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Kinase-targeted libraries: The design and synthesis of novel, potent, and selective kinase inhibitors

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Protein kinases continue to hold tremendous promise for therapeutic intervention, and the search for novel, safe and efficacious kinase inhibitors has intensified over the past decade. Given that most kinases are readily inhibited by organic small molecules and that a wealth of structural data exists on kinase–inhibitor complexes, there has been almost universal success in the design and identification of potent kinase inhibitors. The issues of non-selectivity and congested IP space, however, present formidable challenges for the successful clinical development of these compounds. We describe a systematic approach implemented at Abbott to enable the rapid discovery and design of novel and potent kinase inhibitors that provide additional opportunities for targeting new intellectual property space and achieving acceptable selectivity profiles.

Introduction

Protein kinases have rapidly become one of the most important group of drug targets, second only to G-protein coupled receptors (GPCRs) [1,2]. Since the successful launch of Glivec, Iressa, Nexavar and others, there are now more than 200 kinase inhibitors in clinical development (Prous Science Integrity) and the preclinical target portfolio of nearly every major pharmaceutical company contains a substantial fraction of protein kinases. The attraction of protein kinases is their ubiquitous involvement in cellular signaling and the fact that, as a class, these proteins are readily amenable to inhibition by small organic molecules. The vast majority of known kinase inhibitors bind to the ATP-binding site of the kinase catalytic domain (Figure 1), which is highly conserved between all protein kinases. This has several significant implications for drug discovery efforts. First, achieving specificity amongst the more than 500 protein kinases in the human genome can be a major hurdle for avoiding adverse events due to off-target effects [3,4]. Second, inhibitor chemotypes for different protein kinases can have significant overlap with each other as they target very similar sites on the protein. This has led to a highly congested intellectual property (IP) landscape. In fact, a survey of the US Patent and Trademark Office reveals that, since 2001, more than 10,000 patents and patent applications have been published describing inhibitors of protein kinases. This has dramatically increased the IP risk associated with pursuing protein kinase inhibitors.

High-throughput screening of large corporate compound collections is a powerful and rapid approach to identifying lead inhibitors for kinases. These compound collections, however, typically suffer from two fundamental liabilities with respect to intellectual property. First, a large fraction of the compounds in most corporate repositories have been purchased from external vendors [5]. As a result, several companies screening the same kinase target may, in fact, find the same leads and end up competing for the same chemical space. Second, another significant fraction of most corporate compound collections are derived from historical programs, which may not contain kinase inhibitors or will produce known inhibitors optimized for other targets. Thus, the probability of identifying novel, high-quality kinase inhibitors from many compound repositories is low. In fact, an internal analysis of the Abbott compound collection conducted in 2006 suggested that less than 4% of the compounds in our repository have the potential to yield novel kinase hinge-binders.

In order to address this issue, there have been significant efforts at many large pharmaceutical companies and chemical suppliers to augment existing collections with large numbers of compounds that are potential kinase inhibitors and that do not infringe on the current IP landscape. There are a variety of approaches that have been developed for the identification of candidate structures,

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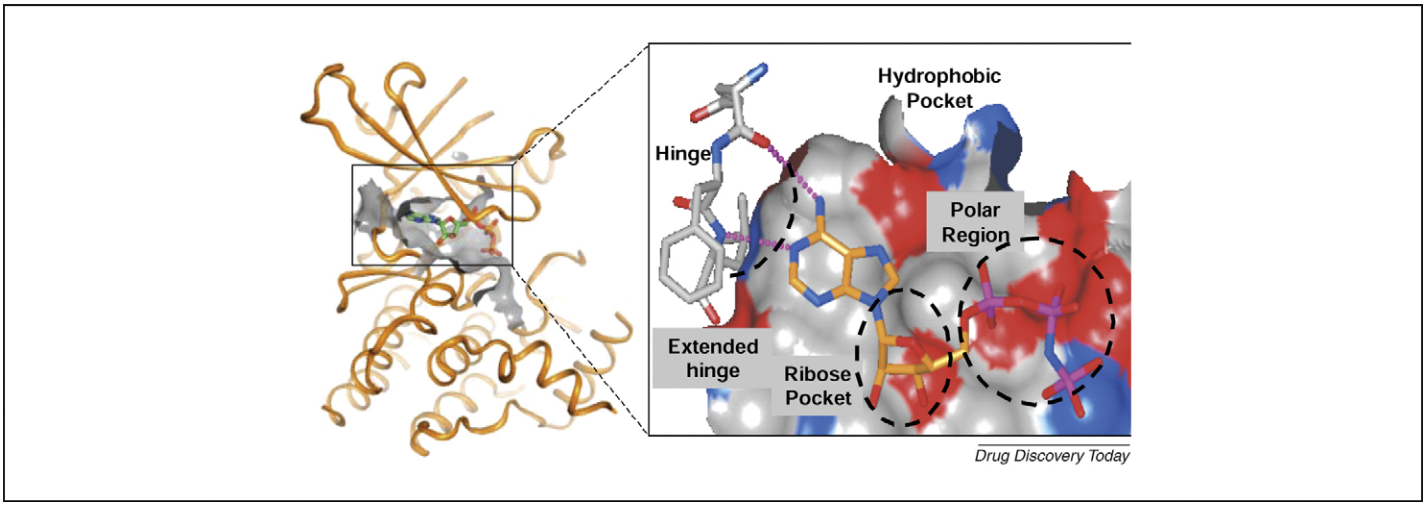

