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Novel N^9-arenethenyl purines as potent dual Src/Abl tyrosine kinase inhibitors

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ABSTRACT

Novel N^9-arenethenyl purines, optimized potent dual Src/Abl tyrosine kinase inhibitors, are described. The key structural feature is a *trans* vinyl linkage at N^9 on the purine core which projects hydrophobic substituents into the selectivity pocket at the rear of the ATP site. Their synthesis was achieved through a Horner–Wadsworth–Emmons reaction of N^9-phosphorylmethylpurines and substituted benzaldehydes or Heck reactions between 9-vinyl purines and aryl halides. Most compounds are potent inhibitors of both Src and Abl kinase, and several possess good oral bioavailability.

Src tyrosine kinase is the prototypical member of a family of kinases (Src Family Kinases, SFKs) that modulate multiple intracellular signal transduction pathways involved in cell growth, differentiation, migration and survival.[1] There is evidence that aberrant Src kinase activity is associated with the invasive phenotype in both early and advanced solid tumors.[2,3] Moreover, recent data have demonstrated that Src inhibitors can enhance the antitumor efficacy of hormonal and cytotoxic agents as well as regulate angiogenesis through the control of VEGF expression.[4,5] Bcr-Abl, the constitutively activated fusion protein product of the Philadelphia chromosome (Ph) is the principal oncogene underlying the pathology of chronic myelogenous leukemia (CML). Imatinib is a potent inhibitor of Bcr-Abl, binding to an inactive confirmation of the protein.[6] Despite its striking success in treating patients in the chronic phase of the disease, responses are much less durable in more advanced phases of the disease due to the emergence of drug resistance. Although the most widely accepted mechanism for imatinib resistance is the presence of kinase domain mutations,[7] cells from patients resistant to imatinib express an activated form of the SFK Lyn.[8] Moreover, Hck (another SFK) and Lyn are over-expressed and activated in CML blast-crisis patients and their up-regulation correlates with disease progression and resistance in cell lines and patients treated with imatinib.[9] Therefore, compounds that can inhibit both Src and Abl might be useful in the treatment of patients who have relapsed on imatinib.

Abl shares significant sequence homology with Src and, in its active conformation, bears remarkable structural resemblance with most SFKs. As a result, ATP-competitive compounds originally developed as Src inhibitors frequently exhibit potent inhibition of Abl kinase.[10,11] Several second generation Bcr-Abl kinase inhibitors target both Src and Abl kinases to combat imatinib resistance, and include: dasatinib, bosutinib, AP23464, PD166326, AZD0530, and CGP70630.[12]

Previously, we had described AP23464 as a potent inhibitor of Src and Abl with IC_{50}s for both kinases <1 nM.[13] A crystal structure of AP23464 bound to the active conformation of Src detailed the molecular interactions responsible for its high potency including: (a) two canonical H-bonds to the hinge region, (b) an extensive network of H-bonds between the hydroxyl group on the phenethyl substituent and amino acid residues in the selectivity pocket, and (c) a water-mediated H-bond between the phosphine oxide and the hydroxyl side chain of Y340.[14] To further probe the purine core as a template for potent Src/Abl inhibitors, alternative 2-atom linkers between the template and a pendant hydrophobic substituent were explored. Herein, we describe a vinyl group as one such linkage leading to a series of stable, synthetically accessible, purine-based dual Src-Abl inhibitors (Fig. 1).

The coordinates from the AP23464:Src crystal structure were used to build a model of Src for docking studies. Docking revealed that a *trans* double bond[15] on N^9 of purine template could properly orient a 2,6-disubstituted phenyl ring in the selectivity pocket in a manner similar to dasatinib[16] and PD166326 (Fig. 2A). Alternatively, a vinyl-linked 4-substituted indazole was predicted to preserve the intricate series of H-bonds observed in the AP23464 crystal structure (Fig. 2B).

