Bioorganic & Medicinal Chemistry Letters 22 (2012) 4044–4048

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and structure–activity relationship of aminopyridines with substituted benzoxazoles as c-Met kinase inhibitors

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ARTICLE INFO

Article history:
Received 6 December 2011
Revised 10 April 2012
Accepted 17 April 2012
Available online 25 April 2012

Keywords:
Cancer
c-Met kinase
Aminopyridine
Benzoxazole

ABSTRACT

A series of hydroxybenzoxazole derivatives was synthesized, and their c-Met kinase inhibitory activity was evaluated. Described herein is a potent c-Met inhibitor by structural modification of the parent benzoxazole scaffold, with particular focus on the hydroxyl substituent of the benzoxazole moiety.

c-Met (mesenchymal-epithelial transition factor) and its ligand hepatocyte growth factor (HGF),[1] also called scatter factor (SF), are attractive targets for cancer therapy.[2] Dysregulation of c-Met signaling has been strongly implicated in invasive growth and tumorigenic transformation. Binding of HGF to the extracellular domain of c-Met results in the dimerization and phosphorylation of the tyrosine residues in the intracellular domains.[3] Tyrosine phosphorylation of the c-Met juxtamembrane, catalytic, and cytoplasmic domains regulates the internalization, catalytic activity, and docking of regulatory substrates, which possibly plays a role in oncogenic signaling cascades such as MAPK, PI3K, and STAT pathways.[4] The multifunctional binding sites of c-Met trigger a broad spectrum of biological responses such as cell proliferation, migration, and invasion.[5] Blocking of the c-Met signaling pathway reduces tumor cell growth and invasion.[6] Activating mutations, gene rearrangements, bypass mechanisms, and interactions with other receptor families are major key factors for acquiring resistance to Met inhibitors.[7]

Recently, we have described c-Met inhibitors with a benzoxazole scaffold, and presented their biological activity.[8] As a part of our research, we identified strongly potent c-Met inhibitors by structural modification of the parent benzoxazole scaffold, with particular focus on the hydroxyl substituent of the benzoxazole moiety. Here, we present the results of our studies on the structure–activity relationship (SAR) of modified benzoxazoles.

A series of benzoxazole derivatives were synthesized, and their biological activity was evaluated. Chlorobenzoxazole derivative **1** was synthesized from 3 (or 4)-chloro-2-aminophenol in place of aminophenol derivatives as the same method previously described.[8a] Chlorobenzoxazole derivatives of **1** were reacted with aryl bromide by a palladium-catalyzed cross-coupling reaction to afford **2**, followed by treatment with trifluoroacetic acid (Scheme 1). Various alkylamine and alkoxy derivatives were synthesized by the Pd-catalyzed cross-coupling reaction, followed by treatment with trifluoroacetic acid.

Hydroxybenzoxazole derivatives of **6** were synthesized by Pd-catalyzed hydroxylation of the corresponding chlorobenzoxazole derivatives of **1**, followed by treatment with trifluoroacetic acid. Aromatics were introduced by following a previously published method,[9] which involved Pd-catalyzed heteroarylation of the corresponding hydroxybenzoxazoles of **4** to afford a C-arylation product **7** (Scheme 2).

Introduction of an amine to the benzoxazole was easily accomplished by starting with the aminonitrophenol to afford **8**. A series of aminobenzoxazole derivatives of **10** were synthesized

