INNOVATION

High-throughput kinase profiling as a platform for drug discovery

David M. Goldstein, Nathanael S. Gray and Patrick P. Zarrinkar

Abstract | To fully exploit the potential of kinases as drug targets, novel strategies for the efficient discovery of inhibitors are required. In contrast to the traditional, linear process of inhibitor discovery, high-throughput kinase profiling enables a parallel approach by interrogating compounds against hundreds of targets in a single screen. Compound potency and selectivity are determined simultaneously, providing a choice of targets to pursue that is guided by the quality of lead compounds available, rather than by target biology alone.

Imatinib (Gleevec; Novartis), the first small-molecule kinase inhibitor to be approved for use in humans, has dramatically changed the prognosis for patients with chronic myeloid leukaemia[1]. The success of imatinib has demonstrated that targeting kinases can be a very effective therapeutic approach, and the approval of additional small-molecule kinase inhibitors has shown that imatinib is not unique[2,3]. Small molecule kinase inhibitors therefore are a new class of drug that will grow significantly as the large number of compounds currently in preclinical and clinical development progress towards marketing approval[3–5].

The central role of kinases in cellular processes that are important in disease, and the discovery of dysregulation of kinase activity in an expanding list of disorders, suggest that the number of kinases with potential as drug targets is significant[6–17], perhaps eclipsing G-protein-coupled receptors as a target class. Importantly, kinases have been implicated not only in oncology, but also in a number of non-oncology indications, including central nervous system disorders[13,18], autoimmune disease[19], post-transplant immunosuppression[20], osteoporosis[7] and metabolic disorders[21]. To fully explore and exploit this opportunity, potent and selective inhibitors will be required for a multitude of kinases, both as tool compounds for target validation and as leads for drug development.

Kinase-inhibitor discovery has traditionally been a largely linear process that addresses one kinase at a time and requires significant investment of time and resources for each target[3] (FIG. 1a). In the traditional process, a library of compounds is screened against a carefully selected individual kinase to identify hits — compounds capable of inhibiting enzymatic activity of the target — that often have weak or modest potency. Hits are then optimized, generally first for potency, to generate lead compounds. Lead compounds are further modified to improve pharmaceutical properties until a candidate for clinical development is identified[22–24]. Kinase selectivity is typically assessed on only a subset of the screening hits, and is monitored only sporadically throughout the lead optimization process.

This strategy has at least two significant drawbacks. First, targets are addressed one at a time, and the entire process has to be repeated for each new target of interest. Second, decisions about which targets to pursue are based on biology alone, with minimal knowledge about the availability or quality of hits against the designated target in the available chemical library. The traditional approach is therefore target-centric. Resource-intensive and time-consuming

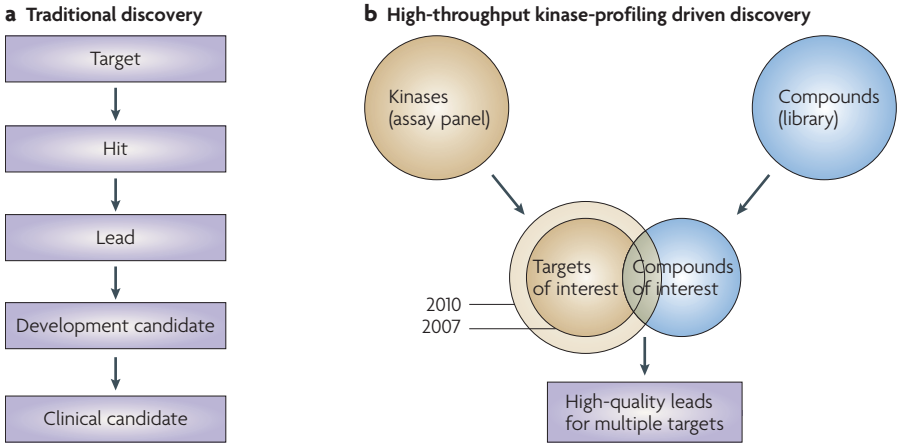


Figure 1 | **A novel approach to kinase-inhibitor discovery.** The ability to efficiently screen compound libraries against many kinases in parallel enables a more systematic approach to drug discovery. **a** | Discovery of new inhibitors traditionally has been a linear, target-centric process, proceeding from target validation, hit identification and lead generation to designation of a candidate molecule for preclinical and clinical development. In this paradigm, decisions about which targets to pursue are based on target biology alone. **b** | A novel, parallel, compound-centric approach to discovery. Profiling of compound libraries against a large panel of kinases allows the efficient identification of the overlap between targets of interest, defined by target biology, and compounds of interest, defined by compound properties and the potency and selectivity results obtained from the screen. Interrogating many targets in parallel reveals those targets for which high-quality leads are available, and focuses medicinal chemistry efforts on projects that are most likely to yield development candidates. Decisions are based on biological and chemical considerations.

