Journal of Medicinal Chemistry

Perspective

pubs.acs.org/jmc

Conformation-Specific Effects of Raf Kinase Inhibitors

Miniperspective

Xiaolun Wang*[,†] and Joseph Kim[‡]

[†]Takeda California, 10410 Science Center Drive, San Diego, California 92121, United States

[‡]Blueprint Medicines, 215 First Street, Cambridge, Massachusetts 02142, United States

INTRODUCTION

The elucidation of signal-transduction networks that regulate most aspects of cell function has led to targeted cancer therapeutics[1] that focus on specific molecular drivers. Kinases have been one of the most intensively studied drug targets in cancer over the past decade,[2] and 14 small molecule kinase inhibitors (Figure 1) have been approved since 2001 as a result of extensive drug discovery efforts in this area. Coincident with this, a significant amount of structural information on human kinases and their interactions with small molecule inhibitors has accumulated. A recent survey[3] revealed that crystal structures of 155 individual kinases among the 518 human protein kinases have been solved, many of them under multiple conformations and/or with numerous distinct inhibitors. This growing database of information has not only expanded our knowledge of kinase structural biology but also facilitated the design of potent and selective kinase inhibitors.

It has been well documented that protein kinases can adopt either active or inactive conformations, which can be targeted by inhibitors classified respectively as type I or type II.[4−6] Kinase inactive conformations can be further divided into two major subclasses based on the position of the DFG-motif or αC-helix, with further inhibitor classification as type IIA and type IIB.[7] Each of these kinase domain conformations has proven to be druggable as is summarized in Table 1 for all currently approved kinase inhibitors. Even for the same disease-relevant kinase, targeting disparate conformations has similarly produced clinically efficacious agents. For example, imatinib (type IIA)[34−36] and dasatinib (type I)[26] bind to the inactive and active conformations of BCR-ABL, respectively, and both are effective against BCR-ABL-driven chronic myeloid leukemia. A second example is the pair of VEGFR inhibitors **1** (sorafenib)[50] and pazopanib,[28] antiangiogenesis agents used for the treatment of renal cell carcinoma. Targeting the inactive conformation has been touted as an approach toward obtaining more selective kinase inhibitors,[8,9] although this has not been borne out for most type II inhibitors.[6] Nevertheless, the ability to target different kinase conformations has provided opportunities[4,6] to medicinal chemists for exploring kinase inhibitor chemical space, modifying inhibitor physical properties, overcoming resistance mutations,[10] and increasing drug−target residence time.[11,42]

B-Raf has been recognized as a promising drug target since the seminal paper "Mutations of the BRAF Gene in Human Cancer" was published by Davies et al. in 2002.[12] Since then, a number of B-Raf inhibitors have been described,[13−15] and recently two novel and selective B-Raf inhibitors, **2** (vemurafenib, PLX4032)[16,17] and *N*-{3-[5-(2-aminopyrimidin-4-yl)-2-(1,1-dimethylethyl)thiazol-4-yl]-2-fluorophenyl}-2,6-difluorobenzenesulfonamide (dabrafenib, GSK2118436),[15,18] have shown impressive results in clinical trials against metastatic melanoma harboring the B-Raf^{V600E} mutant kinase. More recently **2** was approved[19] for unresectable or metastatic melanoma with the BRAF^{V600E} mutation. **2** is well-tolerated both clinically and preclinically,[20] while other B-Raf inhibitors have suffered from toxicities due to proliferative effects in preclinical studies. Recent studies[21−23] suggest that B-Raf inhibitors can activate the RAF-MEK-ERK signaling pathway in cells containing wild type B-Raf through Raf dimerization, producing undesired proliferative effects. Moreover, it has been shown that Raf dimerization and resultant pathway activation are dependent on specific conformations that the inhibitors induce upon binding. This perspective focuses on conformational effects of inhibitor binding to Raf kinase and consequences on MAPK pathway activation in B-Raf^{WT} cells. These conformationally driven effects are highly relevant to the successful clinical path of **2** compared to other potent and selective B-Raf inhibitors.

RAF CRYSTAL STRUCTURES (KINASE DOMAIN)

As of June 2012, there were 25 B-Raf and 1 C-Raf X-ray crystal structures (Table 2, Figure 2) available in the PDB. The crystallographic results to date all reveal Raf as a dimer with a consistent interface, and this is unlikely driven solely by crystal packing. These structures also demonstrate that B-Raf can adopt all three commonly observed ATP binding site conformations (active, DFG-out, and αC-helix out) with various bound inhibitors. Type I inhibitors bind to a Raf active conformation, while type IIA inhibitors bind to the DFG-out conformation. Type IIB inhibitors bind to an αC-helix-out Raf conformation in which the highly conserved salt bridge between Lys483 and Glu501 is broken. Differences in these conformations are illustrated in Figure 3a, an overlay of three crystal structures of B-Raf with a type I (**3**, SB-590885),[45,46] type IIA (**13**),[47,48] and type IIB (**2**)[43] inhibitor. Compound **3** binds to the B-Raf active conformation featuring DFG-in and αC-helix-in configurations and a preserved ATP binding pocket, including the Lys483-Glu501 salt bridge. Figure 3b shows key H-bond interactions in the B-Raf/**3** complex. Compound **13** binds to and stabilizes B-Raf in a DFG-out, inactive conformation in which the ATP pocket is partially filled by Phe595 and Gly596 from the DFG motif. Repositioning of the DFG triad creates a hydrophobic pocket that is occupied by

Received: May 3, 2012
Published: July 18, 2012

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

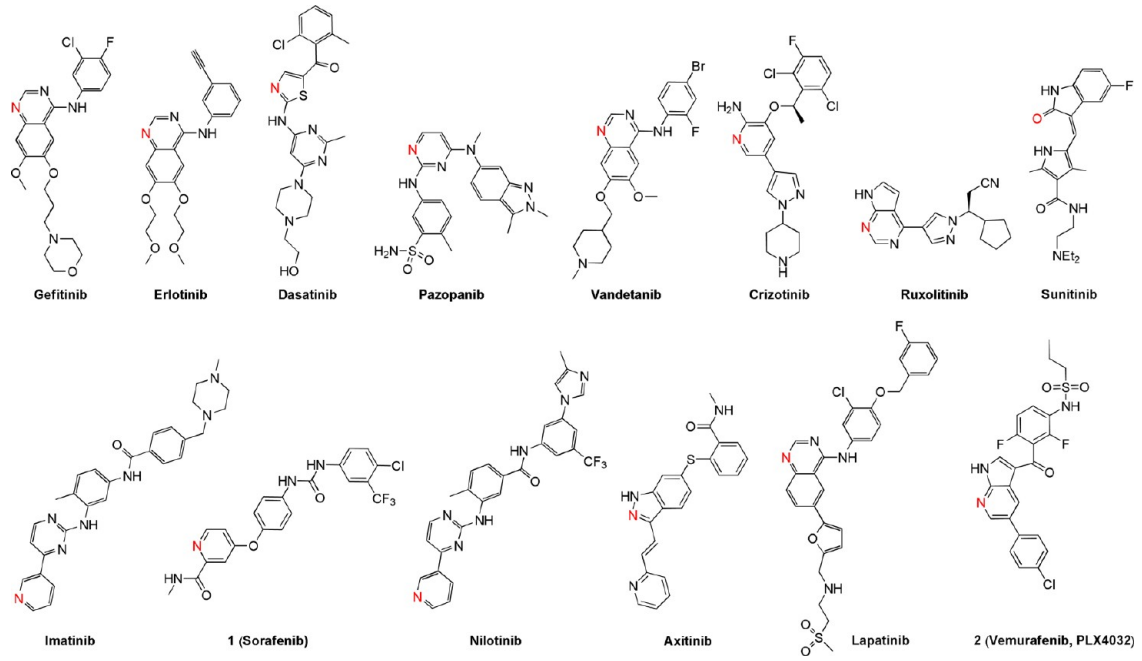

Figure 1. Approved kinase inhibitor drugs.

