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Discovery of amido-benzisoxazoles as potent c-Kit inhibitors

Roxanne K. Kunz [a,*], Shannon Rumfelt [a], Ning Chen [a], Dawei Zhang [a], Andrew S. Tasker [a], Roland Bürli [a], Randall Hungate [a], Violeta Yu [b], Yen Nguyen [b], Douglas A. Whittington [c], Kristin L. Meagher [c], Matthew Plant [d], Yanyan Tudor [e], Michael Schrag [f], Yang Xu [f], Gordon Y. Ng [d], Essa Hu [a]

[a] Department of Medicinal Chemistry, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[b] Department of HTS Molecular Pharmacology, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[c] Department of Molecular Structure, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[d] Department of Inflammation, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[e] Department of Molecular Sciences, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA
[f] Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA

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ABSTRACT

Deregulation of the receptor tyrosine kinase c-Kit is associated with an increasing number of human diseases, including certain cancers and mast cell diseases. Interference of c-Kit signaling with multi-kinase inhibitors has been shown clinically to successfully treat gastrointestinal stromal tumors and mastocytosis. Targeted therapy of c-Kit activity may provide therapeutic advantages against off-target effects for non-oncology applications. A new structural class of c-Kit inhibitors is described, including in vitro c-Kit potency, kinase selectivity, and the observed binding mode.

The receptor tyrosine kinase, c-Kit, is a transmembrane glycoprotein, which is highly expressed on mast cells, as well as hematopoietic stem cells, germ cells, and gastrointestinal tract cells.[1] Ligation of c-Kit to the cytokine stem cell factor (SCF) results in dimerization, transphosphorylation, and initiation of multiple downstream signaling pathways. Deregulation, overexpression, or mutation of c-Kit signaling has been implicated in a variety of human tumors, including mast cell tumors, non-small cell lung tumors, and gastrointestinal stomal tumors.[2,3] Additionally, the interaction between c-Kit and SCF regulates the survival, differentiation, and proliferation of mast cells, which has implications in inflammatory and autoimmune diseases.[4,5]

Gleevec (also known as imatinib mesylate or STI-571) inhibits the tyrosine kinases c-Abl, platelet-derived growth factor (PDGF), and c-Kit, and is approved for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors.[6] For non-oncology applications, however, we desired a potent and more selective c-Kit inhibitor for the treatment of mast cell-related autoimmune or inflammatory diseases. As such, compound **1** was identified through a screen of our kinase-preferred library. Gratifyingly, compound **1** exhibited greater c-Kit enzymatic potency compared to Gleevec (Fig. 1)[7] but was less selective against p38 kinase (14-fold vs 236-fold, respectively), which was selected as our primary counter-screen to avoid acting through the MAP kinase pathway.[8] Preliminary in vivo metabolism studies of compound **1** revealed extensive hydrolysis of the lower trifluoromethyl amide and formation of a potentially toxic aniline metabolite.[9,10] While bis-amide **1** represented a new structural class of c-Kit inhibitor, a more selective compound, which avoided the potential formation of reactive metabolites, was desired.[11]

Based on molecular modeling, we envisioned that the lower amide in compound **1** could be replaced by a bicyclic benzisoxazole moiety. Substitution with this bioisostere would bypass the amide hydrolysis metabolism pathway.[12] We also chose to replace

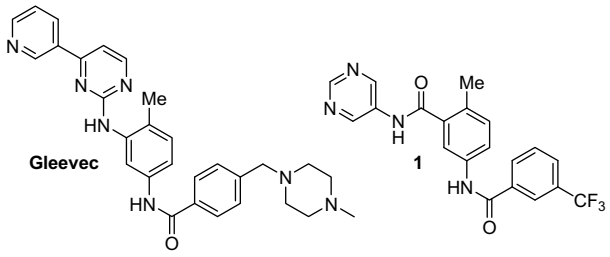

Figure 1. In vitro potencies of Gleevec and bis-amide **1**.

* Corresponding author. Tel.: +1 805 313 5370; fax: +1 805 480 3016.
E-mail address: kunz@amgen.com (R.K. Kunz).