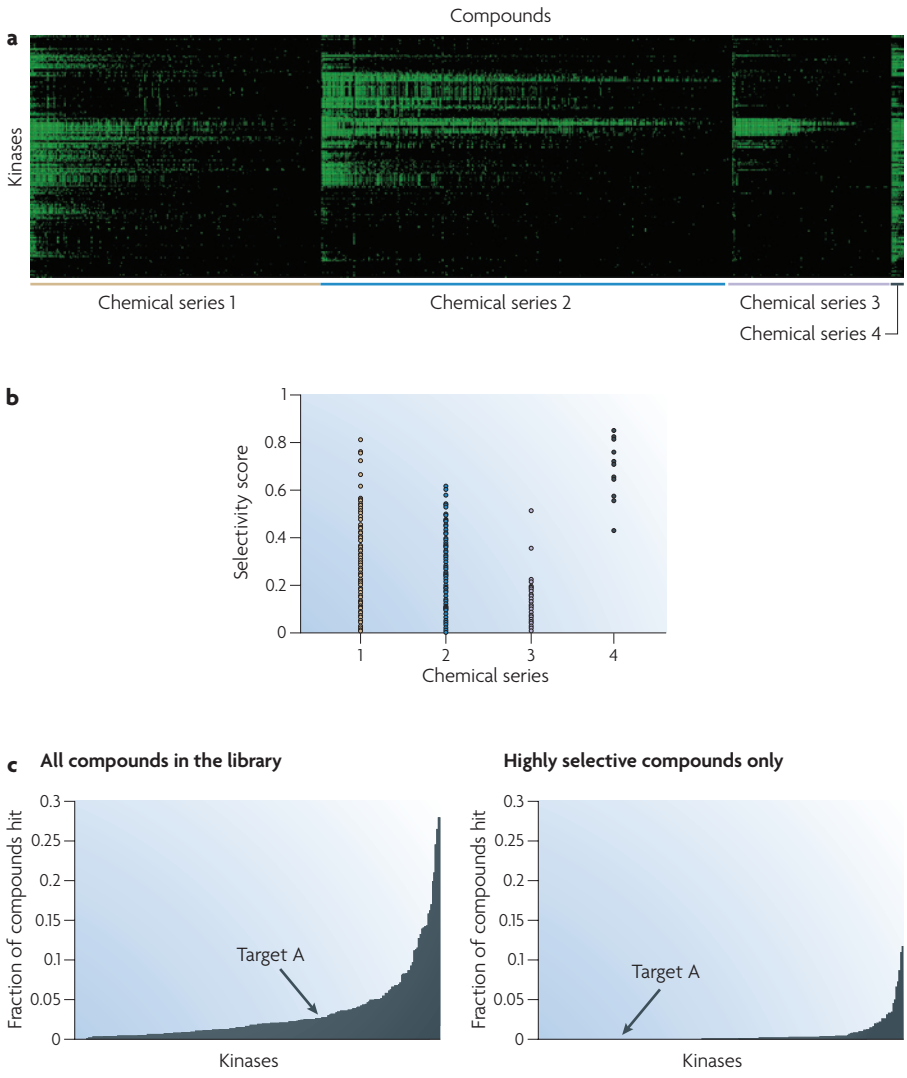


Figure 2 | **Characterization and annotation of compound libraries.** This is an illustrative example of a library of several thousand compounds screened against several hundred kinases. **a** | A heat-map representation of the results, with compounds in columns and kinases in rows. Green features indicate binding interactions. Compounds are sorted by chemical series, and rank-ordered by selectivity within each series. The results reveal the detailed relationship between chemical structure and kinase interaction pattern. **b** | Comparison of selectivity trends for chemical series. Selectivity was quantitated as described[30], and the selectivity score plotted for each compound in a series. Chemical series 4 consists of non-selective compounds, series 3 largely contains selective compounds, and selectivity within series 1 and 2 varies widely. **c** | Annotating the druggability of kinases. Kinases are rank-ordered according to what fraction of compounds in the library they interact with, either considering all compounds screened or only that subset of compounds with good overall selectivity. Target A is a kinase that interacts with a number of compounds in the library; however none of the hits is a selective compound. This analysis focuses attention on the most promising targets, and reveals which targets may be too challenging to pursue with the available compounds.

medicinal chemistry to optimize hits from screens is a major bottleneck in the generation of viable clinical candidates. Here, we describe an alternative, highly parallel, compound-centric strategy for inhibitor discovery that takes advantage of the large number of kinases implicated as potential drug targets (FIG. 1b).