FIGURE 1

Architecture of the hinge. On the left is a ribbon diagram of the catalytic domain of a protein kinase (CDK2, pdb code 1HCK) complexed with ATP at the hinge region (solid surface). An expanded view is shown in the right with labels for the various regions of the binding site as described in the text.

incorporating all available experimental and computational information about known compounds and protein kinase structures [6]. At Abbott, we have implemented a scaffold-based strategy for kinase inhibitor development that capitalizes on our strengths in the areas of fragment-based screening, X-ray crystallography, molecular modeling and synthetic chemistry. Three parallel approaches have been developed, with balanced levels of potential IP risk and required synthetic effort. In this article, we will discuss our strategies for scaffold-based kinase inhibitor design, the implementation in practice, and internal data that validate the approach.

Targeting the hinge

All protein kinases contain a sequence of amino acids that comprises the 'hinge' between the two lobes of the catalytic domain (Figure 1). The backbone atoms of the hinge region contain the critical hydrogen bond donor and acceptor atoms that anchor ATP-binding and allow phosphorylation of the substrate. Blocking ATP-binding to this site is, therefore, a straightforward approach to kinase inhibition. While there has been some recent success targeting regions of a protein kinase other than the hinge-binding region [7], the vast majority of kinase inhibitors make at least one hydrogen bonding interaction with the hinge region and then proceed to gain affinity and specificity by making additional interactions in the ribose and polar region (which are also utilized by ATP) or the extended hinge and hydrophobic/specificity pockets (that are not utilized by ATP) [8].

For kinase inhibitors that target the hinge region, the hinge-binding element is critical for achieving highly potent molecules. For example, inhibitors for three different kinases (KDR, Chk-1 and Akt-1) [9–11] are shown in Figure 2, along with the activity data on the corresponding hinge-binding elements. Two very important insights come from these simple comparisons. First, the 10–15 atoms in the hinge-binding elements contribute 40–60% of the total binding energy of the fully elaborated inhibitor. Second, the binding efficiency [12] of the final inhibitor will likely be slightly lower than the efficiency of the hinge-binding fragment [13]. Thus, the starting potency of the hinge-binding scaffold will in large part dictate the size and potency (e.g. the binding efficiency) of the final compound. This expectation has been substantiated by a recent study [14], in which an analysis of the frequency with which kinases bind certain fragments can provide an understanding of not only the potency, but also the specificity of binding. In addition to potency and specificity, the hinge-binding element is also an important factor in securing novel intellectual property (IP). The three hinge-binding elements shown in Figure 2 are represented in many patents describing kinase inhibitors and thus carry an IP liability. Therefore, the identification of novel, highly efficient and drug-friendly hinge-binding fragments presents a tremendous opportunity for the development of novel kinase inhibitors against a range of protein kinase targets.