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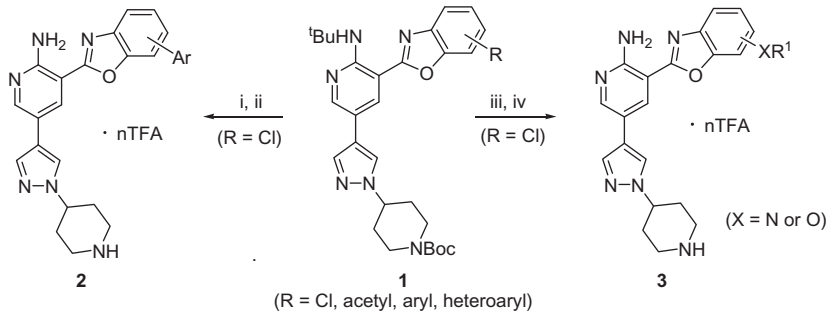
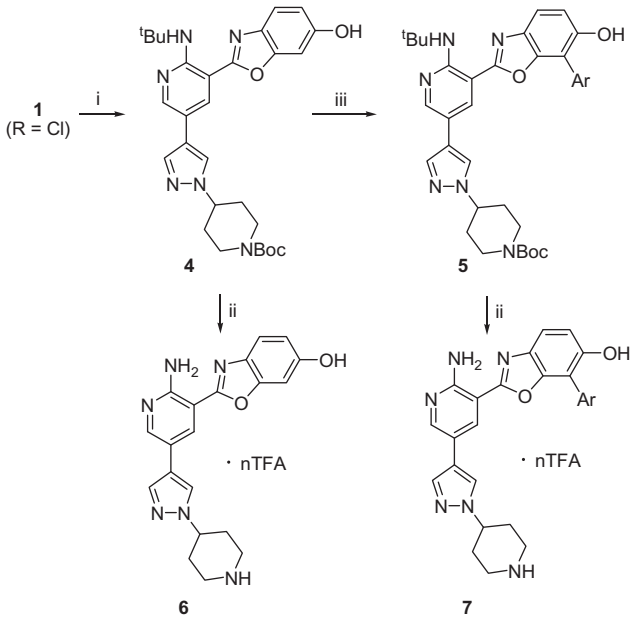
http://dx.doi.org/10.1016/j.bmcl.2012.04.083



Scheme 1. Reagents and conditions: (i) $Pd(PPh_3)_4$, Na_2CO_3, $ArB(OH)_2$, DMF, 120 °C, 65%; (ii) TFA, rt, >95%; (iii) R^1NH_2 or R^1OH, $Pd_2(dba)_3$, X-Phos, $NaOBu^t$, toluene, 100 °C, 67%; and (iv) TFA, 60 °C, >95%.



Scheme 2. Reagents and conditions: (i) Pd_2dba_3, KOH, H_2O, *n*-Bu_3P, 1,4-dioxane, 75%; (ii) TFA, 60 °C, >95%, (iii) ArBr, Pd_2dba_3, X-Phos, K_3PO_4, toluene, 100 °C, 13 h, 45%.

by reduction of **8**, followed by treatment with trifluoroacetic acid (Scheme 3).

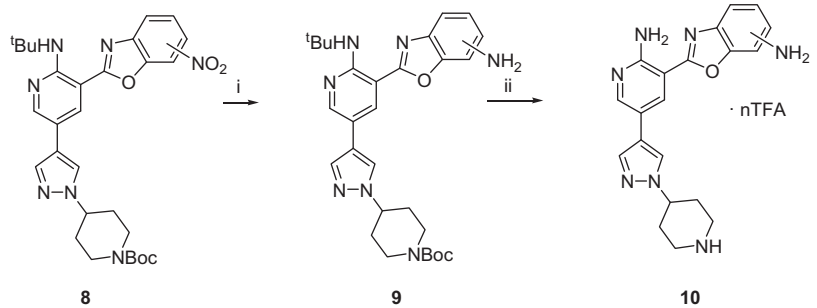
Next, we investigated the substituent effects of the amine functional group. Ureas **11** were synthesized by treating **9** with the corresponding isocyanates. Sulfonyl isocyanates, sulfonyl chlorides, benzoyl chlorides, and aryl bromides were used for the synthesis of amides **12**, sulfonamides **13**, alkylamines **14**, and sulfonylureas **15**, respectively (Scheme 4).

Modifications to the pyrazole moiety were accomplished by cross coupling **16** with suitable pyrazole derivatives. Treatment of **17** with trifluoroacetic acid afforded **18** (Scheme 5).