Initially, a Horner–Wadsworth–Emmons (HWE) approach between N^9 phosphine oxides and substituted benzaldehydes was pursued to obtain the target molecules.[17] The synthesis (Scheme 1)[18] was initiated from readily accessible 6-chloro-2-iodo-9-THP-

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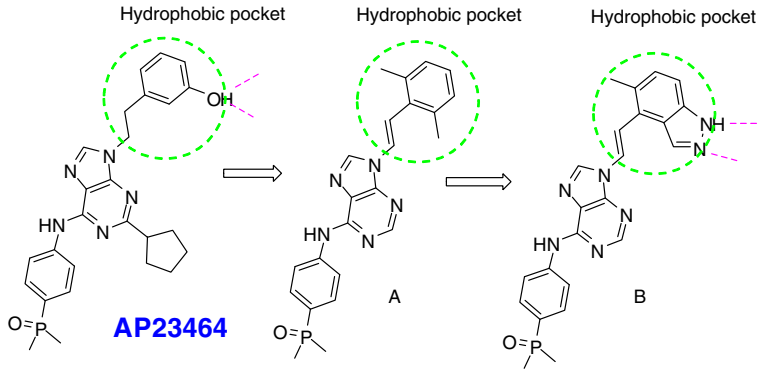


Figure 1. Evolution of novel 9-arenethenyl purines as dual Src/Abl inhibitors. Dotted lines indicate H-bonds.

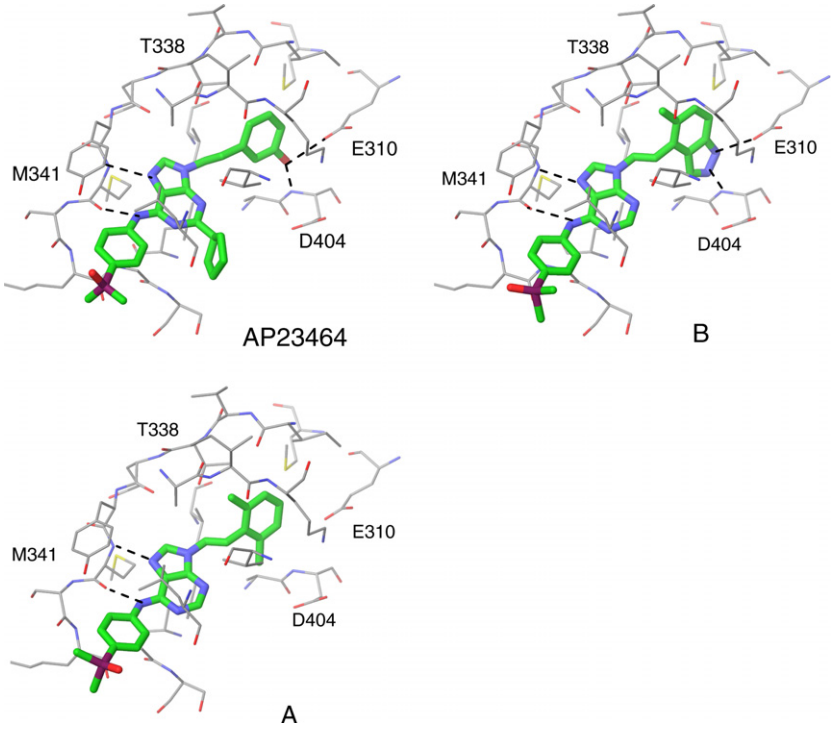


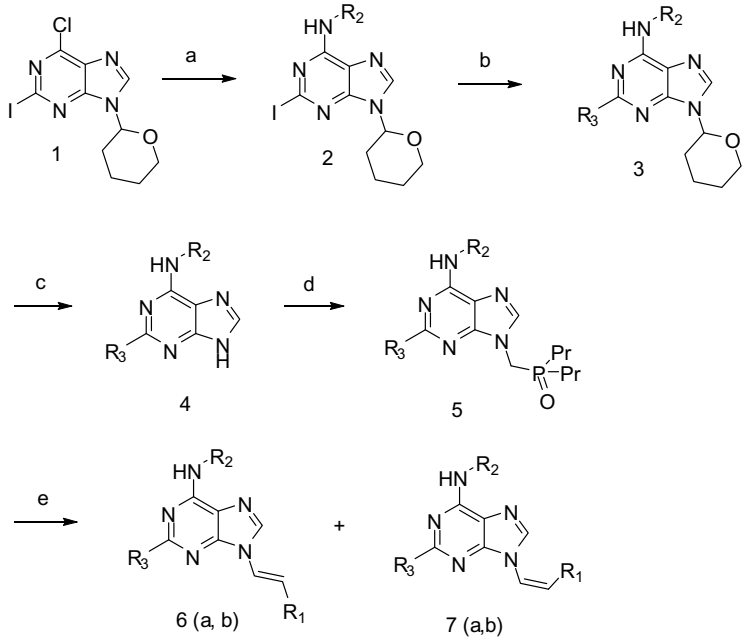
Figure 2. Models of AP23464, compound A, compound B docked in Src. Green, carbon; blue, nitrogen; red, phosphorus. Dotted lines indicate H-bonds.

