require a 'DFG-out' conformation of the activation loop and do not contact this pocket, vary widely in overall selectivity. Several type I inhibitors are among the most selective, whereas two type II inhibitors are among the least selective compounds tested. This shows that selectivity may be achieved with a type I binding mode and is not guaranteed with a type II binding mode. The compound set contains

selective inhibitors for the majority of the 28 kinases that represent the intended, primary targets of the compounds tested. This suggests that it is indeed possible to develop selective inhibitors for a diversity of kinases. Furthermore, a quantitative analysis of selectivity across the major kinase groups or subfamilies reveals a class of 'group-selective' compounds that interact broadly with one kinase group, but are selective outside of the targeted group. Selectivity within a kinase subfamily or group is therefore not always predictive of overall selectivity. Therefore, testing compounds against kinases closely related to the primary, intended target, as has frequently been done to estimate compound selectivity, does not reliably address global selectivity.

RESULTS

A comprehensive assay set for protein kinases

We used competition binding assays³ to undertake a 10-year effort to develop a biochemical assay panel that would enable comprehensive and direct testing of compounds across the kinome. The primary emphasis was on building assays for catalytically active human protein kinase domains in the eight major 'typical' groups, as defined¹². Due to their high therapeutic and biological relevance, we also included assays for PI3K-family lipid kinases, several atypical protein kinases such as mTOR, and kinases from human pathogens, as well as disease-linked mutant variants and noncatalytic kinase domains. The effort has yielded 442 assays representing >80% of catalytic, nonatypical human protein kinase domains (363 distinct kinase domains, not counting mutant variants). The panel also includes seven atypical kinases, 11 lipid kinases (not counting mutant variants), two kinases from *Plasmodium falciparum* and one from *Mycobacterium tuberculosis*, 7 activation-state variants, 49 disease-relevant mutant variants and two kinase domains believed to be noncatalytic (Supplementary Table 1).

We screened a diverse set of 72 known kinase inhibitors against the assay panel to generate a robust overview of what types of

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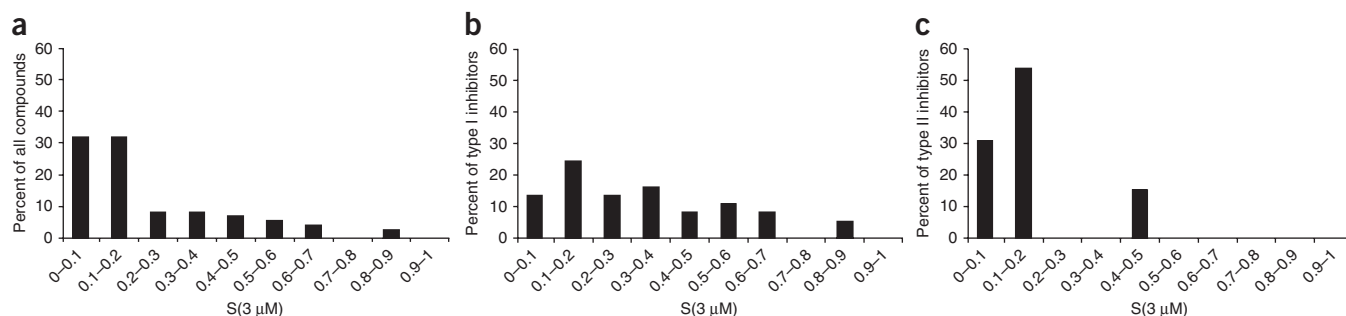


Figure 1 Quantitative distribution of kinome-wide selectivity of compounds. A kinome selectivity score ($S(3 \mu\text{M})$) was calculated for each compound as described in the text, and compounds were binned according to their scores. (a) Distribution of selectivity for the entire set of 72 compounds. (b) Distribution of selectivity for 37 compounds classified as type I inhibitors based on relative binding affinity for phosphorylated and nonphosphorylated variants of ABL1 (Supplementary Table 5). (c) Distribution of selectivity for 13 compounds classified as type II inhibitors based on relative binding affinity for phosphorylated and nonphosphorylated variants of ABL1 (Supplementary Table 5).

small molecule–kinome interaction patterns can be observed (Supplementary Table 2). At least 28 different kinases, representing six of the eight ‘typical’ kinase groups as well as the atypical and lipid kinase subfamilies, are among the primary, intended targets of these compounds (Supplementary Table 3). The compound set therefore represents a cross-section of inhibitors optimized for activity against a variety of kinases. We initially screened each compound against the panel at a single concentration (10 μM) to identify candidate kinase targets, and determined a quantitative dissociation constant (K_d) for each interaction observed in this primary screen (Supplementary Table 4). Data for 40 of the compounds against smaller assay panels have been published previously^{7,13} and are included once more here in the interest of presenting a single, systematic and unified data set that may readily be accessed and used as a resource for further studies and analyses. The updated and extended data set represents close to a 2.5-fold increase in the number of data points available compared to the previously published results, assessed by the number of compound/kinase combinations queried. As before⁷, the binding constants measured here generally agree well with published values determined using biochemical enzyme activity assays (Supplementary Table 3 and Supplementary Fig. 1).