A novel discovery approach

Large panels of kinase assays. Over the past several years, large and growing panels of assays for up to hundreds of human kinases have become available[25–32]. The development of these panels has mainly been motivated by the desire to assess kinase-inhibitor selectivity as comprehensively as possible, and the technologies applied include measurements of inhibitor binding, inhibition of enzymatic activity, and cellular activity. Knowledge of off-target activities can be important for understanding and interpreting biological activity[26,33–35] (particularly for non-selective compounds[26]), anticipating potential toxicities[36,37], as well as leading to novel and unanticipated uses of existing compounds[38–40]. The main application of these panels has therefore been to produce kinase interaction maps for individual compounds[25,26,29,30]. Until recently, it has been technically difficult, expensive and time-consuming to systematically screen large numbers of compounds against significant numbers of kinases. However, steady improvements in technology have enhanced the efficiency of screening and are continuing to decrease costs to the point at which large-scale kinase profiling not just of individual compounds, but of entire libraries is becoming increasingly viable. Several examples of systematic kinase screens of compound collections have been published[28,32], and we anticipate that such data sets on progressively larger scales will become common in the future.

Annotating libraries and kinases. Pharmaceutical and biotechnology companies have made large investments to design, synthesize, assemble and curate compound libraries, and many have constructed privileged 'kinase-focused' sublibraries of thousands to several tens of thousands of compounds. These libraries are the foundation for kinase-inhibitor discovery efforts, but, although they are chemically well characterized, their functional annotation and understanding of kinome-wide inhibition potential is generally limited and is enhanced only slowly as screens against additional individual targets are performed over time. Much of the value inherent in the libraries therefore remains hidden and unexploited, but could be unlocked if a more comprehensive and systematic view of activity against the kinome were available. High-throughput kinase profiling of compound libraries provides this systematic view by defining multidimensional structure–activity relationships against hundreds of targets simultaneously. The interdependence between chemical structure and kinase interaction patterns reveals the consequences of structural modifications within each compound series, as well as functional differences between series (FIG. 2a,b; BOX 1).

Although kinase inhibitors have a propensity to crossreact with multiple kinases, not all kinases are equally likely to interact

with small molecules. Some kinases can be inhibited by a wide range of chemical scaffolds (BOX 2), others are quite selective, and for some it has been difficult to identify inhibitors[28]. Profiling of diverse chemical libraries against large panels of kinases annotates the druggability of kinases and provides insight into the chemical preferences of each enzyme. The data guide discovery efforts by revealing which kinases can be accessed with compounds in a given collection (FIG. 2c), and whether the difficulty of identifying inhibitors for certain kinases may be due to the biased nature of kinase-focused libraries or is an inherent property of these particular enzymes.

Identifying new scaffolds. A major consideration for discovery and development is the ability to create intellectual property to cover novel inhibitors. This is becoming increasingly difficult for kinase inhibitors because of the relatively small number of distinct chemical scaffolds already known to target kinases[41]. Novel kinase inhibitor scaffolds therefore need to be discovered, and this will require screening of chemically diverse libraries that are distinct and orthogonal to kinase-focused libraries. As even the largest structurally diverse libraries cover chemical space only sparsely, profiling libraries against a panel of hundreds of kinases will be more likely to identify scaffolds that are capable of inhibiting kinases than a traditional screen against one or a small number of targets will. It may be sufficient to initially identify novel scaffold leads that target any kinase and provide a starting point for modification and optimization to yield inhibitors with a range of kinase interaction patterns.

