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Discovery of aminopyridines substituted with benzoxazole as orally active c-Met kinase inhibitors

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ABSTRACT

We report the synthesis and biological evaluation of aminopyridines substituted with benzoxazole. The SAR of the aminopyridines was explored to improve the inhibitory activity against c-Met and to decrease hERG affinity. These studies led to the discovery of amide **24** which showed good c-Met inhibitory potency, low affinity to hERG and favorable pharmacokinetic properties in rats.

c-Met is a receptor tyrosine kinase and plays important roles in cell survival, proliferation, migration, and angiogenesis under physiological conditions. Upon the binding of the hepatocyte growth factor/scatter factor (HGF/SF), c-Met dimerizes, autophosphorylates and induces activation of a number of downstream signal transducers such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), signal transducers and activators of transcription (STAT), phosphoinositide-3-kinase (PI3K), Akt and focal adhesion kinase (FAK).[1–3] c-Met has recently attracted considerable interest as a therapeutic target based on the discovery that aberrant c-Met activity leads to the formation of various cancers including lung, gastric, renal, ovarian, prostate, and liver cancers.[2,3] HGF/SF and epidermal growth factor (EGF) induce synergistic phosphorylation of c-Met, Akt, and ERK1/2,[4] and resistance to an epidermal growth factor receptor (EGFR) inhibitor (gefitinib) was developed by c-Met amplification in non-small cell lung cancers.[5]

Activation of c-Met can be regulated by preventing HGF/SF from binding to the receptor with antibodies, truncated HGF fragments, decoy receptors, and ribozyme based treatments, and/or blocking the active site of the kinase domain with small molecules.[2,3] Small molecule c-Met inhibitors could be used broadly to treat cancer patients with deregulated c-Met because they inhibit ligand dependent and independent activation of c-Met. A number of small molecule inhibitors targeting c-Met have been reported and some of them reached the clinical trials phase.[2] Described herein is a series of aminopyridine-based inhibitors of c-Met kinase, which were substituted with benzoxazole.

The syntheses of **6**–**11** and **13** are shown in Scheme 1. Nicotinoyl chloride **1**, prepared by a procedure explained in the literature,[6] was coupled with aminophenol **2** to provide benzoxazole **3**.[7] Substitution of chloride in benzoxazole **3** with *t*-butylamine furnished bromopyridine **4**.[8] Suzuki coupling of bromopyridine **4** with various boronic acids or boronate afforded diaryl substituted pyridine **5**.[9] Removal of the *t*-butyl group of pyridine **5** by treatment with trifluoroacetic acid gave aminopyridines **6–11**.[10] Piperidine **13** was also obtained from pyrazole **12** in the same conditions for compounds **6–11**. Both the *t*-butyl and Boc groups of compound **12** were removed by treatment with trifluoroacetic acid simultaneously.

Scheme 2 depicts the syntheses of **16** and **18**. Benzoxazole **15**, prepared from commercially available nicotinoyl chloride **14** and aminophenol **2** like benzoxazole **3**, was coupled with boronates to give pyrazoles **16** and **17**. The Boc group of pyrazole **17** was deprotected to produce piperidine **18** by treatment with hydrogen chloride solution.

Aminopyridine **19** was obtained by the replacement of chloride with amines from benzoxazole **3** and converted to 3,5-diaryl-substituted pyridines **20–22** by Suzuki coupling, as shown in Scheme 3. Deprotection of the Boc group transformed pyridine **22** to piperidine **23**.

The syntheses of compounds **24–33** were described in Scheme 4. Piperidine **13** was coupled with carbonyl chlorides to produce aminopyridines **24–32**. Acetate **32** was hydrolyzed to alcohol **33** by treatment with LiOH.

