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Selective Inhibitors of the Mutant B-Raf Pathway: Discovery of a Potent and Orally Bioavailable Aminoisoquinoline

Adrian L. Smith,*[,†] Frenel F. DeMorin,[†] Nick A. Paras,[†] Qi Huang,[†] Jeffrey K. Petkus,[†] Elizabeth M. Doherty,[†] Thomas Nixey,[†] Joseph L. Kim,[‡] Douglas A. Whittington,[‡] Linda F. Epstein,[‡] Matthew R. Lee,[§] Mark J. Rose,[∥] Carol Babij,[¶] Manory Fernando,[¶] Kristen Hess,[¶] Quynh Le,[¶] Pedro Beltran,[¶] and Josette Carnahan[¶]

Departments of [†]Medicinal Chemistry, [§]Molecular Structure, [∥]Pharmacokinetics and Drug Metabolism, and [¶]Oncology Research, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320-1799, and [‡]Department of Molecular Structure, Amgen Inc., 360 Binney Street, Cambridge, Massachusetts 02142

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Abstract: The discovery and optimization of a novel series of aminoisoquinolines as potent, selective, and efficacious inhibitors of the mutant B-Raf pathway is presented. The N-linked pyridylpyrimidine benzamide **2** was identified as a potent, modestly selective inhibitor of the B-Raf enzyme. Replacement of the benzamide with an aminoisoquinoline core significantly improved kinase selectivity and imparted favorable pharmacokinetic properties, leading to the identification of **1** as a potent antitumor agent in xenograft models.

B-Raf is a Ras-activated serine/threonine protein kinase that plays a central role in transducing signals from cell surface receptors to the nucleus through the mitogen-activated protein kinase (MAPK[a]) pathway (Ras/B-Raf/MEK/ERK). This pathway has long been associated with human cancers due to frequent oncogenic mutations identified in its members.[1] Ras mutations occur in ∼15% of cancers, and B-Raf is also mutated at high frequency in certain cancers including melanoma (∼66%), thyroid cancer (35−70%), colorectal cancer (5−20%), and ovarian cancer (∼30%).[2] Over 45 B-Raf mutations have been described, but by far the most common mutation (∼90%) is Val600 → Glu in the activation loop. This mutation renders the kinase constitutively active. Given the high incidence of B-Raf mutations in melanoma, together with few effective treatments currently being available for metastatic melanoma, selectively targeting the mutant B-Raf pathway offers clinicians an attractive new approach to treating this deadly disease.[3] There have been few reports to date of selective inhibitors of the mutant B-Raf pathway in an in vitro cellular setting that demonstrate robust antitumor efficacy in vivo against tumors driven by the mutant B-Raf pathway.[4] We herein disclose work leading to the discovery of **1** as such an agent.

A high-throughput screen against recombinant ^{V600E}B-Raf led to the identification of a series of pyridylpyrimidine and related aminoquinazoline benzamides that potently inhibit the enzyme and potently inhibit downstream phosphorylation of ERK in the A375 human melanoma cell line bearing the V600E mutation in vitro. Compounds **2** and **3** are representative of these leads (Figure 1).[5] While these leads were strong inhibitors of B-Raf,[6] they also potently inhibited other kinases with smaller gatekeeper residues and known DFG-out binding modes, including p38α, KDR, Lck, and Tie-2 (Table 1).[5,7] In addition, rat pharmacokinetic properties were suboptimal (short $t_{1/2}$, moderate clearance, and generally low to modest bioavailability; Table 1).

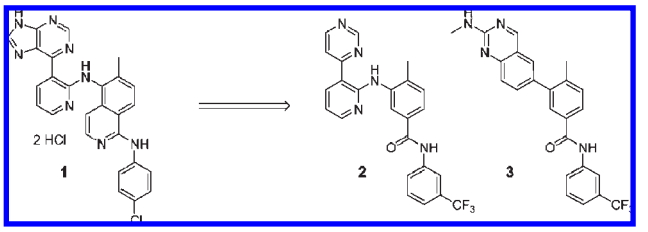

Figure 1. Structures of a potent, selective Raf inhibitor (**1**) and the screening leads **2** and **3** from which it was derived.