Identifying the highest quality leads

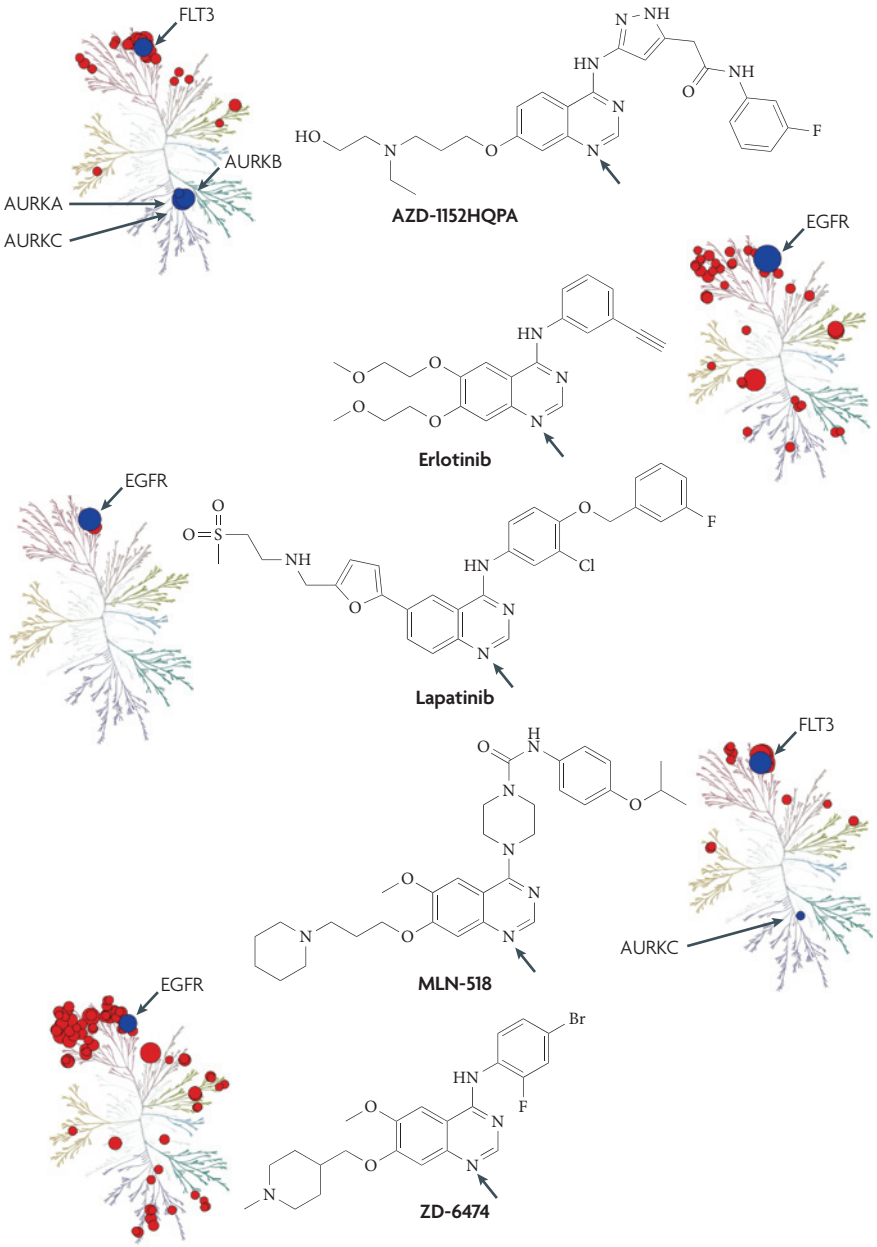
Targets of interest and compounds of interest. The number of kinases that may be good targets for small-molecule intervention is large and growing. Furthermore, for many indications or patient subpopulations the specific kinase or combination of kinases that should be inhibited to provide a therapeutic benefit has not yet been fully defined. For most drug discovery efforts there is, therefore, flexibility about which targets to pursue.

High-throughput kinase profiling takes advantage of this flexibility in target choice. Screening a library of compounds against a panel of kinases reveals potential lead compounds for multiple candidate targets. The quality of hits for each kinase may be considered along with knowledge about target biology in deciding how to prioritize medicinal chemistry and other discovery resources.

Box 1 | Chemical structure, binding modes and kinase interaction patterns: quinazolines

