Bioorganic & Medicinal Chemistry Letters 20 (2010) 2998–3002

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and structure–activity relationships of novel biarylamine-based Met kinase inhibitors

David K. Williams *, Xiao-Tao Chen, Christine Tarby, Robert Kaltenbach, Zhen-Wei Cai, John S. Tokarski, Yongmi An, John S. Sack, Barri Wautlet, Johnni Gullo-Brown, Benjamin J. Henley, Robert Jeyaseelan, Kristen Kellar, Veeraswamy Manne, George L. Trainor, Louis J. Lombardo, Joseph Fargnoli, Robert M. Borzilleri

Bristol-Myers Squibb Research and Development, PO Box 4000, Princeton, NJ 08543-4000, USA

ARTICLE INFO

Article history:
Received 23 October 2009
Revised 8 January 2010
Accepted 11 January 2010
Available online 20 January 2010

Keywords:
c-Met kinase inhibitor
Kinase inhibitor
HGFR inhibitor
Met kinase

ABSTRACT

Biarylamine-based inhibitors of Met kinase have been identified. Lead compounds demonstrate nanomolar potency in Met kinase biochemical assays and significant activity in the Met-driven GTL-16 human gastric carcinoma cell line. X-ray crystallography revealed that these compounds adopt a bioactive conformation, in the kinase domain, consistent with that previously seen with 2-pyridone-based Met kinase inhibitors. Compound **9b** demonstrated potent in vivo antitumor activity in the GTL-16 human tumor xenograft model.

Met (c-Met, HGFR-hepatocyte growth factor receptor) is a glycosylated, heterodimeric receptor tyrosine kinase, which is primarily expressed on epithelial and endothelial cells.[1] The activation of Met is ligand dependent with the natural ligand being HGF/SF (hepatocyte growth factor/scatter factor).[2] The high-affinity binding of HGF/SF to Met results in receptor autophosphorylation and upregulation of Met kinase activity, which in turn activates a complex cell-signaling pathway that stimulates mitogenesis, motogenesis, and morphogenesis. Met signaling is implicated in a wide variety of human malignancies, including: colon, lung, prostate, gastric, and breast cancers.[3] This signaling contributes to oncogenesis and tumor progression in several human cancers and promotes aggressive cellular invasiveness that is strongly linked to tumor metastasis.[4] The activation of Met-mediated oncogenic signaling can occur through aberrant paracrine or autocrine HGF stimulation and/or receptor overexpression. Other modes of activation for Met include: activating mutations, gene rearrangement and/or amplification, as well as the interaction of Met with other receptor families.[4]

We have previously disclosed potent Met kinase inhibitors, which have focused on the utilization of malonamide **1**, acylurea **2**, or pyridone **3** groups tethered to suitable kinase inhibitor templates (Fig. 1).[5–7] These aminopyridine (AP) and pyrrolopyridine (PP) templates have been shown, by X-ray crystal structure analysis, to serve as a replacement for the adenine group of ATP in establishing the critical hydrogen-bonding array to the hinge region of the Met protein. Kinetic analysis has further demonstrated that these compounds bind in the Met kinase domain in a manner that is ATP-competitive. The malonamide and acylurea groups of **1** and **2** approach planarity in the bioactive conformation, due to a hydrogen-bonding arrangement between the phenoxyaniline NH and the distal carbonyl group of either the malonamide or acylurea. Compound **3** appears to engage in a similar arrangement between the aniline NH and the carbonyl oxygen of the pyridone ring. Constraining this region of the molecule, as an aromatic ring, might serve to lock this portion of the molecule into a bioactive conformation. Molecular modeling studies suggested that a biarylamine motif could potentially be used to preorganize the molecule. To adopt the bioactive conformation, however, the aromatic rings would need to approach the coplanarity observed between the

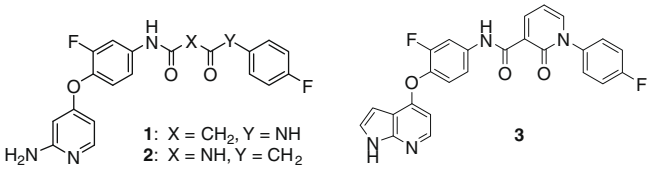


Figure 1. Previously disclosed Met kinase inhibitors.