Modeling of **2** into the B-Raf crystal structure[8] indicated that it is well accommodated in the ATP-binding site of the kinase domain of the protein, where the activation loop folds out (DFG-out)[9] to open up an extended hydrophobic pocket in which the trifluoromethylaniline moiety forms favorable hydrophobic interactions. An examination of the amino acid residues surrounding the inhibitor **2** in B-Raf indicated a small unoccupied cleft in the vicinity of the conserved X-DFG motif at the start of the activation loop adjacent to the amide linkage of the benzamide of **2** (Figure 2). A glycine residue (Gly593) immediately precedes the DFG sequence in B-Raf (X = G), but for many other kinases the corresponding residue is larger (X = L, C, A, and A for p38α, KDR, Lck, and Tie-2, respectively). The resulting side chain from the residue X is directed toward this unoccupied cleft, and modeling suggested that filling this small unoccupied pocket would significantly improve the kinase selectivity of **2**. The aminoisoquinoline **12a** was therefore designed and prepared (Schemes 1 and 2) to achieve this while maintaining key hydrogen bonding interactions through the endocyclic and exocyclic nitrogens of the aminoisoquinoline to Asp594 and Glu501, respectively.[6]

The aminoisoquinoline **12a** exhibited a reduction in activity against the recombinant B-Raf enzyme but was only slightly less active in inhibiting phosphorylation of ERK in an A375 cellular assay (Table 1). It has been noted elsewhere that Raf inhibitors can result in counterintuitive activation of Raf kinase activity;[10] therefore, structure−activity relationships were principally derived from activity in the A375 cellular assay.

Notably, activity of **12a** against p38α and KDR was markedly attenuated, as predicted from the initial modeling studies with **2**. Selectivity against Lck and Tie-2 was more modest as might be predicted from the smaller side chain of the alanine residue preceding the DFG motif in these kinases. However, a comparison of the extended hydrophobic pockets of the B-Raf, Lck, and Tie-2 structures suggested more room in the vicinity of the 4-position of the aniline side chain for

*To whom correspondence should be addressed. Phone: 805-447-4662. Fax: 805-480-3016. E-mail: adrians@amgen.com.

[a]Abbreviations: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; SAR, structure−activity relationship; PD, pharmacodynamic.

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Table 1. Activity of Compounds in B-Raf Enzyme and Cellular Assays, Selected Kinase Counterscreening Data, and Rat Pharmacokinetic Parameters

		enzyme inhibition, IC_{50} (nM)					rat pharmacokinetics[c]			
compd	A375 pERK inh, IC_{50} (nM)[a]	B-Raf[b]	p38α	KDR	Lck	Tie-2	$t_{1/2}$ (h)	CL ((L/h)/kg)	V_{ss} (L/kg)	F (%)
2	15	0.46	12	4.2	3.5	3.6	1.3	2.1	1.9	16
3	27	1.7	3.5	1.0	0.27	1.8	2.3	1.6	3.6	24
12a	53	17	>1600	760	81	47	4.5	1.2	3.7	22
12b	34	3.4	>1600	>25000	280	520	3.5	0.67	2.2	83
13	35	56	>1600	300	12	24	4.7	0.29	1.7	100
14	44	39	>1600	>1000	150	400	2.7	0.11	0.37	100
15	26	18	450	420	210	8.1	2.8	0.73	2.2	76
16	29	110	350	170	23	5.8	2.9	0.33	0.72	100
18	270	5.4	>1600	>25000	110	>1000				
19	22	70	110	230	21	200	3.9	1.8	5.5	24
20	27	21	51	240	78	>1000	4.8	0.65	4.3	55
1	1.8	1.6	>1600	1000	83	120	3.5	0.40	1.9	80

[a] In-cell Western assay performed using LiCOR Odyssey scanner; inh = inhibition. [b] Recombinant ^{V600E}B-Raf kinase domain. [c] Compounds were dosed at 2 mg/kg iv and 5 mg/kg po in male Sprague–Dawley rats. $t_{1/2}$ is the plasma elimination-phase half-life (iv).