Selectivity of type I and type II inhibitors

To provide an overview of global kinome selectivity, we prepared kinome interaction maps (Supplementary Fig. 2) and calculated selectivity scores for each compound by dividing the number of kinases bound with $K_d < 3 \mu\text{M}$ by the total number of distinct kinase domains queried (386, after excluding mutant and activation state variants), as described previously⁷ ($S(3 \mu\text{M})$). For the majority of compounds (46 of 72, or 64%), $S(3 \mu\text{M}) < 0.2$, indicating that they bind <20% of the kinases tested (Fig. 1a and Supplementary Table 5). The scores for most of the remaining compounds are broadly distributed between 0.2 and 0.7, with the exception of two highly promiscuous outliers with scores >0.8. The outliers are the staurosporine analog CEP-701 and staurosporine itself, which is known to interact with a large fraction of kinases. The lowest selectivity scores, and therefore the greatest selectivity, were observed for the MEK inhibitors AZD-6244/ARRY-886 and CI-1040, the MET inhibitor SGX-523, the CSF1R inhibitor GW-2580 and the ERBB2/EGFR inhibitor lapatinib (Tykerb). Each of these highly selective compounds exploits a structural feature that may distinguish the target kinase from most other kinases. The MEK inhibitors bind an allosteric pocket adjacent to the ATP site, distinct from the pocket exploited by type II inhibitors, without contacting the ATP site itself¹⁴; SGX-523 requires a unique inactive conformation of

the kinase activation loop¹⁵; GW-2580 is a type II inhibitor, characterized by requiring an inactive ‘DFG-out’ conformation of the kinase¹¹; and lapatinib, although not a typical type II inhibitor, requires an unusual displacement of the alpha-C helix¹⁶.

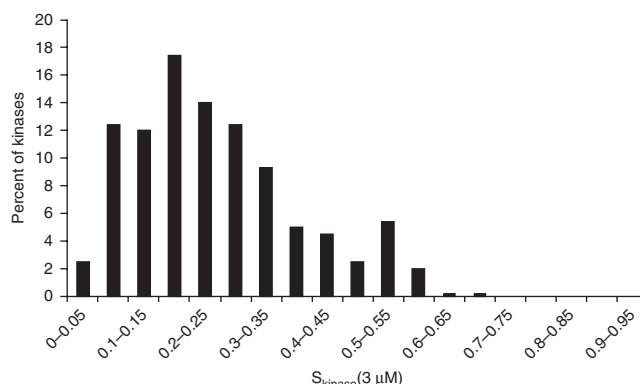
One of the potential advantages frequently noted for type II inhibitors is that there may be greater conformational heterogeneity among inactive kinase states than in the canonical active state, providing opportunities for optimizing selectivity for the inactive-like conformation specific to a target kinase^{11,17}. A broad comparison of selectivity across a diverse set of type I and type II inhibitors has not been performed, however. We have recently shown that differential binding to phosphorylated and nonphosphorylated forms of ABL1 can functionally differentiate compounds that prefer an inactive, ‘DFG-out’ kinase conformation (type II inhibitors) from those that do not (type I inhibitors), even for compounds that are not primarily ABL1 inhibitors but exhibit at least modest affinity for ABL1 or a mutant variant of ABL1 (ref. 18). To functionally classify the inhibitors tested in the current study we therefore used binding affinities measured for the phosphorylated and nonphosphorylated forms of wild-type ABL1 and five ABL1 mutant variants included in our assay panel. Although this classification method is robust, we cannot completely rule out the possibility that some inhibitors have kinase-specific binding modes. Of the 72 compounds tested, 50 (69%) bind at least one of the paired ABL1 variants with $K_d < 3 \mu\text{M}$ and could be classified based on these data. Of these 50 compounds, 37 exhibit little or no preference for the nonphosphorylated state and were classified as type I inhibitors. The remaining 13 have a marked preference for the nonphosphorylated state and therefore were classified as type II inhibitors (Supplementary Table 5). We then plotted the distribution of selectivity scores separately for type I and type II inhibitors (Fig. 1b,c). The scores for type I inhibitors are fairly evenly distributed across the range observed for the compound set as a whole. In contrast, all but two of the type II inhibitors had scores <0.2. A similar pattern was observed when the analysis was repeated using selectivity scores based on a 300 nM affinity cutoff ($S(300 \text{ nM})$) (Supplementary Table 5 and Supplementary Fig. 3). The type II inhibitors therefore are largely responsible for the bias toward selective compounds observed for the compound set as a whole (Fig. 1a). Importantly, there are two type II inhibitors, EXEL-2880/GSK1363089 and AST-487, with $S(3 \mu\text{M})$ of 0.44 and 0.49, respectively, which interact with a large number of kinases. These two compounds remain outliers when the analysis is repeated with a range of affinity cutoffs for calculating selectivity scores. Understanding why these compounds behave differently from other type II inhibitors will likely require structural studies, and