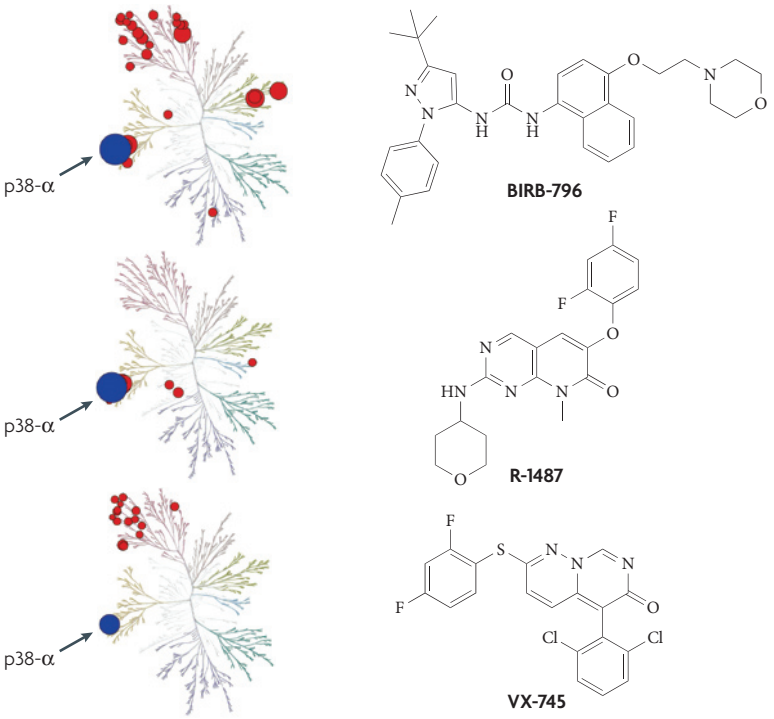
The quinazoline ring has frequently been used as a core scaffold to occupy the adenine ring region of the ATP binding site. The kinase interaction maps for five quinazoline compounds — AZD-1152HQPA, erlotinib (Tarceva; OSI/Genentech/Roche), lapatinib (Tykerb; GlaxoSmithKline), MLN-518 and ZD-6474 — show that compounds based on the quinazoline scaffold can target a range of kinases with varying degrees of selectivity[30]. A superposition of the binding conformation of these inhibitors reveals that the quinazoline is in approximately the same position and forms a key hydrogen bond between the quinazoline N1 (see arrows in the accompanying figures) and the kinase hinge region. Lapatinib is the most selective compound in this set and only interacts with the epidermal growth factor receptor (EGFR) subfamily. Presumably this is due to the ability of lapatinib to recognize an inactive conformation of EGFR[47] that is distinct from the classical active conformation recognized by the type I inhibitors erlotinib[50] and ZD-6474 (REF. 51). Erlotinib and ZD-6474 show considerably broadened kinase interaction profiles.

AZD-1152HQPA[52] and MLN-518 (REF. 53) are type II inhibitors[54] that are predicted to recognize an inactive conformation in which the activation loop blocks substrate binding, known as the DFG-out conformation. The interaction map reveals that AZD-1152HQPA binds with high affinity to FLT3, anticipating the possibility of optimizing for selective FLT3 inhibitors based on the quinazoline scaffold, such as MLN-518. Conversely, the crossreactivity between MLN-518 and Aurora kinase C (AURKC) would suggest that quinazoline-based AURK inhibitors could be developed.



Box 2 | **Chemical structure, binding modes and kinase interaction patterns: p38 inhibitors**

The chemical structures and kinase interaction maps for three chemically distinct inhibitors of p38-α — BIRB-796, VX-745, and R-1487 — illustrate both the variety of scaffolds that are capable of binding with high affinity to the ATP binding pocket of p38-α, and the diversity of selectivity profiles among compounds optimized for p38 inhibition[29]. The three inhibitors bind in a unique fashion, but they all form a hydrogen bond with methionine 109 in the kinase hinge region, and occupy a key hydrophobic pocket defined by the selectivity residue threonine 106. The most selective inhibitor in this set is R-1487, which binds to both inactive and active forms of the kinase, whereas less selectivity is achieved by BIRB-796, which binds allosterically to the inactive DFG-out conformation[55].



By contrast, a conventional single-target screen only reveals hits for one target, and it remains unknown whether significantly higher quality hits for another, potentially equally interesting, target remain hidden in the library. In comparison to conventional single-target screening, high-throughput kinase profiling therefore allows the more efficient allocation of discovery resources to the most promising projects.

The integration of existing knowledge about targets and compounds with the results of library profiling requires a novel conceptual framework for the efficient identification of the most promising potential projects (FIG. 1b). A subset of compounds in any library may be considered as compounds of interest based on multiple criteria. These criteria include potency against target kinases of interest, overall selectivity and detailed kinase interaction patterns, which can all be revealed by the screen. For example, knowledge of off-target activities can be used to prioritize the hits with a view to either avoid potential toxicities[36,37] or to broaden pharmacological activity. These criteria can also include pre-existing knowledge, including drug-likeness, ease of synthesis, intellectual property position, cell permeability, and pharmacokinetic and pharmaceutical properties of specific compounds or compound series. Likewise, a subset of the kinases represented in any assay panel may be considered as targets of interest based on known biological function or validation as a target, such as the presence of activating mutations in disease, as well as any additional relevant considerations.