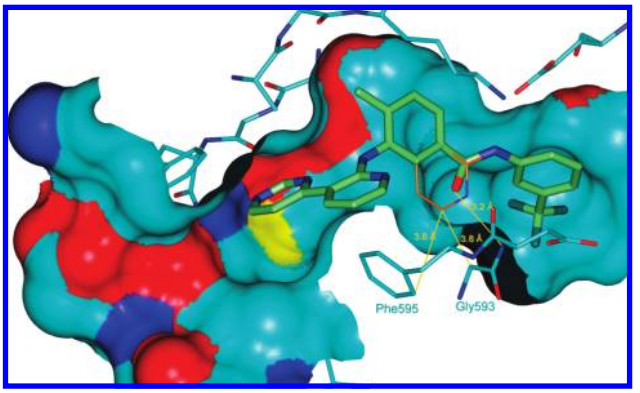
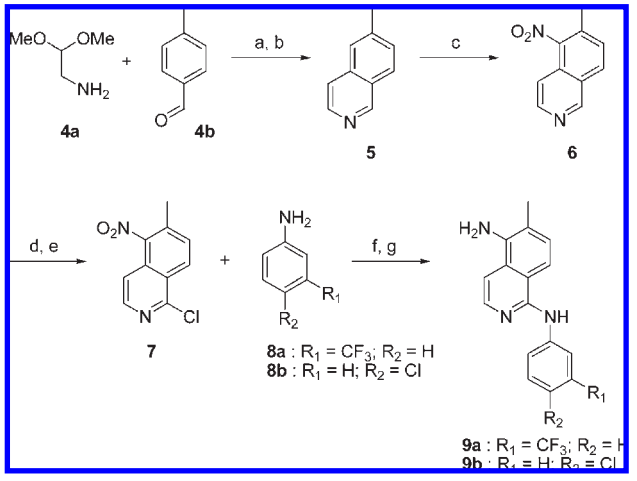


Figure 2. Model of **2** docked into the DFG-out structure of B-Raf. The isoquinoline core is overlaid onto the structure of **2**, where it occupies a small hydrophobic cleft unavailable in other kinases containing any residue larger than a glycine at the position corresponding to Gly593.

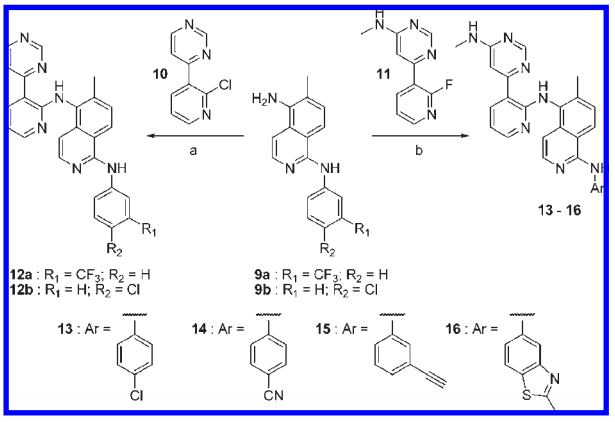
Scheme 1. Preparation of Aminoisoquinoline Intermediates[a]



[a] Reagents and conditions: (a) $CHCl_3$, reflux; (b) EtOCOCl, $P(OEt)_3$, $CHCl_3$, 0 → 22 °C, 23 h, then $TiCl_4$, $CHCl_3$, reflux, 12 h, 78% (2 steps); (c) KNO_3, H_2SO_4, 0 °C, 1.5 h, 95%; (d) mCPBA, CH_2Cl_2, 0 °C, 4 h; (e) $POCl_3$, CH_2Cl_2, 70 °C, 3 h, 86% (2 steps); (f) ^{i}PrOH, microwave, 170 °C, 16 min, 79–84%; (g) $SnCl_2$, EtOH, 75 °C, 16 h, 66–72%.

