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Design and synthesis of 3-substituted benzamide derivatives as Bcr-Abl kinase inhibitors

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Abstract—A series of 3-substituted benzamide derivatives structurally related to STI-571 (imatinib mesylate), a Bcr-Abl tyrosine kinase inhibitor used to treat chronic myeloid leukemia (CML), was prepared and evaluated for antiproliferative activity against the Bcr-Abl-positive leukemia cell line K562. About ten 3-halogenated and 3-trifluoromethylated benzamide derivatives were identified as highly potent Bcr-Abl kinase inhibitors. One of these, NS-187 (**9b**), is a promising new candidate Bcr-Abl inhibitor for the therapy of STI-571-resistant chronic myeloid leukemia.

Chronic myeloid leukemia (CML) is caused by uncontrolled signals from a constitutively activated Bcr-Abl protein tyrosine kinase.[1] STI-571 (imatinib mesylate, Gleevec® or Glivec®; Fig. 1), a specific inhibitor of Bcr-Abl kinase, produces clinical remission in CML patients with minimal toxicity.[2,3] This selective inhibition of Bcr-Abl kinase by STI-571 has been a successful therapeutic strategy for CML because of the high efficacy and mild side effects of this compound.[4,5] Crystallographic studies have revealed that STI-571 binds to the kinase domain of c-Abl only when the domain adopts the inactive 'closed' conformation, which is required for optimal inhibition of the kinase activity.[6,7] However, despite the good hematological and cytogenetic responses obtained, primary refractory disease and secondary resistance still occur with STI-571, particularly in patients with advanced-phase disease.[8] Although several mechanisms have been proposed to account for this resistance, including increased expression of Bcr-Abl protein, amplification of the Bcr-Abl gene, and overexpression of the multidrug resistance P-glycoprotein,[9–12] point mutations in the Bcr-Abl gene itself account for most cases of resistance. To date, more than 30 different clinically relevant point mutations, such as the one at position 255 within the kinase domain, have been identified.[13–16]

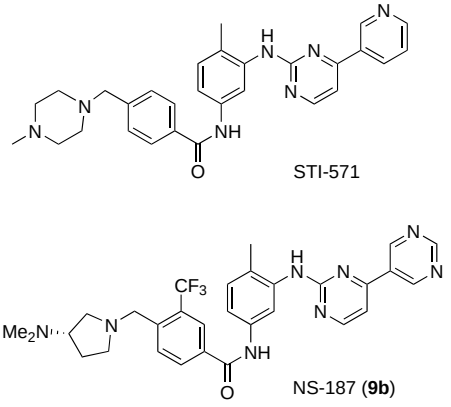

Figure 1. Chemical structures of STI-571 and NS-187 (**9b**).

Nagar et al.[7] have already reported the crystal structure of the kinase domain of c-Abl with STI-571 bound. They showed that STI-571 forms six hydrogen bonds with the protein, and that the majority of contacts are mediated by van der Waals interactions, which lock the c-Abl kinase into its inactive conformation. When we closely examined the published crystal structure, we found a hydrophobic pocket formed by amino acid residues Ile-293, Leu-298, Leu-354, and Val-379 around the

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phenyl ring adjacent to the piperazinylmethyl group of STI-571. As part of an effort to identify compounds with improved antiproliferative activity against Bcr-Abl-positive leukemia cell lines, we first focused on this hydrophobic pocket, and we introduced various hydrophobic substituents at the above-mentioned phenyl ring in STI-571. We found that 3-halogenated and 3-trifluoromethylated benzamides displayed significantly increased activity compared to unsubstituted STI-571. In this report, we describe structure–activity studies of 3-substituted benzamide derivatives, including a potent Bcr-Abl kinase inhibitor, NS-187 (**9b**; Fig. 1).