Figure 2 Quantitative distribution of compound selectivity of kinases. A selectivity score ($S_{\text{kinase}}(3 \mu\text{M})$) was calculated for each of the 442 kinases in the assay panel as described in the text, based on the number of compounds each kinase binds, and kinases binned according to their scores.

should provide important insights into the interplay between binding mode, kinase conformation and selectivity. Our analysis therefore confirms that, in general, type II inhibitors are more likely to be selective than type I inhibitors. However, the data highlight that a type II binding mode does not guarantee high selectivity, nor is it required to achieve selectivity. Several type I inhibitors, including CP-690550 (tofacitinib) and BIBW-2992, are as selective as any of the type II inhibitors, whereas the type II inhibitors EXEL-2880/GSK1363089 and AST-487 are among the least selective compounds tested here.

Selectivity of kinases

It is apparent from the data set that just as some compounds are selective and others are broadly reactive, there are some kinases that interact with many of the compounds tested, whereas others interact with only one or two. To quantify these observations, we calculated selectivity scores for each kinase by dividing the number of compounds bound with $K_d < 3 \mu\text{M}$ by the total number of compounds screened ($S_{\text{kinase}}(3 \mu\text{M})$) (Fig. 2 and Supplementary Table 1). The overall distribution of kinase selectivities is fairly narrow, with >60% of kinases interacting with 10–40% of the compounds tested, and each kinase interacting with at least one compound. Three kinases, ERK1, ERK2 and TRPM6, bind only one compound each with $K_d < 3 \mu\text{M}$, and are the least frequently hit, whereas LCK and YSK4 each interact with >60% of the compounds tested, and are the most frequently hit. A similar pattern was observed when the analysis was repeated using a 300 nM affinity cutoff to calculate $S_{\text{kinase}}(300 \text{ nM})$ (Supplementary Table 1). There is generally good agreement between our results and



the kinase selectivities observed previously in single-concentration primary screens of very different collections of unoptimized compounds against much smaller panels of kinases^{8,9}. Differences in the frequencies with which individual kinases are hit in these studies likely reflect the different nature of the compounds tested and the scale of the experiments.

Selective inhibitors for many kinases

One major question that has been difficult to address is whether it is possible to develop reasonably selective inhibitors for most kinases, or whether there is a significant subset of kinases for which it is difficult to identify selective inhibitors. The compounds used here are, with the exception of staurosporine, mature inhibitors that in most cases are the result of significant optimization against an intended, primary target. As such, they are well suited to begin to address this question. There are 28 distinct kinases that collectively may be considered to represent the primary targets of the compound set tested here (Primary Target 1 in Supplementary Table 3). Of these, 27 are