Finding fragments

As shown in Figure 2, low molecular weight (MW) compounds that interact with the hinge region of a protein kinase are likely to bind in the mid to high μM range and will not typically be detected using conventional biochemical assay conditions that use maximal compound concentrations of 10–30 μM. Thus, multiple approaches were taken for the identification of new hinge-binding compounds (Figure 3). First, one- and two-dimensional NMR screens [15] were performed against 14 different protein kinases. These screens allowed the detection of candidate core molecules with affinities as high as 1000 μM. Second, biochemical assays for 10 kinases were tuned to enable compound evaluation at high compound concentrations (100 μM). With these assays in hand, more than 15,000 diverse fragment leads from our SAR by NMR [16] library were assessed for their potential to bind to the hinge region of protein kinases. This diversity collection was augmented with several thousand picks from a virtual screening campaign, where a dataset of more than 50,000 low MW compounds were docked and scored against an ensemble of protein kinase crystal structures. All hits were then funneled into our panel of high concentration activity assays. From these exercises, more than 500 low molecular weight (MW < 300) candidate scaffold molecules with confirmed enzymatic activity (IC_{50} < 100 μM) were identified.

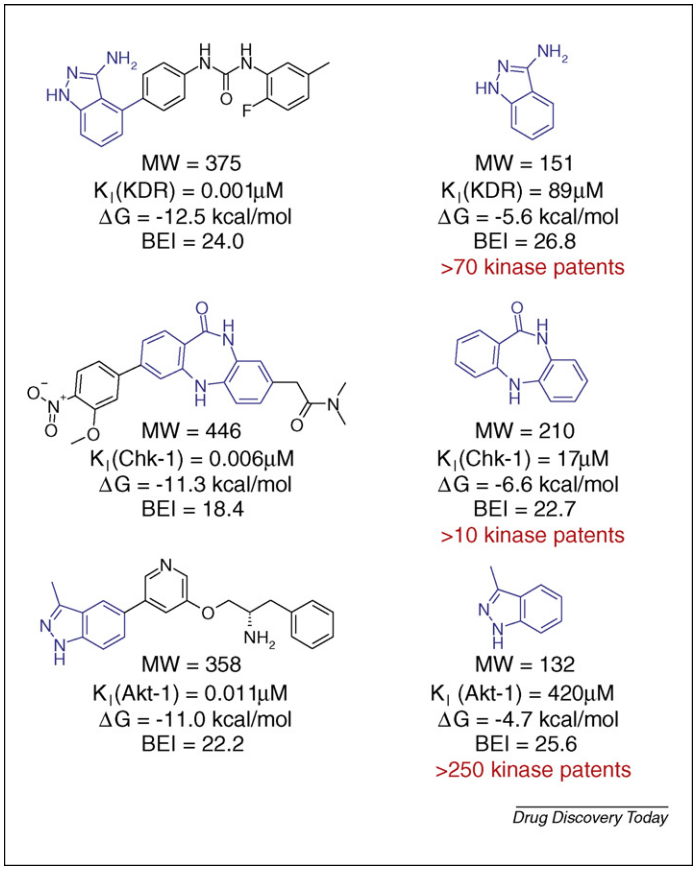

FIGURE 2

Binding energetics. Some inhibitory and other data on representative optimized kinase inhibitors and their corresponding hinge-binding fragments (highlighted in blue).