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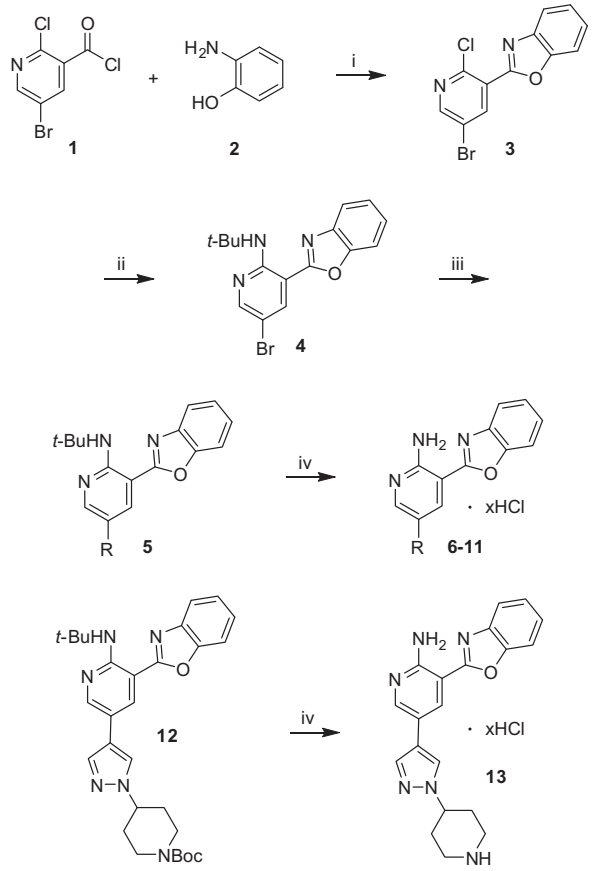
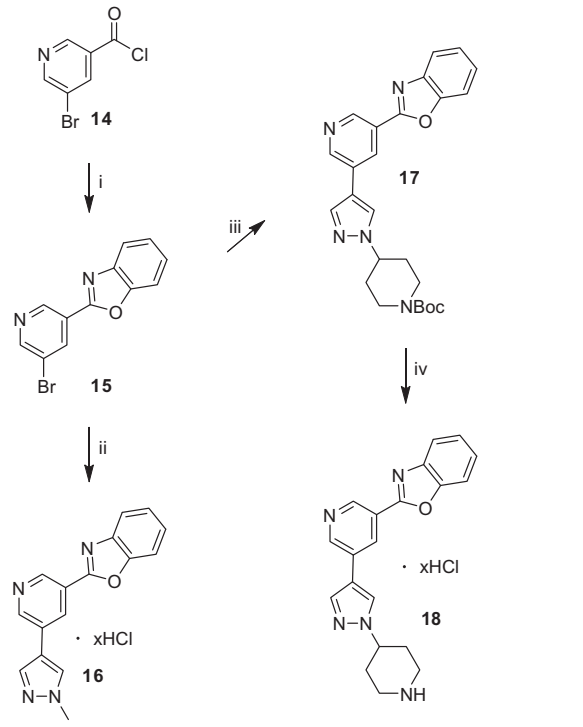


Scheme 1. Reagents and conditions: (i) Et_3N, PPTS, xylene, rt, 3 h then reflux 24 h, 45%; (ii) *t*-$BuNH_2$/DMF (1:1), 70 °C, 72 h, 89%; (iii) $Pd(Ph_3P)_2Cl_2$, $RB(OH)_2$ or $RB(OR^1)_2$, dioxane, rt, 30 min then addition of 1 M aqueous Na_2CO_3, reflux, 3 h, 65–85%; (iv) CF_3COOH, 60 °C, overnight then 4 M HCl in dioxane, MeOH, 70–95%.

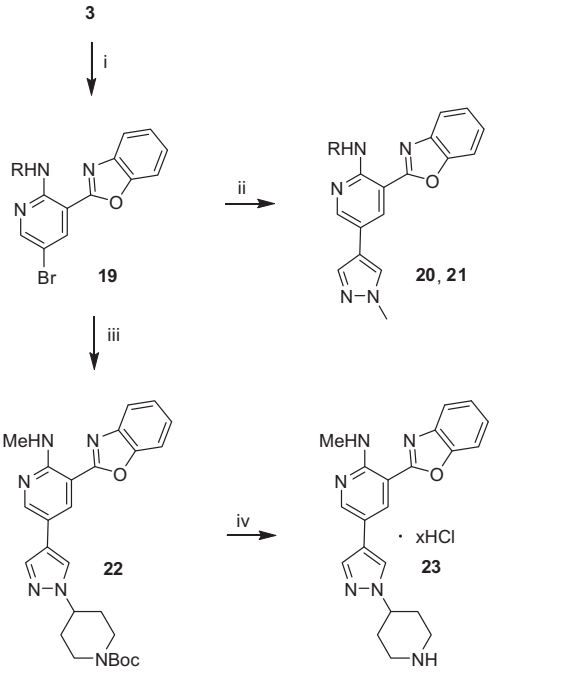
To find novel inhibitors targeting ATP-binding sites of c-Met, we designed several inhibitors based on the X-ray structure of c-Met complexes with known inhibitors.[11–13] Initially aminopyridines substituted with benzoxazole **6–9** were designed and synthesized (Table 1). Aminopyridines **7** and **9** showed low micromolar activity in enzymatic assay in vitro.[14] To figure out the structure–activity relationship (SAR) of aminopyridine **9** we first modified the amino group in the pyridine ring and found that the amino group was essential for activity (Table 2). Removal of the amino group decreased c-Met inhibitory activity very much. Substitution of one hydrogen of the amino group with methyl or benzyl group also abolished the c-Met inhibitory activity. The secondary amino group may not form bidentate H-bonds with a c-Met hinge residue due to an intramolecular hydrogen bonding with the N or O of benzoxazole ring.

