PERSPECTIVE

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Rational design of inhibitors that bind to inactive kinase conformations

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The majority of kinase inhibitors that have been developed so far—known as type I inhibitors—target the ATP binding site of the kinase in its active conformation, in which the activation loop is phosphorylated. Recently, crystal structures of inhibitors such as imatinib (STI571), BIRB796 and sorafenib (BAY43-9006)—known as type II inhibitors—have revealed a new binding mode that exploits an additional binding site immediately adjacent to the region occupied by ATP. This pocket is made accessible by an activation-loop rearrangement that is characteristic of kinases in an inactive conformation. Here, we present a structural analysis of binding modes of known human type II inhibitors and demonstrate that they conform to a pharmacophore model that is currently being used to design a new generation of kinase inhibitors.

There are 518 kinases encoded in the human genome, and they have been demonstrated to play pivotal roles in virtually all aspects of cellular physiology[1]. Dysregulation of kinase activity has been implicated in pathological conditions ranging from neuronal disorders to cellular transformation in leukemias[2]. It is currently estimated that over a quarter of all pharmaceutical drug targets are protein kinases—an assessment that drives an eager search for new chemical scaffolds that have the potential to become drugs[3].

The highly conserved kinase domain consists of a bilobed structure, with Mg-ATP situated in a deep cleft located between the N- and C-terminal lobes (**Fig. 1a**). The majority of small-molecule kinase inhibitors that have been developed so far target the ATP binding site, with the kinase adopting a conformation almost identical to that used to bind ATP (the active conformation). This is not surprising, as historically most inhibitors have been discovered using biochemical screens of highly active, activation loop–phosphorylated recombinant kinase catalytic domains at very low concentrations of ATP, conditions in which hydrophobic compounds are most likely to interact with the active conformation of the ATP cleft. Fortunately, serendipity combined with structure-activity relationship (SAR)–guided medicinal chemistry has allowed the identification of a second class of kinase inhibitors whose members preferentially bind to an inactive conformation of the kinase, thereby preventing activation[4]. Type II inhibitors use the ATP binding cleft and an adjacent hydrophobic pocket created by the activation loop (which contains the conserved DFG motif) being in an 'out' conformation (**Fig. 1b**). So far, eight distinct inhibitors in the public domain have been crystallographically proven to be type II inhibitors: imatinib (**1**)[5], BIRB796 (**2**)[6], sorafenib (**3**)[7], AAL993 (**9**)[8], diaryl urea (**4**)[6], indole amide (**8**)[9], anilinoquinazoline (**10**)[10] and 4-aminopyrimidinoquinazoline (**11**)[11].

Type I inhibitors bind to the ATP binding site through the formation of hydrogen bonds to the kinase 'hinge' residues and through hydrophobic interactions in and around the region occupied by the adenine ring of ATP (**Fig. 1a**,**c**)[12]. Type II inhibitors typically use the ATP binding site, but they also exploit unique hydrogen bonding and hydrophobic interactions made possible by the DFG residues of the activation loop being folded away from the conformation required for ATP phosphate transfer (**Fig. 1b**,**d**). Inspection of the type II inhibitors that have been described in recent articles and patents reveals that numerous new kinase inhibitors are being designed that have functionality capable of forming extensive hydrophobic and hydrogen bonding interactions with both the adenine and allosteric binding site[10,11,13,14]. Examination of the binding features of known type II inhibitors leads us to propose a pharmacophore model that can describe the new generation of structurally diverse type II inhibitors that are being synthesized.

Type I and type II kinase inhibitor binding modes

Type I kinase inhibitors are defined as those that bind in and around the region occupied by the adenine ring of ATP (known as the adenine region) and do not require the DFG motif in the activation loop to adopt a 'DFG-out' conformation for binding. Type I inhibitors typically form ~1–3 hydrogen bonds with the kinase hinge residues that link the N- and C-terminal kinase domains; these hydrogen bonds mimic those normally formed by the exocyclic amino group of adenine (**Fig. 1a**,**c**). The region occupied by type I inhibitors can be further divided into subregions: hydrophobic regions I and II, the adenine region, the ribose region and the phosphate-binding region (**Fig. 1a**)[12]. Although the adenine region is invariably occupied by all type I inhibitors, the ability of the kinase inhibitors to present diverse functionality to other regions can form the basis for inhibitor selectivity among different kinases[15,16]. For example, the type I triarylimidazole p38 inhibitor SB203580 is selective for p38α and p38β over the closely related p38γ and p38δ based on a single gatekeeper residue difference in hydrophobic region I (threonine in p38α and p38β, methionine in p38γ and p38δ)[17,18].