* Corresponding author.
E-mail address: david.williams@bms.com (D.K. Williams).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.042

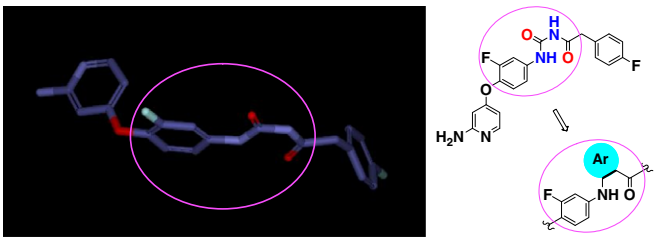


Figure 2. X-ray structure of acylurea **2** in the Met binding domain.

fluorophenyl ring and attached acylurea group seen in compound **2** (Fig. 2). Further analysis suggested that utilization of a diphenylamine in this region, would force the rings out-of-plane by approximately 30°. Incorporation of an aromatic nitrogen ortho to the diarylamino nitrogen, was proposed to relieve this potential conformational energy strain.

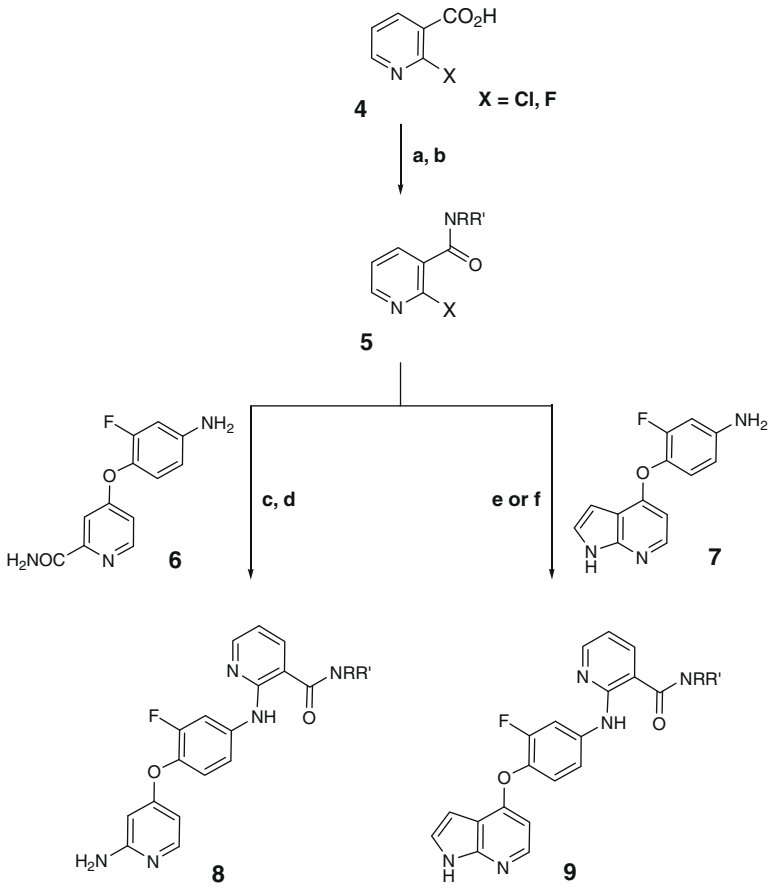
Herein, we describe the synthesis, structure–activity relationships, and in vivo activity of novel biarylamine-based Met kinase inhibitors.

The synthesis of compounds in these series centered upon the acid-catalyzed addition of an appropriate 2-fluoro-4-aryloxyaniline to a desired 2-halonicotinamide (Scheme 1). Thus, 2-chloro- or 2-fluoronicotinamide **5** could be readily generated from the precursor nicotinic acid via generation of the acid chloride and coupling that acid chloride with a suitable amine. Addition of the resultant halonicotinamide to 4-(4-amino-2-fluorophenoxy)picolinamide **6**,[8] in the presence of *p*-toluenesulfonic acid monohydrate,[9] followed by treatment with bis(trifluoroacetoxy)iodobenzene and pyridine afforded compound **8**.[10]