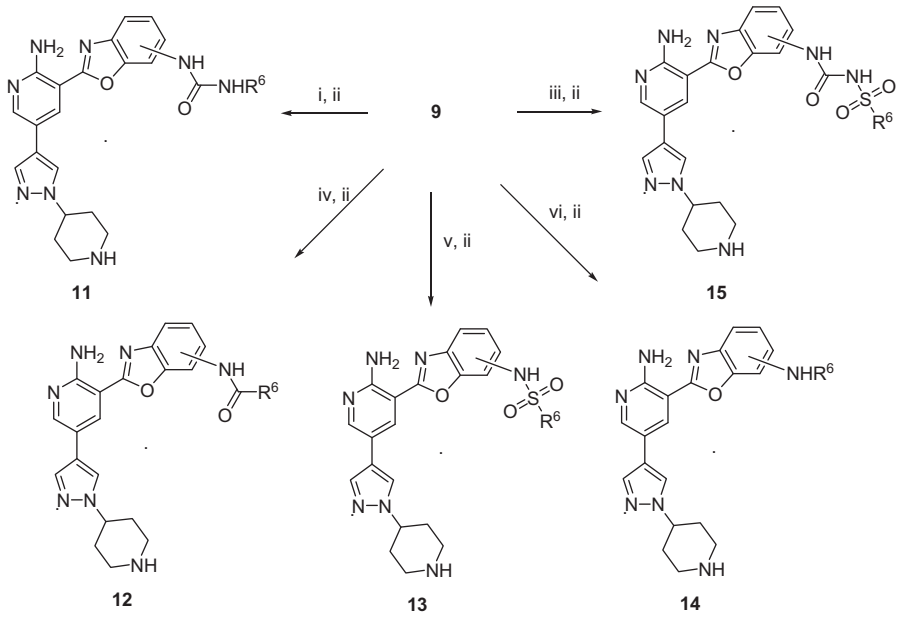
The in vitro c-Met kinase inhibitory activity was tested for all the compounds. As an initial assessment of the SAR focusing on the phenyl ring of the benzoxazole, select functional groups such as halogens, amines, and heteroaryl-oxy were introduced into the phenyl ring; the results are summarized in Table 1. We first examined the effect of halogen substitution on the benzoxazole phenyl. Introduction of chlorine or fluorine (**1b**, **1c**) to the R^2 or R^4 position of benzoxazole did not significantly increase the inhibitory activity, compared to the compounds not subjected to substitution (**1a**).

Substitution of alkylamines (**3a**, **3b**, **3d**) at the benzoxazole ring generally resulted in decreased inhibitory activity compared to the compounds not subjected to substitution (**1a**).[10] Loss of potency was observed with acetyl (**1d**) and 1-hydroxyethane (**1e**) substitution at R^3; these groups should have been equivalent hydrogen bond acceptor and donor as the hydroxyl group. A small increase in inhibitory activity was observed when hetero-aromatic compounds (**2a**, **3f**, **3g**), such as pyridines and O-linked pyrimidine derivatives as hydrogen bond acceptors, were introduced at R^3. A significant increase of inhibitory activity of **2c** was observed when 2-pyridine was substituted at R^4. A possible explanation for increase was illustrated by pi-stacking interaction of aromatic group with Y1230 (vide infra).

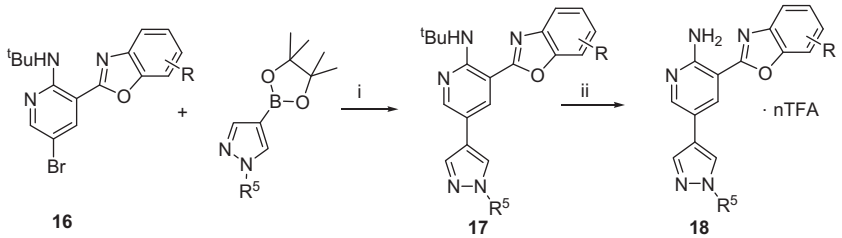
A systematic study of the benzoxazole revealed that inhibitory activity dramatically improved when a hydroxyl group was introduced (Table 2). Introduction of a hydroxyl at the R^2 and R^3 of benzoxazole (**6a**, **6b**) increased the potency by 2- to 3-folds; however, addition of the hydroxyl group at the R^4 position decreased inhibitory activity (**6c**). Significant improvement in potency was observed when a hydroxyl substituent and a substituent adjacent to the hydroxyl group were introduced. These compounds exhibited a nano- to pico-molar range of inhibitory activities. Excellent results were observed when hydroxyl and methyl groups were



Scheme 3. Reagents and conditions: (i) Pd/C, hydrazine hydrate, EtOH, 2 h, rt, 85%; (ii) TFA, 60 °C, >95%.