Table 1. Classification of Marketed Kinase Inhibitors

name	targets	targeted conformation	type	crystal structure of kinase (PDB code)	ref
gefitinib	EGFR	DFG-in/αC-in	I	EGFR (2ITY)	24
erlotinib	EGFR	DFG-in/αC-in	I	EGFR (1M17)	25
dasatinib	BCR-ABL, SRC	DFG-in/αC-in	I	ABL (2GQG), SRC (3G5D)	26, 27
pazopanib	VEGFRs	DFG-in/αC-in	I	two close analogs: VGEFR2 (3CJF, 3CJG)	28
vandetanib	VEGFRs, RET, EGFR	DFG-in/αC-in	I	RET (2IVU)	29
crizotinib	c-MET, ALK	DFG-in/αC-in	I	c-MET (2WGJ), ALK (2XP2)	30
ruxolitinib	JAK1, JAK2	DFG-in/αC-in[a]	I[a]	has not been reported	31
sunitinib	PDGFRs, VEGFRs, c-KIT, etc.	DFG-in or -out/αC-in	I or IIA	I: ITK (3MIY). IIA: KIT(3G0E), KIT^{D816H} (3G0F)	32, 33
imatinib	BCR-ABL, c-KIT, PDGFRs	DFG-out/αC-in	IIA	ABL (1IEP/2HYY/1OPJ/3K5V), KIT (1T46), ABL2 (3GVU)	34−38
sorafenib	VEGFRs, PDGFRs, c-KIT, Raf	DFG-out/αC-in	IIA	B-Raf (1UWH), B-Raf^{V600E} (1UWJ)	39
nilotinib	BCR-ABL	DFG-out/αC-in	IIA	ABL (3CS9)	40
axitinib	VEGFRs	DFG-out/αC-in	IIA	structure with a close analogue has been reported	41
lapatinib	EGFR, HER2	DFG-in/αC-out	IIB	EGFR (1XKK)	42
vemurafenib	B-Raf	DFG-in/αC-out	IIB	B-Raf (3OG7)	43

[a]Ruxolitinib is structurally similar to tofacitinib, which binds to the active conformations[44] of JAK1 (3EYG) and JAK2 (3FUP).

Table 2. Conformations of Inhibitor-Bound B-Raf Kinases

PDB	ligand	inhibitor type	protein conformation	key H-bonding residues	related PDB[a]
2FB8	**3**	I	DFG-in, αC-helix-in	K483, E501, C532, D594	3D4Q, 3PPJ, 3PPK, 3PRF, 3PRI, 3PSB, 3Q4C, 3PSD, 3OMV(C-Raf), 4E26
3IDP[b]	**13**	IIA	DFG-out, αC-helix-in	K483, E501, C532, D594	1UWH, 1UWJ,[b] 3II5, 3Q96, 4DBN
3OG7[b]	**2**	IIB	DFG-in, αC-helix-out	Q530, C532, D594, G596	3C4C, 3C4D,[b] 3SKC, 3TV4, 3TV6, 4E4X, 4EHG, 4EHE

[a]References are available at http://www.rcsb.org/. [b]B-Raf^{V600E}.

the inhibitor *p*-chlorophenyl group (Figure 3c). As is typical for type IIA binders, the Lys-Glu salt bridge remains intact. **2** binds to a DFG-in, αC-helix-out, inactive B-Raf conformation, similar to binding of the type IIB EGFR/HER2 kinase inhibitor lapatinib to EGFR. The key salt bridge between Lys483 and Glu501 in B-Raf is broken, and the propyl group of **2** inserts into a newly formed hydrophobic pocket between the αC-helix and the N-lobe β-sheets (Figure 3d). Interestingly, one protomer of the B-Raf/**2** dimer is unoccupied and exists in an active conformation, which may have relevance to models described recently for inhibitor driven, activated Raf dimers.

■ ENZYME ACTIVITY AND SELECTIVITY OF B-RAF INHIBITORS

Enzyme potencies and kinase selectivity profiles for a set of representative B-Raf inhibitors are summarized in Table 3. This set of inhibitors exhibits high potency against all Raf isoforms (A-Raf data not shown). Both type I inhibitors of the

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

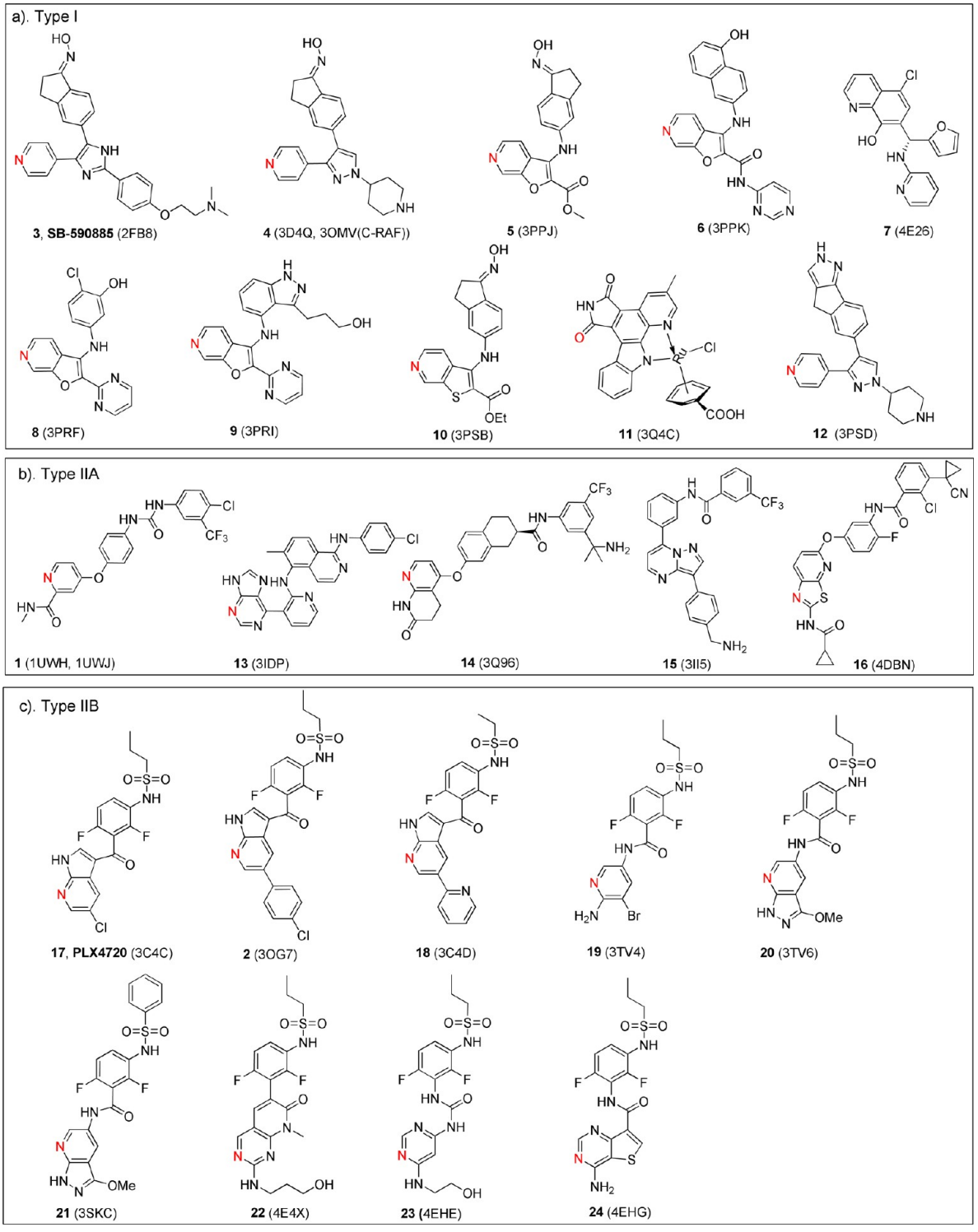

Figure 2. B-Raf inhibitors with X-ray crystal structures.

imidazole/pyrazole class and the type IIB inhibitors show a high degree of kinase selectivity, while the type IIA examples appear less selective, harboring some degree of tyrosine kinase activities. Nevertheless, all three classes of inhibitors show similar affinities for V600E and wild type B-Raf and minimal selectivity against C-Raf. A recent publication[22] revealed that the $K_{M,ATP(app)}$ of full length B-Raf^{V600E} is 13-fold higher than that of wild type full length B-Raf. Therefore, while IC_{50} values of some inhibitors suggest varying degrees of selectivity[43] for B-Raf^{V600E}, their K_i values do not support this (Table 3). For example, the affinities (K_i) of compound **17** (PLX4720)[22,52] for B-Raf^{WT} and B-Raf^{V600E} are roughly equal, although the IC_{50} against B-Raf^{V600E} is 12-fold lower than that against B-Raf^{WT}. Thus, selectivity for mutant B-Raf over wild type as measured at the enzyme level is unlikely the major reason for the relatively benign toxicity profile exhibited by **2** (K_i values for **2** have not been reported).