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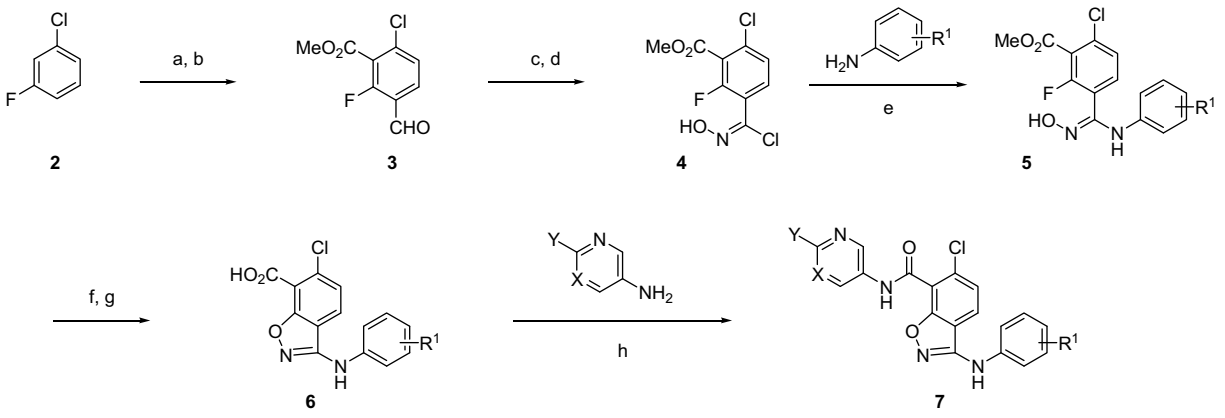


Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, methylchloroformate, −78 to 23 °C (b) LDA, DMF, THF, −78 to 23 °C (50%, two steps); (c) hydroxylamine, EtOH; (d) *N*-chlorosuccinimide, DMF, reflux (90%, two steps); (e) aniline, THF, 23 °C (54–78%); (f) NaH, DMF, 0 °C (40–100%); (g) LiOH, dioxane, reflux (13–100%); (h) aniline, HATU, Et_3N, DMF, reflux (~1–97%).

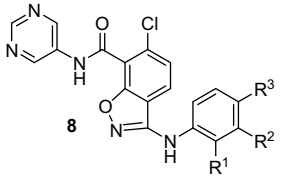
the C-6 methyl group of compound **1** with a chloro group based on our in vivo metabolism studies, which showed benzylic hydroxylation and subsequent oxidation to an aldehyde metabolite. The synthesis of amido-benzisoxazoles (i.e., **7**, Scheme 1) began with lithiation of 1-chloro-3-fluorobenzene (**2**) followed by treatment with methylchloroformate to access the benzoate ester. *ortho*-Lithiation and formylation led to aldehyde **3**, which was condensed with hydroxylamine to give the intermediate oxime. Subsequent chlorination with *N*-chlorosuccinimide gave compound **4** in good yield for the four steps. Various anilines were introduced by displacement of the chloride to provide hydroxyamidine **5**. Closure of the benzisoxazole ring[13] was effected by treatment with sodium hydride, which was followed by ester hydrolysis to give acid **6**. Finally, a series of arylamides were prepared utilizing standard coupling conditions.[14]

Initial structure–activity relationship (SAR) studies began with substituted anilines (Table 1). A class of *para*-substituted compounds was prepared (**9**–**15**), and it was found that increasing the alkyl chain length from ethyl (**9**) to *n*-pentyl (**12**) resulted in a 24-fold decrease in potency. Replacement of the ethyl group with *t*-butyl, trifluoromethyl, or trifluoromethoxy (**13**–**15**) maintained c-Kit potency, although p38 selectivity was diminished. Analogs incorporating *meta*-substitution were examined next (**16**–**19**). Although the intrinsic potency of *m*-nitrile compound **17** was similar to that of Gleevec, the overall fold selectivity against p38 was lower (90-fold vs 236-fold, respectively). Compound **19** was found to have the best combination of c-Kit potency (3 nM) and fold selectivity (113-fold). Compared to the original screening hit **1**, **19** was nearly 5-fold more potent against c-Kit and 8-fold more selective against p38. Disubstitution did not lead to an increase in potency or selectivity (**20**).

Having identified the optimal arylamino-benzisoxazole fragment, the *m*-trifluoromethyl group was kept constant while varying the aryl amide (Table 2). Substitution of the original pyrimidine ring with an amino or acetamido-pyrimidine led to a decrease in p38 selectivity (**24 and 25**). Similarly, amino- or acetamido-pyridines resulted in significant erosion in selectivity (**22 and 23**). Unsubstituted pyridine (**26**) or chloro-pyrimidine (**27**) also did not yield an advantage over the parent pyrimidine compound **19**.

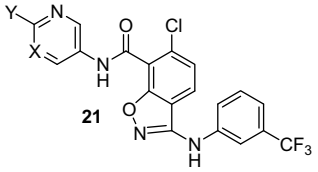
An extended kinase profile of benzisoxazole **19** was undertaken to assess potential for off-target activity (Fig. 2). In addition to p38, good selectivity over KDR was achieved (118-fold), as well as Tie-2 (>5 μM). Moderate (20-fold or greater) selectivity against Src, Lck, and cFMS was observed. Compound **19** was equipotent against the most closely related receptor tyrosine kinase PDGF-α (4 nM). This result is not entirely surprising given the sequence homology between the active site residues of c-Kit and PDGF.[15] Indeed, Gleevec is nearly equipotent against PDGF as compound **19** and it has been suggested that Gleevec may have added potential for treatment of diseases regulated through PDGF signaling.[16]