Pyrrolo[2,3-*b*]pyridine analog **9** was prepared in a similar manner. Thus, microwave-assisted reaction between 4-(1*H*-pyrrolo[2,3-*b*]pyridine-4-yloxy)-3-fluorobenzenamine[7] and the desired 2-chloro- or 2-fluoro-nicotinamide, in the presence of *p*-toluenesulfonic acid monohydrate, afforded compound **9**, albeit in low (18–27%) yield. A subsequent optimization study on the synthesis of the pyrrolopyridine analogs determined preferred conditions, utilizing 4 N HCl in dioxane and the desired 2-fluoronicotinamide, under thermal conditions, which afforded compound **9b** in 72% yield.[11a]

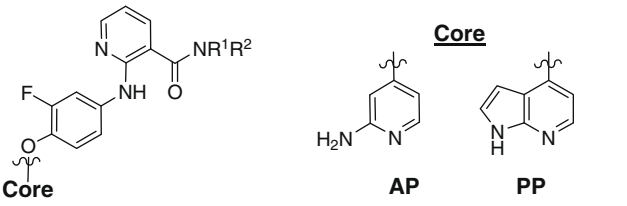
Initial structure–activity relationship (SAR) studies centered on determining the effect of substitution on the nicotinamide nitrogen, with both the aminopyridine (AP) and pyrrolopyridine (PP) based scaffolds (Table 1). Two assays were employed to evaluate the activity of the compounds. A Met kinase substrate based assay was used to assess the ability of these compounds to inhibit the enzymatic activity of the Met receptor.[6] Compounds exhibiting sufficient activity were evaluated for their ability to inhibit the proliferation of the GTL-16 human gastric carcinoma cell line, which expresses high levels of constitutively-activated Met kinase.[6] Studies have shown this cell line to be very sensitive to small molecule inhibitors of Met.[12] As such, it was utilized as the primary in vitro cell line for the screening of Met inhibitors.

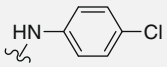
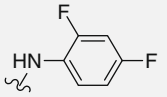
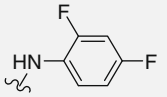
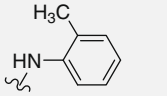
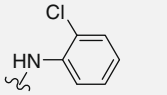
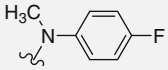
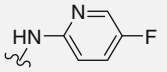
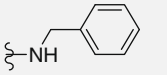
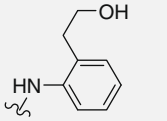
In the Met kinase assay, utilization of the aminopyridine core with an initial *p*-chlorophenyl amide (**8a**) resulted in a compound demonstrating potent activity. Incorporating a 2,4-dihalogenated amine, in the form of the 2,4-difluorophenylamide (**8b**), afforded



Scheme 1. Reagents and conditions: (a) $(ClCO)_2$, THF, DMF, reflux; (b) RR'NH, DMF, pyr, 55–100% (c) *p*-TsOH·H_2O, NMP, MW (300 W), 160 °C, 31–66%; (d) $PhI(OCOCF_3)_2$, H_2O, pyr, DMF 25–93%; (e) *p*-TsOH·H_2O, NMP, MW (300 W), 160 °C, 18–27%; (f) 4 N HCl/dioxane (4 equiv), NMP, 110 °C, 60–72%.

Table 1
SAR of 2-aminopyridine (AP) and 1*H*-pyrrolo[2,3-*b*]pyridine (PP) nicotinamides[a]



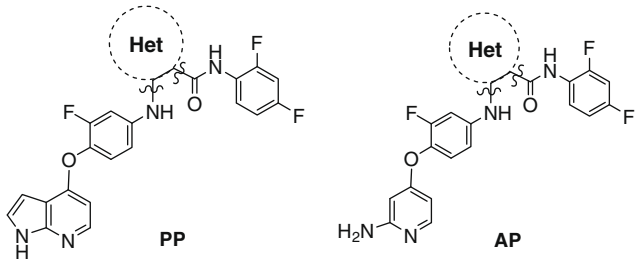
Compound–core	–NR^1R^2	IC_{50}, μM	
		Met	GTL-16
8a–AP		0.011	1.16
8b–AP		0.008	0.90
9b–PP		0.007	0.21
9c–PP		0.006	0.42
9d–PP		0.024	1.20
9e–PP		0.19	5.58
9f–PP		0.034	0.27
9g–PP		0.021	0.46
9h–PP		0.009	0.40

