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BAFETINIB

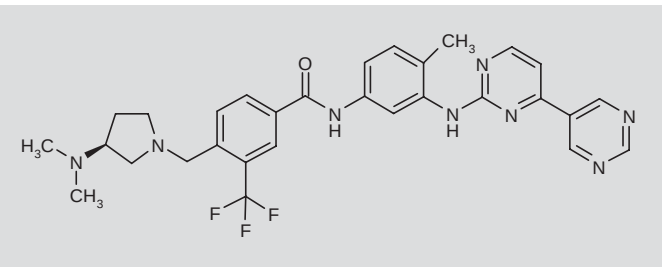
Rec INN; USAN

Dual BCR/ABL and Lyn Tyrosine Kinase Inhibitor
Treatment of Chronic Myeloid Leukemia

CNS-9
INNO-406
NS-187

N-[3-(4,5'-Bipyrimidin-2-ylamino)-4-methylphenyl]-4-[3(*S*)-(dimethylamino)pyrrolidin-1-ylmethyl]-3-(trifluoromethyl)benzamide

InChI=1/C30H31F3N8O/c1-19-4-7-23(13-27(19)39-29-36-10-8-26(38-29)22-14-34-18-35-15-22)37-28(42)20-5-6-21(25(12-20)30(31,32)33)16-41-11-9-24(17-41)40(2)3/h4-8,10,12-15,18,24H,9,11,16-17H2,1-3H3,(H,37,42)(H,36,38,39)/t24-/m0/s1



$C_{30}H_{31}F_3N_8O$
Mol wt: 576.6153
CAS: 859217-05-3
CAS: 859212-07-0 (as hydrochloride)
EN: 387065

ABSTRACT

The treatment of chronic myeloid leukemia (CML) has changed dramatically with the emergence of the ABL tyrosine kinase inhibitor (TKI) imatinib mesilate. However, primary and secondary imatinib resistance has been frequently reported, particularly in patients with advanced-stage disease. To override imatinib resistance, three second-generation ABL TKIs, i.e., dasatinib, nilotinib and bosutinib, were developed. Bafetinib (INNO-406, NS-187) is a dual ABL/Lyn inhibitor developed by our team at Kyoto University Hospital in collaboration with Nippon Shinyaku. Bafetinib was 25-55 times more potent than imatinib in blocking BCR/ABL autophosphorylation, while otherwise retaining specificity for ABL and Lyn. Bafetinib had antiproliferative effects against cells bearing wild-type or most mutated BCR/ABL proteins, except T315I, and also inhibited BCR/ABL-positive leukemic cell growth in the central nervous system. A phase I study on bafetinib was completed and the agent was well tolerated and demonstrated clinical activity across a range of doses. Responses occurred even in the setting of a heavily pretreated population, thus making bafetinib a viable option for CML therapy.

SYNTHESIS*

Heating of 5-acetylpyrimidine (I) with *N*,*N*-dimethylformamide dimethylacetal (II) affords 3-(dimethylamino)-1-(5-pyrimidinyl)-2-propen-1-one (III), which is cyclized with 1-(2-methyl-5-nitrophenyl)guanidine —prepared in situ by adding NaOH to the guanidine nitrate (IV) in 2-propanol at 120 °C— to provide 1-methyl-4-nitro-2-[4-(5-pyrimidinyl)pyrimidin-2-ylamino]benzene (V). The nitro group of compound (V) is then reduced to the corresponding aniline (VI) by catalytic transfer hydrogenation in the presence of formic acid and Pd/C in THF/MeOH. Benzylic bromination of 4-methyl-3-(trifluoromethyl)benzoic acid (VII) using sodium bromate and sodium bisulfite in isopropyl acetate gives 4-(bromomethyl)-3-(trifluoromethyl)-benzoic acid (VIII), which is converted to the acid chloride (IX) by treatment with $(COCl)_2$ and catalytic DMF in CH_2Cl_2. Acid chloride (IX) is then coupled with aniline (VI) in the presence of K_2CO_3 in dioxane to yield the 4-(bromomethyl)benzamide (X), from which the bromide group is finally displaced with (–)-(*S*)-3-(dimethylamino)pyrrolidine (XI) in the presence of K_2CO_3 in anhydrous DMF (1, 2). Scheme 1.