Table 1
SAR of substituted arylamino-benzisoxazoles represented by general structure **8**

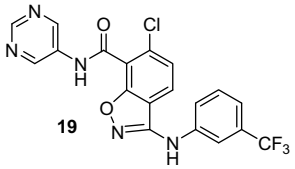


Compound	R^1	R^2	R^3	c-Kit IC_{50} (μM)	p38 IC_{50} (μM)	Fold selectivity
9	H	H	Et	0.007	0.399	57
10	H	H	Pr	0.007	0.189	27
11	H	H	Bu	0.028	0.296	11
12	H	H	Pent	0.170	1.83	11
13	H	H	*t*-Bu	0.008	0.190	24
14	H	H	CF_3	0.011	0.217	20
15	H	H	OCF3	0.011	0.187	17
16	H	*t*-Bu	H	0.005	0.050	10
17	H	CN	H	0.059	5.34	91
18	H	OCF_3	H	0.004	0.156	39
19	H	CF_3	H	0.003	0.339	113
20	H	CF_3	F	0.011	0.265	24

Table 2
SAR of *m*-CF_3 amido-benzisoxazoles represented by general structure **21**



Compound	X	Y	c-Kit IC_{50} (μM)	p38 IC_{50} (μM)	Fold selectivity
19	N	H	0.003	0.339	113
22	CH	NH_2	0.002	0.010	5
23	CH	NHAc	0.001	0.013	13
24	N	NH_2	0.001	0.015	15
25	N	NHAc	0.004	0.182	46
26	CH	H	0.003	0.065	22
27	N	Cl	0.020	0.285	14



Kinase	IC_{50} (μM)
cKit	0.003
p38	0.339
KDR	0.353
Tie-2	> 5.00
Src	0.117
Lck	0.075
cFMS	0.071
PDGF-α	0.004

Figure 2. Kinase selectivity of benzisoxazole **19**.

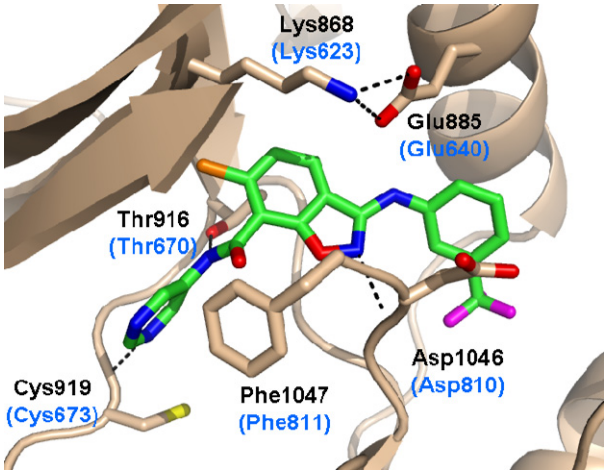


Figure 3. Co-crystal structure of compound **19** and V916T KDR mutant enzyme. Oxygen atoms, red; nitrogen atoms, blue; chlorine atoms, orange; fluorine atoms, magenta; sulfur, yellow. The KDR mutant residues are labeled in black, and the corresponding wild-type c-Kit residues are shown in blue.

To gain a better understanding of the binding mode of compound **19**, a co-crystal structure was obtained with a KDR mutant protein where the gatekeeper residue was mutated from valine to threonine to mimic the active site of c-Kit. The 2.9-Å resolution structure is shown below in Fig. 3.[17] Consistent with the Gleevec/c-Kit co-crystal structure,[18,19] benzisoxazole **19** binds to the DFG-out form of the enzyme. The gatekeeper threonine is engaged in a 3.2 Å hydrogen bonding interaction with the amide hydrogen, similar to the Gleevec aminopyrimidine–Thr-670 hydrogen bond in c-Kit. The pyrimidine ring forms a hydrogen bond with the backbone amide adjacent to the Cys-673 residue in the linker region, which mirrors the hydrogen bond formed between this residue in c-Kit and the pyridine nitrogen of Gleevec. The third contact formed between compound **19** and the KDR mutant is a 2.9-Å hydrogen bond between the benzisoxazole nitrogen and the backbone amide of Asp810. Although the potential exists for a hydrogen bond between Glu-640 and the arylamino-benzisoxazole hydrogen, it is not observed in the KDR mutant co-crystal structure. The equivalent interaction is present in the c-Kit/Gleevec structure (with the amide hydrogen of Gleevec) and this discrepancy could be a result of subtle structural differences between the KDR mutant and wild-type c-Kit.

In summary, a new class of c-Kit inhibitors has been described, which exhibits superior in vitro potencies compared to Gleevec. Compound **19**, exhibiting the greatest selectivity against p38, was nearly 20-fold more potent against c-Kit as compared to Gleevec. Improvement in off-target selectivity against p38 was achieved by modification of the arylamino group.

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