B-Raf compared with Lck and Tie-2 and therefore offered a potential way of further improving the kinase selectivity of **12a**. The corresponding 4-chloroaniline **12b** was therefore prepared (Scheme 2). Not only did **12b** demonstrate an improvement in activity against B-Raf while maintaining excellent selectivity against p38α and KDR, selectivity against Lck and Tie-2 was also markedly improved.[6] A significant improvement in rat pharmacokinetics was noted in going from the benzamide **2** to the aminoisoquinoline **12a** (Table 1). The aniline substituent was identified as a major source of oxidative metabolism/clearance, and changes to the aniline substitution pattern (**12a** → **12b**) resulted in very favorable pharmacokinetic profiles (Table 1).

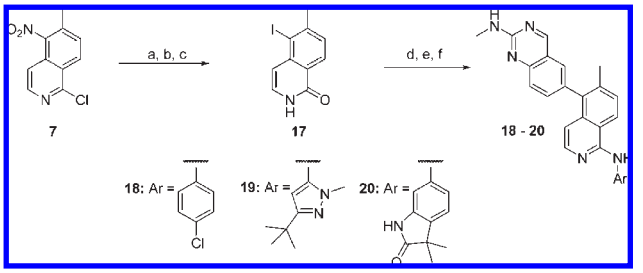
Scheme 2. Preparation of Aminoisoquinolines[a]



[a] Reagents and conditions: (a) LiHMDS, $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, THF, 70 °C, 16 h, 29–56%; (b) LiHMDS, THF, 45 °C, 1 h, 72%.

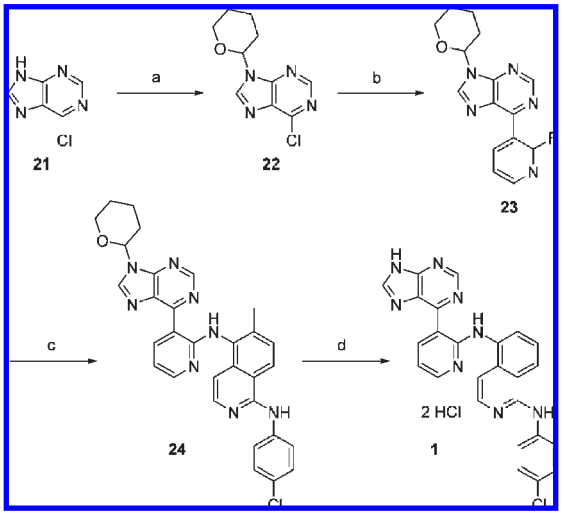
To improve potency, attention was focused on the pyrimidine portion of the molecule. This forms a bidentate hydrogen bonding interaction in the hinge region of the B-Raf protein (i.e., between the pyrimidine N1 and Cys532 (NH) and between the pyrimidine C2–H and Gln530 (C=O)).[11] It was reasoned that an additional hydrogen bond to Cys532 (C=O) would be accessible with the aminomethyl group of **13** (Scheme 2). This turned out to be detrimental to activity against the B-Raf enzyme but interestingly maintained potency in the A375 pERK cellular assay. However, this change was detrimental to selectivity, particularly against Lck and Tie-2. Further SAR exploration of the aniline substituent of **13** using analogous chemistry demonstrated the ability to modulate kinase selectivity with **14**–**16** while retaining equivalent B-Raf/A375 cellular activity and favorable pharmacokinetic properties (Table 1).

Scheme 3. Preparation of Aminoquinazoline Derivatives[a]



[a] Reagents and conditions: (a) THF/5 N HCl (1:1), reflux, 14 h, 87%; (b) H_2, Pd/C, EtOH, 94%; (c) $NaNO_2$, conc HCl, then KI, 88%; (d) *N*-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinazolin-2-amine, $Pd(PPh_3)_4$ (cat.), Na_2CO_3, 1,4-dioxane/water, microwave, 160 °C, 15 min, 52%; (e) $POCl_3$, neat, 100 °C, 2 h, 82%; (f) Ar-NH_2, $Pd_2(dba)_3$ (cat.), DavePhos, LiHMDS, 1,4-dioxane, microwave, 150 °C, 10 min, 40−59%.