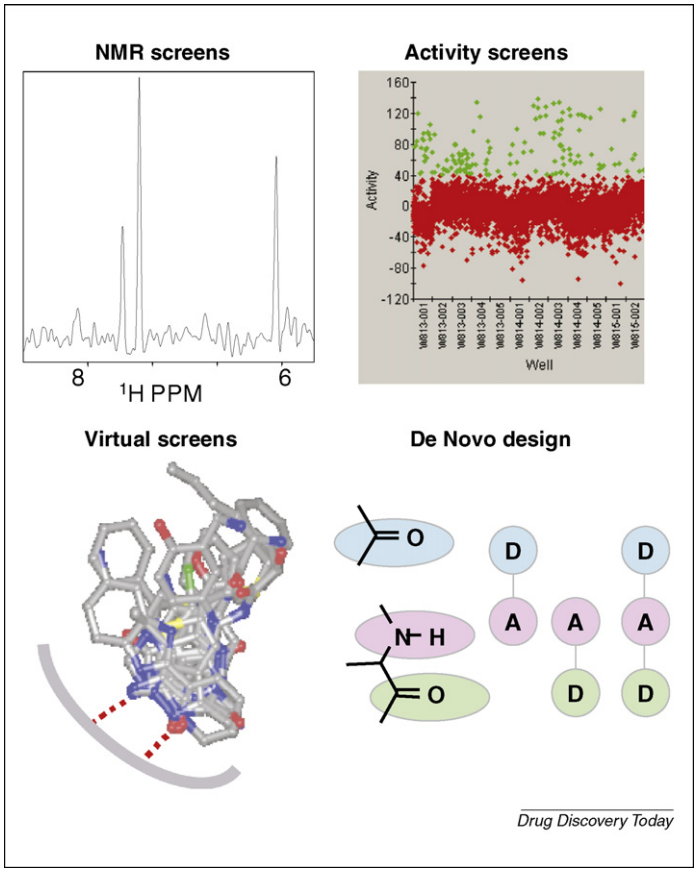

FIGURE 3

Integrated fragment discovery. Multiple methods were employed to identify hinge-binding elements, including NMR (top left), high concentration biochemical screens (top right), virtual screens against the structures of known kinase–inhibitor complexes (bottom left) and *de novo* design based on targeting the available hydrogen bond interactions with novel ring systems (bottom right).

The large-scale screening exercises described above were performed on compounds that were available from our corporate repository or could be purchased from external vendors. Thus, while these collections yielded a large number of active scaffolds, the novelty of most of these hits was relatively low. In fact, a limited patent search indicated that nearly 80% of these candidate hinge-binding fragments carried an IP liability (e.g. the substructure was disclosed in at least 1 patent describing kinase inhibitors). In order to augment our collection of hinge-binding elements, novel kinase scaffolds were designed *de novo*. The basic design principles involved the conception of relatively flat heterocycles that maintain one or more of the key hydrogen bonds to the hinge-region of the ATP-binding site (Figure 3). A thorough analysis of a large collection of literature kinase inhibitors revealed certain structural patterns that are repeatedly found in the parts of the inhibitor interacting with the hinge region of kinases. Common motifs include fragments containing hydrogen donor–acceptor–donor atoms at certain distances from each other in the molecule as well as donor–acceptor and acceptor–donor pairs. The architectures of adenosine and staurosporine (a pan-kinase inhibitor) also provided a general framework for the overall design. Several different approaches were taken. New scaffolds were designed based on known scaffolds (e.g. adenine, amino-indazole, etc.) by ring addition, elimination, expansion or contraction strategies, heterocycle switching and isosteric replacements. In addition a systematic enumeration of all possible 5–5, 5–6, 6–6 and 6–7 ring systems containing key hinge interacting elements such as nitrogens and oxygens was performed. Candidates for synthesis were initially triaged by novelty and synthetic accessibility. The synthesis of molecules unknown in the literature carries many difficulties, such as large upfront synthetic methodology development, unexpected and sometimes undesirable physicochemical properties and possible instability issues. In addition, it is imperative for the new scaffolds to contain key functional group in the right parts of the molecule for further functionalization in order to improve on potency and selectivity. Throughout the course of this initiative, dozens of novel hinge-binders were designed and synthesized. An example is given in Figure 4. An isosteric replacement of the phenyl ring of the known indazole scaffold yielded a thienopyrazole inhibitor, which at the time of its design was not known in the kinase literature. It is interesting to note that in subsequent years, fifteen patents and patent applications containing this substructure were published – highlighting the competitive nature of this endeavor.

Critical for the subsequent utilization of any of these kinase fragments is their validation as bona fide hinge-binders that can be effectively utilized in lead generation and optimization. To this end, extensive validation of the candidate scaffolds is performed through activity measurements, X-ray structural analysis, and