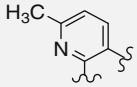
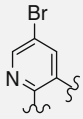
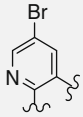
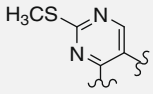
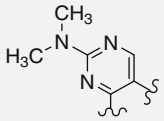
[a] Assay conditions are detailed in Ref. 6.

similar activity in both assays. With a change to the pyrrolopyridine core (**9b**), potency in the Met kinase assay was maintained, while a greater than fourfold improvement in activity in the GTL-16 cellular proliferation assay was observed. Monosubstitution in the 2-position of the aniline (**9c** and **9d**) afforded compounds that, while still potent in the Met kinase assay, exhibited somewhat decreased cellular activity, compared to **9b**. Methylation of the nicotinamide nitrogen (**9e**) resulted in compounds with significantly reduced activity in both assays. As expected, smaller substitutions on the nicotinamide nitrogen, such as the primary carboxamide or the *N*-methyl nicotinamide, also resulted in compounds with reduced activity in the Met kinase assay (data not shown). Heteroaromatic replacement of the phenyl ring, in the form of *N*-pyridin-2-yl-nicotinamide **9f**, afforded potent cellular activity. Extension of the terminal phenyl via *N*-benzyl-nicotinamide (**9g**) and incorporation of polar substituents, such as hydroxyethyl (**9h**) also resulted in compounds exhibiting potency against Met kinase as well as cellular activity.

For the purpose of exploring the SAR about the central heterocycle, the terminal 2,4-diflourophenyl group was held constant while varying the central heterocycle (Table 2). The synthesis of

Table 2
SAR of heterocyclic portion of the diarylamine[a]

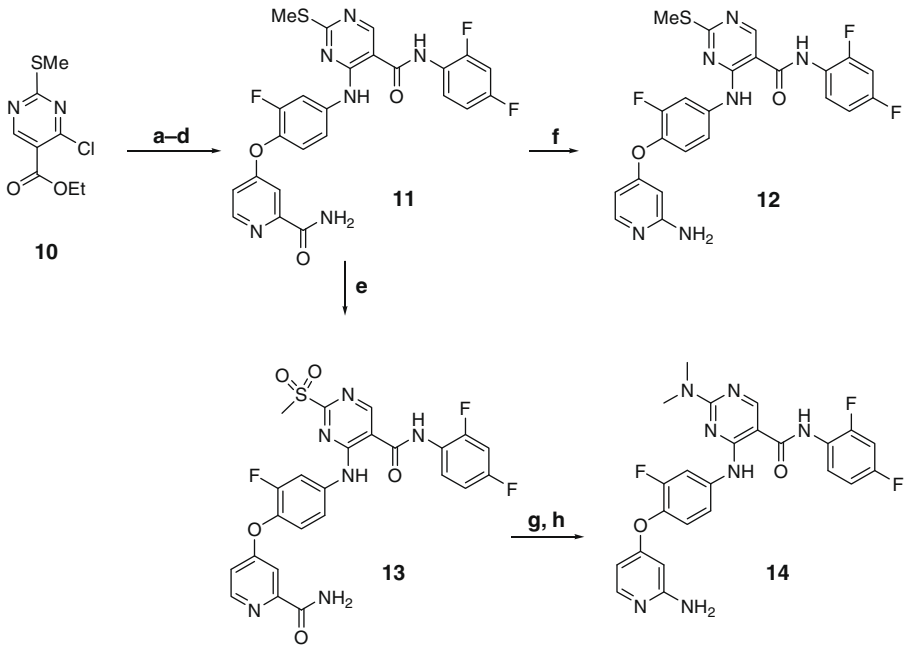


Compound–core	Het	IC_{50}, μM	
		Met	GTL-16
15–PP		0.018	2.40
16–AP		0.013	0.59
17–PP		0.009	0.64
18–AP		3.50	–
19–PP		0.27	>10.00
20–AP		0.069	2.73
21–PP		0.055	7.15
22–AP		0.022	1.93
12–AP		0.009	0.44
14–AP		0.12	>10.00

[a] Assay conditions are detailed in Ref. 6.