The most promising opportunities are defined by the overlap between targets of interest and compounds of interest. This intersection reveals compounds from desirable chemical structural series that have potent activity against at least one potentially valuable kinase target, as well as appropriate selectivity. Many potentially valuable targets are queried in parallel, and a single library profile may therefore yield high-quality hits for multiple kinases (FIG. 3). Further validation is provided by rapid follow-up in cellular assays of hits from *in vitro* screens[26]. Decisions about which projects to pursue could then be based on both compound properties and interaction patterns, and target biology. Compounds highlighted by the analysis may be directly suitable for use as tool compounds to further investigate target biology. Moreover, they may require significantly less medicinal chemistry optimization to produce a candidate for clinical development than typical hits from conventional high-throughput screens against individual targets. As a result, development efforts are focused on those projects most likely to produce a high-quality candidate against an important target in the shortest period of time.

Rapid exploitation of novel targets. In addition to guiding the choice of projects for kinase targets of interest in the present, library profiling also provides a foundation for the future (FIG. 1b). Major efforts in university and industrial laboratories are focused on identifying kinases that drive disease, leading to a steady stream of discoveries that suggest novel targets for small-molecule intervention[6,7,13,14,18]. Many of these targets are already represented in large kinase assay panels. Although kinases newly identified as important in disease may not have been designated as targets of interest during the initial analysis of profiling results, they would be screened as part of a systematic library-profiling campaign. Upon identification of a new target of interest, existing profiling data may therefore be queried to instantaneously reveal whether high-quality hits are available off the shelf. This therefore facilitates the decision of whether to pursue the new target and enables the immediate initiation of a medicinal chemistry programme. The speed with which new targets can be pursued provides a significant competitive advantage compared with the traditional discovery paradigm, which would require the building of an assay and screening a library (and therefore committing resources to the new programme without knowing whether or not high-quality hits will be identified), before hit optimization can even begin.

Multitargeted inhibitors

For many indications, inhibition of a single kinase may not be sufficient to affect the disease process significantly. Indeed, for many kinase inhibitors it is anticipated that their

optimal effect will require combination with cytotoxics or other conventional therapy. Even when inhibition of a single target might be effective, the understanding of cellular signalling networks is generally not quantitative enough to clearly define which kinase in a disease-implicated pathway may be the weak link and the most appropriate target, although the presence of activating mutations or gene amplification can be suggestive[42–44]. There is, therefore, increasing interest in combining inhibitors that selectively target individual kinases, and in compounds that inhibit multiple kinases[4,5,45,46] (BOX 3). Inhibition of multiple kinases affords the ability to combine more than one mechanism of action in a single molecule, such as combined inhibition of angiogenesis and cell-cycle progression, or inhibition of multiple kinases in a single pathway or several intersecting pathways. Concurrent inhibition of multiple targets may also make it more difficult for genetically unstable tumours to develop resistance, or would allow the same compound to be used for more than one indication with different relevant targets.

Most kinase inhibitors that are marketed or in development inhibit multiple kinases[5,30]. However, there are very few examples of compounds that seem to have been deliberately developed to have potent activity against multiple predetermined targets while being selective against the kinome as a whole (BOX 3). One such example is lapatinib (Tykerb; GlaxoSmithKline), a potent inhibitor of human epidermal growth factor receptor (HER) kinases that is also highly selective[30,47].

Profiling of compound libraries against large panels of kinases might enable a new strategy to find true multitargeted yet non-promiscuous inhibitors that could be leads for further optimization, and tool compounds to investigate the utility of specific target combinations in cellular and animal models (FIG. 4). Instead of selecting combinations of two or more individual kinases to target upfront, kinases might be assigned to bins that represent distinct mechanisms of action or pathways; for example, angiogenesis, cell cycle or signal transduction. As the library profile yields the activity of compounds against each of the individual targets in the different bins, as well as their overall selectivity, a computational search of the profiling data might then identify compounds with desirable interaction patterns. By varying the parameters of the search, all compounds in the library with any potentially valuable multitarget activity might be identified systematically.

This cannot be accomplished through traditional screens against one or a small number of targets, as the total number of kinases in the designated bins is likely to be significant, and because a robust measure of overall selectivity is also required. In the absence of thresholds for overall selectivity, a search for potent inhibitors of multiple targets will predominantly yield indiscriminately promiscuous compounds (FIG. 3), and it would be impossible to distinguish these from true multitarget inhibitors. The