Scheme 4. Reagents and conditions: (i) R^6NCO, THF, rt, 65%; (ii) TFA, 60 °C, 85%; (iii) R^6SO_2NCO, THF, rt, 57%; (iv) R^6COCl, NEt_3, CH_2Cl_2, 75%; (v) R^6SO_2Cl, NEt_3, CH_2Cl_2, 55%; and (vi) ArBr, $Pd(PPh_3)_4$, Na_2CO_3, $PhB(OH)_2$, DMF, 120 °C, 57%.



Scheme 5. Reagents and conditions: (i) $Pd(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, 61% and (ii) TFA, 60 °C, 83%.

introduced at the R^2 and R^3 positions of benzoxazole (**6d** and **6e**, respectively). Interestingly, a hydroxyl group at the R^3 position of benzoxazole significantly improved inhibitory activity (**6h**), in contrast to the results for **6g**.

To investigate the SAR of the substituents at the R^4 position of benzoxazole, we introduced 3-pyridinyl (**7a**), 2-pyrazinyl (**7b**), and phenyl (**7c**) in place of the methyl group on **6h**. No significant improvement in potency was observed. Introduction of an amine in place of the hydroxyl group exhibited noticeable potency increase (**10a**, **10b**), which were almost equivalent to the activities of OH group. We next investigated the size and electronic requirements for the formation of H-bond of the moieties at the R^3 position of benzoxazole (**11**, **13**, **14, 15**). Introduction of urea (**11**), sulfonylurea (**15**), and sulfonamide (**13a, 13b**) in place of the hydroxyl group at the R^3 position of benzoxazole did not improve potency.

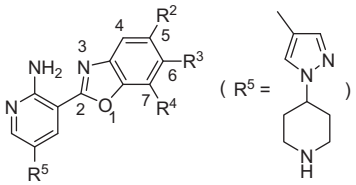
Preliminary SAR studies were focused on the substituents of pyrazole for the parent compound **1a** (Table 3). N-Substitution of piperidine with methyl (**18a**) or *N*-Me-4-piperidinyl (**18b**) did not improve potency. For **18f** and **18g**, loss of potency was observed with N-substitution of piperidine with acetyl, even if the hydroxyl group was at the R^3 position of benzoxazole.

Modeling studies were performed using the Glide software contained in the Maestro 9.1 software with standard-precision (SP) options.[11] The crystal structure of c-Met (PDB code: 3F66[12]) was used with a slight modification in the conformation of Y1230 to accommodate the bulky substituents at the R^4 position. The bound ligand configuration was generated with a good root mean square displacement (RMSD) of 0.5 Å.

Data from the modeling study suggests that aminopyridine compounds bind c-Met by many different strong interactions (Fig. 1). The strong interaction of aminopyridine with c-Met is attributed to the two hydrogen bonds between the aminopyridine moiety and the hinge residues M1160 and P1158, as well as to the partial pi-stacking interactions between the benzoxazole moiety and Y1230. The nitrogen atom in the piperidine ring makes additional hydrogen bonds with the backbone carbonyl of K1161. Hydrophobic interactions of the aminopyridine and benzoxazole moieties with M1211 also possibly contribute to the tight binding. Introduction of a hydrogen donating groups in the R^2 or R^3 [–OH (**6b**) or $–NH_2$ (**10b**) in R^3, –OH in R^2 (**6d**, **6e**, **6f**)] position makes additional strong hydrogen bonds with the carbonyl oxygen of D1222 and N1209, which greatly enhances the potency. A small methyl group adjacent to the hydroxyl group does not make any significant collisions (**6d–6h**), but bulky alkyl chains in the R^2 position make unfavorable interactions with the side chains of A1226 and D1222 in the activation loop, and decrease the potency (**3b, 3c**). Introduction of aromatic groups at the R^4 position (**2c**, **7a**, **7b**) makes partial pi-stacking interactions with Y1230, but methyl groups (**6h**) give better van der Waals interactions with M1211, D1164, N1209 and Y1230 giving the best potency.