various biarylamine targets, wherein the heterocycle was defined as a pyridine ring, were generally carried out similarly to the route previously described in Scheme 1.[8] In the case of the pyrimidine analogs a slightly different route was employed (Scheme 2).[8]

Ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate was treated with 4-(4-amino-2-fluorophenoxy)-picolinamide, under acidic conditions, to give ethyl 4-(4-(2-carbamoylpyridin-4-yloxy)-3-fluoro-phenylamino)-2-(methylthio)-pyrimidine-5-carboxylate. Saponification with NaOH, afforded the carboxylate salt which was then coupled with 2,4-difluoroaniline to give intermediate **11**. Treat-



Scheme 2. Reagents and conditions: (a) **6**, 4 N HCl/dioxane, NMP, 93%; (b) 4 N NaOH, MeOH/H_2O, 83%; (c) $SOCl_2$, reflux; (d) 2,4-difluoroaniline, 45%; (e) *m*-CPBA, DMF, 81%; (f) $PhI(OCOCF_3)_2$, H_2O, pyr, DMF, 53%; (g) dimethylamine, THF, 78%; (h) $PhI(OCOCF_3)_2$, H_2O, pyr, DMF, 36%.

ment with bis(trifluoroacetoxy)iodobenzene afforded the 2-(methylthio)pyrimidine product **12**.[11b] Alternatively, intermediate **11** was oxidized with *m*-CPBA to afford sulfone **13**, which was then displaced by a variety of nucleophiles, including dimethylamine. Subsequent treatment with bis(trifluorocetoxy)iodobenzene afforded compound **14**.

The SAR about the heterocyclic portion of the biarylamines is shown in Table 2. Compounds which incorporated a nitrogen in the heterocycle, ortho to the amine juncture, showed preferred activity in the Met kinase assay. Consistent with the activity observed for the 2-aminonicotinamides **8b** and **9b**, compounds substituted in the 5- or 6-position (**15**–**17**) also exhibited potency against Met kinase regardless of the core being utilized. The 4-aminonicotinamides, **18** and **19**, were less potent in the kinase assay, presumably due to the necessity of that pyridine ring to lie out-of-plane with the fluorophenylamino ring. Replacement of the pyridine ring of the nicotinamide with pyrazine (**20** and **21**), led to an eightfold drop in kinase activity, relative to **8b** and **9b**. A decrease in the cellular activity was also observed. Kinase and cellular activities observed with pyrimidine substitution (**22**, **12**, and **14**) were found to be sensitive to the type of group located in the 2-position of the pyrimidine. Attempts to improve the solubility of these compounds, by incorporating a 2-position substituent having a basic nitrogen, resulted in a decrease in activity.

The X-ray crystal structure of **8b**[13] revealed the compound resides in the Met kinase domain (Fig. 3) with a conformation consistent with that of pyridone **3**.[7] Of particular note is how the biarylamine rings adopt a conformation in which they approach planarity mimicking the hydrogen-bond between the amide NH and the 2-pyridone carbonyl oxygen in **3**.

Based on its potency against Met kinase,[14] as well as its activity in the GTL-16 cellular proliferation assay, **9b** was evaluated in a preliminary pharmacokinetic study in mice. The compound demonstrated reasonable exposures in an 8 h mouse oral exposure study at 50 mpk (C_{max} = 9.3 μM, $AUC_{0-8\ h}$ = 42 μM h) and was subsequently taken into the GTL-16 human gastric carcinoma model.[5] In the study, anthymic mice are implanted with GTL-16 xenografts. The compound was administered orally at various dose levels, once

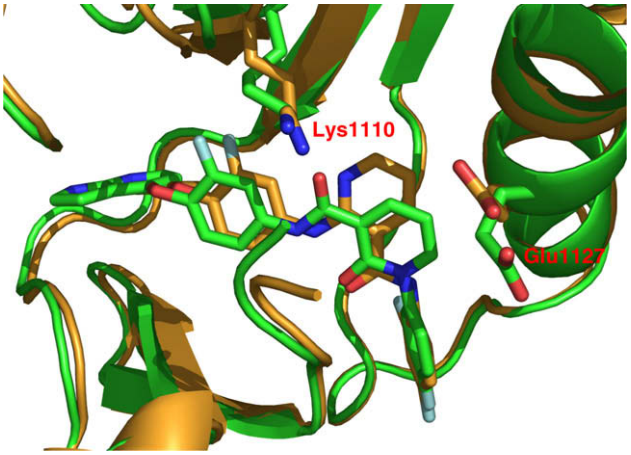


Figure 3. Comparison of X-ray co-crystal structures of compounds **3** (green) and **8b** (orange) bound to the Met Kinase Domain [Met(1067-1378)/Y1212F, Y1252F, Y1253D].