purine **1**.[19] S_NAr reaction of **1** at C6 with 4-(dialkylphosphoryl)-benzeneamine[20] using standard conditions yielded intermediate **2**. A subsequent Negishi coupling reaction at C2 with isopropylzinc chloride furnished **3**. Deprotection of the THP group yielded intermediate **4** which was alkylated with dipropyl tosylmethyl phosphine oxide to give the HWE reagent **5**. Coupling of **5** with various aldehydes produced the desired *trans* isomers together with small amount of the *cis* isomers. Although successful, the limited availability of structurally diverse benzaldehydes, tedious preparation of HME reagents **5**, together with low yields of the HWE reaction, prompted us to pursue an alternative synthetic strategy.

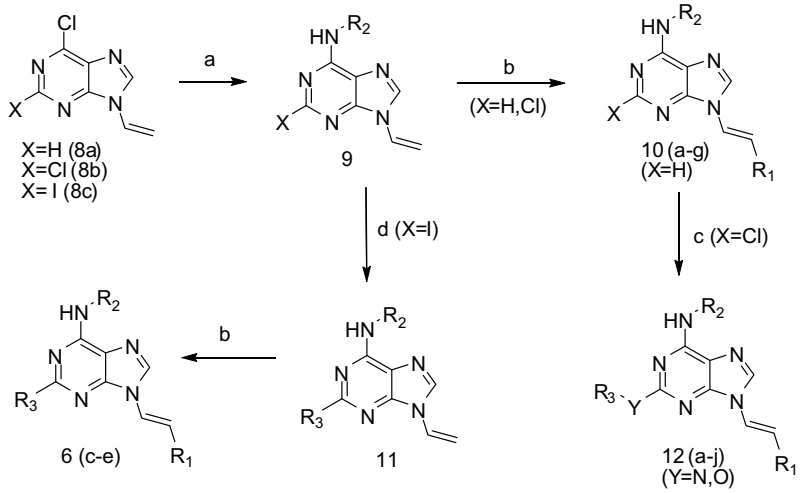
Ultimately, a Heck coupling reaction between substituted 9-vinylpurines and a series of diverse aryl halides was developed to achieve the synthesis of 9-arenethenyl purines.[21] Briefly, 9-vinylpurines **8**[18] were reacted with anilines in the presence of *i*-Pr_2NEt in DMF, to yield intermediate vinylpurines **9**. Subsequent Heck coupling of **9** (X = H, Cl) with aryl halides under standard conditions furnished exclusively the desired *trans* isomer. This reaction is high yielding and quite robust, tolerating a variety of R_1 and R_2 groups, and without the need for protection and deprotection strategies. Compound **10** could be further elaborated by substitution at C2 (X = Cl) with amines or alcohols, thus generating compounds **12a–j** with either an N or O linkage. Where X = I, intermediate **9** was first reacted with alkylzinc reagents then subsequently underwent a Heck reaction to generate **6c–e**. Compounds **6c–e** have C-linked R_3 substitutions at C2. Direct Heck coupling of iodo intermediates **9** with aryl halides was avoided due to the concern that 2-iodopurines might compete with aryl halide substrates (Scheme 2).

All aryl halides employed in the Heck coupling step were commercially available except for target compound **10g** which incorporated a substituted indazole. In this case, the required 4-bromo-5-methylindazole intermediate was synthesized from 2,5-dimethylbromobenzene according to Scheme 3.[22] The synthesis of dialkyl anilinophenylphosphine oxides has been previously reported.[20]

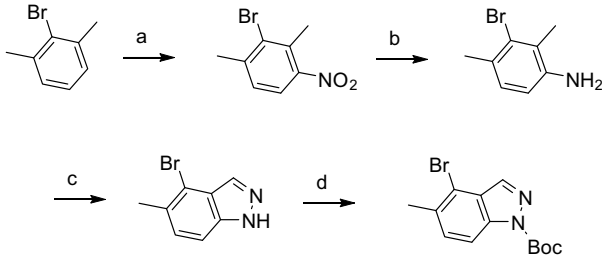
Compounds were then evaluated for their Src[23] and Abl[24] kinase inhibitory activity as previously described. Comparison of compounds **6a**, **b** versus **7a**, **b** confirmed that the desired *trans* isomers were more potent than the corresponding *cis* compounds against Src and Abl enzymes by at least 10-fold (Table 1).