BACKGROUND

The Philadelphia (Ph) chromosome results from a reciprocal translocation between chromosomes 9 and 22 and generates the BCR/ABL fusion protein, which is the cause of chronic myeloid leukemia (CML) and Ph^+ acute lymphoblastic leukemia (ALL) (Fig. 1A) (3, 4). Imatinib mesilate (Gleevec™, Glivec™) is a 2-phenylaminopyrimidine-based ATP-competitive inhibitor of ABL tyrosine kinase (TK) (Fig. 1B) that is not only highly effective in treating CML and Ph^+ALL, but also generally produces only mild adverse effects (5, 6). As a result, the first-line therapy for CML dramatically changed within a few years of the introduction of imatinib to the clinic, and CML therapy is now described as being in the "imatinib era" (7, 8). However, a small percentage of these patients, as well as most patients with advanced-

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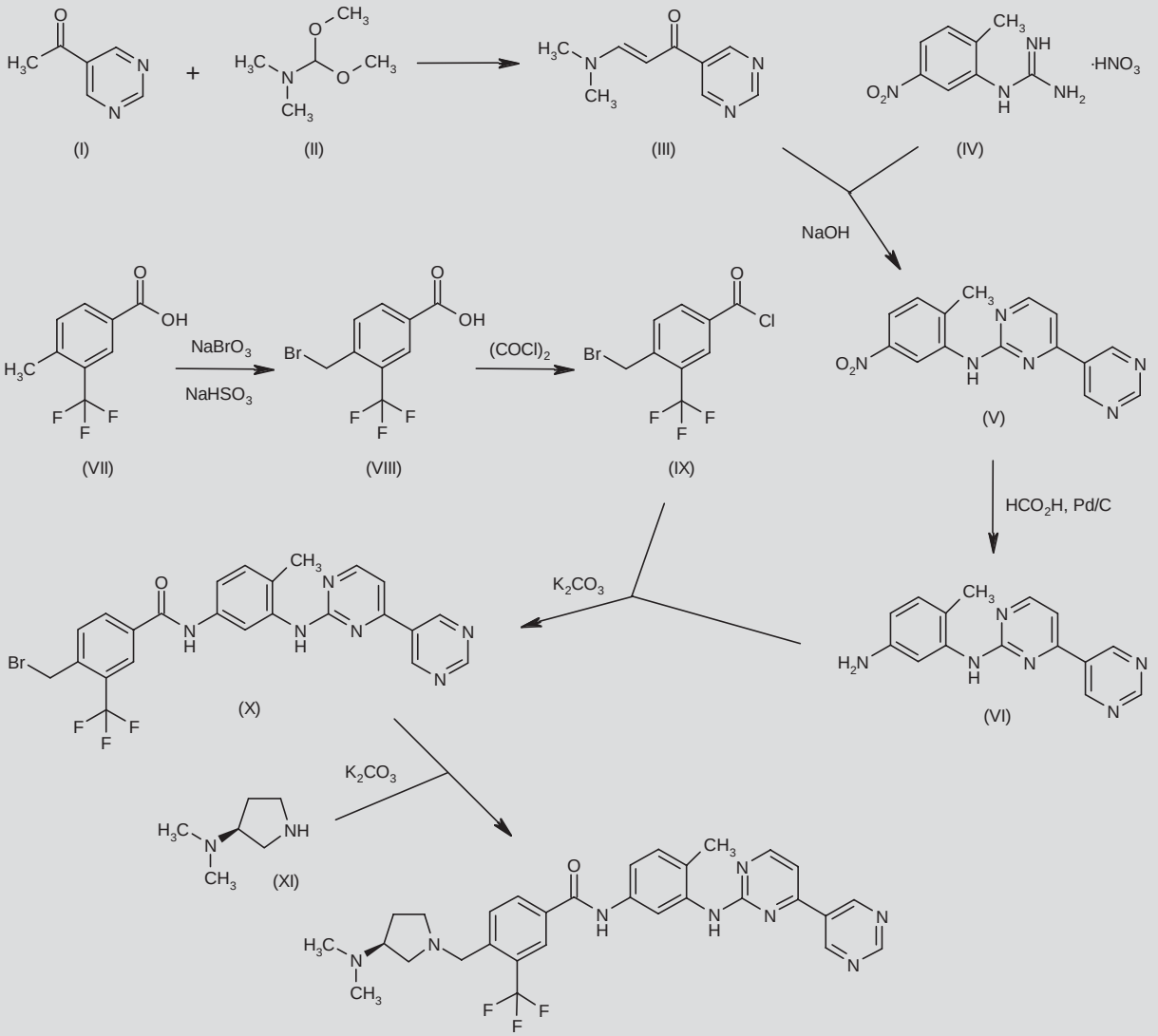
*Synthesis prepared by R. Castañer, J. Bolós. Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Bafetinib