daily over 15 days. Figure 4 illustrates the dose-dependent tumor growth inhibition of compound **9b** in the GTL-16 gastric carcinoma model. The compound demonstrated activity at multiple dose levels, resulting in complete tumor stasis at the lowest dose tested (25 mg/kg).

In summary, biarylamine-based inhibitors of Met kinase were identified. This template was found to serve as an effective surrogate for the hydrogen-bonded array previously observed in acylureas, malonamides, and pyridones. When suitably tethered to an aminopyridine or pyrrolopyridine, these compounds demonstrated potent activity in the kinase and cellular proliferation assays. Based on the X-ray crystal structure, compound **8b** was shown to adopt a conformation consistent with that previously seen for compound **3**, in the Met kinase binding domain. Additionally, in vivo studies involving compound **9b** demonstrated that these compounds can exhibit potent antitumor activity.

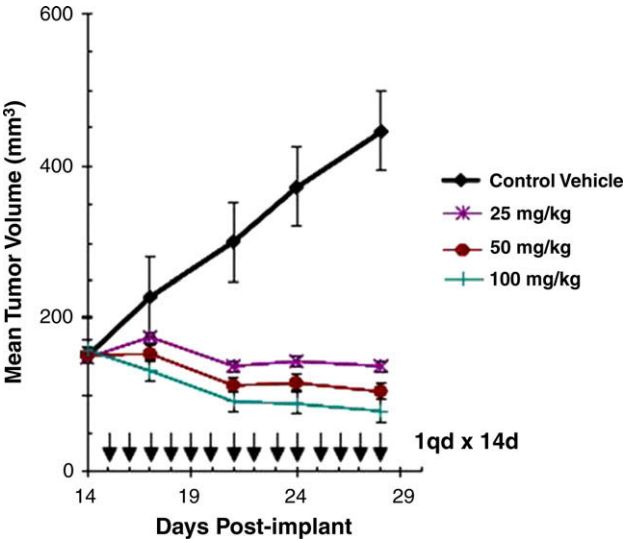


Figure 4. In vivo activity of compound **9b** versus GTL-16 xenografts implanted in anthymic mice (arrows denote days of dosing).