Next, we sought to replace the *N*-methyl group in the pyrazole ring of aminopyridine **9** with other groups (Table 1). The introduction of the hydroxyethyl group or pyran in the pyrazole ring (compounds **10** and **11**) increased the potency by a few fold. Moreover, the c-Met inhibitory activity of aminopyridine **9** was improved by more than one order of magnitude by the substitution of the *N*-methyl group with piperidine. To further confirm the importance of the amino group in the aminopyridines, we prepared piperidine derivatives **18** and **23**. As expected, both compounds **18** and **23** were inactive in a c-Met kinase assay (Table 2).[14]

Recently some small molecules were withdrawn from the market because they induced the prolongation of the QT interval of the surface electrocardiogram (ECG) by blocking the human ether-a-go-go-related gene (hERG) potassium channel.[15] Blockade of the

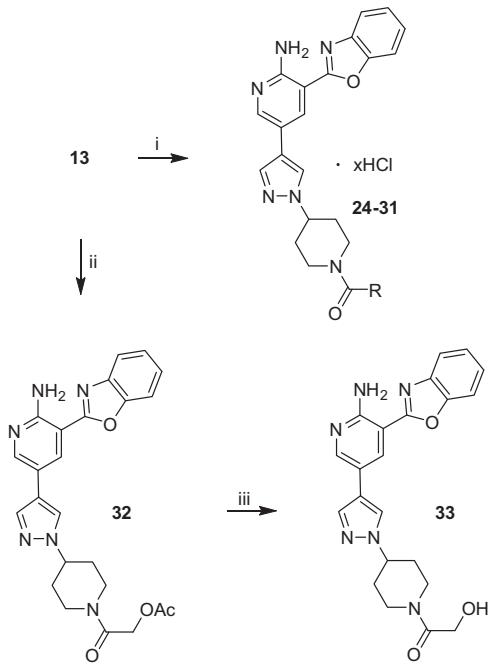


Scheme 2. Reagents and conditions: (i) Et_3N, PPTS, 2-aminophenol (**2**), xylene, rt, 3 h then reflux 24 h, 46%; (ii) $Pd(Ph_3P)_2Cl_2$, 1-methylpyrazole-4-boronic acid pinacolester, dioxane, rt, 30 min then addition of 1 M aqueous Na_2CO_3, reflux, 3 h, 75%; (iii) $Pd(Ph_3P)_2Cl_2$, 1-(4-*N*-Boc-piperidine)pyrazole-4-boronic acid pinacolester, dioxane, rt, 30 min then addition of 1 M aqueous Na_2CO_3, reflux, 3 h; (iv) 4 M HCl in dioxane, MeOH, 0 °C, 3 h, 74% for two steps.



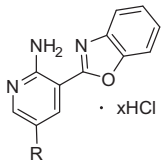
Scheme 3. Reagents and conditions: (i) RNH_2/DMF, 70 °C, 72 h, 65–70%; (ii) $Pd(Ph_3P)_2Cl_2$, 1-methylpyrazole-4-boronic acid pinacolester, dioxane, rt, 30 min then addition of 1 M aqueous Na_2CO_3, reflux, 3 h, 80–95%; (iii) $Pd(Ph_3P)_2Cl_2$, 1-(4-*N*-Boc-piperidine)pyrazole-4-boronic acid pinacolester, dioxane, rt, 30 min then addition of 1 M aqueous Na_2CO_3, reflux, 3 h, 91%; (iv) CF_3COOH, 60 °C, overnight then 4 M HCl in dioxane, MeOH, 72%.