phase CML and Ph⁺ALL, relapse on imatinib therapy (9, 10). Several mechanisms were proposed to explain the cases of refractory disease and relapse, including point mutations within the ABL kinase domain, amplification of the *BCR/ABL* gene, overexpression of the corresponding mRNA (11-14), increased drug efflux from the target cells mediated by P-glycoprotein (15), and activation of Lyn, a kinase from the Src family (16-18).

To overcome imatinib resistance, higher doses of imatinib and combination therapy with other agents were used, with some efficacy. However, these strategies are limited in their application and effectiveness, especially for patients with mutations in the ABL kinase domain (19-21). Thus, the four ATP-competitive-type second-generation ABL TK inhibitors (TKIs) dasatinib (Sprycel™) (22), nilotinib (Tasigna™) (23), bosutinib (SKI-606) (24) and bafetinib (INNO-406, NS-187) (25) were developed. These four ABL TKIs are grossly divided into two groups; one includes dasatinib and bosutinib, which were originally developed as Src kinase inhibitors, and the other includes nilotinib and bafetinib, which have a similar structure to imatinib, i.e., 2-phenylaminopyrimidine-based agents. Bafetinib was developed preclinically by our team at Kyoto University Hospital in collaboration with Nippon Shinyaku. This review describes the preclinical and clinical development of bafetinib and the comparison of five ABL TKIs, including imatinib and four second-generation ABL TKIs.