Scheme 1. Reagents and conditions: (a) R_2–NH_2,[22] *i*-Pr_2NEt, DMF; (b) R_3-ZnX, $PdCl_2(PPh_3)_2$, Cs_2CO_3, dioxane; (c) TFA, rt, 15 min; (d) $TsOCH_2P(O)Pr_2$, NaH, DMF, rt, o/n; (e) R_1-CHO, NaH, DMF, rt, o/n.



Scheme 2. Reagents and conditions: (a) R_2–NH_2, *i*-Pr_2NEt, DMF, 100 °C, 2 h; (b) halobenzene (R_1X), $Pd(OAc)_2$, P(*o*-tol)$_3$, *i*-Pr_2NEt, DMF, μM 120 °C/10 min. (c) i—R_3-YH (Y = N), *i*-Pr_2NEt, DMF; (ii) R_3-YH (Y = O), NaOEt, DMF; (d) R_3ZnX, $PdCl_2(PPh_3)_2$, DMF, μM 60 °C/5 min.



Scheme 3. Reagents and conditions: (a) HNO_3, AcOH, 90 °C, 1 h; (b) Fe/AcOH, EtOH, 105 °C, 2 h; (c) $NaNO_2$, HBF_4 (40% aqueous solution), KOAc, 18-Crown-6, water, $CHCl_3$; (d) Boc_2O, DMAP, THF.

Compound **10a**, an early prototype and devoid of any R_3 substitution, was found to potently inhibit Src and Abl with IC_{50}s of 8.31 and 3.58 nM, respectively. Utilizing **10a**, various modifications at R_1 were then evaluated including a simple chlorine atom substitution (**10a** vs **10b**) which was equipotent. Deletion of the 6-methyl group from **10b** confirmed the requirement for 2,6-disubstitution as a key driver of potency (**10b** vs **10c**) as **10c** was >10-fold less potent against both enzymes. We then evaluated the possibility of mimicking the H-bonding network observed in the AP23464:Src co-crystal structure by systematically adding in the observed hydrogen bonds (**10d** → **10e** → **10f**). While compounds **10d** showed marked decreases in potency against both enzymes relative to **10b**, the indazole derivative **10e**, was essentially equipotent (see Table 1). Moreover, when we incorporated a 5-methyl substituent, the resulting compound (**10f**) was at least 10-fold more potent than **10e**. Extensive docking studies with **10f** revealed that it could mimic the hydrogen bonding network observed in the AP23464 co-crystal structure and that the *ortho* methyl substituent

Table 1
IC_{50} values for 9-arenethenyl purines

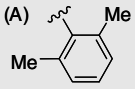
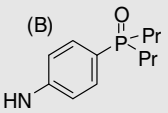
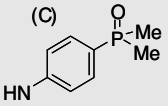
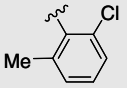
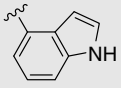
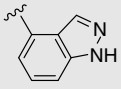
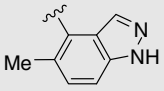
Compound	R_1	R_2	R_3 or R_3-Y	IC_{50} (nM)			
				Src	Abl	K562	Ba/F3(WT)
6a			*i*-Pr	1.78	5.93	115	122
7a	A	B	*i*-Pr	6.58	54.3	964	
6b	Ph	B	*i*-Pr	31.8	73.3	>1000	
7b	Ph	B	*i*-Pr	376	626	>1000	
10a	A		H	8.31	3.58	109	168
10b		C	H	5.56	2.77	109	200
10c		C	H	126	69.4	1394	2006
10d		C	H	198	16.7	954	1541
10e		C	H	17	2.36	355	850
10f		C	H	<0.46	<0.46	10.5	37.3
10g	A	B	H	4.0	17.3	368	286
12a	A	C	Me_2N	3.74	17.6	217	269
12b	A	C	1-Pyrrolidine	1.43	19.5	89.1	123
12c	A	C	1-Morpholine	<0.23	3.02		20.8
12d	A	C	4-Methyl-1-piperazine	<0.23	6.8	23.5	73.7
12e	A	C	1-Imidazole	<0.46	1.63	55.7	
12f	A	C	MeO	<0.46	1.8	52.4	32.7
12g	A	C	*i*-PrO	<0.46	7.32	40.6	34.8
12h	A	C	$MeOCH_2CH_2O$	<0.46	1.57	26.7	51.7
12i	A	C	4-(N-Me-piperidine)	<0.46	<0.46	28.0	285
12j	A	C	3-PyO	<0.46	0.50	8.5	35.1
6c	A	C	*i*-Pr	0.89	15.8	22.7	27.1
6d	A	C	Cyclopentyl	1.29	32.3	224	
6e	A	C	$NCCH_2CH_2$	<0.46	<0.46	15.8	38.6