Scheme 4. Preparation of Pyridylpurine Aminoisoquinoline **1**[a]



[a] Reagents and conditions: (a) 3,4-dihydro-2*H*-pyran, TsOH (cat.), EtOAc, reflux, 1 h, 99%; (b) 2-fluoropyridin-3-ylboronic acid, A-Phos (cat.), KOAc, EtOH, water, 80 °C, 2 h, 96%; (c) **9b**, LiHMDS, THF, 0 °C, 1 h; (d) HCl, water, reflux, 1 h, 73% (two steps).

Replacement of the aminopyridylpyrimidine portion of **13** with the aminoquinazoline fragment from the screening lead **3** gave the corresponding analogue **18** (Scheme 3). While **18** retained good activity against the B-Raf enzyme accompanied with good kinase selectivity, a notable drop in A375 cellular activity was observed. Cellular activity could be restored by varying the aniline substituent, for example with **19** and **20**, but at the expense of kinase selectivity (Table 1).

Rationalizing that rotational freedom of the aminomethyl group of **13**−**16** may have introduced an entropic penalty for binding to B-Raf, it was conformationally locked to form a purine. The corresponding pyridylpurine **1** was therefore prepared (Scheme 4). This resulted in a significant improvement in potency against the B-Raf enzyme and in the A375 pERK cellular assay (Table 1). A cocrystal structure of **1** with B-Raf revealed that the purine not only forms an additional hydrogen bonding interaction with Cys532 but also allows a favorable π-stacking interaction with the side chain of Trp531 which is probably responsible for the bulk of the improvement in potency (Figure 3).[12] Additionally, kinase selectivity relative to **13** was greatly increased and **1** displayed a very favorable rat pharmacokinetic profile.

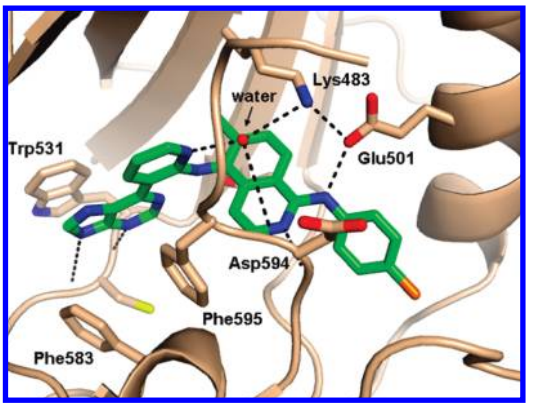


Figure 3. Crystal structure of **1** solved in complex with B-Raf at 2.7 Å resolution.

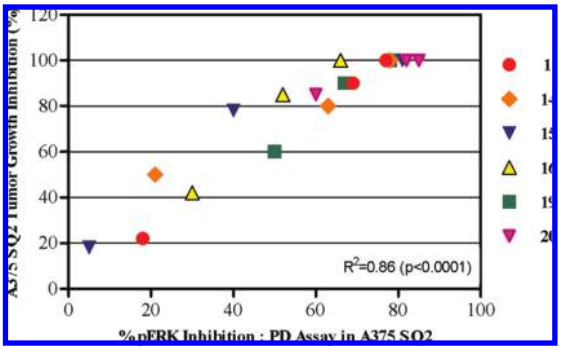


Figure 4. Strong correlation observed between in vivo ^{V600E}B-Raf MAPK pathway signaling inhibition 6 h postdose orally and A375 tumor growth inhibition in A375 SQ2 tumor-bearing mice dosed orally for 14 days (same doses).

A pharmacodynamic (PD) assay in mouse measuring tumor biochemical target coverage (in a dose and time dependent manner) in A375 tumor lysate was performed to help in designing dosing schedule for compounds in efficacy studies.[13] The 6 h time point following oral dosing across multiple doses and compounds was found to be highly predictive of in vivo efficacy, as illustrated by the significant correlation ($R^2 = 0.86$) observed between in vivo pERK inhibition and A375 tumor growth inhibition across all Raf inhibitors tested (Figure 4).