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Type II kinase inhibitors occupy a hydrophobic site that is directly adjacent to the ATP binding pocket created by a unique conformation of the activation loop (DFG-out) in which the phenylalanine residue of the DFG motif moves more than 10 Å from its position in the kinase active conformation (**Fig. 1b,d**). This unique DFG-out conformation was first observed crystallographically in an inactive conformation of an unliganded insulin receptor kinase[19], but it was not until the structures of Abl in complex with imatinib and analogs were solved that it became clear that this conformation could be exploited by inhibitors[5,20]. The DFG-out conformation creates an additional hydrophobic pocket adjacent to the ATP pocket that is frequently referred to as the 'allosteric site'[6]. Because the amino acids surrounding this pocket are less conserved relative to those in the ATP binding pocket, it has been proposed that it may be easier to achieve kinase selectivity with type II inhibitors[4]. Type II inhibitors typically have potent cellular activity, presumably because they recognize (or induce) the DFG-out conformation, which has a lower affinity for ATP than for the active kinase (**Fig. 2**). Although type II inhibitors are indirectly competitive with ATP, the frequent discrepancy observed between kinase IC_{50}s measured by biochemical versus cellular assays shows that type II inhibitors may recognize different kinase conformations in the two assays. Inspection of known type II inhibitor crystal structures reveals that they all possess a conserved hydrogen-bond pair between the ligand (using an amide or a urea) and the residues in the allosteric site: one hydrogen bond with the side chain of a conserved glutamic acid in the αC-helix and the other with the backbone amide of aspartic acid in the DFG motif. All type II inhibitors also have a hydrophobic moiety that is located immediately after the hydrogen bond donor-acceptor pair and that forms van der Waals interactions with the allosteric site. Although occupying the allosteric site is characteristic of type II inhibitors, they can also extend into the adenine region and form one or two hydrogen bonds with kinase hinge residues in a manner similar to that of type I inhibitors. Type I inhibitors can bind to kinases in both the active and inactive conformations, but so far all type II inhibitors have been co-crystallized with kinases that are not phosphorylated in the activation loop, which assumes the DFG-out conformation (**Fig. 2**). The mechanisms by which type II inhibitors might bind to kinases in which the activation loop is phosphorylated remain unclear. Because the crystallographically observed type II inhibitor binding mode is not compatible with the active conformation[5], there may be another kinase conformation that type II inhibitors use for binding to activation loop–phosphorylated kinases. A further consideration is whether the activation loop can become phosphorylated or dephosphorylated once the kinase is bound by a type II inhibitor. A detailed comparison of the binding features of type I and type II inhibitors can be found in **Table 1**.

First-generation type II inhibitors

The first set of type II inhibitors were all developed by kinase enzyme activity–guided optimization starting from two simple screening hits: a phenylaminopyrimidine (PAP) in the case of imatinib and a biaryl urea in the case of BIRB796 and sorafenib (**Fig. 3a**)[21–23]. Concomitant introduction of the so-called 'flag' methyl and a *meta*-benzamide functionality into imatinib converted the compound, which was intially a scaffold that targeted protein kinase C (PKC) and cyclin-dependent kinases (CDKs), into a compound that preferentially inhibited Abl (**Fig. 3b**)[21]. The researchers obtained imatinib through the addition of a methylpiperazine group to the *para* position of the benzamide, which greatly enhanced the water solubility and physicochemical properties of the compound. Although the medicinal chemistry was based on a molecular model that incorrectly predicted that the phenylaminopyrimidine motif would form the expected hydrogen bonding pair to the kinase hinge, the enzyme-selectivity assays still guided optimization correctly. Once the crystal structure of imatinib in complex with Abl was solved, it became clear that the flag methyl and *meta*-benzamide substitutions had introduced conformational preferences and hydrogen