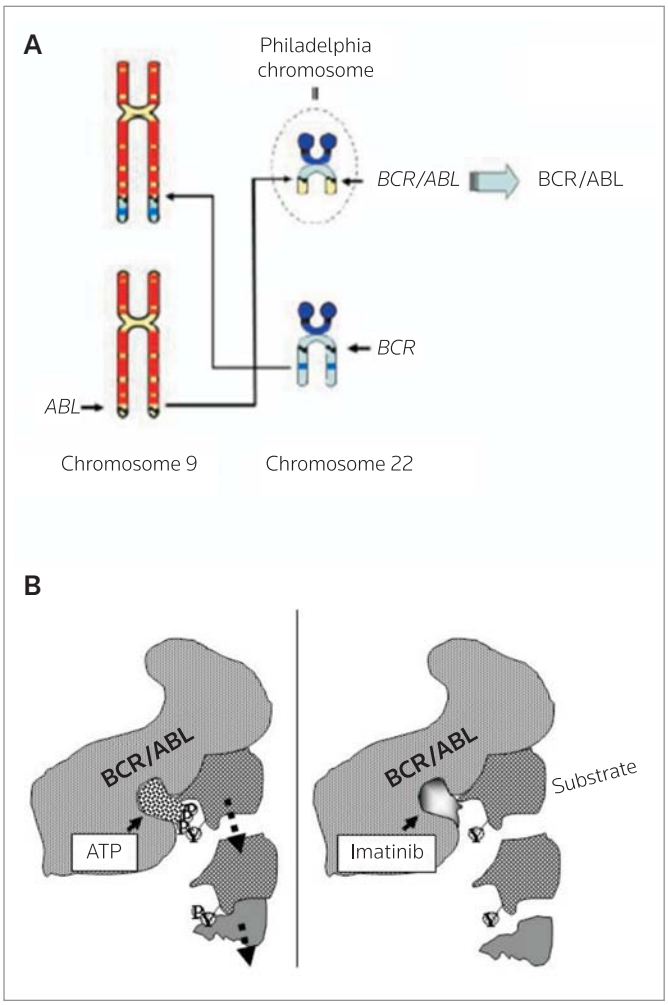


Figure 1. Philadelphia chromosome and the action of imatinib. (**A**) Reciprocal translocation between chromosome 9 and chromosome 22 forms an extra-long chromosome 9 and the Philadelphia chromosome containing the fused *BCR/ABL* gene. The fused *BCR/ABL* gene is translated to BCR/ABL chimeric proteins which cause CML and Ph^+ALL. (**B**) The BCR/ABL tyrosine kinase (TK) is a constitutively active kinase that functions by binding ATP and transferring phosphate (PO_4) from ATP to tyrosine residues on various substrates. This causes excess proliferation of myeloid cells characteristic of chronic myeloid leukemia (CML) and Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL). Imatinib functions by blocking the binding of ATP to the BCR/ABL TK, inhibiting its activity. In the absence of TK activity, substrates required for BCR/ABL function cannot be phosphorylated, and subsequent cellular events are abrogated.

PRECLINICAL PHARMACOLOGY

Nagar et al. (26) reported that imatinib forms six hydrogen bonds with the ATP-binding pocket of the ABL kinase domain, and that the majority of contacts are mediated by van der Waals interactions, which lock the ABL into the inactive conformation. The crystal structure of imatinib revealed a hydrophobic pocket formed by amino acid residues Ile-293, Leu-298, Leu-354 and Val-379 around the phenyl ring adjacent to the piperazinylmethyl groups (25, 27). This hydrophobic pocket was focused on and various hydrophobic substituents were introduced at the above-mentioned phenyl ring in imatinib. All compounds were

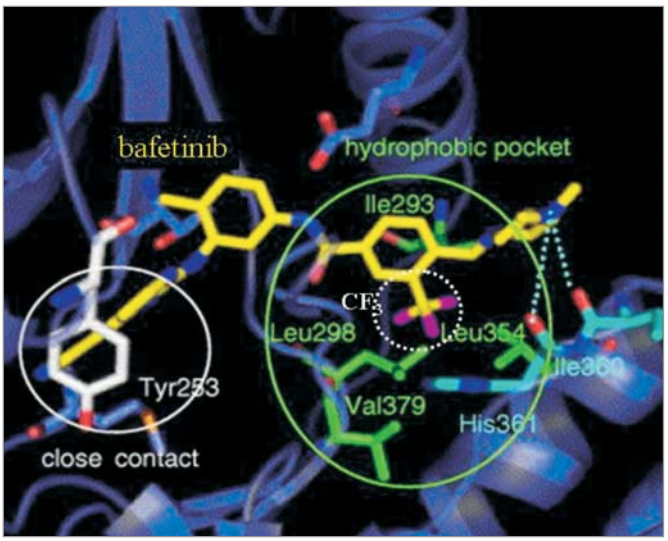


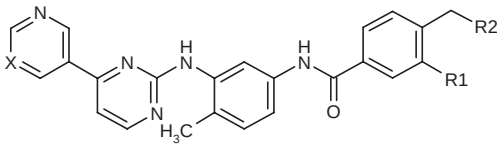
Figure 2. Docking model of ABL in complex with bafetinib. Hydrophobic amino acids are shown in green and hydrogen-bonding interactions are shown as blue broken lines. The amino acid close to the pyrimidine ring of bafetinib is shown in white. The figure was prepared with PyMOL version 0.97 (DeLano Scientific). Adapted from Asaki, T., Sugiyama, Y., Hamamoto, T., Higashioka, M., Umehara, M., Naito, H., Niwa, T. *Design and synthesis of 3-substituted benzamide derivatives as Bcr-Abl kinase inhibitors*. Bioorg Med Chem Lett 2006, 16(5): 1421-5, with permission from Elsevier.

