Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 386–390
Bioorganic & Medicinal Chemistry Letters

Identification of aminopyrazolopyridine ureas as potent VEGFR/PDGFR multitargeted kinase inhibitors

Yujia Dai,* Kresna Hartandi, Niru B. Soni, Lori J. Pease, David R. Reuter, Amanda M. Olson, Donald J. Osterling, Stella Z. Doktor, Daniel H. Albert, Jennifer J. Bouska, Keith B. Glaser, Patrick A. Marcotte, Kent D. Stewart, Steven K. Davidsen and Michael R. Michaelides

Cancer Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6100, USA

Received 30 August 2007; revised 5 October 2007; accepted 8 October 2007
Available online 17 October 2007

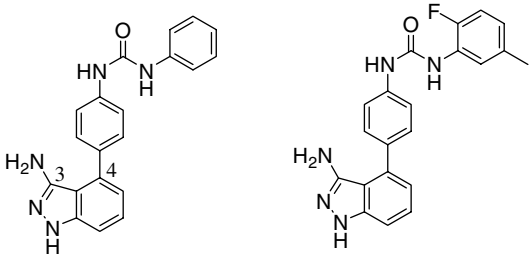
Abstract—Tumor angiogenesis is mediated by KDR and other VEGFR and PDGFR kinases. Their inhibition presents an attractive approach for developing anticancer therapeutics. Here, we report a series of aminopyrazolopyridine ureas as potent VEGFR/PDGFR multitargeted kinase inhibitors. A number of compounds have been identified to be orally bioavailable and efficacious in the mouse edema model.

Angiogenesis, the formation of new vessels from pre-existing vasculature, is a required process in tumor growth and metastasis.[1] Angiogenesis is primarily mediated by vascular endothelial growth factor receptor (VEGFR) tyrosine kinases, a subfamily of receptor tyrosine kinases (RTKs), which include Fms-like tyrosine kinase 1 (FLT1; VEGFR1), kinase insert domain-containing receptor (KDR; VEGFR2), and FLT4 (VEGFR3).[2] Aberrant activation of VEGFR family RTKs and especially of KDR by vascular endothelial growth factors (VEGFs) has been linked to the progression of various human cancers.[3]

Although VEGFR kinases play the major role in tumor angiogenesis, platelet derived growth factor receptor (PDGFR) tyrosine kinases, a structurally related RTK subfamily containing PDGFRα, PDGFRβ, cKit, colony-stimulating factor 1 receptor (CSF1R), and FLT3, are believed to indirectly promote tumor angiogenesis and also directly contribute to tumor growth through various mechanisms.[4] Additionally, it is also well documented that the constitutive activation of FLT3 and cKIT through mutation is directly associated, respectively, with the progression of acute myeloid leukemia (AML)[5] and gastrointestinal stromal tumor (GIST).[6]

Due to the vital roles of VEGFR/PDGFR signaling in tumor angiogenesis, inhibition of VEGFR/PDGFR activation has become a compelling approach for developing targeted cancer therapies. Both selective KDR agents and multitargeted kinase inhibitors have been developed.[7]

In our efforts to identify potent and novel small-molecule kinase inhibitors, we discovered that 3-aminoindazole could serve as a novel kinase hinge-binding template



1
KDR: IC_{50} = 64 nM

2 (ABT-869)
KDR: IC_{50} = 4 nM

Figure 1. Aminoindazole urea VEGFR/PDGFR kinase inhibitors.

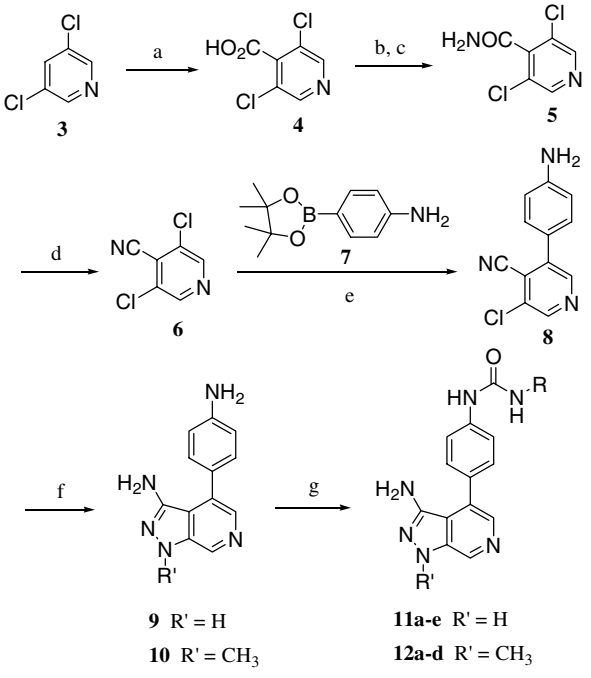
Keywords: Pyrazolopyridines; KDR; VEGFR/PDGFR kinase inhibitors.
* Corresponding author. E-mail: yujia.dai@abbott.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.018