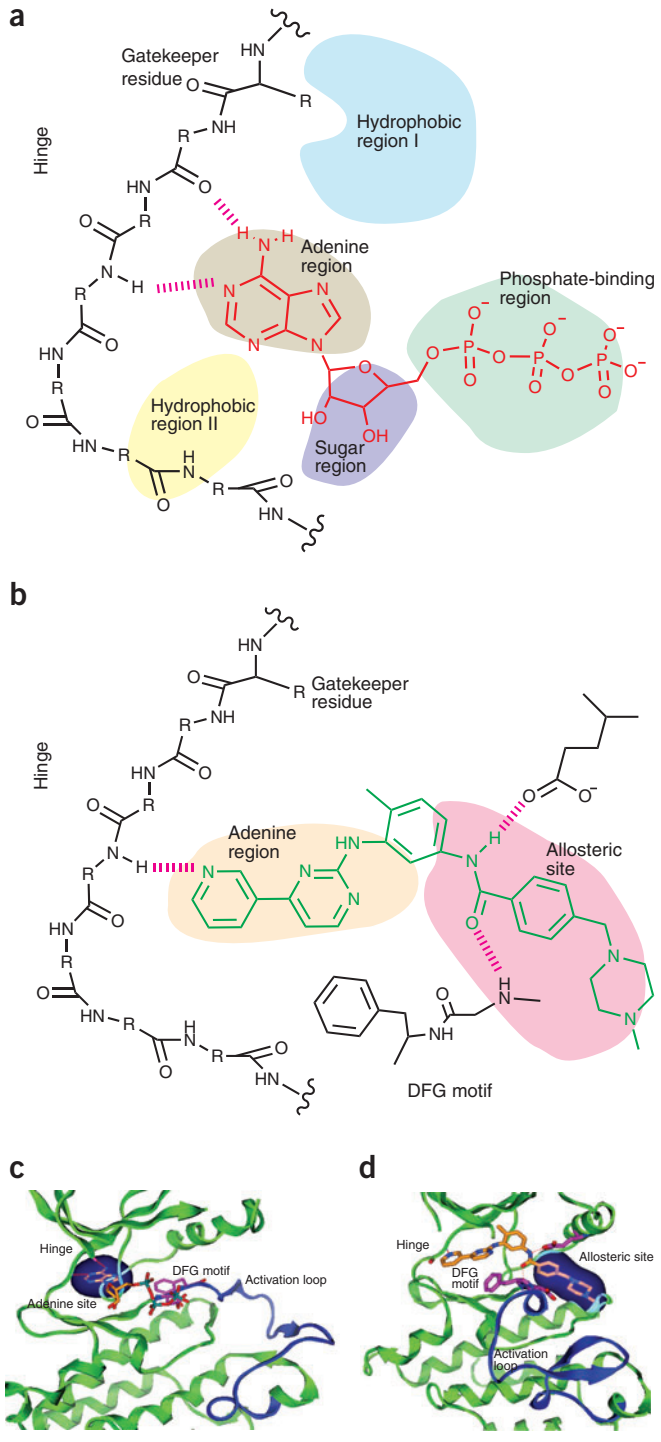

Figure 1 Binding modes of kinase inhibitors. (**a**) Schematic representation of the ATP binding site divided into subregions. (**b**) Schematic representation of the allosteric binding site. (**c**) Ribbon diagram of ATP binding site with a DFG-in activation-loop conformation (active conformation). (**d**) Ribbon diagram of a representative of type II binding mode showing the DFG-out activation-loop conformation (inactive conformation).

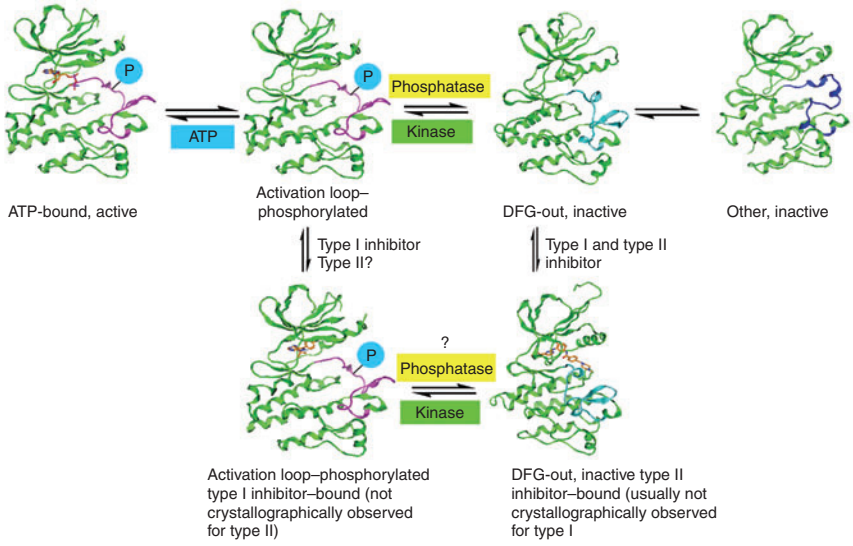

Figure 2 Schematic representation of the equilibrium between active, inactive, apo and type I and II ligand-bound kinase conformations.

bonding interactions that gave the PAP scaffold the ability to stabilize the unexpected DFG-out conformation of Abl. Consistent with the observed binding mode, imatinib preferentially inhibits the enzymatic activity of the unphosphorylated Abl ($K_i = 37$ nM) over the phosphorylated form ($K_i = 7$ μM)[5]. The inability of PKCs and CDKs to assume the DFG-out conformation combined with the energetic penalty resulting from imatinib's assumption of the pyrimidine *cis* conformation, which is required for hinge-region binding, may provide an explanation for the selectivity of imatinib (**Fig. 1d**). However, recently imatinib has been crystallographically demonstrated to bind to Syk, a target that it inhibits only very weakly ($IC_{50} > 10$ μM), by using the pyrimidine *cis* conformation[24]. Though no experimental techniques are available for determining which conformation is relevant intracellularly, the available crystal structures highlight the plasticity of the kinase active site, which might suggest the existence of a dynamic equilibrium among a population of kinase-inhibitor complexes (**Fig. 2**).