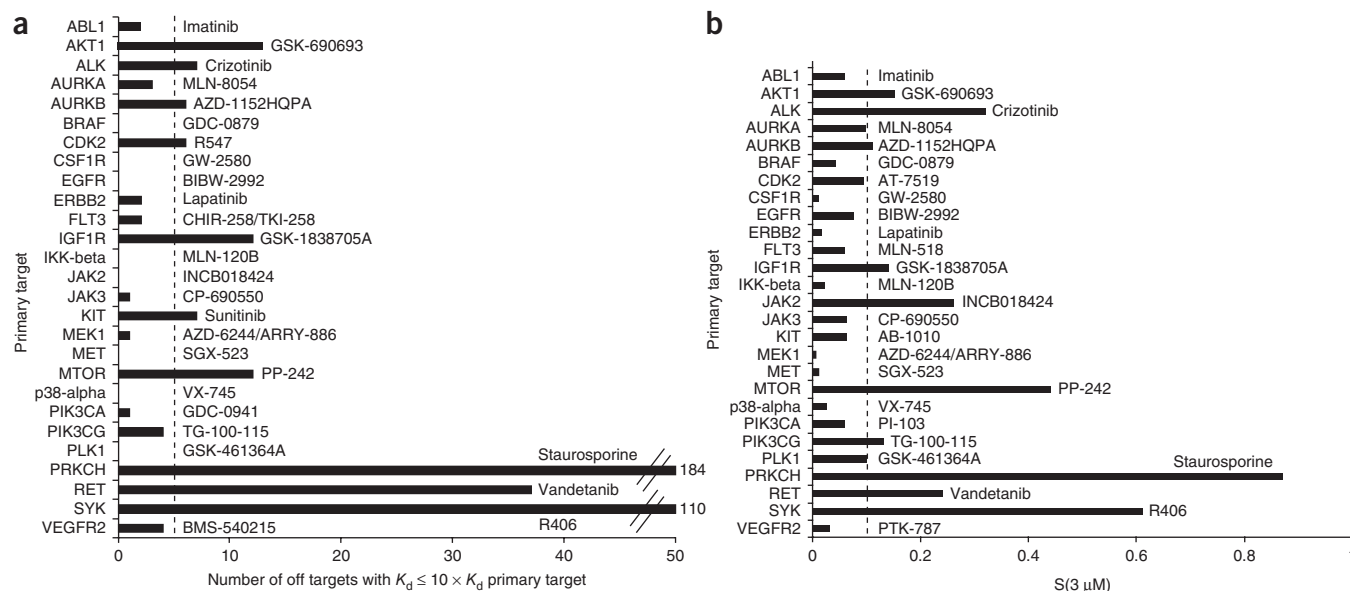


Figure 3 Selective inhibitors for primary targets. Each kinase on the y axis is a primary, intended target of one or more compounds in the set tested here (Supplementary Table 3). The most selective compound for each target is shown. (a) Relative selectivity. For each of these primary targets, the compound with the greatest relative selectivity for that target was identified by counting the number of kinases bound with a K_d within tenfold or better of the K_d for the primary target for each compound targeting the kinase. The number of kinases bound with K_d within tenfold of that for the primary target is shown for the most selective compound targeting each of the kinases shown. (b) Absolute selectivity. For each of the primary targets the compound with the greatest absolute selectivity for that target was identified, using the $S(3 \mu\text{M})$ as a measure of absolute selectivity (Supplementary Table 5). The $S(3 \mu\text{M})$ is shown for the most selective compound targeting each of the kinases shown.