References and notes

1. For recent reviews on Met kinase see: (a) Migliore, C.; Giordano, S. *Eur. J. Cancer* **2008**, *44*, 641; (b) Peruzzi, B.; Bottaro, D. P. *Clin. Cancer Res.* **2006**, *12*, 3657; (c) Christensen, J. G.; Burrows, J.; Salgia, R. *Cancer Lett.* **2005**, *225*, 1; (d) Ma, P. C.; Maulik, G.; Christensen, J.; Salgia, R. *Cancer Metastasis Rev.* **2003**, *22*, 309.
2. (a) Maulik, G.; Shrikhande, A.; Kijima, T.; Ma, P. C.; Morrison, P. T.; Salgia, R. *Cytokine Growth Factor Rev.* **2002**, *13*, 41; (b) Bottaro, D. P.; Rubin, J. S.; Faletto, D. L.; Chan, A. M.; Kmiecik, T. E.; VandeWoude, G. F.; Aaronson, S. A. *Science* **1991**, *251*, 802.
3. A tabular summary of the role of HGF/SF and Met in various cancers, with links to appropriate references, is available at: http://www.vai.org/met/.
4. Danilkovitch-Miagkova, A.; Zbar, B. *J. Clin. Invest.* **2002**, *109*, 863.
5. Cai, Z.-W.; Wei, D.; Schroeder, G. M.; Cornelius, L. A. M.; Kim, K.; Chen, X.-T.; Schmidt, R. J.; Williams, D. K.; Tokarski, J. S.; An, Y.; Sack, J. S.; Manne, V.; Kamath, A.; Zhang, Y.; Marathe, P.; Hunt, J. T.; Lombardo, L. J.; Fargnoli, J.; Borzilleri, R. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3224.
6. Schroeder, G. M.; Chen, X.-T.; Williams, D. K.; Nirschl, D. S.; Cai, Z.-W.; Wei, D.; Tokarski, J. S.; An, Y.; Sack, J.; Chen, Z.; Huynh, T.; Vaccaro, W.; Poss, M.; Wautlet, B.; Gullo-Brown, J.; Kellar, K.; Manne, V.; Hunt, J. T.; Wong, T. W.; Lombardo, L. J.; Fargnoli, J.; Borzilleri, R. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1945.
7. Kim, K. S.; Zhang, L.; Schmidt, R.; Cai, Z.-W.; Wei, D.; Williams, D. K.; Lombardo, L. J.; Trainor, G. L.; Xie, D.; Zhang, Y.; An, Y.; Sack, J. S.; Tokarski, J. S.; Darienzo, C.; Kamath, A.; Marathe, P.; Zhang, Y.; Lippy, J.; Jeyaseelan, R.; Wautlet, B.; Henley, B.; Gullo-Brown, J.; Manne, V.; Hunt, J. T.; Fargnoli, J.; Borzilleri, R. M. *J. Med. Chem.* **2008**, *51*, 5330.
8. Borzilleri, R. M.; Chen, X.-T.; Williams, D. K.; Tokarski, J. S.; Kaltenbach, R. F. U.S. Patent Application 2007/0078140, 2007.
9. (a) Galeeva, R. N.; Gavrilov, M. Y.; Nazmetdinov, F. Y.; Drovosekova, L. P.; Kolla, V. E.; Kon'shin, M. E. *Khim. Farm. Zh.* **1998**, *32*, 31; (b) Kermack, W. O.; Weatherhead, A. P. *J. Chem. Soc.* **1942**, 726.
10. Yu, C.; Jiang, Y.; Liu, B.; Hu, L. *Tetrahedron Lett.* **2001**, *42*, 1449.
11. (a) *Representative analytical data for:* **9b** (HCl salt): $_1$H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 10.75 (s, 1H), 10.52 (s, 1H), 8.28–8.41 (m, 3H), 8.15 (dd, 1H, J = 13.6, 2.2 Hz), 7.32–7.56 (m, 5H), 7.09–7.13 (m, 1H), 6.98–7.01 (m, 1H), 6.72 (d, 1H, J = 6.5 Hz), 6.48–6.49 (m, 1H); HRMS for $C_{25}H_{17}F_3N_5O_2$ $(M+H)^+$: calcd: 476.1334, found: 476.1350.; (b) **12** (HCl salt):^{1} H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 10.51 (s, 1H), 8.94 (s, 1H), 8.12 (dd, 1H, J = 13.0, 2.3 Hz), 7.98 (d, 1H, J = 7.2 Hz), 7.90 (br s, 2H), 7.61–7.40 (m, 4H), 7.18 (m, 1H), 6.74 (dd, 1H, J = 7.3, 2.5 Hz), 6.18 (d, 1H, J = 2.4 Hz), 2.57 (s, 3H); MS(ESI$^+$) m/z 499 $(M+H)^+$.
12. Christensen, J. G.; Schreck, R.; Burrows, J.; Kuruganti, P.; Chan, E.; Le, P.; Chen, J.; Wang, X.; Ruslim, L.; Blake, R.; Lipson, K. E.; Ramphal, J.; Do, S.; Cui, J. J.; Cherrington, J. M.; Mendel, D. B. *Cancer Res.* **2003**, *63*, 7345.
13. The atomic coordinates have been deposited in the RCSB Protein Data Bank (PDB code: 3L8V).
14. Compound **9b** was further evaluated in an in-house kinase selectivity panel, where it was found to be more than 17-fold selective for Met versus Flt-3 (IC_{50} = 0.12 μM) and greater than 50-fold selective against TrkA (IC_{50} = 0.35 μM). Other kinases tested in the assay, including Lck, IFGR-1R, InsR, MK2, CDK2E and PKC-α, were found to have IC_{50} values of greater than 50 μM.