Table 2
PK properties of selected compounds

Compound	C_{max} (iv)[a] (ng/mL)	$t_{1/2}$ (iv) (h)	V_{dss} (L/kg)	C_{max} (po)[b] (ng/mL)	$t_{1/2}$ (po) (h)	*F*%
10a	4325	3.93	0.98	1277	5	20
10b	4589	2.74	2.74	1683	3.3	17
10f	7082	1.2	1.9	22	NA	0
10g	5142	1.2	2.7	NA	NA	0
6c	1224	7.5	5.4	478	5.1	25

[a] Formulation: 50% DMA, 50% 9:1 Peg400/Tween 80; Conc: 2.5 mg/mL; Dose: 5 mg/kg.

fits in a small hydrophobic pocket formed by residues A293, K295 and T338. The presence of the methyl group should also introduce bias (orthogonality) toward the preferred binding conformation.

Next, we constructed a small focused library at C2 to establish the SAR of various linkages including N, O, and C-linked derivatives. In general, a wide variety of cyclic and acyclic derivatives yielded potent, low nanomolar inhibitors against both Src and Abl, although substitution tended to increase potency primarily against Src. For example, comparison of **10a**, where C2 is unsubstituted (Src and Abl IC_{50}s of 8.31 and 3.58 nM, respectively) to compounds **12c**–**12j** revealed that Src potencies increased by ~10-fold where Abl IC_{50}s remained the same or were improved only slightly. Analysis of the Src/AP23464 crystal structure[14] revealed that R3 substituents can make good hydrophobic contact with several residues on the P-loop lining the ribose pocket. By contrast, the P-loop in Abl is folded differently, such that the side chain of Tyr272 partially fills the ribose pocket, potentially clashing with larger R_3 substituents.[25]

Table 3
Kinase selectivity profile of compound **10a**

Kinase	Enzyme IC_{50} (nM)	Kinase	Enzyme IC_{50} (nM)
Src (SFK)	<0.3	Kit	414
Abl	1	Flt1	591
Fms	3	Abl(T315I)	>1000
EphB1	6	Aurora	>1000
c-Raf	21	CDK2/CyclinA	>1000
EphB4	30	EphA7	>1000
Flt4	151	Flt3	>1000
PDGFRα	221	InsR	>1000

To assess the cellular activities of these compounds, we used both K562 (a Bcr-Abl positive human derived CML cell line) and Ba/F3 cells transfected with wild-type (WT) Bcr-Abl.[26] Compound **10a** inhibited the proliferation of K562 and Ba/F3 cell lines with IC_{50}s of 109 and 168 nM, respectively, which is similar to imatinib (272 and 650 nM, respectively). The cellular activities of the compounds with R_1 or R_3 modifications generally track well with their Abl enzyme inhibitory potencies. The only exceptions are compounds **10e** and **12b**; their cellular data are more in line with their Src inhibitory activities rather than that of Abl, the reason for which is currently unknown. One of the most potent kinase inhibitors of this series, compound **10g**, demonstrated a 10-fold increase in cellular potencies relative to **10a** (Table 1).

To probe the ADME properties of this series we evaluated several compounds in a rat pharmacokinetics model. Interestingly, despite our early concerns about the potential metabolic instability of the vinyl linkage, several compounds possessed good PK properties. Compounds **10a**, **10b**, and **6c** were all characterized as having moderate to high volumes of distribution (V_{dss}) and when dosed orally, they were rapidly absorbed and demonstrated favorable half-lives ($t_{1/2}$). On average, their oral bioavailability (*F*%) in these studies was 20%. Compounds **10e** and **10f**, both of which contain an indazole ring, were not orally bioavailable, presumably due to the rapid bioconjugation of the indazole NH (Table 2).