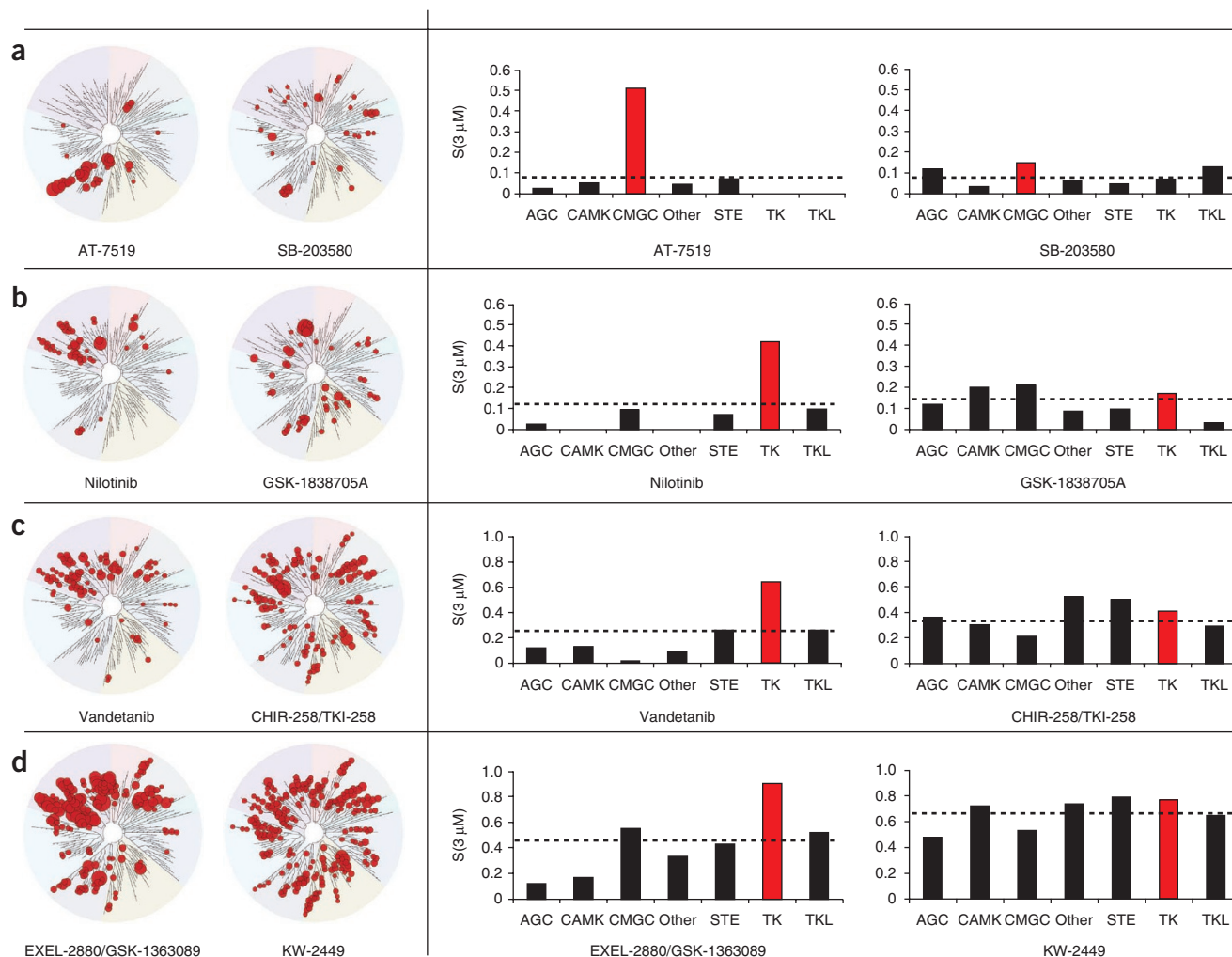


Figure 4 Group-selective compounds. Compounds were divided into selectivity bins based on their overall selectivity ($S(3 \mu\text{M})$ 0–0.1, 0.1–0.2, 0.2–0.4, >0.4), and selectivity scores ($S(3 \mu\text{M})$) were calculated for each compound for the kinase groups for which more than fifteen kinases are represented in the assay panel (thereby excluding atypical, lipid and CK1 kinases). Shown here are the kinase interaction maps and kinase group fingerprints for one group-selective and one non-group-selective compound from each selectivity bin. (a) Compounds from the $S(3 \mu\text{M}) = 0$ –0.1 bin. (b) Compounds from the $S(3 \mu\text{M}) = 0.1$ –0.2 bin. (c) Compounds from the $S(3 \mu\text{M}) = 0.2$ –0.4 bin. (d) Compounds from the $S(3 \mu\text{M}) > 0.4$ bin. The interaction maps were generated using TREEspot software (<http://www.kinomescan.com/>) and display a circular representation of the kinase family tree based on kinase domain sequence. The bars in each bar graph indicate $S(3 \mu\text{M})$ for the individual kinase groups. Red bars indicate the kinase group containing the primary target for each compound. Dashed lines signify the overall $S(3 \mu\text{M})$ for each compound.

represented in the assay panel. To determine which of these 27 kinases are targeted selectively by compounds in our set, we used two approaches. First, we counted for each compound the number of kinases bound with K_d within tenfold of the K_d for the compound's primary target, and thereby identified for each primary target the compound with the greatest relative selectivity for that target (Fig. 3a). For 17 of the 27 primary target kinases, there was at least one inhibitor in our set that bound fewer than five other kinases with affinities comparable to that for the intended, primary target. Second, we determined for each primary target the compound with the lowest overall selectivity score ($S(3 \mu\text{M})$), and thereby identified for each primary target the compound with the greatest absolute selectivity (Fig. 3b). For 16 of the 27 primary target kinases, there was at least one inhibitor in our set with $S(3 \mu\text{M}) < 0.1$. For 15 of the 27 primary target kinases, there was at least one compound that featured both fewer than five off-targets (kinases other than the primary target) with affinity comparable to that for the primary target, and an $S(3 \mu\text{M}) < 0.1$.

Although the set of 27 primary targets examined here certainly is not an unbiased selection of kinases, these results nevertheless suggest that it is possible to develop reasonably selective inhibitors for a diversity of kinase targets.