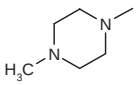
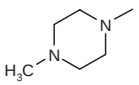
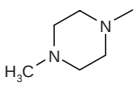
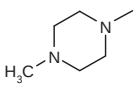
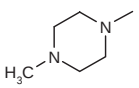
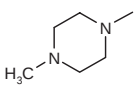
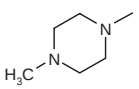
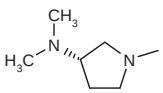
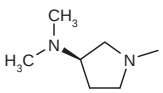
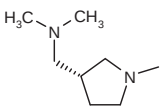
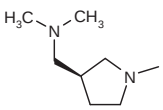
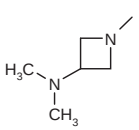
evaluated for antiproliferative activity against BCR/ABL-positive cells (Table I) and bafetinib was selected based on pharmacokinetics and toxicity, as determined in animal studies (1, 25).

Bafetinib was manually docked into the binding site of ABL by using the published coordinates of ABL complexed with imatinib (Fig. 2). Since bafetinib and imatinib are structurally similar, the ABL/bafetinib binding model was assumed to be similar to that of imatinib and typical hydrogen-bonding interactions were retained, although the most striking structural characteristic of bafetinib was its trifluoromethyl (CF_3) group at the 3-position of the benzamide ring. The presence of the CF_3 group increased the hydrophobic interactions of the molecule with the ABL hydrophobic pocket. Another advantage of the inserted CF_3 at the 3-position of the benzamide ring might fix the conformation of the compound by hindering its rotation at the 4-position because the 3-substituent is located adjacent to the methylpiperazine group. Presumably, these two factors work cooperatively to enhance the activity of the compound (1, 25, 27). The x-ray structure of bafetinib bound to ABL was recently solved (Fig. 3A). For comparison, the x-ray structure of imatinib bound to ABL is shown in Figure 3B. The two x-ray structures resemble each other very closely; only slight differences between the complexes were observed in the positions of the ligands and the side-chains and backbones of the kinases (28). It is clear that bafetinib and imatinib interact with ABL in very similar ways, our prediction coinciding with the docking model (25).

Bafetinib was 25-55 times more potent than imatinib in blocking BCR/ABL autophosphorylation (Fig. 4A). Bafetinib inhibited the phosphorylation of Crk-like protein and ERK, which are the downstream mediators of BCR/ABL in CML cells, at much lower concentrations than imatinib. It is well known that imatinib inhibits not only ABL but

Table I. *Activity of 3-substituted benzamide derivatives.*



Compound	X	R1	R2	IC_{50} (nM)	
				K-562	U-937
Imatinib	CH	H		182	14,000
5a	CH	F		63	8000
5b	CH	Cl		10	9000
5c	CH	Br		7	5000
5d	CH	I		10	4000
5e	CH	CF_3		5	5000
9a	N	CF_3		4	5000
9b	N	CF_3		11	10,000
9c	N	CF_3		4	9000
9d	N	CF_3		11	20,000
9e	N	CF_3		9	> 100,000
9f	N	CF_3		17	> 100,000

Adapted from Asaki, T., Sugiyama, Y., Hamamoto, T., Higashioka, M., Umehara, M., Naito, H., Niwa, T. *Design and synthesis of 3-substituted benzamide derivatives as Bcr-Abl kinase inhibitors*. Bioorg Med Chem Lett 2006, 16(5): 1421-5, with permission from Elsevier.

also platelet-derived growth factor receptor (PDGF-R) and Kit (SCFR). Bafetinib suppressed PDGF-R and Kit phosphorylation with IC_{50} values very similar to those of imatinib (Fig. 4A). The ranking of IC_{50} for imatinib was PDGF-R > Kit > BCR/ABL, while the ranking for bafetinib was BCR/ABL > PDGF-R > Kit. Furthermore, the specificity of bafetinib was examined against 79 tyrosine kinases. At a concentration of 0.1 μM, bafetinib inhibited only 4 of the 79 tyrosine kinases, i.e., ABL, ABL2 (ARG), Fyn and Lyn. Notably, at 0.1 μM, bafetinib did not inhibit PDGF-R-α, PDGF-R-β, BLK, Src or Yes. In contrast, 10 μM imatinib inhibited 9 tyrosine kinases (ABL, ABL2, BLK, FLT3, Fyn, Lyn, PDGF-R-α, PDGF-R-β and p70-S6K). These results indicated that bafetinib was much more potent and specific against BCR/ABL than imatinib. This specificity of bafetinib for ABL and Lyn was supported by the docking model, as described elsewhere (25).