BIRB796 was discovered using a combination of traditional and combinatorial lead-optimization strategies starting from a simple biaryl urea compound (**4**; **Fig. 3b**)[22]. BIRB796 is a picomolar-level inhibitor of p38 kinase with a dissociation constant 12,000-fold lower than that of the original high-throughput screening hit. Evaluation of binding kinetics demonstrates that, unlike ATP-competitive inhibitors, BIRB796 shows slow binding and even slower dissociation from the kinase. For example, the calculated half-life for the dissociation of BIRB796 from p38 kinase is 23 h; for the classic ATP-competitive compound SK&F86002, it is 0.1 s (ref. 6). Slow dissociation rates are often linked with protein conformational changes upon ligand binding, as seen in the epidermal growth factor receptor (EGFR) kinase inhibitor lapatinib[25]. Once the crystal structure of BIRB796 bound to p38 was solved, the unusually slow binding could be rationalized based on the need for movement of the activation loop to achieve the DFG-out conformation. A similar biaryl urea compound was used as a starting point for the development of sorafenib, an inhibitor of b-Raf and vascular endothelial growth factor receptor II (VEGFRII), also known as kinase insert domain receptor (KDR)[23]. Lead optimization was facilitated by combinatorial chemistry, and a library of about 1,000 ureas was screened. The pyridyloxy group substituted at the *para* position of the urea was found to improve activity by almost 1,000-fold, a modification that may not have been discovered using a traditional medicinal chemistry approach. The co-crystal structure of sorafenib bound to b-Raf revealed that, like BIRB796, the compound binds to the DFG-out conformation, and the two ureas make identical pairs of hydrogen bonding contacts with equivalent residues. Both inhibitors also make a single contact to a hinge residue: BIRB796 uses the morpholine oxygen and sorafenib uses the pyridyl nitrogen.

One feature common to first-generation type II inhibitors is that their binding affinity is mainly derived from a combination of hydrophobic and hydrogen bonding interactions with the allosteric site created by the DFG-out conformation. All the type II inhibitors shown in **Figure 3** contain a hydrogen bond donor-acceptor pair (urea or amide) and a hydrophobic 'tail' moiety (**Fig. 3a**) that interact with the allosteric site. The majority of type II inhibitors also contain a 'head' group (**Fig. 3a**), which extends to the adenine region and forms a single hydrogen bond with the kinase hinge residue. For example, addition of the ethoxymorpholine head onto the naphthyl group of BIRB796 only decreases the dissociation constant (K_i) by 11-fold[6]. The binding affinity contributed by the head portion in these inhibitors is relatively small, and the head groups are themselves incapable of kinase inhibition.

Table 1 Comparison of the general properties of type I and type II kinase inhibitors

	Type I inhibitors	Type II inhibitors
Activation state of the kinase inhibited	Active or inactive	Inactive
Require specific DFG-out conformation?	No	Yes
Sensitive to phosphorylation state?	Usually no	Usually yes
Can apply to every kinase?	Yes	No, only to those with DFG-out conformation available
Kinase-binding region	ATP site	ATP site and allosteric site
Hydrogen bond to hinge?	Yes for almost all inhibitors	Not required but usually yes
ATP competitive?	Yes	Yes, indirectly
Selectivity	Usually low, but very selective inhibitors have been identified	Advantage; the allosteric site provides another handle for tuning kinase selectivity
Inhibitor resistance?	Yes; usually mutations occur in the ATP site	Yes; mutations occur both in and out of the ATP site
Intellectual propriety position	Disadvantage; extremely crowded patent space	Advantage; more chemical space to exploit

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Second-generation type II inhibitors

Extensive crystallographic and associated molecular modeling efforts have allowed much kinase inhibitor potency and selective activity to be rationalized. For example, the binding modes of well-known type I inhibitors such as oxindole, quinazoline and phenyl amino pyrimidine can be predicted with high accuracy and used to guide lead optimization. However, examination of recent articles and patents relating to kinase inhibitors reveals that many inhibitors have been disclosed that contain a well-known type I scaffold attached to a large substituent that would be predicted to be incompatible with the structure-activity relationships associated with the original type I scaffold. For example, the head portion of compounds **10**[10], **11**[11] and **12**[13] contains a quinazoline moiety (**Fig. 4**) that is itself a kinase inhibitor, even without the large tail substituent (**Fig. 4**). The structure of a quinazoline inhibitor in complex with p38 has been solved (**Fig. 5a**)[26]; when the quinazoline moiety is kept in the same binding pose, it is sterically impossible to dock compound **10** into this p38 structure. The structural similarity between the tail portions of compound **10** and imatinib suggests that compound **10** may also bind to the DFG-out conformation of p38. This idea is supported by the fact that compound **10** can be easily docked into the DFG-out BIRB796-p38 co-crystal structure. In this model, the quinazoline N1 forms a hydrogen bond with the amide of Met109 (a hinge contact), and the carboxamide of **10** forms a pair of hydrogen bonds with the side chain of Glu71 and the amide backbone of Asp168. The structure of **10** bound to p38 was solved, and indeed the kinase adopts a DFG-out inactive conformation to accommodate **10** (**Fig. 5b**)[10]. Like imatinib, pyrimidinoquinazoline (**11**) possesses a benzamide tail that cannot be accommodated within any of the known ATP sites of previously known Aurora kinase co-structures. The Aurora A–(**11**) co-structure again demonstrates that the kinase adopts the inactive DFG-out conformation[11]. No co-crystal structures are available for the other inhibitors in **Figure 4**, but they can all be modeled to fit well into the DFG-out kinase conformation by using the type I portion to bind to the ATP pocket and by accommodating the aryl amides in the allosteric pocket (**12–19**)[13,14,27–32].