A quantitative fingerprint of interaction patterns

We have previously noted that the pattern of interactions across the various kinase groups or subfamilies can vary widely, even among compounds that share a primary target⁷. To describe the interaction patterns of compounds quantitatively, we calculated individual selectivity scores, again using a $3 \mu\text{M}$ affinity cutoff, for each of the major kinase groups¹². The relative pattern, or fingerprint, of these group-specific selectivity scores reveals whether a compound preferentially targets one or more kinase subfamilies, or whether the interaction pattern is distributed across the kinome. This quantitative approach provides an objective description of compounds' kinase group preferences that is difficult to obtain

from a more qualitative visual assessment of interaction patterns, or from screening a limited number of kinases.

For many compounds, the selectivity scores for the individual kinase groups are relatively similar, and close to the overall kinome-wide score. For a subset of compounds, however, the score for one kinase group (generally the group that includes the compounds' primary target) is substantially higher both than that for the remaining groups, and than the overall score (Fig. 4). These compounds can thus be considered 'kinase group selective', but are not necessarily selective for their specific target. Group-selective inhibitors may have broad reactivity against members of the primary targeted kinase group, and testing kinases closely related to the primary target is therefore unlikely to yield an accurate assessment of their overall selectivity. Group-selective inhibitors included compounds with a wide range of overall selectivity (Fig. 4), both type I and type II inhibitors, and compounds with diverse chemical structures. These observations suggest that group selectivity is not governed by gross binding mode, chemical scaffold or global propensity to interact with a range of kinases. Three of the four cyclin-dependent kinase (CDK) inhibitors tested (AT-7519, R547, BMS-387032/SNS-032) were strongly group selective (Supplementary Fig. 2). This may reflect similar strategies taken to optimize selectivity for CDKs over non-CMGC kinases, or may signal a more fundamental structural feature that distinguishes CMGC kinases from kinases in other groups that each of these compounds exploits.

DISCUSSION

Our data set represents the most detailed comprehensive assessment of the reactivity of known and clinical kinase inhibitors across the kinome published to date. The assay panel approaches near-complete coverage of the human protein kinome and, together with the diversity of chemical scaffolds and of primary targets represented by the compound collection tested, yields a broad overview of how optimized small-molecule inhibitors interact with the kinome.

An assessment of overall selectivity of the compounds tested here by compound class shows that, as a class, type II inhibitors are more likely to be selective than type I inhibitors, and that type I inhibitors can have a wide range of selectivities. This observation is consistent with the general assumption that the inactive conformation preferred by type II inhibitors is more kinase-specific than an active conformation that can accommodate typical type I inhibitors. However, the data also demonstrate that several type II inhibitors exhibit poor selectivity, whereas a number of type I inhibitors are quite selective. Therefore, inhibitor type does not dictate selectivity. A common theme for the most selective compounds, regardless of inhibitor type, is that they exploit structural features or kinase conformations that can help distinguish the target kinase from other kinases. The data also show that for at least 15 of the 27 kinases that are the primary, intended targets for the compounds tested and that are represented in the assay panel, selective inhibitors, as assessed by both absolute selectivity across the kinome and selectivity relative to the primary target, are among the 72 tested here. Although the number of primary targets and compounds assessed is still limited, these results nevertheless provide an initial encouraging suggestion that it may be possible to develop selective inhibitors for a majority of kinases.

Small-molecule inhibitors are valuable tools to study the biology and therapeutic potential of specific kinases. Nonetheless, dedicated inhibitors are available for only a very small fraction of protein kinases. Our data set reveals a large number of previously undescribed activities of known and available inhibitors, along with the overall selectivity

and interaction pattern for each compound. The information may enable the use of compounds in the set studied here as tools for kinases for which no specifically targeted inhibitors are currently available. In some cases, it may suggest possible novel applications to explore for known drugs or starting points for the development of optimized inhibitors targeting novel kinases. Interesting novel activities include the high affinity of sunitinib (Sutent) for RET harboring gatekeeper mutations (RET(V804L/M)), which are not bound with high affinity by the approved RET inhibitor vandetanib (Zactima)¹⁹; the interaction of PKC-412, a compound in late-stage clinical development, with EGFR(T790M), which is a major resistance mutation for EGFR inhibitors in lung cancer and against which no drugs are currently available; and the interaction of PFCDPK1 from the malaria parasite with PLX-4720, a compound closely related to the recently approved drug vemurafenib (Zelboraf).