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

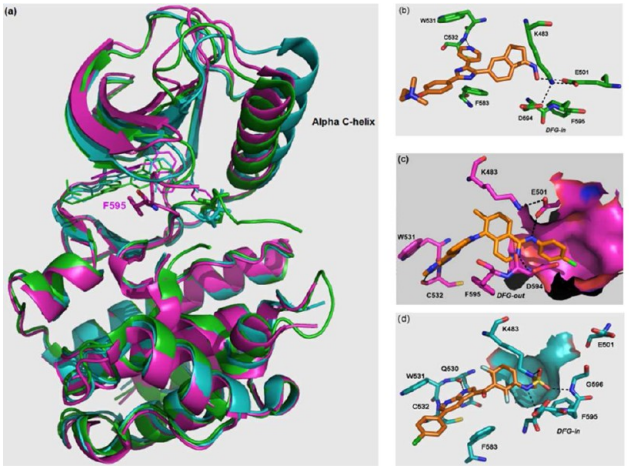

Figure 3. (a) Overlay of three inhibitor-bound conformations: DFG-in and αC-helix-in (green, 2FB8); DFG-out and αC-helix-in (purple, 3IDP, F595 as shown); DFG-in and αC-helix-out (cyan, 3OG7). (b) Binding site of 2FB8. (c) Binding site of 3IDP. (d) Binding site of 3OG7.

CELL SELECTIVITY/ACTIVITY AND TOXICITY

Type I and type IIB B-Raf inhibitors show high apparent selectivity (Table 4a) toward cell lines harboring B-Raf^{V600E}, as measured by viability and by inhibition of ERK phosphorylation. Type IIA inhibitors such as **1** and **13** additionally exhibit low micromolar inhibition against wild type B-Raf cell lines, which may be due to off-target kinase activities or alternatively to pan-Raf inhibition, including Raf dimers. A number of these inhibitors have demonstrated efficacy in B-Raf^{V600E} tumor models with commensurate inhibition of ERK phosphorylation in tumor tissue (Table 4a). While selective B-Raf inhibitors possess no or minimal growth inhibitory activity against cell lines containing wild type B-Raf, many have been shown to activate the RAF-MEK-ERK pathway in such lines, as was first reported for a Raf inhibitor in 1999.[53] The former feature of B-Raf inhibitors distinguishes them significantly from MEK inhibitors, which largely suppress MAPK pathway signaling in normal cells and may thereby provide B-Raf inhibitors with a larger therapeutic window relative to MEK inhibitors.[23,46] The latter property, however, is likely the cause of undesired proliferative effects that have been associated with B-Raf inhibitors preclinically. For example, the type I selective inhibitor **25** (GDC-0879, Figure 4)[22,49] induces skin acanthosis and hyperkeratosis in mice while another type I B-Raf inhibitor causes epithelial inflammation and hyperplasia in dogs.[54,55] Similarly, the type IIA inhibitor **13** induced proliferation and hyperplasia in normal tissues of mice.[48,56] Moreover, both type I and type IIA inhibitors can promote wild type B-Raf tumor growth correlated with enhanced ERK phosphorylation in mouse xenograft models (Table 4b). Conversely, the closely related type IIB inhibitors **2** and **17** are well tolerated in animal models[43] and demonstrate no measurable in vivo growth enhancement of B-Raf^{WT} tumors, although increases in ERK phosphorylation have been observed in in vitro studies (Table 4b).

Table 4. Activity, Selectivity, And Effects of B-Raf Inhibitors

(a) Activity and Selectivity of Representative B-Raf Inhibitors against Tumors

	cell IC_{50} (μM)				
inhibitor (type)	A375[a] proliferation	A375 p-ERK	HCT-116[b] proliferation	in vivo tumor growth inhibition model[c]	ref
3 (type I)	0.37	0.29	4.6	A375	46
25 (type I)	<0.5	<1	>20	A375	22, 49
1 (type IIA)	2.4	na[d]	2−4	Colo205,[a] A549[b]	50, 58
13 (type IIA)	0.31	0.002	0.72	A375, M24met[e]	47, 48
17 (type IIB)	0.5	0.046	27	Colo205	52
2 (type IIB)	0.31	na[d]	>2	A375	20, 59, 60

(b) Effects of B-Raf Inhibitors on B-Raf^{WT} Tumors

inhibitor (type)	cell lines with p-ERK enhancement (pathway activation)	in vitro cell growth promotion	in vivo tumor growth promotion	ref
26 (885-A)[f] (type I)	MM415,[g] WM852[g]	not reported	not reported	21
25 (type I)	H2122,[b] MeWo[b]	H226,[h] CaCo-2[h]	LXFA983,[b] LXFA1041[b]	22
1 (type IIA)	less common: Calu-6,[g] MIA PaCa-2[f]	not reported	not reported	23, 48
13 (type IIA)	MIA PaCa-2[b]	MIA PaCa-2	MIA PaCa-2	48
17 (type IIB)	Calu-6, H2122, MeWo,	SW48[h]	not reported	22, 23, 61
2 (type IIB)	HCT-116, SkMel-31[h]	WM1382,[h] YULOVY[g]	not reported	60, 62

[a]B-Raf^{V600E}; RAS^{WT}. [b]B-Raf^{WT}, KRAS^{MT}. [c]All were correlated with pERK inhibition except **1** against A549. [d]Not available. [e]B-Raf^{WT}, NRAS^{MT}. [f]A close analogue of **3** (Figure 4). [g]B-Raf^{WT}, NRAS^{MT}. [h]B-Raf^{WT}, RAS^{WT}.

Table 3. Enzymatic Activity and Selectivity of Representative B-Raf Inhibitors

	K_i/IC_{50} (nM)			kinase selectivity profile		
inhibitor (type)	B-Raf^{WT}	B-Raf^{V600E}	C-Raf	other kinase hits	S (1 μM)[c]/panel size[d]	ref
3 (type I)	0.3[a]	0.16[a]	1.72[a]	clean	0.00/46	45, 46
25 (type I)	0.17[a]	0.19[a]	0.54[a]	clean (only slightly hits CK1-δ)	0.01/139	22, 49
1 (type IIA)	22[b,e]	38[b,e]	6[b,e]	RET, KIT, VEGFRs, PDGFRs, TIE1, etc.	0.14/314	50, 51
13 (type IIA)	1.0[a]	1.0[a]	0.3[a]	LCK, TIE1/2, VEGFRs, JAK2, KIT, etc.	0.10/350	47, 48
17 (type IIB)	2.6[a]/160[b,f]	4.0[a]/13[b,f]	3.3[a]/6.5[b,f]	BRK	0.02/65	22, 52
2 (type IIB)	100[b,f]	31[b,f]	48[b,f]	SRMS, ACK1, FGR, LCK, BRK, etc.	0.04/289	43

[a]K_i. [b]IC_{50}. [c]Selectivity score defined as the number (IC_{50} < 1 μM) of hits divided by the panel size. [d]Raf kinases are excluded. [e][ATP] = 5 μM. [f][ATP] = 100 μM.

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

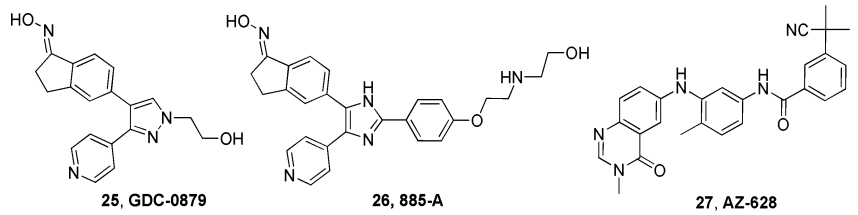

Figure 4. Structures of other B-Raf inhibitors.