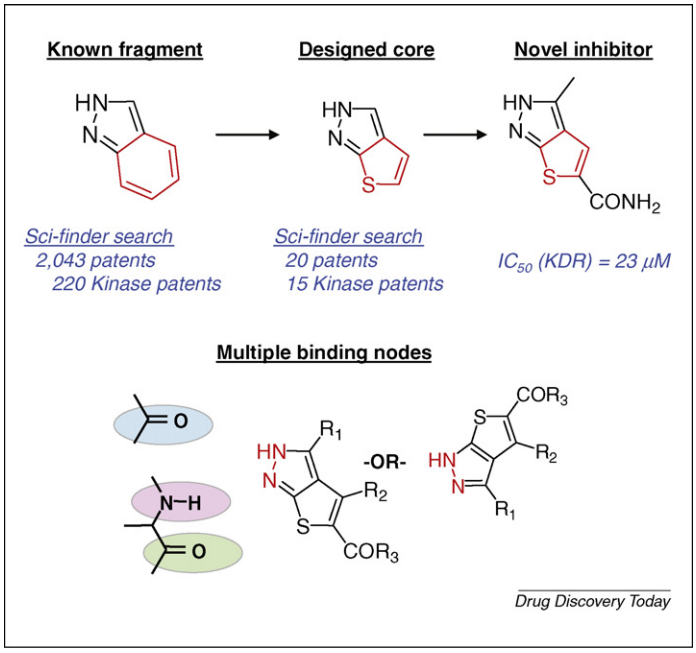

FIGURE 4

De novo design. An example of the *de novo* design of a kinase core by starting with indazole (which is represented in more than 200 kinase patents) and synthetically pursuing the thienopyrazole analog, which was not known as a kinase hinge element at the time of its inception but is now represented in 15 kinase patents. A representative molecule synthesized to evaluate the utility of this core is shown in the right, from which multiple compound libraries were derived. Multiple binding modes were experimentally observed for this chemotype (schematically illustrated at the bottom) based on tautomerization of the pyrazole ring.

synthetic modification. More than 30 X-ray crystal structures have been obtained on candidate scaffolds, and this information has been used not only to reveal the mode of binding to the ATP site, but also to guide modification of the existing scaffolds and aid in the design of new kinase cores. Crystallization experiments were typically performed on a small number (2–3) of protein kinases that readily crystallized and yielded high-resolution data. Surprisingly, in several cases, the same fragment was able to adopt different binding modes with different kinases. In addition, elaboration of a particular core in different ways could induce a change in the binding mode even for the same kinase. This is shown schematically in Figure 4, where tautomerization of the pyrazole ring allowed two distinct hinge-binding modes for this thienopyrazole core based on the substitution pattern. These results illustrate the complexity and subtlety of kinase–inhibitor interactions. These observations, however, also provide new opportunities for multiple design pathways to improve both potency and specificity.

Swapping fragments

The fragments identified, as described above, serve as excellent starting points for the generation of new leads against a variety of protein kinases. A powerful approach for the utilization of these novel kinase scaffolds is in lead modification and optimization. In this strategy, the hinge-binding element of a given kinase lead is replaced, based on structural information, with the novel scaffold. One example of this is given in Figure 5 in the design of novel inhibitors for KDR kinase [17]. Many highly potent KDR inhibitors exist, including the aminoindazole urea at the top of Figure 5. Deconstruction of this inhibitor reveals that the hinge-binding element exhibits an IC_{50} value of 5 μM for this kinase – which serves as a benchmark for any potential mimetics. We designed