One of the most compelling applications of comprehensive kinase assay panels is the screening of large compound collections to efficiently identify novel inhibitors and starting points for drug discovery^{8–10,20–22}. Identifying compounds of interest from the large data sets generated by this application requires computational handles for classifying compounds and revealing the most promising and interesting hits. The approach we describe here of calculating selectivity scores for each of the major kinase groups or subfamilies provides such a handle by generating a quantitative and numeric description of not only the overall kinome selectivity of compounds, but their detailed interaction pattern across kinase groups. This kinase group fingerprint makes it possible to systematically search large data sets to identify compounds with specific interaction patterns without the need to manually examine large numbers of qualitative interaction map images.

A major rationale for sequencing the human genome was the promise that the genome sequence would facilitate and enable drug discovery. The most commonly assumed path from genome to drugs is through the identification of novel gene-disease associations and of potential new drug targets. An alternative path by which the genome can affect drug discovery is through enabling the development of technologies that directly facilitate drug discovery, rather than target discovery. This path is exemplified by the application of high-throughput kinase profiling to drug discovery. The genome sequence was essential to enumerate the human protein kinome¹², which in turn has been essential to systematically build panels of kinase assays to interrogate the kinome with small molecules, and for understanding how complete and representative the assay panels were^{2–4,6}. Profiling of known inhibitors, including approved drugs, across the assay panels has revealed many previously unrecognized activities, and has yielded a more complete understanding of how these compounds may affect biology^{2–4,6,7,23–27}. The assay panels further have enabled a novel approach to kinase inhibitor discovery, based on screening entire libraries of compounds against panels of kinases, which has resulted in the discovery of several promising new inhibitors^{8,9,13,20–22,28,29}. At least one of these inhibitors is currently in clinical trials and has exhibited efficacy in patients³⁰. This direct path from genome sequence to kinome, from kinome to kinase profiling-based drug discovery, and from kinase profiling to novel drugs and a greater understanding of existing drugs illustrates one way in which the genome sequence is living up to the promise of improving human health.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturebiotechnology/>.

Note: Supplementary information is available on the Nature Biotechnology website.

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AUTHOR CONTRIBUTIONS

M.I.D. coordinated development of the assay panel, J.P.H. developed technology to enhance the efficiency of compound screening, S.H. analyzed data, M.I.D., J.P.H., P.C. and L.M.W. developed binding assay technology and performed assay development, G.P. coordinated and executed the measurement of K_d values, M.H. synthesized compounds, D.K.T. conceived the technology, designed assay development strategies, and supervised technology and assay development, S.H. and D.K.T. contributed to preparation of the manuscript, P.P.Z. designed the study, supervised the project, analyzed data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/nbt/index.html>.

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1. Bain, J., McLauchlan, H., Elliott, M. & Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **371**, 199–204 (2003).
2. Bantscheff, M. *et al.* Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat. Biotechnol.* **25**, 1035–1044 (2007).
3. Fabian, M.A. *et al.* A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **23**, 329–336 (2005).
4. Melnick, J.S. *et al.* An efficient rapid system for profiling the cellular activities of molecular libraries. *Proc. Natl. Acad. Sci. USA* **103**, 3153–3158 (2006).
5. Patricelli, M.P. *et al.* Functional interrogation of the kinome using nucleotide acyl phosphates. *Biochemistry* **46**, 350–358 (2007).
6. Fedorov, O. *et al.* A systematic interaction map of validated kinase inhibitors with Ser/Thr kinases. *Proc. Natl. Acad. Sci. USA* **104**, 20523–20528 (2007).
7. Karaman, M.W. *et al.* A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **26**, 127–132 (2008).
8. Bamborough, P., Drewry, D., Harper, G., Smith, G.K. & Schneider, K. Assessment of chemical coverage of kinome space and its implications for kinase drug discovery. *J. Med. Chem.* **51**, 7898–7914 (2008).
9. Posy, S.L. *et al.* Trends in kinase selectivity: insights for target class-focused library screening. *J. Med. Chem.* **54**, 54–66 (2011).
10. Metz, J.T. *et al.* Navigating the kinome. *Nat. Chem. Biol.* **7**, 200–202 (2011).