Compound **1** was profiled in the A375 SQ2 xenograft model[13] in mouse based on its in vitro potency, kinase selectivity, and favorable rat pharmacokinetic profile. Pharmacokinetics in mouse were similar to those observed in rat (CD1 mouse: $t_{1/2}$ = 3.3 h; CL = 0.19 (L/h)/kg; V_{ss} = 1.1 L/kg; F = 79%). The ability of **1** to modulate ERK phosphorylation levels in the tumor was demonstrated in the A375 PD assay, where a 2.5 mg/kg dose administered orally resulted in 69% inhibition of ERK phosphorylation 6 h following a single dose, and a 5.0 mg/kg dose resulted in a corresponding 77% inhibition. Repeat oral dosing in an established A375 xenograft model in mouse[13,14] resulted in tumor growth inhibition with an ED_{50} of 1.3 mg/kg QD (AUC_{0-24h} = 2.7 μg·h/mL) and 85% tumor regression at 5 mg/kg QD (AUC_{0-24h} = 14 μg·h/mL) after dosing for 14 days.

In summary, a novel series of selective inhibitors of the mutant B-Raf pathway is disclosed. Identification of the lead compounds **2** and **3** as multikinase inhibitors that potently inhibited B-Raf kinase, combined with an understanding of how they bind in the target and off-target kinases, allowed a structure-based approach to guide the medicinal chemistry team. A relatively uncommon G-DFG motif in Raf provided

an opportunity for kinase selectivity that was exploited successfully with an aminoisoquinoline core. Additional selectivity was exploited through subtle differences in the extended hydrophobic pockets of the kinases, and potency was optimized through changes in the hinge-binding region through the introduction of a purine moiety. This led to the identification of **1** as a potent, highly selective inhibitor of Raf[6] that demonstrates potent antitumor activity in an in vivo melanoma model driven by the mutant B-Raf pathway. Although **1** was apparently well tolerated, this group will present elsewhere data that suggest potent Raf inhibitors can lead to compensatory activation of the MAPK pathway with undesirable functional consequences observed in normal tissues and certain other non-B-Raf mutant tumor models in vivo.

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Note Added after ASAP Publication. There was an error in the Abbreviations in the version of this paper published on September 18, 2009. The revised version was published on September 22, 2009.

Supporting Information Available: Synthesis details for compounds described herein, analytical data, X-ray crystallographic data, kinase counterscreening data for **1**, and PD-efficacy data for the compounds shown in Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Dibb, N. J.; Dilworth, S. M.; Mol, C. D. Switching of kinases: oncogenic activation of BRAF and the PDGFR family. *Nat. Rev. Cancer* **2004**, *4*, 718–727. (b) Chen, Z.; Gibson, T. B.; Robinson, F.; Silvestro, L.; Pearson, G.; Xu, B.; Wright, A.; Vanderbilt, C.; Cobb, M. H. MAP kinases. *Chem. Rev.* **2001**, *101*, 2449–2476.

(2) 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.

(3) (a) Thompson, N.; Lyons, J. Recent progress in targeting the Raf/MEK/ERK pathway in cancer drug discovery. *Curr. Opin. Pharmacol.* **2005**, *5*, 350–356. (b) Sebolt-Leopold, J. S.; Herrera, R. Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nat. Rev. Cancer* **2004**, *4*, 937–947. (c) Chang, F.; Steelman, L. S.; Shelton, J. G.; Lee, J. T.; Navolanic, P. M.; Blalock, W. L.; Franklin, R.; McCubrey, J. A. Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway. *Int. J. Oncol.* **2003**, *22*, 469–480.

(4) Plexxikon filed an IND in 2006 for PLX-4032. Exelixis filed an IND in 2006 for XL-2819. Both compounds are claimed to be selective B-Raf inhibitors. For examples of potent and selective B-Raf inhibitors, see: (a) 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. (b) 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. (c) Tang, J.; Hamajima, T.; Nakano, M.; Sato, H.; Dickerson, S. H.; Lackey, K. E. Knowledge-based design of 7-azaindoles as selective B-Raf inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4610–4614. (d) 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 $BRAF^{V600E}$ mutational status and sustained extracellular signal-regulated kinase/mitogen-activated protein kinase pathway suppression. *Cancer Res.* **2009**, *69*, 3042–3051.