More than 90 point mutations within the ABL kinase domain have been reported (29, 30). Bafetinib at physiologically obtainable concentrations inhibited the phosphorylation of 12 (M244V, G250E, Q252H, Y253F, E255K, E255V, F317L, M351T, E355G, F359V, H396P and F486S) of 13 mutated BCR/ABLs, except T315I (Fig. 4B). For all mutants except T315I, the IC_{50} for imatinib was at least five times as high as the corresponding value for bafetinib (25). The effects of bafetinib on seven of the mutants were also examined in whole cells by assessing the phosphorylation of BCR/ABL with the Ba/F3 cell line expressing wild-type p210 BCR/ABL (wt) and the M244V, G250E, Q252H, Y253F, T315I, M351T or H396P mutants. Bafetinib at physiologically obtainable concentrations inhibited the growth of these cell lines, except BCR/ABL T315I. Imatinib, on the other hand, was much less active against all cell lines tested. These data indicated that bafetinib greatly inhibited not only the intracellular phosphorylation of most mutated BCR/ABLs, but also the proliferation of cells expressing those kinases. In addition, imatinib inhibited the kinase activity of the Tyr-393-unphosphorylated form of the ABL kinase domain with an IC_{50} of 35 nM, but had little effect on the phosphorylated form. In contrast, bafetinib effectively inhibited the kinase activity of both Tyr-

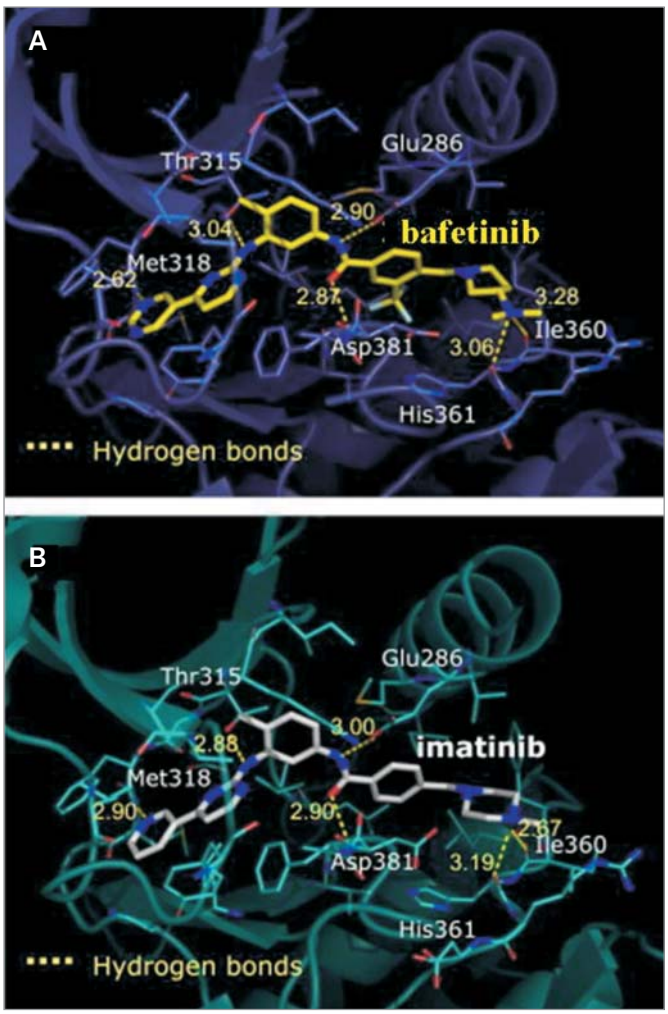


Figure 3. X-ray structures of bafetinib/ABL (**A**) and imatinib/ABL complexes (**B**). Adapted from Horio, T., Hamasaki, T., Inoue, T., Wakayama, T., Itou, S., Naito, H., Asaki, T., Hayase, H., Niwa, T. *Structural factors contributing to the Abl/Lyn dual inhibitory activity of 3-substituted benzamide derivatives*. Bioorg Med Chem Lett 2007, 17(10): 2712-7, with permission from Elsevier.