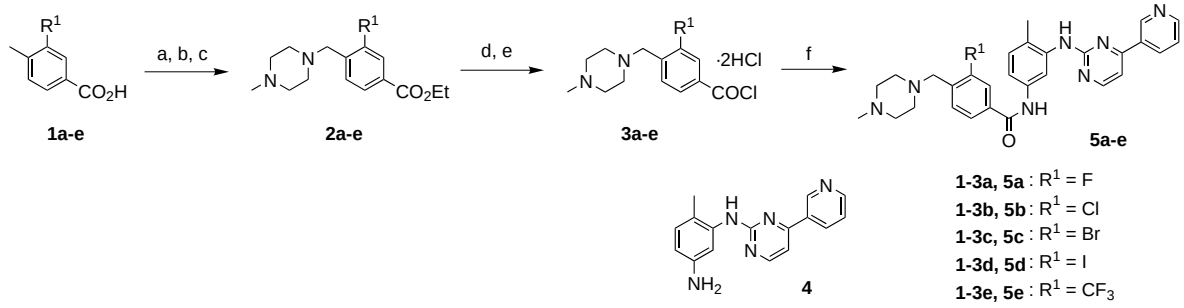
All 3-substituted benzamides reported herein were synthesized as described below. The synthesis of pyridine-tailed derivatives **5a**–**e** is illustrated in Scheme 1. Esterification of the commercially available carboxylic acids **1a**–**e**, subsequent α-bromination with *N*-bromosuccinimide, and coupling with 1-methylpiperazine afforded compounds **2a**–**e**. Acid chlorides **3a**–**e** were obtained by hydrolysis of the ethyl esters **2a**–**e** followed by reaction with thionyl chloride. The benzoylation of the aniline **4**[17] with **3a**–**e** in pyridine solvent afforded the corresponding amides **5a**–**e**.

Pyrimidine-tailed derivatives **9a**–**f** were synthesized as shown in Scheme 2. α-Bromination of 4-methyl-3-trifluoromethylbenzoic acid **1e** by $NaBrO_3/NaHSO_3$ reagent[18] followed by reaction with thionyl chloride yielded acid chloride **6**. Compound **6** was converted to the benzyl bromide **8** through condensation with the aniline **7**, which was prepared from 5-acetylpyrimidine as previously described.[17] Displacement reaction of **8** with various cyclic amines afforded the desired final products **9a**–**f**.

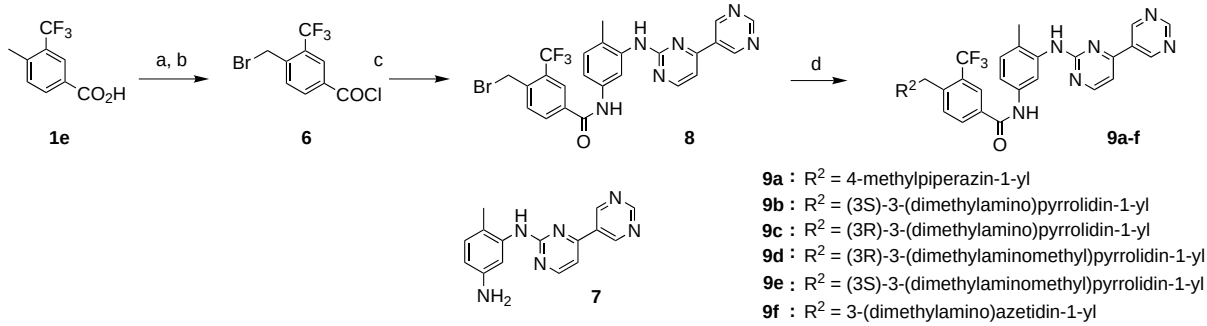
The antiproliferative activities of the 3-substituted benzamides synthesized were evaluated in vitro against Bcr-Abl-positive and Bcr-Abl-negative leukemia cells (Table 1). Bcr-Abl-positive K562 cells and Bcr-Abl-negative U937 cells were plated at 1×10^3 cells/well and 5×10^3 cells/well, respectively, in 96-well plates and incubated with serial dilutions of the compounds for 3 days. Antiproliferative activity was determined by the assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT),[19,20] and the IC_{50} values were estimated by fitting the data to a logistic curve.

All compounds tested showed more-potent antiproliferative activity against K562 cells than did STI-571, whereas they had only weak antiproliferative activity against U937 cells, with IC_{50} values of micromolar order and generally about the same as that of STI-571. These data indicate that our newly synthesized compounds inhibited Bcr-Abl more specifically than did STI-571.

The introduction of a fluoro substituent at the 3-position of the phenyl ring (**5a**; IC_{50} = 63 nM against K562 cells) yielded an approximately 3-fold improvement in antiproliferative activity compared to STI-571 (IC_{50} = 182 nM). To our surprise, the 3-chloro, 3-bromo, and 3-iodobenzamide analogues (**5b**–**d**) had even higher potency. The 3-bromophenyl analogue **5c**, in particular, exhibited an IC_{50} value as low as 7 nM, 26-fold lower than that of STI-571. The most potent derivative

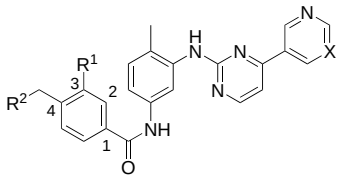


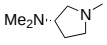
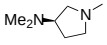
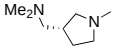
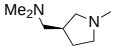
Scheme 1. Reagents and conditions: (a) H_2SO_4, EtOH, reflux; (b) NBS, cat. $(PhCO)_2O_2$, CCl_4, reflux; (c) 1-methylpiperazine, K_2CO_3, THF, rt; (d) 1 N NaOH, reflux, then aq HCl; (e) $SOCl_2$, reflux; (f) **4**, pyridine, rt.