We consider all the inhibitors shown in **Figure 4** to be second-generation type II inhibitors because they possess structural features that are distinct from those of the first generation of type II inhibitors shown in **Figure 3a**. The major distinction is that whereas the hinge region–interacting head groups of the

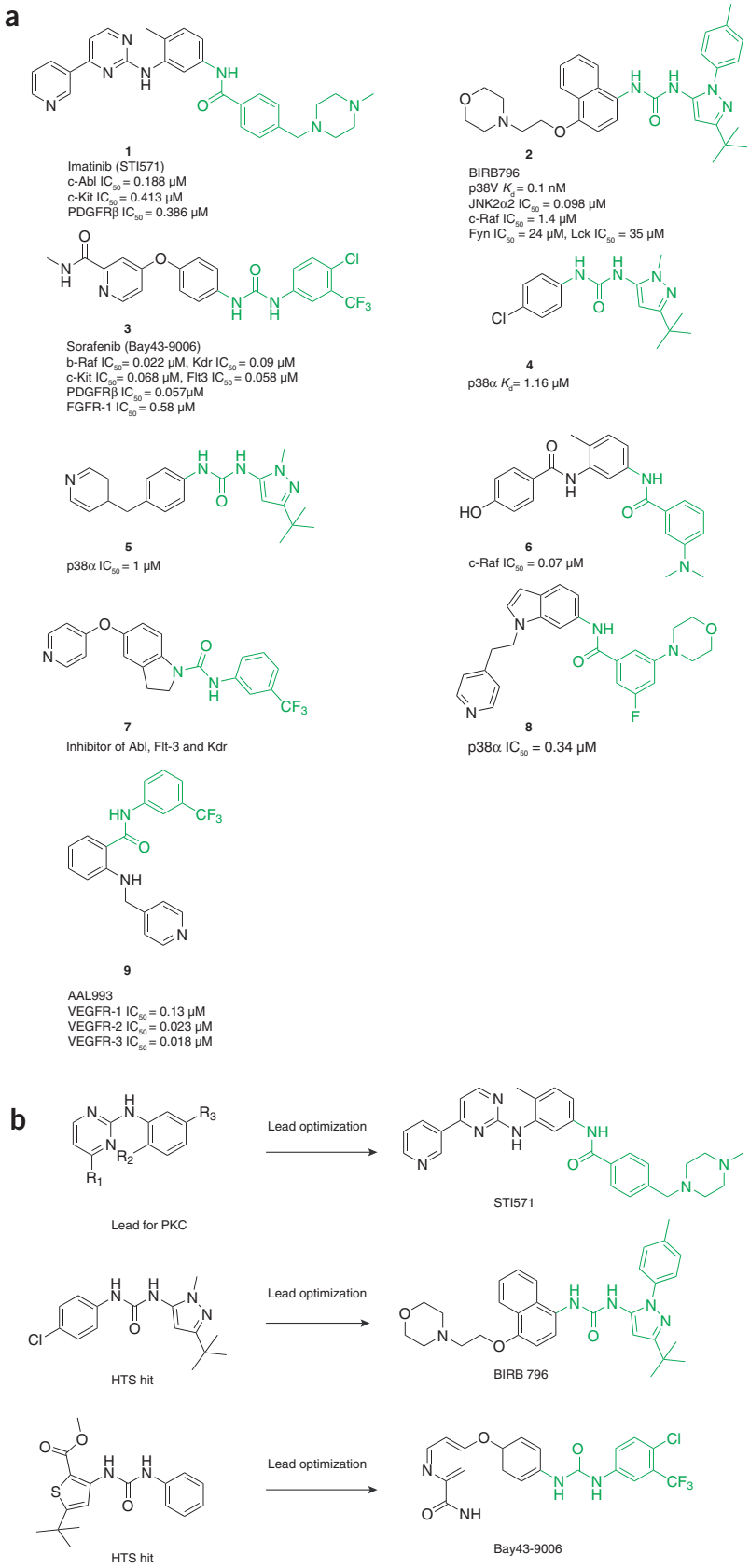

Figure 3 The first-generation type II kinase inhibitors. (**a**) Representative structures of the first-generation type II kinase inhibitors. Tail moieties are shown in green; head groups are black. (**b**) Origins of the first-generation type II kinase inhibitors STI571, BIRB796 and Bay43-9006.

first generation of type II inhibitors do not notably contribute to the binding affinity of the whole molecule, the head groups of the second generation of type II inhibitors are themselves capable of functioning as type I inhibitors and therefore contribute substantially to the binding affinity of the entire molecule. The inhibitors depicted in **Figure 4** are only a few representatives of an ever-growing class of compounds.

Rational design of DFG-out kinase inhibitors

Although there are frequent reports of new kinase inhibitors, the number of examples of structurally unique compounds or compounds that exploit a unique binding pose is actually quite small. One reason for this is that the majority of kinase inhibitors exclusively target the ATP binding site, which has a predilection to accommodate flat, hydrophobic, heterocyclic compounds that satisfy many of the interactions that ATP also satisfies. The availability of an allosteric site created by the unique DFG-out conformation opens a new space in a much less highly conserved region of the kinase that can be used in the design of novel kinase inhibitors. Most of the first generation of type II inhibitors were stumbled across during the lead-optimization process, although a fragment-based lead generation method, which led to the synthesis of a new type II p38 inhibitor (**8**), was recently described[9]. Clearly a structure-based design strategy that allowed for the rational generation of new type II inhibitors would be highly valuable. Based on a retrospective analysis of the second generation of type II inhibitors, we have constructed a 'hybrid-design' method to convert type I inhibitors into type II inhibitors.