2 was the first selective B-Raf inhibitor advanced to clinical trials. In the phase III clinical trial for metastatic melanoma patients with B-Raf^{V600E} mutation, **2** gave an impressive response rate of 48% with corresponding MAPK pathway inhibition.[16,17] While **2** was generally well tolerated, 18−31% of the patients developed skin squamous-cell carcinoma with "very low invasive potential and no metastatic potential".[16,17] This proliferative effect is very likely related to the MAPK pathway activation potential of Raf inhibitors in B-Raf^{WT} cells. Table 5 summarizes the cell selectivity and preclinical toxicities for three types of selective B-Raf inhibitors. Type IIB B-Raf inhibitors appear to have a unique ability to inhibit B-Raf^{V600E} driven disease while minimizing adverse proliferative effects. Recent publications[21−23,57] shed light on why this is so.

Table 5. Cell Selectivity and Preclinical Toxicities of Three Types of B-Raf Inhibitors

type	selective toward B-Raf mutant cells	activation of MAP pathway	toxicity in animal models
I	yes	yes	hyperkeratosis, acanthosis, hyperplasia, inflammation
IIA	dependent on the kinase selectivity	yes	hyperplasia
IIB	yes	yes but weak	no proliferative effects observed

DIMERIZATION OF RAF KINASES

In 1996, back-to-back publications revealed that homodimerization of two molecules of C-Raf leads to their functional activation.[63,64] Five years later the heterodimerization of C-Raf and B-Raf in cells harboring activated Ras was discovered[65] and elevated activity of this B-Raf/C-Raf heterodimer was confirmed.[66,67] Recently, Rajakulendran et al.[57] proposed a C2 symmetric, side-by-side model for Raf kinase dimers, recognizing that all six known B-Raf crystal structures published at that time displayed a common dimer interface. Their proposal that this dimer interface was indeed a biologically relevant interface was supported by site-directed mutagenesis studies of Raf kinase in *Drosophila* Schneider S2 cells. Figure 5 shows the overlay of three representative B-Raf and one C-Raf homodimers, illustrating a conserved juxtapositioning between two Raf protomers. As mentioned earlier, this structural feature exists in all known Raf crystal structures and, relevant to binding modes of type IIB inhibitors, involves part of the αC-helix. The Raf dimer interface is highlighted in Figure 6.[22,57] Hydrophobic and hydrogen binding interactions are formed throughout the interface. Arg509 (B-Raf numbering), located near the center of the interface, plays a critical role in dimerization, interacting with Thr508, Arg506, and Phe516 from the associated protomer. Mutation of this residue to histidine (R509H) or mutation of the analogous residue in C-Raf (R401A/H) abolishes the formation of Raf dimers,

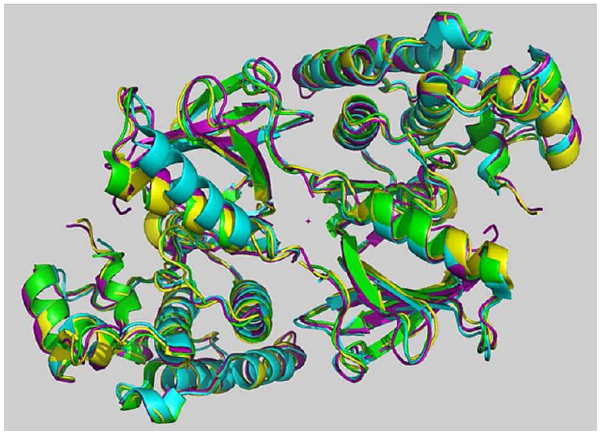

Figure 5. Overlay of B-Raf crystal structures (green, 2FB8; purple 3IDP; cyan 3OG7) and C-Raf crystal structure (yellow, 3OMV).

confirming the importance of this residue and the crystallographically observed interface in Raf dimerization and activation.[22,23]

While the studies of Rajakulendran et al. demonstrated the association between Raf dimerization and Raf activation, no inhibitor was involved in their work. It was not until 2010 that Heidorn et al.[21] first described inhibitor-induced Raf dimerization and ERK activation in B-Raf^{WT} cells. Shortly thereafter, papers by Hatzivassiliou et al.[22] and Poulikakos et al.[23] reported detailed studies regarding Raf inhibitor induced activation of MAPK pathway signaling. Figure 7 shows a simplified version of their mechanisms.[23] Inhibitor binding to a Raf monomer can induce homo/heterodimerization with a second Raf protomer in the presence of Ras-GTP. Alternatively, inhibitor binding may stabilize pre-existing Raf homo/heterodimers. The paired Raf protomer appears to have considerably lower affinity for inhibitor (or much higher affinity for ATP) and significantly elevated kinase activity, activating MEK and downstream effectors until a second inhibitor can bind, typically at much higher concentration. All three papers[21−23] revealed that inhibitor-induced Raf dimerization is reliant on the presence of activated Ras and that B-Raf^{V600E} cells maintain low levels of activated Ras, an important distinction between the two genotypes. Low Ras-GTP levels in B-Raf^{V600E} cells disfavor dimer mediated MAPK pathway activation and, coupled with the high $K_{m,ATP}$ of B-Raf^{V600E}, render these lines more sensitive to B-Raf inhibitors. Consequently, tumor growth driven by B-Raf^{V600E} is effectively thwarted by B-Raf inhibitors.

All three classes of B-Raf inhibitors can induce Raf dimerization and MAPK activation in B-Raf^{WT} cells, but there are notable differences among them (Table 6). Type I B-Raf inhibitors such as compound **25** are potent activators of the MAPK pathway. **25** promotes not only C-Raf homodimerization but also B-Raf/C-Raf heterodimerization.[22] In animal studies, hyperplasia and inflammation have been linked to type

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

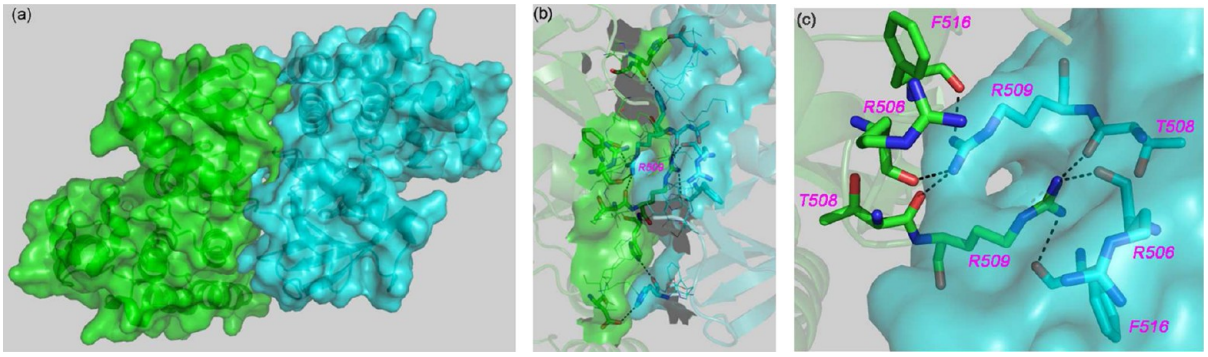

Figure 6. Interactions between two B-Raf protomers in 2FB8.

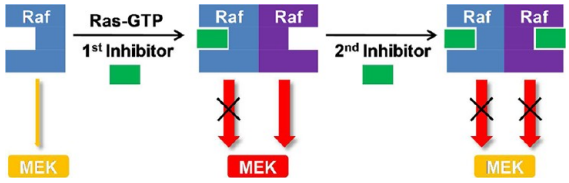

Figure 7. Mechanism of Raf activation in B-Raf^{WT} cells.