Scheme 2. Reagents and conditions: (a) $NaBrO_3$, $NaHSO_3$, EtOAc; (b) $(COCl)_2$, cat. DMF, CH_2Cl_2, rt; (c) **7**, K_2CO_3, dioxane, rt; (d) cyclic amines, K_2CO_3, DMF, rt.

Table 1. Antiproliferative activity of 3-substituted benzamide derivatives



Compound	X	R^1	R^2	IC_{50}[a] (nM)	
				K562 cells	U937 cells
STI-571	CH	H		182	14,000
5a	CH	F		63	8000
5b	CH	Cl		10	9000
5c	CH	Br		7	5000
5d	CH	I		10	6000
5e	CH	CF_3		5	4000
9a	N	CF_3		4	5000
9b	N	CF_3		11	10,000
9c[b]	N	CF_3		4	9000
9d[b]	N	CF_3		11	20,000
9e[b]	N	CF_3		9	>100,000
9f[b]	N	CF_3		17	>100,000

[a] IC_{50} values represent the concentration which inhibits cell proliferation by 50%.

[b] The biological activity of the monohydrochloride salts was evaluated.

in this series was the 3-trifluoromethylbenzamide **5e**, which, with an IC_{50} value of 5 nM, was 36-fold more potent than STI-571 in the cellular activity profile.

To elucidate the structural factors responsible for enhancing the inhibitory activity of STI-571, we studied the correlation of the antiproliferative activity of STI-571 and **5a**–**e** with various physicochemical parameters of the 3-substituents with Excel2000 (Microsoft). We found a high correlation of activity with the hydrophobic substituent parameter π.[21]

$$pIC_{50} = 1.280(\pm 0.732)\pi - 2.059(\pm 0.542) \quad (1)$$
$$n = 6,\ s = 0.263,\ r = 0.925,\ F = 23.6$$

In this and the following equations, n is the number of compounds, s is the standard error, r is the correlation coefficient, F is the ratio of the variances of the calculated and observed values, and the figures in parentheses are the 95% confidence intervals. According to Eq. 1, hydrophobic 3-substituents increase the activity, indicating the existence of hydrophobic interactions between the 3-substituent and the hydrophobic amino acids, such as Ile-293, Leu-298, Leu-354, and Val-379. The activity of the compounds was also highly correlated with Verloop's steric parameter, B1,[21] which represents the minimum width of the substituent:

$$pIC_{50} = 1.337(\pm 0.592)B1 - 3.549(\pm 1.039) \quad (2)$$
$$n = 6,\ s = 0.210,\ r = 0.953,\ F = 39.3$$

According to Eq. 2, the inhibitory activity of the compounds increases with the minimum width of the 3-substituent. Since the 3-substituent is located adjacent to the methylpiperazine group, its steric effect appears to restrict the rotation of the methylpiperazine group and thereby increase the activity. Presumably, these two factors work cooperatively to enhance the activity of the compound.

To compensate for the increased hydrophobicity caused by the introduction of the trifluoromethyl group in **5e**, the distal pyridine ring was selected for further modification. According to the crystal structure of the c-Abl kinase domain with bound STI-571, Tyr-253 is located very close to the distal pyridine ring, and the resulting interaction stabilizes the inactive form of the kinase; so that a structural modification that increases the bulk in this region would be expected to be unfavorable. The pyridine ring was therefore replaced by the more hydrophilic pyrimidine ring. Pyrimidine-tailed derivative **9a** displayed activity (IC_{50} = 4 nM) similar to that of the original pyridine-tailed derivative **5e**, which suggests that pyrimidinyl substitution is compatible with high activity.