Selected compounds were tested against Flt3 (Fms-like tyrosine kinase 3) kinase and in an Hs746T cell proliferation assay; the results are summarized in Table 4. Flt3 receptor tyrosine kiase, a

Table 1
SAR of substituents on benzoxazole



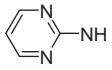
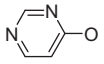
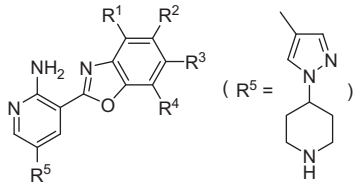
Entry	R^2	R^3	R^4	c-Met IC_{50} (μM)
1a	H	H	H	0.080
1b	Cl	H	H	0.053
1c	Cl	H	F	0.041
1d	H	Me(CO)	H	0.8
1e	H	Me(OH)CH	H	0.13
2a	H	3-Pyridinyl	H	0.028
2b	H	3-Pyrazoly	H	0.21
2c	H	H	2-Pyridinyl	0.0016
2d	H	H	4-MeO-Ph	0.077
3a	H	H	Me_2CHNH	0.13
3b	NHMe	H	H	0.16
3c	MeCONH	H	H	0.54
3d	H	$CH_3CH_2CH_2NH$	H	0.15
3e	H		H	0.063
3f	H		H	0.034
3g	H		H	0.023

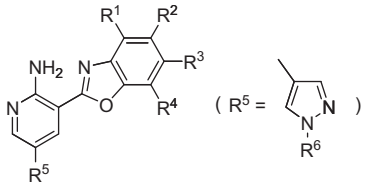
Table 2
SAR of hydroxyl or amine substituents on benzoxazole



Compound	R^1	R^2	R^3	R^4	c-Met IC_{50} (μM)
6a	H	OH	H	H	0.022
6b	H	H	OH	H	0.019
6c	H	H	H	OH	0.11
6d	H	OH	Me	H	0.027
6e	Me	OH	Me	H	0.003
6f	H	OH	Et	H	0.009
6g	H	Me	OH	H	0.18
6h	H	H	OH	Me	0.0002
7a	H	H	OH	3-Pyridinyl	0.004
7b	H	H	OH	2-Pyrazinyl	0.008
7c	H	H	OH	Ph	0.048
10a	H	NH_2	H	H	0.056
10b	H	H	NH_2	H	0.014
11	H	H	PhNH(CO)NH	H	0.13
12	H	NHCOMe	H	H	0.54
13a	H	H	$MeSO_2NH$	H	0.088
13b	H	H	4-F-$PhSO_2NH$	H	0.067
14	H	H	4-F-PhNH	H	0.41
15	H	H	4-Me-$PhSO_2NH(CO)NH$	H	0.045

family of PDGFR (platelet-derived growth factor receptor), is constitutively active in many case of acute myeloid leukemia (AML) found

Table 3
SAR of R^4 and R^6



Entry	R^1	R^2	R^3	R^4	R^6	c-Met IC_{50} μM)
18a	H	H	Cl	H	Me	1.7
18b	H	H	F	H	N-Me-4-piperidinyl	0.24
18c	H	H	F	H	N-Hydroxyethyl-4-piperidinyl	0.09
18d	H	H	Cl	H	N-Acetyl-4-piperidinyl	0.22
18e	H	H	H	Cl	N-(N,N′-Dimethylacetyl)-4-piperidnyl	0.027
18f	H	OH	Me	H	N-Acetyl-4-piperidinyl	0.051
18g	Me	OH	Me	H	N-Acetyl-4-piperidinyl	0.028
18h	H	H	H	Pyridin-2-yl	Hydroxyethyl	0.053
18i	H	H	H	Pyridin-2-yl	Me	0.014
18j	H	H	H	Pyridin-2-yl	N-(N,N′-Dimethylacetyl)-4-piperidnyl	0.076

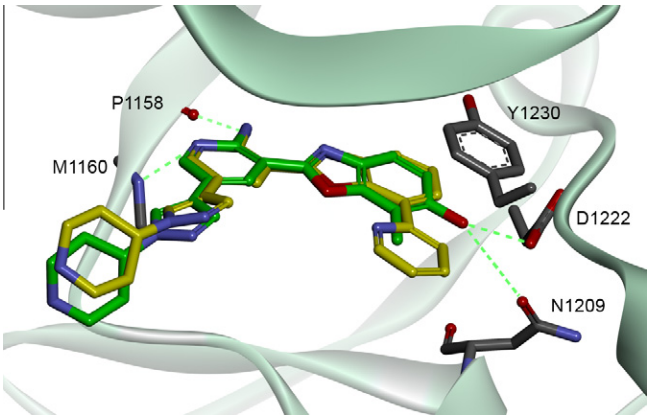


Figure 1. A proposed structure for the c-Met complexed with **2c** (yellow) and **6h** (apple green): Hydrogen bonding between each compound and c-Met is shown in green dotted lines.