Second-generation type II inhibitor molecules can be broken down into two portions (**Fig. 6a**). The head portion is a normal type I inhibitor that has hydrogen bonding interactions with the kinase hinge residues and hydrophobic interactions in and around the adenine region of the ATP binding pocket. The tail portion bears a moiety similar to that seen on the known type II inhibitors that contains a pair of hydrogen bond donating and accepting groups. In addition, the tail contains a hydrophobic substitution that occupies the pocket formed by the shift of phenylalanine from the DFG motif. The 'hybrid-design' approach consists of appending a type II tail onto a type I scaffold (**Fig. 6b**). The first step is to select a type I scaffold based on its kinase activity and selectivity and based on the ease with which a linker can be installed to serve as anchor point to attach the type II tail. The second step is to attach the type II tail moiety, which consists of a hydrogen bond donor-acceptor pair and a hydrophobic motif. The resulting potential type II molecule can then be docked to an available DFG-out kinase structure or to a homology model to verify that the type I head, the linker and the type II tail can fit into the ATP and allosteric pocket and form the expected hydrogen bonding and hydrophobic interactions.

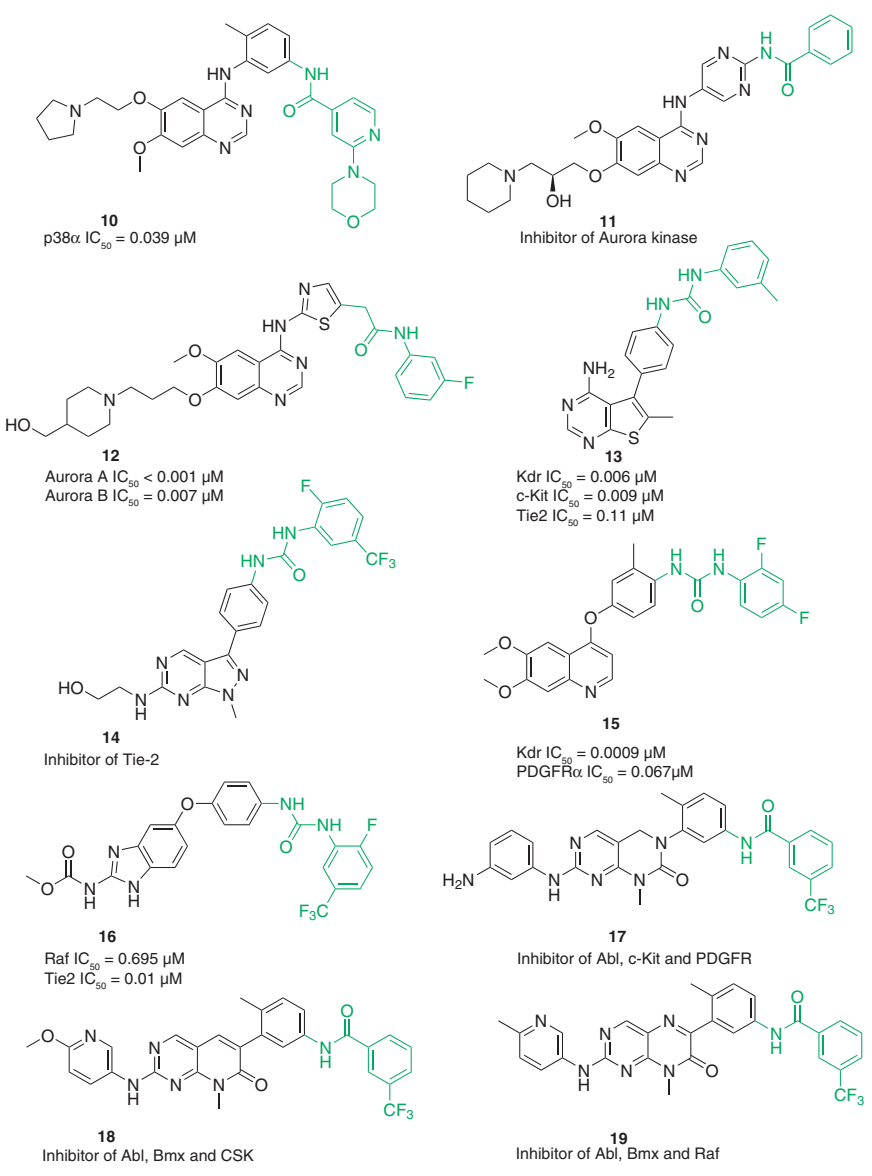

Figure 4 Representative structures of the second-generation type II kinase inhibitors. Tail moieties are shown in green; head groups are black.