Table 6. Raf Dimerization Induced by Three Types of Inhibitors

type	example	B- and C-Raf heterodimer[a]	C-Raf homodimer	MAPK pathway activation	ref
I	**25**, **26**	yes	yes	yes, significant	22, 23
IIA	**1**, **27**	yes	yes	mixed results	21−23
IIB	**2**, **17**	weak	yes	yes, moderate	22, 23

[a]It has been reported that the Raf heterodimer possesses a higher kinase activity than the homodimer.[67]

I inhibitors, accompanied by sustained levels of phosphorylated ERK.[22,55] Results for type IIA inhibitors are less consistent among different chemotypes. It has been shown that compound **27** (AZ628)[22] strongly induces Raf dimerization but does not elicit the enhanced phosphorylation of MEK and ERK characteristic of inhibitor-induced dimerization. The authors proposed that tight binding and a slow off rate help **27** occupy both ATP binding pockets of the dimer and prevent signal transduction. Unfortunately no preclinical toxicity studies for **27** have been reported to determine if the observed lack of MAPK pathway activation by this agent limits proliferative effects in vivo. Raf dimerization induced by **1** has also been reported.[23] While Heidorn et al.[21] reported no MAPK activation in cells treated with 10 μM **1**, Poulikakos et al.[23] found that **1** at lower concentrations can activate the MAPK pathway. In line with this finding, cutaneous squamous cell carcinoma is an observed side effect in patients taking **1**.[68] Finally, hyperplasia in a mouse model has been reported for the potent and selective type IIA inhibitor **13**.[48,56]

In contrast, type IIB inhibitors only weakly induce Raf dimerization and do not readily promote the translocation of Raf kinases to the membrane, steps considered important in Raf activation.[22] Some B-Raf crystal structures with type IIB inhibitors show two protomers existing in different conformations, with one protomer lacking ligand or being only partially occupied (Table 7).[69] Movement of the αC-helix to an out position by type IIB inhibitor binding appears to disfavor Raf dimerization,[22] consistent with the αC-helix forming part of the dimerization interface (Figure 8). This conformational effect of

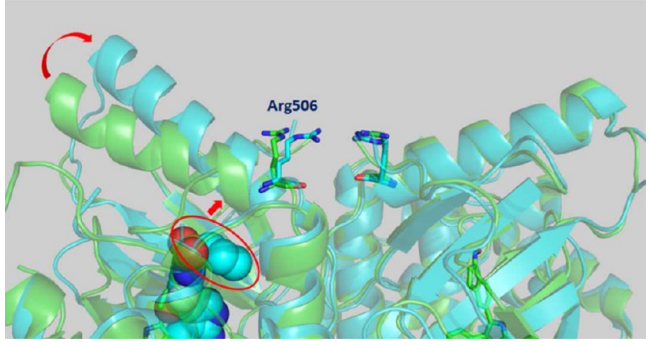

Figure 8. Effect of αC-helix-out conformation on the Raf dimerization (green, 2FB8; cyan, 3OG7). The positively charged side chain of the Arg506 from the αC-helix-out protomer is pushed close to that of the Arg506 from the other protomer.

type IIB inhibitors on Raf binding likely explains their minimal proliferative and favorable toxicity profile in preclinical studies, which allowed their progression into clinical trials. Lack of significant MAPK activation or inhibition in B-Raf^{WT} cells seemingly allows for dosing to greater target coverage in B-Raf mutant tumor without significant proliferative or antiproliferative effects in normal tissues, much to the benefit of large numbers of patients.

Table 7. Conformations of Protomers for Type IIB Inhibitors

	protomer 1			protomer 2		
PDB code	ligand	conformation	salt bridge[a]	ligand	conformation	salt bridge
3OG7	**2**	DFG-in, αC-helix-out	no	none	DFG-in, αC-helix-in	yes
3C4C	**17**	DFG-in, αC-helix-out	no	**17**, 60% occupancy[b]	DFG-out, αC-helix-in	yes
3C4D	**18**	DFG-in, αC-helix-out	no	none	DFG-out, αC-helix-in	yes

[a]If the salt bridge between Lys483 and Glu501 is maintained. [b]This suggests **17** is a weak type IIA inhibitor.

dx.doi.org/10.1021/jm300613w | J. Med. Chem. 2012, 55, 7332−7341

CONCLUSION AND PERSPECTIVES

The drug discovery industry has made significant progress toward agents targeting B-Raf since recognizing its important role in cancer progression.[13,14] These drug candidates have proven to be critical tools in unraveling previously underappreciated intricacies of the MAPK signaling pathway.[70−72] Recent publications[21−23] have disclosed a novel mechanism for Raf inhibitor-induced activation of the MAPK pathway in B-Raf wild type cells. Inhibitors binding to different Raf kinase conformations display differential abilities in promoting Raf dimerization and MAPK activation and now provide a guide for designing the next generation of B-Raf inhibitors that may mitigate the adverse effects linked to pathway activation. Also, it was recently shown that combination of a Raf and MEK inhibitor can eliminate undesired proliferative effects in mouse models[48] and preliminary results from a phase I/II clinical trial combining a B-Raf inhibitor and a MEK inhibitor indicated no cutaneous squamous cell carcinoma incidences while producing a high objective response rate.[73] This combination not only may alleviate proliferative side effects but also might also address some of the resistance mechanisms emerging in patients treated with **2**.[74] For example, Poulikakos et al.[75] recently found that melanoma patients treated with **2** develop a variant of B-Raf^{V600E} lacking the Ras binding domain, which can form constitutive dimers, reactivate the MAPK pathway absent Ras-GTP, and escape inhibition by **2**. The authors suggested that a combination of a B-Raf inhibitor with a MEK inhibitor might delay the onset of this resistance, since they did not observe this B-Raf^{V600E} variant at a detectable level in **2** naive patients and because these **2** resistant cells are still sensitive to MEK inhibition. As more details of MAPK signaling biology are being elucidated,[76,77] the desired profile for a B-Raf inhibitor will also evolve, eventually leading to better therapeutics for the treatment of B-Raf mutant cancers, including possible combinations with other mechanism-based agents.

AUTHOR INFORMATION

Corresponding Author

*Phone: (858) 731-8983. E-mail: xxw104@gmail.com.

Notes

The authors declare no competing financial interest.

Biographies

Xiaolun Wang obtained his Ph.D. in Organic Chemistry from Columbia University, NY, under the supervision of Prof. James Leighton and then joined Wyeth at Pearl River in 2005. He moved to the Cambridge site after the acquisition of Wyeth by Pfizer. In 2011, he joined Takeda California. He has led the chemistry teams for multiple projects in the immunology and oncology therapeutic areas. His main areas of expertise are structure based drug design, kinase inhibitor design, and fragment based drug discovery.

Joseph Kim obtained his Ph.D. in Biophysical Chemistry at Yale University, CT, and undertook postdoctoral studies in X-ray crystallography at The Rockefeller University, NY, under the direction of Dr. Stephen Burley. Dr. Kim joined Vertex Pharmaceuticals in 1995, where his accomplishments included solving the crystal structures of three novel proteins. In 1998 he joined Kinetix Pharmaceuticals and commenced work on structure-based design and optimization of protein kinase inhibitors. Upon acquisition of Kinetix by Amgen, he joined the Amgen Massachusetts site, continuing structure-based inhibitor efforts around numerous kinase targets, including VEGFR-2, Tie-2, and B-Raf. In 2011 Dr. Kim joined Blueprint Medicines, a company discovering selective agents against genetic drivers of cancer.

ACKNOWLEDGMENTS

We thank Drs. Derek Cole, Matthew Marx, Patrick Vincent, Steve Wenglowsky, and Christoph Lengauer for their insightful suggestions for our manuscript. We also thank Drs. Joachim Rudolph and Walter Voegtli for providing valuable information regarding B-Raf crystallization conditions.

ABBREVIATION USED

ACK1, activated CDC42 kinase 1; ALK, anaplastic lymphoma kinase; BCR-ABL, breakpoint cluster region Abelson kinase; BRK, breast tumor kinase; CK1-δ, casein kinase I isoform δ; c-MET, MNNG HOS transforming gene; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HER2, human epidermal growth factor receptor 2; LCK, lymphocyte-specific protein tyrosine kinase; MEK, mitogen activated protein kinase kinase; PDGFR, platelet derived growth factor receptor; RET, rearranged during transfection; SRMS, src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites; VEGFR, vascular endothelial growth factor receptor

REFERENCES

(1) Hait, W. N.; Hambley, T. W. Targeted cancer therapeutics. *Cancer Res.* **2009**, *69*, 1263−1267.

(2) Li, R.; Stafford, J. A. *Kinase Inhibitor Drugs*, 1st ed.; Wiley: Hoboken, NJ, 2009.

(3) Taylor, S. S.; Kornev, A. P. Protein kinases: evolution of dynamic regulatory proteins. *Trends Biochem. Sci.* **2011**, *36*, 65−77.

(4) Alton, G. R.; Lunney, E. A. Targeting the unactivated conformations of protein kinases for small molecule drug discovery. *Expert Opin. Drug Discovery* **2008**, *3*, 595−605.