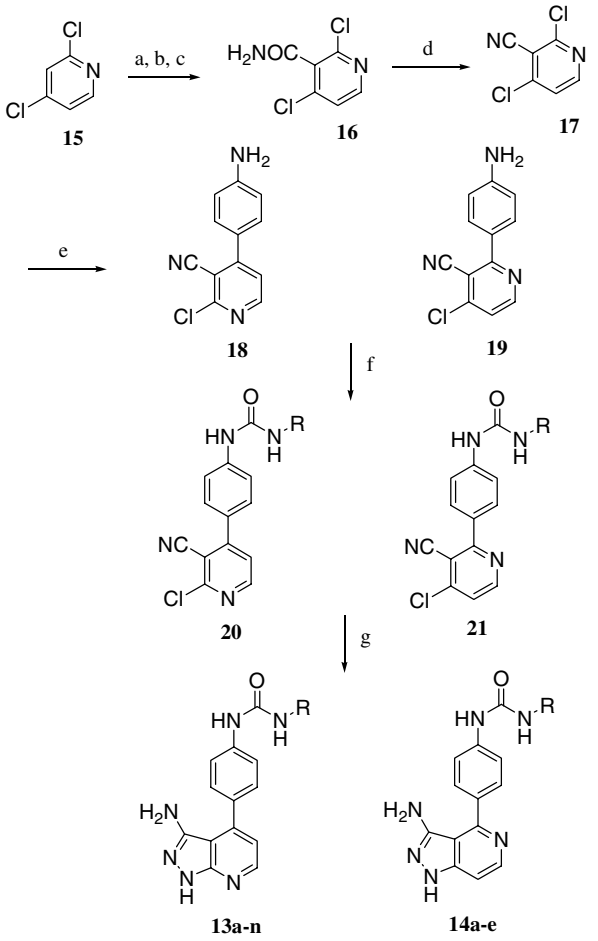
for kinase inhibitors. By incorporating a diaryl urea unit at the C4-position, a series of potent and orally bioavailable VEGFR/PDGFR inhibitors were generated (Fig. 1). Optimization of this series led to the identification of a clinical candidate (**2**, ABT-869)[8,9] that is currently in phase II trials. Encouraged by the promising in vitro and in vivo properties of this series of compounds, we expanded our investigation into 3-aminopyrazolopyridine systems. We were, on one hand, interested in how the incorporated pyridine nitrogen would impact the activity of these inhibitors; on the other hand, we reasoned that the increased basicity would likely make these compounds more soluble than their indazole analogs, which exhibited very poor aqueous solubility. In this paper, we report in brief the synthesis, structure–activity relationships (SARs), and preliminary characterization of these new inhibitors.

The chemical synthesis of the 3-aminopyrazolo[3,4-*c*]pyridine ureas **11a**–**e** and **12a**–**d** is outlined in Scheme 1. The Suzuki coupling reaction between chloride **6** and 4-aminophenyl boronic acid pinacol ester (**7**) afforded aniline **8**. The aniline was then heated with hydrazine monohydrate to form 3-aminopyrazolo[3,4-*c*]pyridine **9**. Similarly, *N*-methyl 3-aminopyrazolo[3,4-*c*]pyridine **10** was obtained by using methylhydrazine instead of hydrazine. Reaction of **9** and **10** with various isocyanate generated ureas **11a**–**e** and **12a**–**d**, respectively.

As shown in Scheme 2, 3-aminopyrazolo[3,4-*b*]pyridine ureas **13a**–**n** and 3-aminopyrazolo[4,3-*c*]pyridine ureas **14a**–**e** were synthesized utilizing a strategy similar to



Scheme 1. Reagents and conditions: (a) LDA, THF, −78 °C, 1 h; then ground dry ice, −78 °C → rt, 56%; (b) oxalyl chloride, cat. DMF, rt, 3h; (c) concentrated aqueous NH_4OH, THF, 86%; (d) $POCl_3$, 100 °C, 4h, 87%; (e) $Pd(dppf)Cl_2$, Na_2CO_3, DME, H_2O, 35%; (f) NH_2NH_2–H_2O or CH_3NHNH_2, *n*-BuOH, 110 °C, 5 h; (g) RNCO, DMF.