The crystal structure of the kinase domain of c-Abl in complex with STI-571 reported by Nagar et al.[7] reveals that the piperazine moiety in STI-571 interacts with the carbonyl oxygen atoms of Ile-360 and His-361 through hydrogen bonding. In the light of the importance of this interaction, we focused on replacement of the piperazine moiety in **9a** with other cyclic amines in an effort to identify even-more-potent compounds. The optically pure 3-(dimethylamino)pyrrolidine derivatives **9b** and **9c**, and 3-(dimethylaminomethyl)pyrrolidine derivatives **9d** and **9e**, and the 3-(dimethylamino)azetidine derivative **9f**, which may function as piperazine isosteres,[22] were synthesized and evaluated for antiproliferative activity. The pyrrolidine derivative **9c** had excellent potency (IC_{50} = 4 nM), comparable to that of **9a**. Compound **9b**, the enantiomer of **9c**, as well as other pyrrolidine derivatives **9d** and **9e**, also had superior antiproliferative activity profiles (IC_{50} = 11, 11, and 9 nM, respectively),

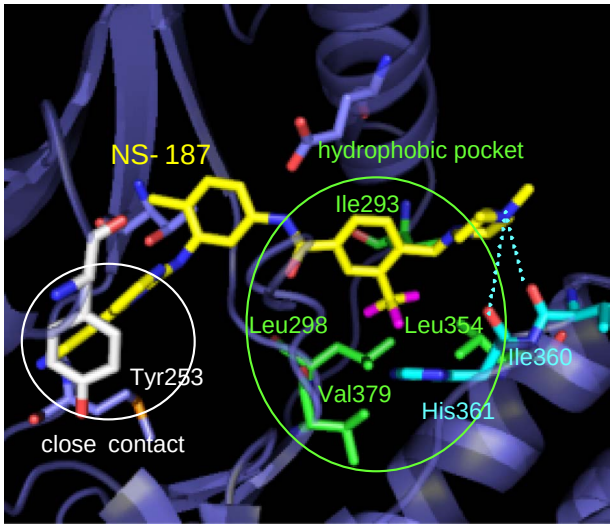


Figure 2. Docking model of Abl in complex with **9b** (NS-187). Hydrophobic amino acids are shown in green, and hydrogen-bonding interactions are shown as blue broken lines. The amino acid close to the pyrimidine ring of **9b** is shown in white. The figure was prepared with PyMOL version 0.97 (DeLano Scientific).

while azetidine **9f** had a slightly lower potency (IC_{50} = 17 nM). These results suggest that the position of the terminal amino function has a significant impact on the antiproliferative activity.

Among the compounds synthesized, we selected **9b** (NS-187) as a promising candidate for development, judging from its overall characteristics, including its pharmacokinetics and toxicity as determined in animal studies. Figure 2 depicts a docking model of **9b** and Abl kinase. Compound **9b** was manually docked into the binding site of Abl by using the published coordinates of Abl complexed with STI-571.[7] The energy of this model was minimized by using the MMFF94 force field[23] with MOE version 2003.01 (Chemical Computing Group Inc.). Conformational changes of **9b** and the nearby amino acids were small during minimization, and the mode of binding of **9b** was very similar to that of STI-571. The trifluoromethyl group interacts well with the hydrophobic pocket formed by the Ile-293, Leu-298, Leu-354, and Val-379, shown in green in Figure 2. An automatic docking study with GLIDE version 3.0 (Schrödinger Inc.) also suggested the existence of favorable interactions of the trifluoromethyl group with these hydrophobic amino acids (data not shown). Tyr-253 is located close to the distal pyrimidine, showing that our use of a pyrimidine instead of a pyridine ring did not alter the important role of Tyr-253 in stabilizing the inactive form of the kinase. Hydrogen-bonding interactions are shown as blue broken lines in Figure 2; the nitrogen atom of the dimethylamino group interacts with the carbonyl oxygen atoms of Ile-360 and His-361 through hydrogen bonding. These results confirmed the validity of our strategy for improving the activity of STI-571.

In conclusion, we have synthesized some 3-substituted benzamide derivatives and evaluated them as Bcr-Abl tyrosine kinase inhibitors. We found 3-halogenated and 3-trifluoromethylated benzamide derivatives to be much more potent than unsubstituted STI-571. We identified the clinical candidate **9b** (NS-187) as a highly potent Bcr-Abl kinase inhibitor. Compound **9b** also showed a potent inhibitory effect against E255K Bcr-Abl. Furthermore, **9b** was a potent inhibitor of Lyn kinase, whose overexpression is also associated with STI-571 resistance.[24,25] These detailed pharmacological properties of **9b** have already been published.[20] We expect that **9b** will advance to clinical trials.

Acknowledgments

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