To evaluate its selectivity profile, compound **10a** was screened in an enzymatic assay against a panel of 35 tyrosine and serine–threonine kinases.[27] The data confirmed the potencies against both Src (SFK) and Abl, and revealed low to double digit nM potencies against Fms, EphB1, c-Raf and EphB4. The compound was inactive against those kinases with larger gate-keeper residues such as Aurora (Leu), CDK2 (Phe), InsR (Phe), and Abl containing the T315I point mutant (Table 3).

In conclusion, a novel series of 9-arenethenyl purines possessing a *trans* double bond were identified as potent inhibitors of Src and Abl tyrosine kinases. Several compounds in this class potently inhibited the proliferation of cell lines driven by Bcr-Abl with at least 10-fold greater potency than imatinib. Interestingly, the N-linked double bond was not rapidly metabolized, yielding compounds with good pharmacokinetic properties.

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23. Src enzymatic assay protocol. Inhibition of Src activity by ARIAD Pharmaceuticals, Inc. compounds (AP compounds) was measured in a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) assay using partially purified full length human Src from a baculovirus expression system (Upstate Biotechnology Inc.) and a biotinylated phosphokinase substrate peptide (amino acid Sequence—Biotin-KVEKIGEGTYGVVYK-NH_2) as the substrate peptide (Pierce). Kinase reactions were carried out in complete LANCE kinase buffer (LKB); 20 mM Na–Hepes, pH 7.4, 0.1 mg/mL BSA, 1 mM ATP, 10 mM $MgCl_2$, 0.41 mM DTT, in round-bottomed black 96-well Microfluor plates (Dynex Technologies Inc.) pre-blocked with 1% BSA in PBS at 4 °C overnight. For the kinase reaction, compound dilutions were incubated with Src (165 pM) and substrate peptide (50 nM) for 2 h at 37 °C in a total volume of 0.1 mL LKB. The kinase reaction was terminated by addition of 0.05 mL of a kill/detection solution containing 15 μM of a potent ARIAD kinase inhibitor, 6 nM Europium-labeled anti-phosphotyrosine mAb PT66 and 60 nM allophycocyanin-labeled streptavidin (LANCE™ reagents from Perkin-Elmer Inc.). Assay plates were incubated at room temperature in the dark for 20 min prior to reading fluorescence at 615 and 665 nm on a Victor2V plate reader (Perkin-Elmer). Positive and negative controls and a standard curve for phosphorylated PKS1 peptide were included on each plate. Data values were transferred to an Excel spreadsheet and IC_{50}s were calculated from the fluorescence at 665 nm by interpolation between the averaged duplicate well data on 3-fold serial dilutions of AP Compounds. No compound effects on the 615 nm fluorescence were observed.
24. The Abl enzymatic assay protocol is described in Ref. 15.
25. The Abl/**10f** structure was resolved and will be published elsewhere.
26. K562 and Ba/F3 cell assay format. For the cell proliferation assay, the K-562 human wild-type Bcr-Abl CML cell line and the murine pro-B Ba/F3 cell line stably transfected with a construct expressing wild-type Bcr-Abl were used. K-562 was obtained from the American Type Culture Collection (ATCC) and maintained in Iscove's modified Dulbecco's medium with 10% FBS. The Ba/F3

cell line was obtained from Brian J. Druker (Howard Hughes Medical Institute, Oregon Health and Science University, Portland, OR, USA) and was maintained in RPMI 1640 growth medium with 10% FBS. All cells were incubated at 37 °C in 5% CO_2. After the compounds were incubated with the cells for 3 days, the number of viable cells in each well in 96-well plate was measured using an MTS assay (Promega). This assay is a colorimetric method for determining the number of viable cells through measurement of their metabolic activity, which can be detected by absorbance at 490 nm. MTS reagent was added to all wells and then the plates were returned to the incubator at 37 °C for 2 h. The absorbance in each well was then measured at 490 nm using a Wallac Victor2V plate reader. The IC_{50} was calculated by determining the concentration of compound required to decrease the MTS signal by 50% in best-fit curves using Microsoft XLfit software, by comparing with baseline, the DMSO control, as 0% inhibition.

27. The KinaseProfiler™ assay service from Millipore was used. Assay protocols are available at http://www.millipore.com/drug discovery/svp3/profilerxl.