Selectivity of type II inhibitors

Protein kinases have a high degree of sequence and structural similarity in the kinase domain. The sequence and structural conservation in the ATP binding pockets is even greater because of the need to recognize ATP and catalyze phosphotransfer. This makes designing highly selective type I kinase inhibitors difficult. In contrast, there is considerably more conformational variability among kinases in their inactive state that can be exploited by type II inhibitors. The DFG-out conformation does not seem to be accessible for every kinase (although this may not be observed without the appropriate inhibitor). Finally, type II inhibitors can achieve selectivity by exploiting energetic differences among the activation-loop conformations of different kinases. Therefore, occupying an additional site with type II inhibitors provides another handle for tuning kinase selectivity, which provides an advantage over type I inhibitors. Although a comprehensive selectivity comparison of a large number of type I and type II inhibi-

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tors has not been published, the available data suggest that type II inhibitors can achieve a high degree of selectivity. For example, imatinib, which was first synthesized when there were only a handful of kinase assays available, has proven to be highly selective—inhibiting only the platelet-derived growth factor receptor (PDGFR), Abl-related kinase (ARG) and the stem cell–factor receptor tyrosine kinase (c-Kit)—with potencies comparable to that of Abl, despite having its selectivity profiled across large enzyme panels (http://www.invitrogen.com/downloads/SelectScrn_Brochure.pdf) and by kinase-domain phage display[33]. Notably, imatinib does not inhibit the highly related Src kinase, with which it shares 50% sequence identity in the kinase domain and 75% identity in the ATP binding site. BIRB796 is also a highly selective p38 kinase inhibitor: out of 15 kinases it only shows weak activity towards JNK2α2 and c-Raf (and shows no activity towards the others: ERK-1, SYK, IKK2β, ZAP-70, EGFR, HER2, PKA, PKC-α, PKC-β, PKC-γ, Fyn and Lck). Almost all of the type II kinase inhibitors shown in **Figure 3** are claimed to be selective[10,13,28]. A general conclusion that can be drawn from kinase-domain phage-display screening is that it is relatively easy to make type I inhibitors very unselective (in one study, a general type I inhibitor, staurosporine, bound 104 of 113 kinases, and an oxindole type I kinase inhibitor, SU11248, bound 73 of 113 kinases), whereas none of the type II inhibitors can be made as promiscuous as type I inhibitors (the most promiscuous type II inhibitor tested, Bay43-9006, bound 35 of 113 kinases)[33]. However, the most selective compound discovered from this screening approach is GW-2016 (lapatinib), a quinazoline-based type I EGFR inhibitor. As type II inhibitors are only now being systematically explored through rational design, it is premature to make a general conclusion about whether type I or type II inhibitors are likely to be more selective. In general, to achieve the highest degree of selectivity the preferred binding mode may be both inhibitor and kinase specific.

Because second-generation type II inhibitors have a type I inhibitor as the hinge-binding element, it is interesting to compare the selectivity of the second-generation type II inhibitors with their corresponding type I inhibitors. Compound **12** inhibits Aurora A kinase with an IC_{50} value of less than 1 nM and is highly selective against a panel of 12 kinases, with weak activity only observed against EGFR kinase (IC_{50} = 800 nM)[13]. Compound **10** is a potent inhibitor of p38α and β (IC_{50} = 39 nM against p38α), and an analog of compound **10** (obtained by replacing pyrrolidine in **10** with dimethyl amine) was selective for p38α and β (IC_{50} = 35 nM against p38α) when tested against a panel of 24 kinases[10]. It is notable that compounds **10** and **12** achieve a high degree of selectivity, but they do not achieve this selectivity through inhibiting a subset of kinases that are targeted by their corresponding type I inhibitors. Both compounds **10** and **12** possess a quinazoline scaf-

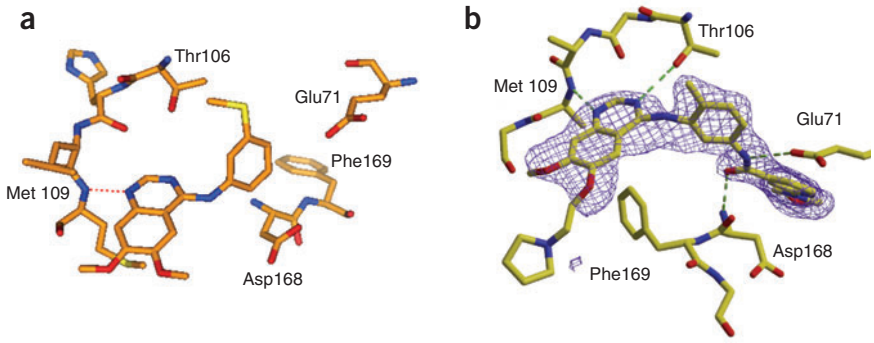

Figure 5 Binding modes of p38 with quinazoline-derived type I and type II kinase inhibitors. (**a**) An anilinoquinazoline compound binds to p38 using the type I binding mode. The DFG motif is in an 'in' conformation (based on crystal structure 1DI9.pdb). (**b**) An anilinoquinazoline compound with a type II tail binds to p38 using the type II binding mode. The DFG motif is in an 'out' conformation (adapted from reference[10] with permission from Elsevier).