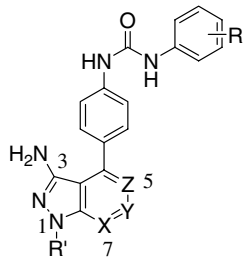


Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 1 h; then ground dry ice, −78 °C → rt, 70%; (b) oxalyl chloride, cat. DMF, rt, 3 h; (c) concentrated aqueous NH_4OH, THF, 100%; (d) $POCl_3$, 100 °C, 4 h, 54%; (e) 2, $Pd(dppf)Cl_2$, Na_2CO_3, DME, H_2O; (f) RNCO, CH_2Cl_2, 50 °C; (g) NH_2NH_2–H_2O, *n*-BuOH, 110 °C, 5 h.

that used in the preparation of 3-aminopyrazolo[3,4-*c*]pyridine analogs. The Suzuki coupling between chloride **17** and **7** yielded a mixture of **18** and **19** in a ratio of 2:1. Attempts to separate them using flash column chromatography were unsuccessful. Consequently, the mixture was directly used for the subsequent urea formation and cyclization. The final products **13a**–**n** and **14a**–**e** were then separated using reverse phase HPLC.

Expected to be ATP-competitive KDR inhibitors, the synthesized 3-aminopyrazolopyridine ureas were evaluated in the presence of a high concentration (1.0 mM) ATP.[10] We first looked at the 3-aminopyrazolo[3,4-*b*]pyridine ureas and the results are shown in the upper portion of Table 1. These compounds proved to be highly potent KDR inhibitors and compared favorably to their aminoindazole counterparts. For instance, both **13a** and **13j** are, respectively, more potent than **1** and **2** (Fig. 1). Introduction of a range of mono- or bis-substituents on the urea terminal phenyl is generally well tolerated. In fact, all the substituted ureas of the 3-aminopyrazolo[3,4-*b*]pyridine except *o*-Me analog **13b** in Table 1 showed an IC_{50} value of <7 nM. The *o*-Me analog **13b** (KDR IC_{50} = 35 nM) is 7-fold less potent

Table 1. Inhibition of KDR in vitro and mouse uterine edema in vivo



Compound	R′	X	Y	Z	R	KDR IC_{50}[a] (nM)	KDR (cell) IC_{50}[a] (nM)	UE[b] % inhibition
13a	H	N	CH	CH	H	4.5	4.0	39
13b	H	N	CH	CH	*o*-Me	35	2.2	<5
13c	H	N	CH	CH	*m*-Me	2.0	1.0	
13d	H	N	CH	CH	*p*-Me	2.9	16	<5
13e	H	N	CH	CH	*m*-Cl	1.0	1.5	23
13f	H	N	CH	CH	*m*-CF_3	1.7	2.6	
13g	H	N	CH	CH	*m*-OMe	3.8	3.9	
13h	H	N	CH	CH	3,5-di-F	6.3	8.1	
13i	H	N	CH	CH	3,5-di-Me	1.0	1.1	77
13j	H	N	CH	CH	2-F-5-Me	2.0	1.0	66
13k	H	N	CH	CH	2-F-5-CF_3	2.4	7.9	
13l	H	N	CH	CH	4-F-3-Me	1.1	1.2	108
13m	H	N	CH	CH	4-F-3-CF_3	2.0	3.8	
13n	H	N	CH	CH	3-Cl-4-F	1.8	5.1	55
11a	H	CH	N	CH	*m*-Me	0.7	0.6	50
11b	H	CH	N	CH	*m*-Cl	1.2	3.1	<5
11c	H	CH	N	CH	2-F-5-Me	2.1	1.0	98
11d	H	CH	N	CH	2-F-5-CF_3	1.0	1.3	35
11e	H	CH	N	CH	4-F-3-Me	0.7	0.7	19
12a	Me	CH	N	CH	*m*-Me	4.6	1.8	79
12b	Me	CH	N	CH	*m*-Cl	1.5	8.8	52
12c	Me	CH	N	CH	2-F-5-Me	7.7	3.0	72
12d	Me	CH	N	CH	4-F-5-CF_3	3.7	13	59
14a	H	CH	CH	N	*m*-Cl	12	46	
14b	H	CH	CH	N	*m*-CF_3	7.1	99	<5
14c	H	CH	CH	N	2-F-5-Me	18	61	<5
14d	H	CH	CH	N	3,5-di-Me	12	65	<5
14e	H	CH	CH	N	4-F-3-Me	20	34	<5