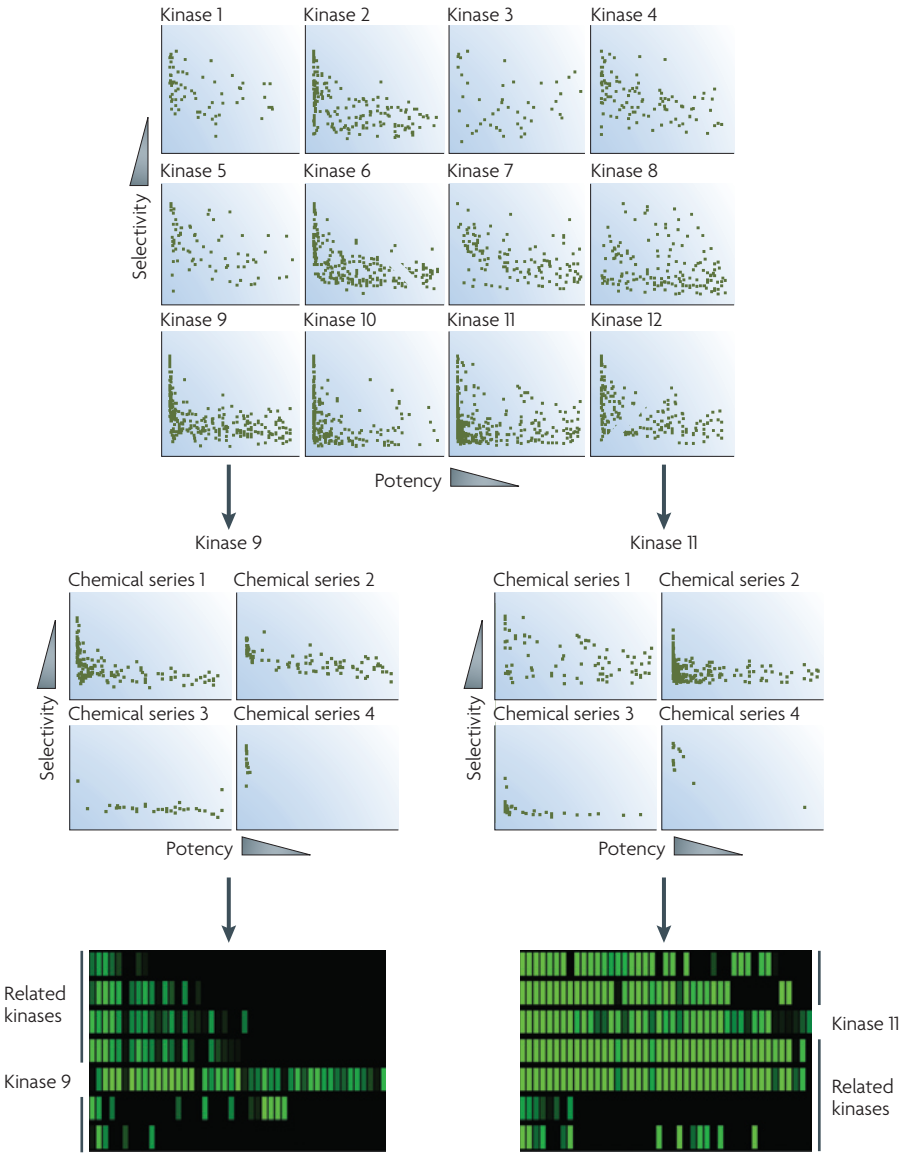


Figure 3 | **Identification of high-quality lead compounds.** In this illustrative example of a library profile, a two-dimensional view of library activity across multiple targets is obtained by plotting potency and selectivity for every hit against the targets of interest. Individual compounds of interest are highlighted when the results are analysed by compound series. In the example shown, Kinase 9 favours chemical series 1, whereas Kinase 11 favours chemical series 2. Furthermore, for each primary drug target there usually are related kinases that need to be considered. For example, for ABL1 inhibitors it is important to know activity against drug-resistant ABL1 mutant forms and SRC family kinases, and for JAK2 inhibitors, activity against JAK1 and JAK3. The heatmaps illustrate that the detailed interaction pattern of each hit for a primary target against these related kinases is available from the screening results and further functionally differentiates hits, independent of overall selectivity. Traditional one-target screens, by comparison, provide only a one-dimensional (potency only) view for a single target. Collectively, information from high-throughput kinase profiling provides a comprehensive assessment of library activity from two perspectives — compounds and targets — that may be used to identify the most promising starting points for compound optimization.

Box 3 | **Challenges for multitargeted kinase inhibitors**

Most compounds that have been called multitargeted kinase inhibitors were developed as inhibitors of a primary target, and activity against secondary targets was either tolerated or initially not recognized, and was discovered and exploited opportunistically later, as was done for imatinib (Gleevec; Novartis) and dasatinib (Sprycel; Bristol–Myers Squibb)[40,56]. For some compounds, it is not completely understood whether clinical activity is due to inhibition of one or of multiple targets. For example, the clinical efficacy of sorafenib (Nexavar; Bayer/Onyx) is probably not primarily due to inhibition of RAF, the initial primary target for which the compound was developed[57].