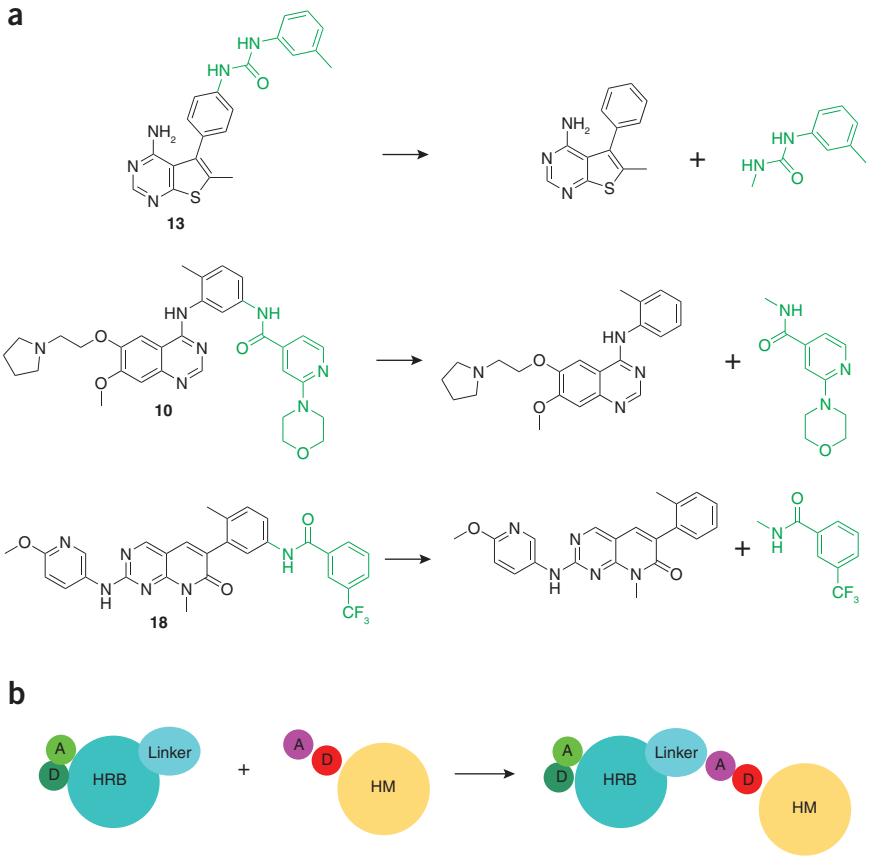

Figure 6 A general pharmacophore model for rational design of type II inhibitors. (**a**) The second-generation type II kinase inhibitors can be broken down into a type I head (black) attached to a type II tail (green). (**b**) A schematic representation of the rational design of new type II kinase inhibitors. A, hydrogen bond acceptor; D, hydrogen bond donor; HRB, hinge-region binding; HM, hydrophobic motif.

fold that has been proven to be a very versatile type I inhibitor, and quinazoline-based inhibitors have been developed against EGFR (for instance, erlotinib, gefitinib and lapatinib), Src and Lck. However, no potent quinazoline-based type I inhibitors have been reported for the Aurora and p38 kinases. The fact that the quinazoline scaffold can be used to generate highly potent and selective Aurora and p38 type II kinase inhibitors demonstrates that the second-generation type II inhibitors not only can achieve a high degree of selectivity but also have a different spectrum of selectivity than their type I counterparts. This observation may allow the recycling of many well-explored type I scaffolds to create new type II inhibitors.

Future directions

Tremendous efforts have been invested in the discovery of protein kinase inhibitors, and five distinct small-molecule kinase inhibitors have received US Food and Drug Administration approval for the treatment of specific cancers[34]. However, given that the majority of kinases are still not targeted by an inhibitor with a useful level of selectivity, there is a compelling need to expand the chemical space available for synthesizing new, potent and selective kinase inhibitors. So far, most of the kinase inhibitors that have been developed are of type I, targeting the ATP binding pocket of active kinases. With the large amount of accumulated structure-activity and crystallographic data for type I inhibitors[35,36], the application of the hybrid-design strategy outlined in this paper could quickly convert many of these type I inhibitors into their type II counterparts. Because the new type II inhibitors target the unique DFG-out inactive kinase conformation, they are likely to possess greater cellular potency and altered selectivity relative to their type I counterparts. The extremely high affinity of second-generation type II inhibitors that is generated as a result of interactions with both the ATP pocket and the allosteric site may provide an avenue to overcome mutations that induce resistance to the first generation of type II kinase inhibitors. The DFG-out conformation has been observed crystallographically for Abl, p38, b-Raf, EGFR, Kdr, c-Kit and Aurora A kinase, and it is not clear why the DFG-out conformation has not been observed for other kinases, such as Cdks, for which there are a multitude of reported liganded and unliganded crystal structures. We speculate that rationally designed inhibitors based on the type II pharmacophore will allow the generation of high-affinity inhibitors that can stabilize the DFG-out conformation of many other kinases for which this conformation has not yet been observed. In addition to serving as drug discovery lead compounds and as tools to investigate signaling pathways, these new type II inhibitors will allow the structural plasticity of the kinase active site to be more fully explored.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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