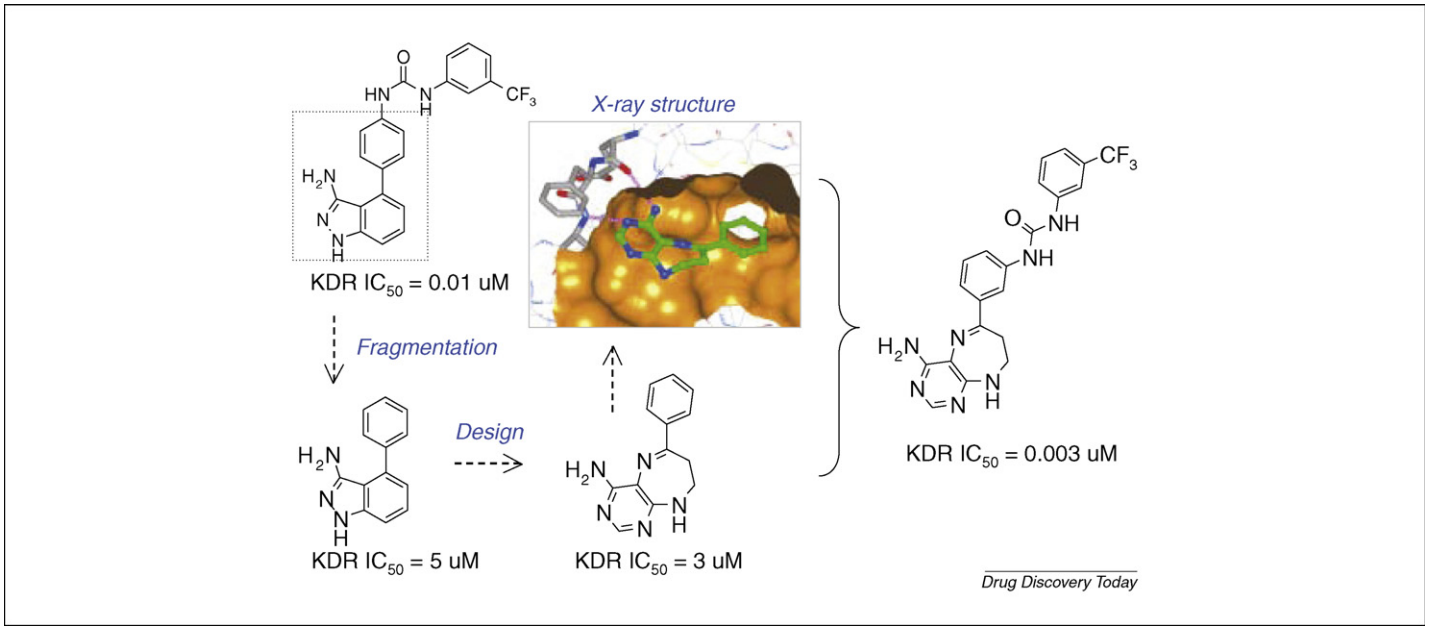

FIGURE 5

Fragment-swapping. Starting with a potent KDR kinase inhibitor (top left), a pyrimido-diazapinone analog (bottom center) of the amino-indazole core of the parent inhibitor (bottom left) exhibited comparable potency and was confirmed to bind to the hinge region by X-ray crystallography (top center shows complex of the pyrimido diazapinone core with the kinase PAK4). Structure-based design was then used to guide the placement of the phenyl–urea moiety onto the diazapinone core to yield highly potent, novel inhibitors.

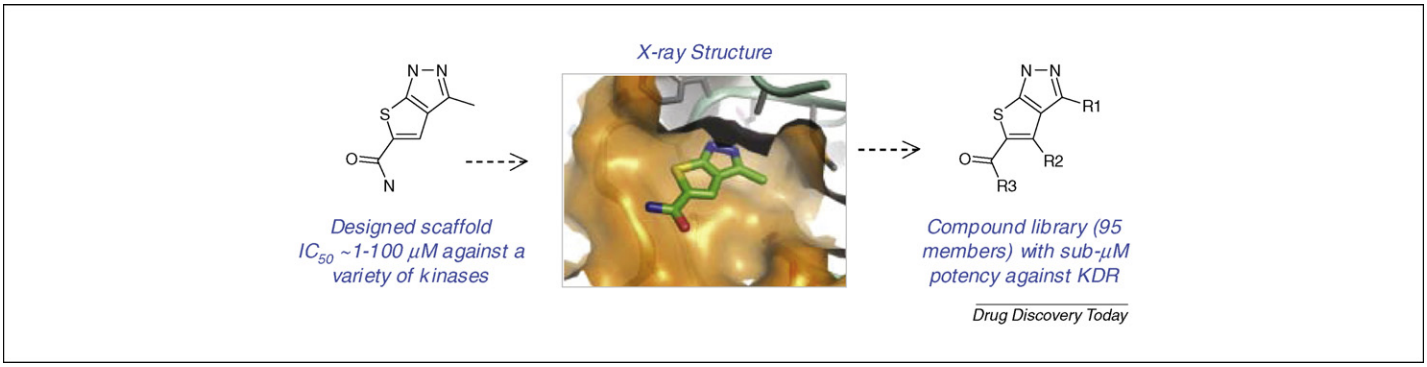

FIGURE 6

Fragment elaboration. The substituted thienopyrazole core (left) exhibited reasonable potency and was confirmed to bind to the hinge region by X-ray crystallography (center shows complex of the thienopyrazole core with the kinase PAK4). The structural information was then used to guide the design of compound libraries around this core, resulting in sub-μM kinase inhibitors.

and synthesized a novel pyrimido diazepine analog of this hinge element that exhibits comparable potency, with an IC_{50} value of 3 μM. X-ray structural information on this core, and subsequent modeling, led to the incorporation of the phenylurea functionality, resulting in the rapid generation of potent lead compounds (IC_{50} values less than 5 nM) with strong IP positions. This concept of scaffold swapping is, in principle, applicable to virtually every kinase inhibitor program, provided that structural information can be generated on both the original series and the scaffolds of interest. Thus, the novel heterocycles identified and validated through this work serve as a molecular tool-kit that can guide the medicinal chemist in making significant but rational modifications to a lead series with a high probability of maintaining or even increasing potency.