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.
[b] Dosed orally at 10 mg/kg.

than **13a** and >10-fold less potent than its *meta*- and *para*-analogs (**13c** and **13d**). The negative impact on KDR potency by introduction of a substituent other than a fluorine at the *ortho*-position is consistent with the SAR observed in the aminoindazole series.

The potent inhibitory activity of these 3-aminopyrazolo[3,4-*b*]pyridine ureas against KDR is well reflected at the cellular level. Table 1 also displays the activity of these compounds in the inhibition of VEGF-induced KDR phosphorylation in 3T3 murine fibroblast cells that were engineered to express human KDR.[11] Except **13d**, all the 3-aminopyrazolo[3,4-*b*]pyridine ureas in Table 1 exhibited a single-digit nanomolar IC_{50} value against cellular KDR phosphorylation.

Moving the pyridine nitrogen from the 7-position to the 6-position (see the numbering on the structure in Table 1) has little impact on the KDR affinity. All the ureas of the 3-aminopyrazolo[3,4-*c*]pyridine system compare well to their corresponding analogs of the 3-aminopyrazolo[3,4-*b*]pyridine both enzymatically and cellularly. Additionally, introduction of a methyl group on the pyrazole ring NH is well tolerated. Compounds **12a**–**d** all exhibited a single-digit nanomolar IC_{50} value against KDR.

While almost all the tested diaryl ureas of both the pyrazolo[3,4-*c*]pyridine and the pyrazolo[3,4-*b*]pyridine displayed low nanomolar potency against KDR and compared well to the indazole analogs, the corresponding analogs of pyrazolo[4,3-*c*]pyridine are generally less active. For instance, **14c** is about 9-fold less potent than both **13j** and **11c**, and about 4-fold less potent than **2**.

To interpret the difference in potency observed for the regioisomeric pyrazolopyridines, we looked into the binding mode of these compounds to KDR. Figure 2 is a model of **14a** bound to KDR generated by molecu-

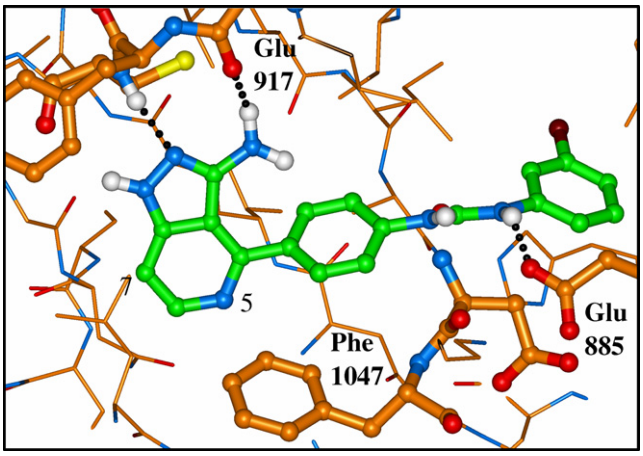


Figure 2. Binding model of **14a** in an inactive form of KDR (DFG-out). Two hinge H-bonds to Glu 917 and Cys 919 and urea H-bond to Glu 885 are shown as dotted lines.

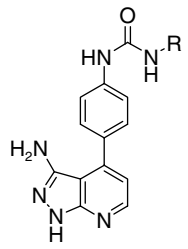
lar modeling.[12] As this model suggests, these 3-aminopyrazolopyridine ureas bind to the ATP-site of KDR in the same fashion as 3-aminoindazole ureas.[8] Specifically, the 3-amino pyrazolopyridine core is anchored to the KDR hinge region via two H-bonds. The kinase adopts an inactive confirmation (DFG-out) so that the urea portion occupies the back hydrophobic pocket with additional H-bond interactions. This model also indicates that the pyrazole ring NH does not form a direct H-bond interaction with the kinase, which is confirmed by the observation that the *N*-methylated compounds are still very potent against KDR as discussed before.