(5) (a) Hodous, B. L.; Geuns-Meyer, S. D.; Hughes, P. E.; Albrecht, B. K.; Bellon, S.; Bready, J.; Caenepeel, S.; Cee, V. J.; Chaffee, S. C.; Coxon, A.; Emery, M.; Fretland, J.; Gallant, P.; Gu, Y.; Hoffman, D.; Johnson, R. E.; Kendall, R.; Kim, J. L.; Long, A. M.; Morrison, M.; Olivieri, P. R.; Patel, V. F.; Polverino, A.; Rose, P.; Tempest, P.; Wang, L.; Whittington, D. A.; Zhao, H. Evolution of a highly selective and potent 2-(pyridin-2-yl)-1,3,5-triazine Tie-2 kinase inhibitor. *J. Med. Chem.* **2007**, *50*, 611–626. (b) DiMauro, E. F.; Newcomb, J.; Nunes, J. J.; Bemis, J. E.; Boucher, C.; Buchanan, J. L.; Buckner, W. H.; Cee, V. J.; Chai, L.; Deak, H. L.; Epstein, L. F.; Faust, T.; Gallant, P.; Geuns-Meyer, S. D.; Gore, A.; Gu, Y.; Henkle, B.; Hodous, B. L.; Hsieh, F.; Huang, X.; Kim, J. L.; Lee, J. H.; Martin, M. W.; Masse, C. E.; McGowan, D. C.; Metz, D.; Mohn, D.; Morgenstern, K. A.; Oliveira-dos-Santos, A.; Patel, V. F.; Powers, D.; Rose, P. E.; Schneider, S.; Tomlinson, S. A.; Tudor, Y.-Y.; Turci, S. M.; Welcher, A. A.; White, R. D.; Zhao, H.; Zhu, L.; Zhu, X. Discovery of aminoquinazolines as potent, orally bioavailable inhibitors of Lck: synthesis, SAR, and in vivo anti-inflammatory activity. *J. Med. Chem.* **2006**, *49*, 5671–5686.

(6) No selectivity was observed for these compounds between ^{V600E}B-Raf and wild type B-Raf or C-Raf in enzyme assays in vitro.

(7) Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Biol.* **2002**, *9*, 268–272.

(8) Cancer Genome Project. 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.

(9) For references comparing DFG-in and DFG-out binding, see: (a) 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. (b) 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.

(10) (a) 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. (b) Hall-Jackson, C. A.; Goedert, M.; Hedge, P.; Cohen, P. Effect of SB 203580 on the activity of c-Raf in vitro and in vivo. *Oncogene* **1999**, *18*, 2047–2054.

(11) This type of heteroaromatic C−H···O═C interaction with the hinge region backbone has been observed with other kinase inhibitor complexes. See: (a) Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H. Y.; Lu, S.; Qian, L.; Han, W. C.; Shan, W.; Mitt, T.; Cai, Z. W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C. Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kellar, K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. Discovery of aminothiazole inhibitors of cyclin-dependent kinase 2: synthesis, X-ray crystallographic analysis, and biological activities. *J. Med. Chem.* **2002**, *45*, 3905–3927. (b) Pierce, A. C.; Sandretto, K. L.; Bemis, G. W. Kinase inhibitors and the case for CH···O hydrogen bonds in protein−ligand binding. *Proteins: Struct., Funct., Genet.* **2002**, *49*, 567–576.

(12) The cocrystal structure of B-Raf with **1** has been deposited in the Protein Data Bank with accession code 3IDP.

(13) Human A375 cells were selected in vivo by passaging twice subcutaneously in CD1 nu/nu mice to optimize for tumor take and growth. This cell line was designated A375 SQ2.

(14) Compound was dosed QD in a randomized, blinded experiment for 14 days starting 17 days postimplantation.