Elaborating fragments

The fragment-swapping strategy described above is ideal for rapidly modifying existing inhibitors. As the resultant molecules have been optimized for a particular kinase, however, they are less suited as initial leads for future kinase targets. Thus, an alternative strategy for exploiting these hinge-binding elements has been the design of small libraries (typically 10–100 members) with the aim of significantly improving potency, ideally to IC_{50} values less than 1–10 μM. This not only serves the purpose of validating the lead (demonstrating that meaningful SAR and potency gains can be obtained with a given scaffold), but also positions these novel leads on a par with typical hits that come from HTS. This concept is again illustrated with the thienopyrazole inhibitor (Figure 6). While the initial core exhibited a potency of 23 μM against KDR, the enumerated library contained compounds with sub-μM potency against this target (along with potency against a variety of other kinase targets) [18]. The nature of the substituents used in library synthesis, as well as their orientation, was carefully designed based on X-ray crystallography data when possible. In addition to the designed extensions, general diversity libraries were also synthesized to allow for unexpected activities. For all of the active cores identified in this initiative, exhaustive searches of commercially available compounds were conducted to enable library design. Criteria for these searches required that the molecules (1) contain a functional group compatible with high-throughput organic synthesis (e.g. acids, amines, aryl bromides, etc.) [19]; (2) which, when enumerated, result in libraries that would be lead-like [20] and have no highly similar compounds in our corporate repository (we have used a Daylight fingerprint Tanimoto of 0.85 as a cut-off for 'highly similar'); (3) are not represented in any known or patented kinase inhibitor; and (4) are available in multi-gram quantities. For those fragments for which chemistry-enabled cores were not available (e.g. especially for the *de novo* designed scaffolds), internal synthetic efforts were initiated.

Lessons learned

Throughout the course of this initiative, more than 5000 compounds have been synthesized around more than 50 novel kinase scaffolds. Several important lessons have emerged from the subsequent evaluation of these compounds. First, it is apparent that both potency and specificity against a broad range of kinases can be achieved not only with the elaborated libraries, but also with the fragments themselves. Shown in Figure 7 is an activity heat map of more than 250 fragments ($\langle MW \rangle = 250$) and 1000 compounds derived from a subset of these fragments ($\langle MW \rangle = 420$) against a panel of 11 different kinases representing a broad sampling of the human kinome. For the fragments (Figure 7a), the typical potency ranged from 1 to 100 μM, while sub-micromolar potency was obtained against every kinase on this panel in the set of elaborated compounds (Figure 7b). While some fragments appeared to be weakly active against essentially all kinases on the panel (bottom of Figure 7a), most of the fragments exhibited at least some level of selectivity – hitting only one or two of the kinases represented. This pattern is very similar to the larger, more potent compounds (Figure 7b), where a small percentage was active against many kinases but most compounds potently inhibited only one or two kinases. This supports the concept that some level of selectivity in kinase inhibition can be achieved even through targeting the highly conserved hinge region. Interestingly, while guidance from crystallography and/or modeling was critical in driving initial potency gains for many of the starting fragments, exploiting the subtle differences between the kinases structures to achieve selectivity could only be achieved in some cases by fine-tuning of substitution patterns through an empirical medicinal chemistry approach.