(5) Backes, A.; Zech, B.; Felber, B.; Klebl, B.; Müller, G. Small-molecule inhibitors binding to protein kinases. Part I: Exceptions from the traditional pharmacophore approach of type I inhibition. *Expert Opin. Drug Discovery* **2008**, *3*, 1409−1425.

(6) Backes, A.; Zech, B.; Felber, B.; Klebl, B.; Müller, G. Small-molecule inhibitors binding to protein kinases. Part II: The novel pharmacophore approach of type II and type III inhibition. *Expert Opin. Drug Discovery* **2008**, *3*, 1427−1449.

(7) Type IIB has been also called type I 1/2 (ref 10). In ref 6 it was included in type II.

(8) Liu, Y.; Gray, N. S. Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* **2006**, *2*, 358−364.

(9) Liao, J. L. Molecular targeting of protein kinases to optimize selectivity and resistance profiles of kinase inhibitors. *Curr. Top Med. Chem.* **2007**, *7*, 1332−1335.

(10) Zuccotto, F.; Ardini, E.; Casale, E.; Angiolini, M. Through the "gatekeeper door": exploiting the active kinase conformation. *J. Med. Chem.* **2010**, *53*, 2681−2694.

(11) Copeland, R. A.; Pompliano, D. L.; Meek, T. D. Drug−target residence time and its implications for lead optimization. *Nat. Rev. Drug Discovery* **2006**, *5*, 730−739.

(12) Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W. C.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. Mutations of the BRAF gene in human cancer. *Nature* **2002**, *417*, 949−954.

(13) Shepherd, C.; Puzanov, I.; Sosman, J. A. B-RAF inhibitors: an evolving role in the therapy of malignant melanoma. *Curr. Oncol. Rep.* **2010**, *12*, 146−152.
(14) Puzanov, I.; Flaherty, K. T. Targeted molecular therapy in melanoma. *Semin. Cutaneous Med. Surg.* **2010**, *29*, 196−201.
(15) Zambon, A.; Niculescu-Duvaz, I.; Niculescu-Duvaz, D.; Marais, R.; Springer, C. J. Small molecule inhibitors of BRAF in clinical trials. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 789−792 . The structure of dabrafenib shown in this reference is incorrect and the correct structure can be found at http://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI%20Thesaurus&code=C82386.
(16) Flaherty, K. T.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthur, G. A.; Sosman, J. A.; O'Dwyer, P. J.; Lee, R. J.; Grippo, J. F.; Nolop, K.; Chapman, P. B. Inhibition of mutated, activated BRAF in metastatic melanoma. *N. Engl. J. Med.* **2010**, *363*, 809−819.
(17) Chapman, P. B.; Hauschild, A.; Robert, C.; Haanen, J. B.; Ascierto, P.; Larkin, J.; Dummer, R.; Garbe, C.; Testori, A.; Maio, M.; Hogg, D.; Lorigan, P.; Lebbe, C.; Jouary, T.; Schadendorf, D.; Ribas, A.; O'Day, S. J.; Sosman, J. A.; Kirkwood, J. M.; Eggermont, A. M. M.; Dreno, B.; Nolop, K.; Li, J.; Nelson, B.; Hou, J.; Lee, R. J.; Flaherty, K. T.; McArthur, G. A. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **2011**, *364*, 2507−2516.
(18) Kefford, R.; Arkenau, H.; Brown, M. P.; Millward, M.; Infante, J. R.; Long, G. V.; Ouellet, D.; Curtis, M.; Lebowitz, P. F.; Falchook, G. S. Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. *J. Clin. Oncol.* **2010**, *28* (Suppl.), Abstract8503.
(19) FDA approved vemurafenib on August 17, 2011. http://www.cancer.gov/cancertopics/druginfo/fda-vemurafenib.
(20) Yang, H.; Higgins, B.; Kolinsky, K.; Packman, K.; Go, Z.; Iyer, R.; Kolis, S.; Zhao, S.; Lee, R.; Grippo, J. F.; Schostack, K.; Simcox, M. E.; Heimbrook, D.; Bollag, G.; Su, F. RG7204 (PLX4032), a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. *Cancer Res.* **2010**, *70*, 5518−5527.
(21) Heidorn, S. J.; Milagre, C.; Whittaker, S.; Nourry, A.; Niculescu-Duvas, I.; Dhomen, N.; Hussain, J.; Reis-Filho, J. S.; Springer, C. J.; Pritchard, C.; Marais, R. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* **2010**, *140*, 209−221.
(22) Hatzivassiliou, G.; Song, K.; Yen, I.; Brandhuber, B. J.; Anderson, D. J.; Alvarado, R.; Ludlam, M. J. C.; Stokoe, D.; Gloor, S. L.; Vigers, G.; Morales, T.; Aliagas, I.; Liu, B.; Sideris, S.; Hoeflich, K. P.; Jaiswal, B. S.; Seshagiri, S.; Koeppen, H.; Belvin, M.; Friedman, L. S.; Malek, S. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* **2010**, *464*, 431−435.
(23) Poulikakos, P. I.; Zhang, C.; Bollag, G.; Shokat, K. M.; Rosen, N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* **2010**, *464*, 427−430.
(24) Yun, C.-H.; Boggon, T. J.; Li, Y.; Woo, M. S.; Greulich, H.; Meyerson, M.; Eck, M. J. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell* **2007**, *11*, 217−227.
(25) Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J. Biol. Chem.* **2002**, *277*, 46265−46272.
(26) Tokarski, J. S.; Newitt, J. A.; Chang, C. Y. J.; Cheng, J. D.; Wittekind, M.; Kiefer, S. E.; Kish, K.; Lee, F. Y. F.; Borzillerri, R.; Lombardo, L. J.; Xie, D.; Zhang, Y.; Klei, H. E. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res.* **2006**, *66*, 5790−5797.
(27) Getlik, M.; Grütter, C.; Simard, J. R.; Klüter, S.; Rabiller, M.; Rode, H. B.; Robubi, A.; Rauh, D. Hybrid compound design to overcome the gatekeeper T338M mutation in cSrc. *J. Med. Chem.* **2009**, *52*, 3915−3926.
(28) Harris, P. A.; Boloor, A.; Cheung, M.; Kumar, R.; Crosby, R. M.; Davis-Ward, R. G.; Epperly, A. H.; Hinkle, K. W.; Hunter, R. N.; Johnson, J. H.; Knick, V. B.; Laudeman, C. P.; Luttrell, D. K.; Mook, R. A.; Nolte, R. T.; Rudolph, S. K.; Szewczyk, J. R.; Truesdale, A. T.; Veal, J. M.; Wang, L.; Stafford, J. A. Discovery of 5-[[4-[(2,3-dimethyl-2*H*-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor. *J. Med. Chem.* **2008**, *51*, 4632−4640.
(29) Knowles, P. P.; Murray-Rust, J.; Kjaer, S.; Scott, R. P.; Hanrahan, S.; Santoro, M.; Ibáñez, C. F.; McDonald, N. Q. Structure and chemical inhibition of the RET tyrosine kinase domain. *J. Biol. Chem.* **2006**, *281*, 33577−33587.
(30) Cui, J. J.; Tran-Dubé, M.; Shen, H.; Nambu, M.; Kung, P.-P.; Pairish, M.; Jia, L.; Meng, J.; Funk, L.; Botrous, I.; McTigue, M.; Grodsky, N.; Ryan, K.; Padrique, E.; Alton, G.; Timofeevski, S.; Yamazaki, S.; Li, Q.; Zou, H.; Christensen, J.; Mroczkowski, B.; Bender, S.; Kania, R. S.; Edwards, M. P. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). *J. Med. Chem.* **2011**, *54*, 6342−6363.
(31) Ostojic, A.; Vrhovac, R.; Verstovsek, S. Ruxolitinib: a new JAK1/2 inhibitor that offers promising options for treatment of myelofibrosis. *Future Oncol.* **2011**, *7*, 1035−1043.
(32) Gajiwala, K. S.; Wu, J. C.; Christensen, J.; Deshmukh, G. D.; Diehl, W.; DiNitto, J. P.; English, J. M.; Greig, M. J.; He, Y.-A.; Jacques, S. L.; Lunney, E. A.; McTigue, M.; Molina, D.; Quenzer, T.; Wells, P. A.; Yu, X.; Zhang, Y.; Zou, A.; Emmett, M. R.; Marshall, A. G.; Zhang, H.-M.; Demetri, G. D. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 1542−1547.
(33) Kutach, A. K.; Villaseñor, A. G.; Lam, D.; Belunis, C.; Janson, C.; Lok, S.; Hong, L.-N.; Liu, C.-M.; Deval, J.; Novak, T. J.; Barnett, J. W.; Chu, W.; Shaw, D.; Kuglstatter, A. Crystal structures of IL-2-inducible T cell kinase complexed with inhibitors: insights into rational drug design and activity regulation. *Chem. Biol. Drug Des.* **2010**, *76*, 154−163.
(34) Nagar, B.; Bornmann, W. G.; Pellicena, P.; Schindler, T.; Veach, D. R.; Miller, W. T.; Clarkson, B.; Kuriyan, J. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* **2002**, *62*, 4236−4243.
(35) Cowan-Jacob, S. W.; Fendrich, G.; Floersheimer, A.; Furet, P.; Liebetanz, J.; Rummel, G.; Rheinberger, P.; Centeleghe, M.; Fabbro, D.; Manley, P. W. Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2007**, *63*, 80−93.
(36) Nagar, B.; Hantschel, O.; Young, M. A.; Scheffzek, K.; Veach, D.; Bornmann, W.; Clarkson, B.; Superti-Furga, G.; Kuriyan, J. Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* **2003**, *112*, 859−871.
(37) Zhang, J.; Adrián, F. J.; Jahnke, W.; Cowan-Jacob, S. W.; Li, A. G.; Iacob, R. E.; Sim, T.; Powers, J.; Dierks, C.; Sun, F.; Guo, G.-R.; Ding, Q.; Okram, B.; Choi, Y.; Wojciechowski, A.; Deng, X.; Liu, G.; Fendrich, G.; Strauss, A.; Vajpai, N.; Grzesiek, S.; Tuntland, T.; Liu, Y.; Bursulaya, B.; Azam, M.; Manley, P. W.; Engen, J. R.; Daley, G. Q.; Warmuth, M.; Gray, N. S. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature* **2010**, *463*, 501−506.
(38) Mol, C. D.; Dougan, D. R.; Schneider, T. R.; Skene, R. J.; Kraus, M. L.; Scheibe, D. N.; Snell, G. P.; Zou, H.; Sang, B.-C.; Wilson, K. P. Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. *J. Biol. Chem.* **2004**, *279*, 31655−31663.
(39) Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* **2004**, *116*, 855−867.
(40) Weisberg, E.; Manley, P. W.; Breitenstein, W.; Brüggen, J.; Cowan-Jacob, S. W.; Ray, A.; Huntly, B.; Fabbro, D.; Fendrich, G.; Hall-Meyers, E.; Kung, A. L.; Mestan, J.; Daley, G. Q.; Callahan, L.; Catley, L.; Cavazza, C.; Azam, M.; Mohammed, A.; Neuberg, D.; Wright, R. D.; Gilliland, D. G.; Griffin, J. D. Characterization of

AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* **2005**, *7*, 129−141.
(41) Kania, R. S. In *Kinase Inhibitor Drugs*; Li, R., Stafford, J. A., Eds.; Wiley: Hoboken, NJ, 2009; pp 167−201.
(42) Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib). *Cancer Res.* **2004**, *64*, 6652.
(43) Bollag, G.; Hirth, P.; Tsai, J.; Zhang, J.; Ibrahim, P. N.; Cho, H.; Spevak, W.; Zhang, C.; Zhang, Y.; Habets, G.; Burton, E. A.; Wong, B.; Tsang, G.; West, B. L.; Powell, B.; Shellooe, R.; Marimuthu, A.; Nguyen, H.; Zhang, K. Y. J.; Artis, D. R.; Schlessinger, J.; Su, F.; Higgins, B.; Iyer, R.; D'Andrea, K.; Koehler, A.; Stumm, M.; Lin, P. S.; Lee, R. J.; Grippo, J.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthur, G. A.; Sosman, J. A.; Chapman, P. B.; Flaherty, K. T.; Xu, X.; Nathanson, K. L.; Nolop, K. Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* **2010**, *467*, 596−599.
(44) Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. Dissecting specificity in the Janus kinases: the structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. *J. Mol. Biol.* **2009**, *387*, 219−232.
(45) Takle, A. K.; Brown, M. J. B.; Davies, S.; Dean, D. K.; Francis, G.; Gaiba, A.; Hird, A. W.; King, F. D.; Lovell, P. J.; Naylor, A.; Reith, A. D.; Steadman, J. G.; Wilson, D. M. The identification of potent and selective imidazole-based inhibitors of B-Raf kinase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 378−381.
(46) King, A. J.; Patrick, D. R.; Batorsky, R. S.; Ho, M. L.; Do, H. T.; Zhang, S. Y.; Kumar, R.; Rusnak, D. W.; Takle, A. K.; Wilson, D. M.; Hugger, E.; Wang, L.; Karreth, F.; Lougheed, J. C.; Lee, J.; Chau, D.; Stout, T. J.; May, E. W.; Rominger, C. M.; Schaber, M. D.; Luo, L.; Lakdawala, A. S.; Adams, J. L.; Contractor, R. G.; Smalley, K. S. M.; Herlyn, M.; Morrissey, M. M.; Tuveson, D. A.; Huang, P. S. Demonstration of a genetic therapeutic index for tumors expressing oncogenic BRAF by the kinase inhibitor SB-590885. *Cancer Res.* **2006**, *66*, 11100−11105.
(47) Smith, A. L.; DeMorin, F. F.; Paras, N. A.; Huang, Q.; Petkus, J. K.; Doherty, E. M.; Nixey, T.; Kim, J. L.; Whittington, D. A.; Epstein, L. F.; Lee, M. R.; Rose, M. J.; Babij, C.; Fernando, M.; Hess, K.; Le, Q.; Beltran, P.; Carnahan, J. Selective inhibitors of the mutant B-Raf pathway: discovery of a potent and orally bioavailable aminoisoquinoline. *J. Med. Chem.* **2009**, *52*, 6189−6192.
(48) Carnahan, J.; Beltran, P. J.; Babij, C.; Le, Q.; Rose, M. J.; Vonderfecht, S.; Kim, J. L.; Smith, A. L.; Nagapudi, K.; Broome, M. A.; Fernando, M.; Kha, H.; Belmontes, B.; Radinsky, R.; Kendall, R.; Burgess, T. L. Selective and potent Raf inhibitors paradoxically stimulate normal cell proliferation and tumor growth. *Mol. Cancer Ther.* **2010**, *9*, 2399−2410.
(49) Hoeflich, K. P.; Herter, S.; Tien, J.; Wong, L.; Berry, L.; Chan, J.; O'Brien, C.; Modrusan, Z.; Seshagiri, S.; Lackner, M.; Stern, H.; Choo, E.; Murray, L.; Friedman, L. S.; Belvin, M. Antitumor efficacy of the novel RAF inhibitor GDC-0879 is predicted by BRAFV600E mutational status and sustained extracellular signal-regulated kinase/mitogen-activated protein kinase pathway suppression. *Cancer Res.* **2009**, *69*, 3042−3051.
(50) Wilhelm, S. M.; Carter, C.; Tang, L.; Wilkie, D.; McNabola, A.; Rong, H.; Chen, C.; Zhang, X.; Vincent, P.; McHugh, M.; Cao, Y.; Shujath, J.; Gawlak, S.; Eveleigh, D.; Rowley, B.; Liu, L.; Adnane, L.; Lynch, M.; Auclair, D.; Taylor, I.; Gedrich, R.; Voznesensky, A.; Riedl, B.; Post, L. E.; Bollag, G.; Trail, P. A. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res.* **2004**, *64*, 7099−7109.
(51) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **2008**, *26*, 127−132.
(52) Tsai, J.; Lee, J. T.; Wang, W.; Zhang, J.; Cho, H.; Mamo, S.; Bremer, R.; Gillette, S.; Kong, J.; Haass, N. K.; Sproesser, K.; Li, L.; Smalley, K. S. M.; Fong, D.; Zhu, Y.-L.; Marimuthu, A.; Nguyen, H.; Lam, B.; Liu, J.; Cheung, I.; Rice, J.; Suzuki, Y.; Luu, C.; Settachatgul, C.; Shellooe, R.; Cantwell, J.; Kim, S.-H.; Schlessinger, J.; Zhang, K. Y. J.; West, B. L.; Powell, B.; Habets, G.; Zhang, C.; Ibrahim, P. N.; Hirth, P.; Artis, D. R.; Herlyn, M.; Bollag, G. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3041−3046.
(53) Hall-Jackson, C. A.; Eyers, P. A.; Cohen, P.; Goedert, M.; Boyle, F. T.; Hewitt, N.; Plant, H.; Hedge, P. Paradoxical activation of Raf by a novel Raf inhibitor. *Chem. Biol.* **1999**, *6*, 559−568.
(54) Wang, X.; Berger, D. M.; Salaski, E. J.; Torres, N.; Dutia, M.; Hanna, C.; Hu, Y.; Levin, J. I.; Powell, D.; Wojciechowicz, D.; Collins, K.; Frommer, E.; Lucas, J. Indazolylpyrazolopyrimidine as highly potent B-Raf inhibitors with in vivo activity. *J. Med. Chem.* **2010**, *53*, 7874−7878.
(55) Besteman, E.; Bienvenu, J.-G.; Chaudhary, I.; Cukierski, M.; Marsh, B.; Surprenant, B. Epithelial Hyperplasia Induced by a Selective B-Raf Inhibitor, WYE-130600. *Proceedings of the 101st Annual Meeting of the AACR*; American Association for Cancer Research: Philadelphia, PA, 2010; Abstract 1677.
(56) Wisler, J. A.; Afshari, C.; Fielden, M.; Zimmermann, C.; Taylor, S.; Carnahan, J.; Vonderfecht, S. Raf inhibition causes extensive multiple tissue hyperplasia and urinary bladder neoplasia in the rat. *Toxicol. Pathol.* **2011**, *39*, 809−822.
(57) Rajakulendran, T.; Sahmi, M.; Lefrancois, M.; Sicheri, F.; Therrien, M. A dimerization-dependent mechanism drives RAF catalytic activation. *Nature* **2009**, *461*, 542−545.
(58) Zarrinkar, P. P.; Gunawardane, R. N.; Cramer, M. D.; Gardner, M. F.; Brigham, D.; Belli, B.; Karaman, M. W.; Pratz, K. W.; Pallares, G.; Chao, Q.; Sprankle, K. G.; Patel, H. K.; Levis, M.; Armstrong, R. C.; James, J.; Bhagwat, S. S. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). *Blood* **2009**, *114*, 2984−2992.
(59) Smalley, K. S. M. PLX-4032, a small-molecule B-Raf inhibitor for the potential treatment of malignant melanoma. *Curr. Opin. Invest. Drugs* **2010**, *11*, 699−706.
(60) Joseph, E. W.; Pratilas, C. A.; Poulikakos, P. I.; Tadi, M.; Wang, W.; Taylor, B. S.; Halilovic, E.; Persaud, Y.; Xing, F.; Viale, A.; Tsai, J.; Chapman, P. B.; Bollag, G.; Solit, D. B.; Rosen, N. The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 14903−14908.
(61) Kaplan, F. M.; Shao, Y.; Mayberry, M. M.; Aplin, A. E. Hyperactivation of MEK-ERK1/2 signaling and resistance to apoptosis induced by the oncogenic B-RAF inhibitor, PLX4720, in mutant N-RAS melanoma cells. *Oncogene* **2011**, *30*, 366−371.
(62) Halaban, R.; Zhang, W.; Bacchiocchi, A.; Cheng, E.; Parisi, F.; Ariyan, S.; Krauthammer, M.; McCusker, J. P.; Kluger, Y.; Sznol, M. PLX4032, a selective BRAF(V600E) kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAF melanoma cells. *Pigm. Cell Melanoma Res.* **2010**, *23*, 190−200.
(63) Farrar, M. A.; Alberol-Ila, J.; Perlmutter, R. M. Activation of the Raf-1 kinase cascade by coumermycin-induced dimerization. *Nature* **1996**, *383*, 178−181.
(64) Luo, Z.; Tzivion, G.; Belshaw, P. J.; Vavvas, D.; Marshall, M.; Avruch, J. Oligomerization activates c-Raf-1 through a Ras-dependent mechanism. *Nature* **1996**, *383*, 181−185.
(65) Weber, C. K.; Slupsky, J. R.; Kalmes, H. A.; Rapp, U. R. Active Ras induces heterodimerization of cRaf and BRaf. *Cancer Res.* **2001**, *61*, 3595.
(66) Garnett, M. J.; Rana, S.; Paterson, H.; Barford, D.; Marais, R. Wild-type and mutant B-RAF activate C-RAF through distinct mechanisms involving heterodimerization. *Mol. Cell* **2005**, *20*, 963−969.