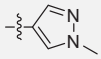
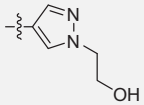
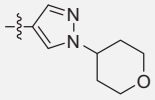
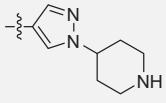
hERG channel is a significant hurdle in lead optimization activity due to its potential cardiac toxicity. Thus, we measured the hERG



Scheme 4. Reagents and conditions: (i) RCOCl, Et_3N, CH_2Cl_2, 0 °C, 1–4 h then 4 M HCl in dioxane, MeOH, 70–99%; (ii) $AcOCH_2COCl$, Et_3N, CH_2Cl_2, 0 °C, 3 h, 84%; (iii) LiOH, THF/H_2O (4:1), rt, 5 h then 4 M HCl in dioxane, MeOH, 99%.

Table 1
Aminopyridines substituted with benzoxazole: c-Met potency and hERG binding inhibition

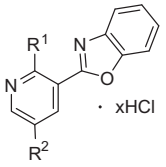


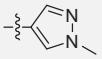
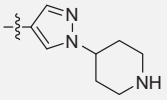
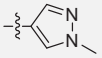
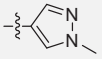
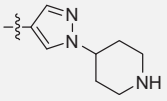
Compound	R	c-Met IC_{50}[a] (μM)	hERG inhibition @ 10 μM[b]
6	Ph	>10	N[c]
7	4-Pyridyl	5.2	N[c]
8	3-Pyridyl	>10	N[c]
9		2.1	15%
10		1.3	N[c]
11		0.43	12%
13		0.08	0.75 μM[d]

[a] For assay conditions, see Ref. 14.
[b] For assay conditions, see Ref. 16.
[c] Not tested.
[d] IC_{50} value.

affinity of some of the aminopyridines prior to further optimization. Piperidine **13** bound to the hERG channel with an IC_{50} value of 0.75 μM, but aminopyridines **9** and **11** did poorly.[16] The results

Table 2
SAR of pyridines substituted with benzoxazole



Compound	R^1	R^2	c-Met IC_{50}[a] (μM)
16	H		>10
18	H		>10
20	NHMe		>10
21	NHBn		>10
23	NHMe		>10

[a] For assay conditions, see Ref. 14.

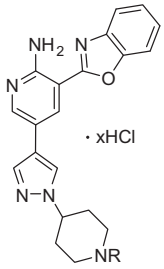
suggest that the piperidine group of compound **13** should be responsible for the high hERG affinity. To improve the inhibitory potency against c-Met and overcome hERG binding, we modified the piperidine group of compound **13**. N-alkylation of the piperidine group did not increase potency but decreased the hERG affinity 10–30-fold (data not shown). Piperidines substituted with benzoyl, carbamyl, carbonate, and sulfonyl groups did not reveal better c-Met inhibitory activity than compound **13** but had less affinity to hERG (Table 3). Gratifyingly, however, the introduction of acetyl, substituted acetyl and propanoyl groups improved the potency and strongly decreased the hERG affinity. Acetamide **24**[17] showed single digit nanomolar c-Met inhibitory activity and no affinity to the hERG channel even at 100 μM.

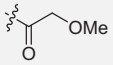
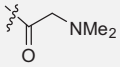
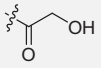
Acetamide **24** fits in the ATP-binding site of c-Met very well as shown in Figure 1. A crystal structure of c-Met complex with K252a was obtained from the protein data bank (pdb entry; 1R0P).[11] Calculations for docking was carried out using LigandFit[18] interfaced with Accelrys DiscoveryStudio2.5. The crystal structure of 1R0P was regenerated very well with −4.58 kcal/mol of binding energy for K252a. We set the O atom and the H atom in the peptide backbone of P1158 and M1160 as interaction sites with default parameters in LigandFit. Acetamide **24** bound c-Met very tightly (−9.56 kcal/mol) when the same docking parameters were used. The strong interaction of acetamide **24** with c-Met is attributed to H-bondings of both the aminopyridine moiety with hinge residues M1160 and P1158, and the acetyl group with Y1230. Nitrogen in the piperidine ring may contribute to the tight binding by forming water-bridged H-bonding with Y1230 or N1167. Interestingly, the binding mode of acetamide **24** is quite different from that of PF-2341066 which is also a 3,5-disubstituted aminopyridine-based c-Met inhibitor in clinical trials.[9b,13,19] Acetamide **24** interacts with Y1230 by a H-bonding instead of a π-stacking interaction. While 2,6-di-chloro-3-fluorophenyl ring of PF-2341066 forms a strong π-stacking interaction with Y1230, a π-stacking interaction is not present between benzoxazole group of acetamide **24** and Y1230.