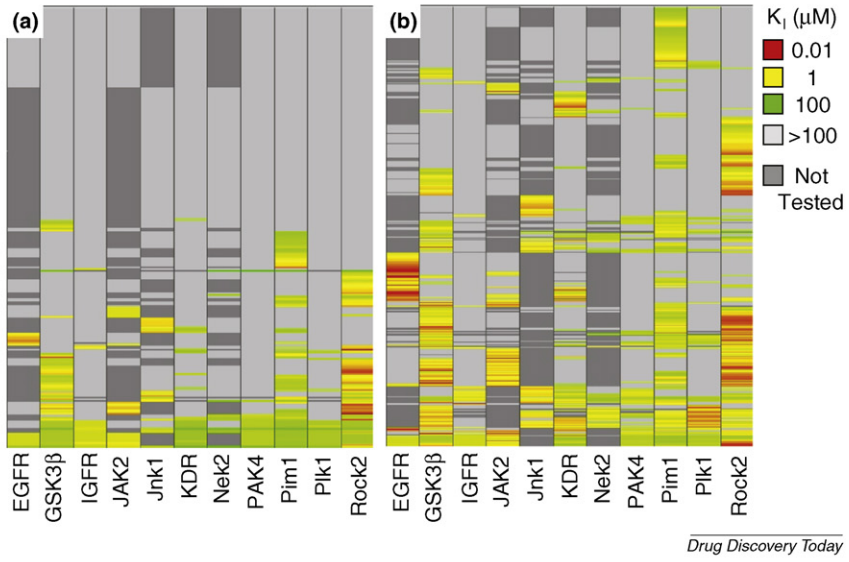

FIGURE 7

Kinase selectivity. Activity heat maps for (a) more than 250 fragments (⟨MW⟩ = 250) and (b) more than 1000 elaborated molecules (⟨MW⟩ = 420) derived from our internal Kinase Initiative against a panel of 11 protein kinases, with inhibitory activities ranging from 1 nM (red) to 100 μM (bright green).

Another lesson from this initiative is the persistent value of adding high-quality, drug-like, biologically relevant compounds to corporate screening collections. Many of the compounds from this initiative have since been incorporated into our HTS decks, and have been evaluated in more than 30 different screens. Gratifyingly, these compounds exhibit an average hit rate against kinases that is more than 10-fold than that observed for our general compound collection (Figure 8). Unexpectedly, however, some of the kinase-targeted libraries also exhibit increased hit rates against non-kinase targets, with an average enhancement of threefold over our entire collection. In retrospect, these compounds are simply small, novel, functionalized adenine mimetics that may well be active against a variety of protein targets, including ATPases, adenosine receptors, nucleotide exchange factors and other nucleotide-binding proteins. The fact that most of the molecules are relatively small in size makes them ideal hits as it is expected that further elaboration would provide potency and selectivity for the target of interest.

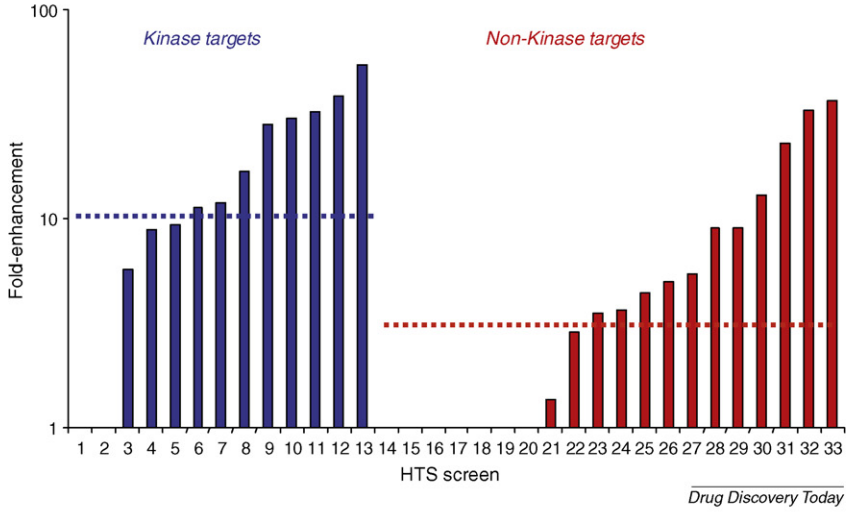

FIGURE 8

Lead generation. A plot of the fold-enrichment in HTS hit-rates for the Kinase Initiative compounds as compared to our general compound collection against 13 kinase targets (blue bars, average of 10-fold enrichment) and 20 non-kinase targets (red bars, average of threefold).

Summary

In summary, we have developed a highly integrated, scaffold-based approach to the discovery of novel and potent inhibitors of protein kinases. Through screening and *de novo* design, a collection of low molecular weight, novel, functionalized heterocycles with confirmed kinase activity has been identified. Using synthetic elaboration, more than 5000 compounds in 50 series have been generated, yielding sub-μM inhibitors of multiple protein kinases. Several current kinase inhibitor programs are pursuing these compounds as back-up lead series. Scaffold-swapping has been utilized to produce multiple novel lead series for KDR, and it is anticipated that this approach can have significant impact on many future kinase inhibitor programs. To facilitate this process, a database has been developed that enables the medicinal chemist to rapidly browse all of the scaffolds produced by the initiative, along with activity data, library information and crystallographic data. Finally, the compounds produced by this initiative have value beyond the search for novel kinase inhibitors, and have begun to produce novel and interesting leads for other targets in Discovery.

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