11. Liu, Y. & Gray, N.S. Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* **2**, 358–364 (2006).
12. Manning, G., Whyte, D.B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912–1934 (2002).
13. Zarrinkar, P.P. *et al.* AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). *Blood* **114**, 2984–2992 (2009).
14. Ohren, J.F. *et al.* Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat. Struct. Mol. Biol.* **11**, 1192–1197 (2004).
15. Buchanan, S.G. *et al.* SGX523 is an exquisitely selective, ATP-competitive inhibitor of the MET receptor tyrosine kinase with antitumor activity in vivo. *Mol. Cancer Ther.* **8**, 3181–3190 (2009).
16. Wood, E.R. *et al.* A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.* **64**, 6652–6659 (2004).
17. Simard, J.R. *et al.* Fluorophore labeling of the glycine-rich loop as a method of identifying inhibitors that bind to active and inactive kinase conformations. *J. Am. Chem. Soc.* **132**, 4152–4160 (2010).
18. Wodicka, L.M. *et al.* Activation state-dependent binding of small molecule kinase inhibitors: structural insights from biochemistry. *Chem. Biol.* **17**, 1241–1249 (2010).
19. Carlomagno, F. *et al.* Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. *Oncogene* **23**, 6056–6063 (2004).
20. Goldstein, D.M., Gray, N.S. & Zarrinkar, P.P. High-throughput kinase profiling as a platform for drug discovery. *Nat. Rev. Drug Discov.* **7**, 391–397 (2008).
21. Kwiatkowski, N. *et al.* Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. *Nat. Chem. Biol.* **6**, 359–368 (2010).
22. Deng, X. *et al.* Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nat. Chem. Biol.* **7**, 203–205 (2011).
23. Hasinoff, B.B. & Patel, D. The lack of target specificity of small molecule anticancer kinase inhibitors is correlated with their ability to damage myocytes in vitro. *Toxicol. Appl. Pharmacol.* **249**, 132–139 (2010).
24. Huang, D., Zhou, T., Lafleur, K., Nevado, C. & Cafilisch, A. Kinase selectivity potential for inhibitors targeting the ATP binding site: a network analysis. *Bioinformatics* **26**, 198–204 (2010).
25. Olaharski, A.J. *et al.* Identification of a kinase profile that predicts chromosome damage induced by small molecule kinase inhibitors. *PLOS Comput. Biol.* **5**, e1000446 (2009).
26. Yang, X. *et al.* Kinase inhibition-related adverse events predicted from in vitro kinome and clinical trial data. *J. Biomed. Inform.* **43**, 376–384 (2010).
27. Remsing Rix, L.L. *et al.* Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. *Leukemia* **23**, 477–485 (2009).
28. Knight, Z.A., Lin, H. & Shokat, K.M. Targeting the cancer kinome through polypharmacology. *Nat. Rev. Cancer* **10**, 130–137 (2010).
29. Miduturu, C.V. *et al.* High-throughput kinase profiling: a more efficient approach toward the discovery of new kinase inhibitors. *Chem. Biol.* **18**, 868–879 (2011).
30. Cortes, J. *et al.* AC220, a potent, selective, second generation FLT3 receptor tyrosine kinase (RTK) inhibitor, in a first-in-human (FIH) phase I clinical trial. *Blood (ASH Annual Meeting Abstracts)* **114**, Abstract 636 (2009).



ONLINE METHODS

Compounds. Inhibitors were either purchased from A.G. Scientific, Calbiochem/EMD Chemicals, Tocris Bioscience, Archerchem, Axon Medchem or SYNthesismedchem, custom synthesized by Qventas, SAI Advantium, CiVentiChem, Shanghai SynCores Technologies, WuXi AppTec, BioDuro, SynChem or synthesized at Ambit Biosciences.

Competition binding assays. Competition binding assays were developed, validated and performed as described previously^{3,18}. Kinases were produced either as fusions to T7 phage³, or were expressed as fusions to NF- κ B in HEK-293 cells and subsequently tagged with DNA for PCR detection¹⁸. In general, full-length constructs were used for small, single-domain kinases, and catalytic domain constructs including appropriate flanking sequences were used for multidomain kinases. Briefly, for the binding assays, streptavidin-coated magnetic beads were treated with biotinylated affinity ligands to

generate affinity resins. The liganded beads were blocked to reduce nonspecific binding and washed to remove unbound ligand. Binding reactions were assembled by combining kinase, liganded affinity beads and test compounds prepared as 100 \times stocks in DMSO. DMSO was added to control assays lacking a test compound. Primary screen interactions were performed in 384-well plates, whereas K_d determinations were performed in 96-well plates. Assay plates were incubated at 25 °C with shaking for 1 h, and the affinity beads were washed extensively to remove unbound protein. Bound kinase was eluted in the presence of nonbiotinylated affinity ligands for 30 min at 25 °C with shaking. The kinase concentration in the eluates was measured by quantitative PCR. K_d s were determined using 11 serial threefold dilutions of test compound and a DMSO control. Kinase interaction maps shown in **Figure 4** were generated using TREEspot software (<http://www.kinomescan.com/>).

False-positive and false-negative rates for single-concentration primary screens have been previously determined⁷.