(67) Rushworth, L. K.; Hindley, A. D.; O'Neill, E.; Kolch, W. Regulation and role of Raf-1/B-Raf heterodimerization. *Mol. Cell. Biol.* **2006**, *26*, 2262.
(68) Dubauskas, Z.; Kunishige, J.; Prieto, V. G.; Jonasch, E.; Hwu, P.; Tannir, N. M. Cutaneous squamous cell carcinoma and inflammation of actinic keratoses associated with sorafenib. *Clin. Genitourin. Cancer* **2009**, *7*, 20−23.
(69) More recent examples of B-Raf/type IIB inhibitor structures show both protomers of the dimer occupied (3SKC, 3TV4, 3TV6, 4E4X, 4EHG, 4EHE). These crystallizations were performed at much lower protein concentrations than those used for the PLX4032 structure, which utilized a highly soluble, 16 site directed mutant protein, and may therefore have allowed for full occupancy of the kinase inhibitor binding sites (W. Voegtli, personal communication).
(70) Dhomen, N.; Marais, R. BRAF signaling and targeted therapies in melanoma. *Hematol. Oncol. Clin. North Am.* **2009**, *23*, 529−545 , ix.
(71) Roskoski, R., Jr. RAF protein-serine/threonine kinases: structure and regulation. *Biochem. Biophys. Res. Commun.* **2010**, *399*, 313−317.
(72) Udell, C. M.; Rajakulendran, T.; Sicheri, F.; Therrien, M. Mechanistic principles of RAF kinase signaling. *Cell. Mol. Life Sci.* **2010**, *68*, 553−565.
(73) Infante, J. R.; Falchook, G. S.; Lawrence, D. P.; Weber, R. F.; Kefford, R. F.; Bendell, J. C.; Kurzrock, R.; Shapiro, G.; Kudchadkar, R. R.; Long, G. V.; Burris, H. A.; Kim, K. B.; Clements, A.; Peng, S.; Yi, B.; Allred, A. J.; Ouellet, D.; Patel, K.; Lebowitz, P. F.; Flaherty, K. T. Phase I/II study to assess safety, pharmacokinetics, and efficacy of the oral MEK 1/2 inhibitor GSK1120212 (GSK212) dosed in combination with the oral BRAF inhibitor GSK2118436 (GSK436). *J. Clin. Oncol.* **2011**, *29* (Suppl.), AbstractCRA8503.
(74) Corcoran, R. B.; Settleman, J.; Engelman, J. A. Potential therapeutic strategies to overcome acquired resistance to BRAF or MEK inhibitors in BRAF mutant cancers. *Oncotarget* **2011**, *2*, 336−346.
(75) Poulikakos, P. I.; Persaud, Y.; Janakiraman, M.; Kong, X.; Ng, C.; Moriceau, G.; Shi, H.; Atefi, M.; Titz, B.; Gabay, M. T.; Salton, M.; Dahlman, K. B.; Tadi, M.; Wargo, J. A.; Flaherty, K. T.; Kelley, M. C.; Misteli, T.; Chapman, P. B.; Sosman, J. A.; Graeber, T. G.; Ribas, A.; Lo, R. S.; Rosen, N.; Solit, D. B. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* **2011**, *480*, 387−390.
(76) Hu, J.; Yu, H.; Kornev, A. P.; Zhao, J.; Filbert, E. L.; Taylor, S. S.; Shaw, A. S. Mutation that blocks ATP binding creates a pseudokinase stabilizing the scaffolding function of kinase suppressor of Ras, CRAF and BRAF. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6067−6072.
(77) McKay, M. M.; Ritt, D. A.; Morrison, D. K. RAF inhibitor-induced KSR1/B-RAF binding and its effects on ERK cascade signaling. *Curr. Biol.* **2011**, *21*, 563−568.