Although it is possible to identify combinations of cellular processes or signalling pathways that might be desirable to modulate, disease biology is generally not sufficiently well defined to confidently know the best combination of individual kinases to target[58]. Several kinases have been implicated as important for angiogenesis, including vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR), TIE2 and others[57,59], and numerous kinases are known to be required for cell-cycle progression, including cyclin dependent kinases (CDKs), Aurora kinases (AURKs), polo-like kinases (PLKs), checkpoint kinases (CHEKs) and others[60]. Both angiogenesis inhibitors and cell-cycle inhibitors may have utility against solid tumours, and a compound that inhibits both angiogenesis and cell-cycle progression would be an attractive agent. But what is the right combination of targets for such a compound? One angiogenesis target and one cell-cycle target? If so, which ones? Should all kinases implicated in either process be inhibited for maximum effect? Does it matter which angiogenesis target is combined with which cell-cycle target? These questions are only beginning to be explored, and the answers may depend on tumour type[46].

systematic identification of selective yet multitargeted inhibitors might not only yield potential drug candidates, but might also provide valuable tool compounds to identify the most promising target combinations.

Conclusions

High-throughput profiling of compound libraries against large panels of kinases is becoming technically feasible and has the potential to significantly affect the productivity of kinase-inhibitor discovery. In contrast to the traditional linear, target-centric approach to discovery, library profiling enables a parallel, compound-centric approach by interrogating many kinases in parallel and revealing which targets can be accessed with available compounds. Development resources are focused on those targets for which the highest quality leads are available, thus shortening the path to clinical candidates. As new knowledge accumulates to validate additional kinases as drug targets, existing profiling results may be queried to determine whether leads for novel targets are already available. Newly validated targets can then be immediately exploited. Combining high-throughput kinase profiling with the systematic profiling of select compounds across large numbers of cell lines may be a particularly powerful approach to reveal potential novel kinase targets for small-molecule intervention, and to correlate kinase inhibition patterns with cellular phenotypes[34].

Library profiles reveal the interplay between chemical structure and kinase interaction patterns. Comparisons of interaction patterns across chemical series, and integration of this information with crystallographic and modelling data, will provide more complete information to medicinal and computational chemists to optimize initial screening hits and design next-generation libraries than single-target screens can. High-throughput kinase profiling represents a practical realization of the promise of protein-family-based chemogenomics[48,49]. The compound-centric approach outlined here provides a means to unlock and exploit the value inherent in existing compound collections, and establishes a foundation for future discovery.

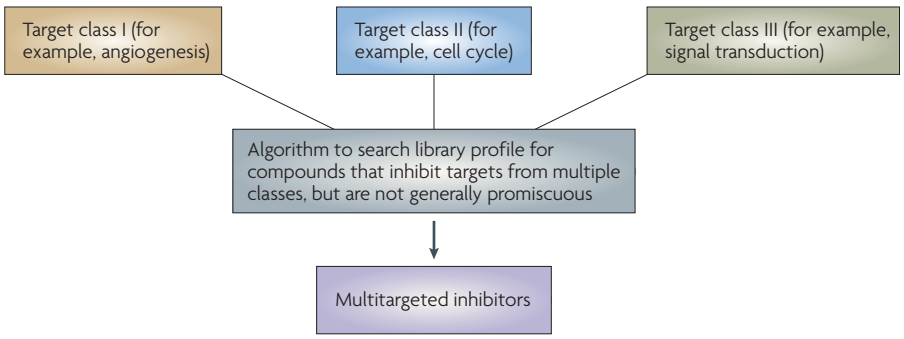


Figure 4 | **Scheme for identifying multitargeted yet selective inhibitors.** Potential targets are classified and sorted into bins or target classes. A computational search of library screening data may then identify compounds that inhibit one or more targets from each bin, but maintain acceptable overall selectivity. The parameters of the search may be adjusted to reveal compounds with a variety of desirable inhibition profiles.

David M. Goldstein is at Roche Palo Alto, 3431 Hillview Avenue, R6-201, Palo Alto, California 94304, USA.

Nathanael S. Gray is at the Dana–Farber Cancer Institute, Harvard Medical School, Seeley G. Mudd Building 628A, 250 Longwood Avenue, Boston, Massachusetts 02115, USA.

Patrick P. Zarrinkar is at Ambit Biosciences, 4215 Sorrento Valley Boulevard, San Diego, California 92121, USA.

Correspondence to P.P.Z.
e-mail: pzarrinkar@ambitbio.com

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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