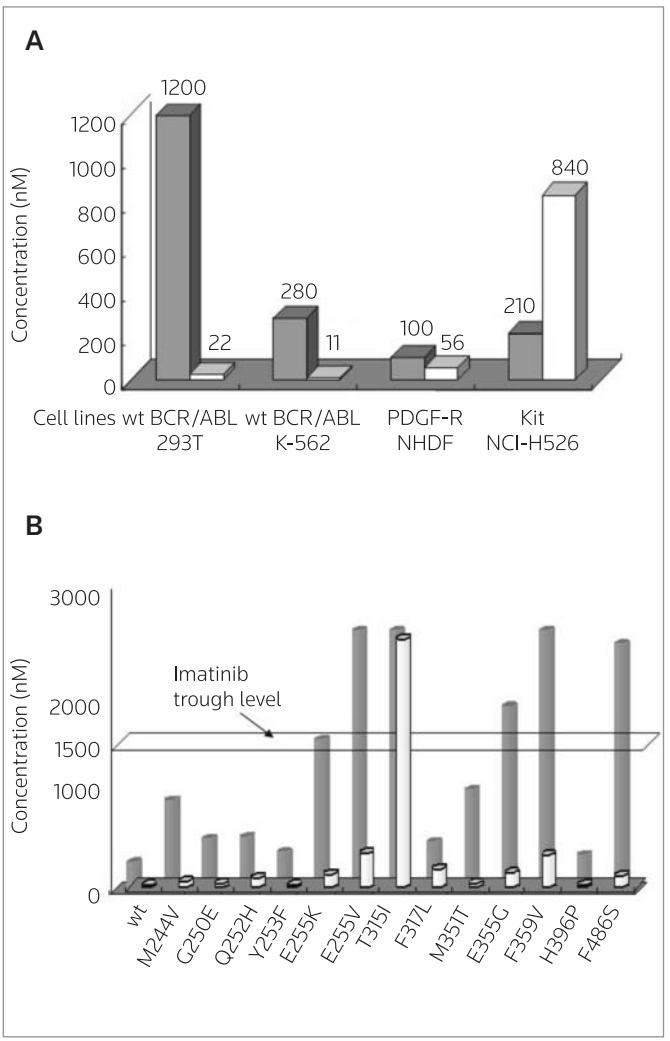


Figure 4. In vitro effects of bafetinib. (**A**) Dephosphorylation of BCR/ABL, PDGF-R and Kit by imatinib (gray column) and bafetinib (white column). (**B**) Effects of imatinib (gray column) and bafetinib (white column) against mutated BCR/ABL kinases. wt, wild-type.

393-phosphorylated and Tyr-393-unphosphorylated forms of ABL with respective IC_{50} values of 72 and 30 nM, suggesting that it might have sufficiently high affinity for BCR/ABL to enable it to bind even to an unfavorable conformation of the kinase (31).

Bafetinib increased the activity of proapoptotic Bcl-2 homology domain 3 (BH3)-only proteins and induced apoptosis in BCR/ABL$^+$ leukemic cells, as evidenced by DNA fragmentation, caspase-3 activation and the loss of mitochondrial outer membrane permeabilization. ABT-737, an inhibitor of Bcl-2 and Bcl-XL, enhanced the apoptosis induced by bafetinib, even in cells with mutated BCR/ABL that were less sensitive to bafetinib, suggesting that Bcl-2 family-regulated intrinsic apoptosis occurred through caspase activation (32). Even in the presence of the pan-caspase inhibitor zVAD-fmk, bafetinib still induced apoptosis in some cells, indicating the additional involvement of a caspase-independent apoptotic pathway. The observation of an increased number of cells showing the hallmarks of autophagy suggested that autophagy participated in the response against BCR/ABL blockade. Inhibition of autophagy by chloroquine significantly enhanced bafetinib-induced cell death (33). These results might be useful in the design of a rational therapeutic approach for efficiently eradicating BCR/ABL$^+$ leukemic cells.