Preliminary pharmacokinetic studies of acetamide **24** in male rats demonstrated favorable pharmacokinetic properties (Table 4).

In summary, we have designed and synthesized a series of aminopyridines substituted with benzoxazole, which targets

Table 3
SAR of piperidines



Compound	R	c-Met IC_{50}[a] (μM)	hERG inhibition @10 μM[b]
24	Ac	0.003	0% @ 100 μM
25		0.007	10%
26	Bz	0.140	63%
27		0.017	0%
28		0.060	0%
29		0.357	46%
30		0.058	40%
31		0.371	1%
33		0.026	13%

[a] For assay conditions, see Ref. 14.
[b] For assay conditions, see Ref. 16.

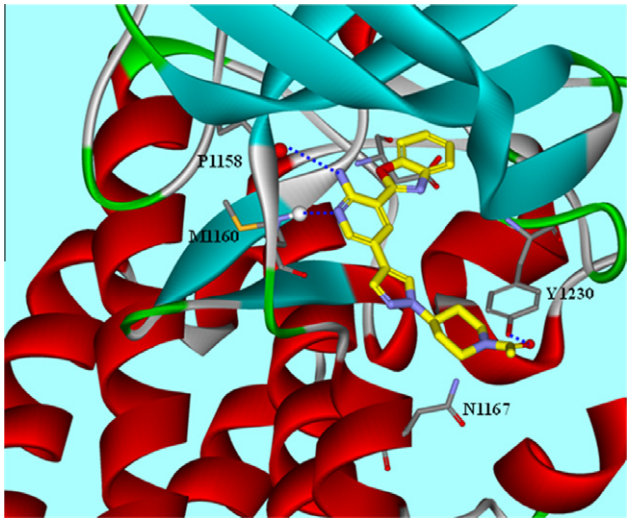


Figure 1. A proposed structure for c-Met complex with acetamide **24** (yellow). H-bonding interactions between the compound and c-Met are shown in blue dotted lines.

c-Met kinase. Some of the compounds showed good c-Met inhibitory activity, low hERG affinity and good oral bioavailability in rats. Currently, we are carrying out both in vitro and in vivo pharmacological evaluation of the compounds, and the results will be reported in a near future.

Table 4
Preliminary pharmacokinetic profile for **24** in rats

Parameter	iv[a]	po[a]
C_{max} (μg/mL)		0.6
T_{max}		2.7
$t_{1/2}$	1.0	1.5
AUC (μg h/mL)	5.6	2.7
CL (L/kg h)	1.8	
V_{ss} (L/kg)	2.4	
% *F*		47

[a] Dose, 10 mg/kg (three rats used).

Acknowledgments

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14. *c-Met 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).
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16. *hERG binding assay*: inhibition profiles were measured in HEK293 cells, which were stably transfected with a hERG channel construct, by a radioligand binding assay with [^{3}H]astemizole.
17. *Characterization data for acetamide* **24**: ^{1}H NMR (500 MHz, CD_3OD) δ 9.06 (d, J = 2.2 Hz, 1H), 8.39 (d, J = 2.2 Hz, 1H), 8.34 (s, 1H), 8.02 (d, J = 0.6 Hz, 1H), 7.87 (ddd, J = 8.0, 1.2, 0.6 Hz, 1H), 7.78 (ddd, J = 8.0, 0.9, 0.9 Hz, 1H), 7.54 (ddd,

J = 7.8, 7.8, 1.3 Hz, 1H), 7.49 (ddd, J = 7.7, 7.7, 1.1 Hz, 1H), 4.69–4.63 (m, 1H), 4.57–4.50 (m, 1H), 4.12–4.06 (m, 1H), 3.37–3.31 (m, 1H), 2.87 (ddd, J = 13.0, 13.0, 2.6 Hz, 1H), 2.26–2.15 (m, 5H), 2.26–2.15 (m, 5H), 2.10–1.90 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.7, 159.4, 151.7, 151.1, 142.0, 141.9, 137.4, 135.5, 128.2, 127.3, 126.8, 121.5, 120.7, 117.4, 112.1, 111.6, 60.4, 41.7, 33.8, 33.1, 21.2; HRMS (EI) m/z calcd for $C_{22}H_{22}N_6O_2$ (M^+) 402.1804, found 402.1801.

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