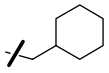
Based on this model, the 7- and 6-positions of pyrazolopyridine cores project toward solvent accessible region. Consequently, insertion of a hydrophilic pyridine nitrogen at either of these two positions should be favored. This is consistent with the improved potency displayed by both the pyrazolo[3,4-*b*]pyridines and pyrazolo[3,4-*c*]pyridines relative to the indazoles. However, a hydrophilic nitrogen at the 5-position would have a negative impact on potency because the 5-position projects to a hydrophobic region comprised of Phe 1047 of the DFG-motif. This prediction is indeed in line with the weaker activity observed for the pyrazolo[4,3-*c*]pyridine sub-series.

We also briefly investigated the replacement of the urea terminal aromatic residue with an aliphatic group (Table 2). Consistent with the SARs in other series of urea class KDR inhibitors,[8,12] a diaryl urea moiety is favored for optimal interaction with the hydrophobic back pocket of KDR kinase.

Given their impressive enzymatic and cellular KDR inhibitory activity, these compounds were subsequently evaluated in an estradiol-induced mouse uterine edema (UE) model, which is based on the finding that the enhanced vascular permeability is a direct response to VEGF-stimulated KDR signaling. Since the tested compounds were given orally, this in vivo functional assay enabled a rapid screening of the oral

Table 2. SARs of replacement of the urea terminal phenyl group



Compound	R	KDR IC_{50}[a] (nM)	KDR (cell) IC_{50}[a] (nM)
13a		4.5	4.0
22		12.7	37
23		115	
24		64	
25		240	

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

activity of KDR inhibitors. The percent inhibition of edema at a 10 mg/kg oral dose is given in Table 1. A number of compounds were found to be orally efficacious in this model. For instance, compounds **13i**, **13j**, **13l**, **13n**, **11c**, and **12a–d** exhibited >50% inhibition at the 10 mg/kg dose. Interestingly, all orally potent compounds are ureas of the pyrazolo[3,4-*b*]pyridines and pyrazolo[3,4-*c*]pyridines; the analogs of the pyrazolo[4,3-*c*]pyridine did not show measurable oral activity at the same dose. Although the pyrazolo[4,3-*c*]pyridines exhibited inferior enzymatic and cellular KDR inhibitory activity in comparison with their analogs of pyrazolo[3,4-*b*]pyridines and pyrazolo[3,4-*c*]pyridines, their lack of activity in the UE model is likely due to their poor oral systemic exposure. Several compounds were evaluated for their mouse pharmacokinetic properties (Table 3). **14d**, a pyrazol-

Table 3. Mouse pharmacokinetic profiles of **12a**, **12c**, **13j**, and **14d**

Compound	iv[a]			po[b]	
	$t_{1/2}$ (h)	V_d (L/kg)	Cl (ml/min kg)	AUC (μM h)	*F* (%)
12a	0.6	0.66	12.7	25.0	71
12c	1.5	1.48	11.5	30.2	81
13j	1.0	1.50	17.3	15.7	62
14d	0.45	1.71	43.7	0.89	9

[a] Dosed at 3 mg/kg.
[b] Dosed at 10 mg/kg.

Table 4. Kinase inhibitory profiles of **12a** and **13f**

Compound	KDR	FLT1	FLT3	cKit	CSF1R	FYN	SRC
				IC_{50}[a] (nM)			
12a	4.6	7	4	7	2	>12,500	>12,500
13f	4	5	2	7	3	11,800	—

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

o[4,3-*c*]pyridine analog displaying little oral UE activity at 10 mg/kg, is in fact barely orally available with an AUC value of less than 1 μM h. In contrast, compounds **12a**, **12c**, and **13j**, which were all potent in the UE model, possess significantly better oral PK profiles (AUCs > 15.7 μM h and Fs > 60%).

Although the discussion so far has been primarily focused upon the inhibition of KDR kinase, these compounds proved to be potent VEGFR/PDGFR multitargeted RTK inhibitors. To demonstrate this, Table 4 gives the kinase inhibitory profiles of **12a** and **13f**. Clearly, **12a** and **13f** not only possess impressive activity against KDR, but also potently inhibit other kinases of VEGFR family as well as the kinases of PDGFR family. However, they were much less active in the inhibition of other structurally non-related kinases such as FYN and SRC.