up to 30% of cases.[13] Compound **2c** exhibited potent c-Met and Flt3 enzyme inhibition and weak inhibition of Hs746T proliferation; the IC_{50} values ranged from 0.012 to 0.45 μM. **6b** exhibited potent inhibitory activities against Flt3 (IC_{50} = 4 nM), mutant Flt3 (D835Y, IC_{50} = 0.3 nM), Ron (IC_{50} = 0.014 μM), and Aurora A (IC_{50} = 0.09 μM). Compound **6h** displayed potent c-Met (IC_{50} = 0.2 nM) and Flt3 IC_{50} = 0.034 μM) inhibitory activity, with moderate activity in the Hs746T proliferation assay (IC_{50} = 0.035 μM). A series of

Table 4
SAR for Flt3 and Hs746T assay[15]

Compound	c-MetIC_{50} (μM)	FLT3 IC_{50} (μM)	Hs746T IC_{50} (μM)
2c	0.0016	0.0006	0.24
6b	0.019	0.004	0.45
6d	0.027	0.002	0.012
6f	0.009	0.004	0.022
6h	0.0002	0.034	0.035
7b	0.008	0.035	0.18
7c	0.048	0.014	0.010
10b	0.014	0.0036	3.7

benzoxazole compounds exhibited a low level of oral exposure and bioavailability (**6b**, F = 17%, data not shown). To overcome the lack of pharmacokinetic properties presumably attributed to intrinsic phenolic properties, we tried to introduce some of bio-isotere[14] group instead of hydroxyl group of benzoxazoles, such as pyrazole (**2b**), H-bond donor (**1e**) or acceptor groups (**1d**, **2c**), and other functional groups (**13a**, **13b**, **15**). Introduction of functional groups other than hydroxyl group at R^2 and R^3 positions could not increase the c-Met kinase inhibitory activities.

In summary, we have identified a series of benzoxazoles with a substituted hydroxyl group as potent c-Met kinase inhibitors. A series of hydroxyl-substituted benzoxazoles exhibited potent c-Met and Flt3 dual kinase inhibitory activities, with excellent inhibition of Hs746T proliferation. Further studies for improving the pharmacokinetic properties of benzoxazoles in order to increase oral bioavailability and anti-tumor activity are underway in our research group.

Acknowledgments

This work was supported by the Korea Research Institute of Chemical Technology and the Basic Science Research Program from NRF (2011-0010374, S.-Y.H.) funded by the government of Korea (MEST).

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15. *c-Met and Flt3 kinase assay*: Inhibition of kinase activity against recombinant c-Met protein was measured using homogeneous time-resolved fluorescence (HTRF) assays. Recombinant proteins containing c-Met kinase domain were purchased from Millipore. Optimal enzyme, ATP, and substrate concentrations were established using HTRF KinEASE kit (Cisbio) according to the manufacturer's instructions. Assays are composed of the c-Met enzyme mixed with serially diluted compounds and peptide substrates in a kinase reaction buffer (250 mM HEPES (pH 7.0), 0.5 mM orthovanadate, 0.05% BSA, 0.1% NaN_3, 5 mM $MgCl_2$, 1 mM DTT). Following the addition of reagents for detection, the Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) signal was measured using an EnVision multi-label reader (Perkin Elmer). Dose–response curves were generated to determine IC_{50} using Prism version 5.01 (GraphPad). Flt3 kinase assay was performed as described above using recombinant Flt3 protein in kinase reaction buffer (250 mM HEPES (pH 7.0), 0.5 mM orthovanadate, 0.05% BSA, 0.1% NaN_3, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM DTT). Reference compounds were included in each assay for plate uniformity (crizotinib for c-Met and lestaurtinib for Flt3) and select compounds were subjected to repeat experiments.
Proliferation assay: Cells were plated in 96-well plates (10,000 cells per well) and serial dilutions of compounds were added. At the end of the incubation period (72 h), cell viability was measured by a tetrazolium dye assay using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] (Dojindo, Japan). IC_{50} was calculated by a nonlinear regression using Prism version 5.01.