A brief study on the aqueous solubility of selected compounds was also conducted. The measured solubility for **11c**, **13e**, and **14c** at pH 7.2 was 5.8 μM, 1.6 μM, and 9.5 μM, respectively. At the same pH, the measured solubility for ABT-869 (**2**) was 0.07 μM. The incorporated pyridine nitrogen led to an improved aqueous solubility for these compounds.

In summary, we have identified a series of potent multitargeted RTK inhibitors by expanding our investigation of the aminoindazole series into the aminopyrazolopyridines. The diaryl ureas of the pyrazolo[3,4-*b*]pyridine and pyrazolo[3,4-*c*]pyridine potently inhibited KDR and other RTKs of the VEGFR family as well as the kinases of the PDGFR family. Their potent activity against KDR is also reflected by their inhibition of VEGF-induced cellular KDR phosphorylation. Further evaluation of these compounds in a VEGF-induced mouse edema model resulted in the identification of a number of orally active compounds. Mouse pharmacokinetic studies of **12a**, **12c**, and **13j** demonstrated that some of these compounds possess reasonable pharmacokinetic profiles and are promising as oral agents. Moreover, a brief investigation of selected compounds revealed that these inhibitors possess improved aqueous solubility in comparison to their aminoindazole analogs.

References and notes

1. (a) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249; (b) Zetter, B. R. *Annu. Rev. Med.* **1998**, *49*, 407.
2. (a) Ullrich, A.; Schlessinger, J. *Cell* **1990**, *61*, 203; (b) Hubbard, S. R.; Till, J. H. *Annu. Rev. Biochem.* **2000**, *69*, 373.
3. Bergland, E. K. *Am. J. Health-Syst. Pharm.* **2004**, *61*(Suppl. 5), S4.
4. (a) Skobe, M.; Fusenig, N. E. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1050; (b) Lindahl, P.; Johansson, B. R.; Leveen, P.; Betsholtz, C. *Science* **1997**, *277*, 242; (c) Lin, E. Y.; Nguyen, A. V.; Russell, R. G.; Pollard, J. W. *J. Exp. Med.* **2001**, *193*, 727.
5. Stirewalt, D. L.; Radich, J. P. *Nat. Rev. Cancer* **2003**, *3*, 650.
6. Demetri, G. D. *Semin. Oncol.* **2001**, *28*, 19.
7. (a) Ferrara, N.; Kerbel, R. S. *Nature* **2005**, *438*, 967; (b) Bilodeau, M. T.; Fraley, M. E.; Hartman, G. D. *Expert Opin. Investig. Drugs* **2002**, *11*, 737; (c) Bergsland, E. K. *Am. J. Health-Syst. Pharm.* **2004**, *61*(Suppl. 5), S12.
8. Dai, Y.; Hartandi, K.; Ji, Z.; Ahmed, A. A.; Albert, D. H.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Harris, C. M.; Hickman, D.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.; Moskey, M. D.; Martin, R. L.; Olson, A. M.; Ostering, D. J.; Pease, L. J.; Soni, N. B.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Reuter, D. R.; Davidsen, S. K.; Michaelides, M. R. *J. Med. Chem.* **2007**, *50*, 1584.
9. Albert, D. H.; Tapang, P.; Magoc, T. J.; Pease, L. J.; Reuter, D. R.; Wei, R.-Q.; Li, J.; Guo, J.; Bousquet, P. F.; Ghoreish-Haack, N. S.; Wang, B.; Bukofzer, G. T.; Wang, Y.-C.; Stavropoulos, J. A.; Hartandi, K.; Niquette, A. L.; Soni, N.; Johnson, E. F.; McCall, J. O.; Bouska, J. J.; Luo, Y.; Donawho, C. K.; Dai, Y.; Marcotte, P. A.; Glaser, K. B.; Michaelides, M. R.; Davidsen, S. K. *Mol. Cancer Ther.* **2006**, *5*, 995.
10. Kinase enzymatic assays were performed utilizing the homogeneous time-resolved fluorescence (HTRF) protocol in the presence of 1.0 mM ATP. For the detailed procedure, see Ref. 8.
11. For the procedure of the KDR cellular assay, see Ref. 8.
12. Dai, Y.; Guo, Y.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Ahmed, A. A.; Albert, D. H.; Arnold, L.; Arries, S. S.; Barlozzari, T.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.; Mosley, M. D.; Pease, L. J.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Wishart, N.; Davidsen, S. K.; Michaelides, M. *J. Med. Chem.* **2005**, *48*, 6066.