Furthermore, a direct comparison of the effects of imatinib, dasatinib, nilotinib (Fig. 5) and bafetinib is shown in Table II (34). Because we had not compared the effects of bosutinib (Fig. 5) directly, the data on bosutinib were obtained from the literature (24). Dasatinib showed the strongest potency against BCR/ABL-positive cells, with little selectivity over Src family kinases. Nilotinib showed weaker affinity than others, but was highly specific for ABL and might be useful for P-glycoprotein-overexpressing leukemic cells. Bafetinib had intermediate affinity between dasatinib and nilotinib and inhibited Lyn in addition to ABL. Bafetinib was more potent than nilotinib against the CML cell line K-562 and its subclones with *BCR/ABL* gene amplification along with elevated levels of its transcript and protein (35). Both nilotinib and bafetinib were potent inhibitors of the dasatinib-resistant T315A, F317L and F317V BCR/ABL mutations (34).

The ability of bafetinib to suppress tumor growth was tested in three murine tumor models. In one model, BALB/c *nu/nu* mice were injected s.c. with leukemia KU812 cells on day 0 and given bafetinib or imatinib orally b.i.d. from day 7 to day 17. At 20 mg/kg/day imatinib slightly inhibited tumor growth, while at 200 mg/kg/day it almost completely inhibited tumor growth. Bafetinib significantly inhibited tumor growth at only 0.2 mg/kg/day, while at 20 mg/kg/day it completely inhibited tumor growth without adverse effects. When mice were treated with bafetinib at 0.2 and 20 mg/kg/day the estimated C_{max} was 4 and 400 nM, respectively, comparable to the concentrations at which in vitro effects of bafetinib were obtained. Bafetinib was therefore at least 10-fold more potent than imatinib in vivo, with complete inhibition of tumor growth as the endpoint and at least 100-fold more potent with partial inhibition as the endpoint. Bafetinib was well tolerated by the mice (25).

In another model, BALB/c *nu/nu* mice injected i.v. with wild-type Ba/F3 cells were given bafetinib or imatinib orally for 11 days starting on day 1. All 7 untreated mice died by day 23 due to leukemic cell expansion, and all mice treated with 400 mg/kg/day imatinib died by day 25. Bafetinib, in contrast, significantly prolonged the survival of the mice in a dose-dependent manner compared with untreated mice (25).

To investigate the efficacy of bafetinib in a mouse model of leukemia, the ability to block the growth of Ba/F3 cells expressing mutated BCR/ABL was tested in BALB/c *nu/nu* mice (31). Mice bearing Ba/F3 cells expressing M244V, G250E, Q252H, Y253F, E255K, T315I, M351T or H396P were treated with bafetinib or imatinib. Mice bearing Ba/F3 cells expressing wild-type BCR/ABL or any mutated BCR/ABLs except T315I showed significant prolongation of survival following treatment with bafetinib at a dose of 200 mg/kg/day, without any apparent signs of toxicity. These in vivo results were consistent with the in vitro findings. On the other hand, imatinib, even at a dose of 400 mg/kg/day, was much less effective. Bafetinib results in the highest observed percentage increase in mean survival in mice bearing Ba/F3 cells expressing wild-type BCR/ABL, Q252H or M351T, in good agreement with the in vitro results. Moreover, the rank order of the IC_{50} values for cell growth inhibition was inversely correlated with the percentage increase in the mean survival of mice treated with bafetinib. In addition, central nervous system (CNS) relapse accompanying the prolonged administration of imatinib has recently become apparent as an impediment to the therapy of Ph$^+$ leukemia. CNS relapse might be explained by the limited penetration of imatinib mesilate into the cerebrospinal fluid (CSF) because of the presence of P-glycoprotein at the blood–brain barrier. Bafetinib, like imatinib mesilate, is a substrate for P-glycoprotein and the concentrations of bafetinib in the CNS are about 10% of those in the plasma However, this residual concentration was sufficient to inhibit the growth of Ph$^+$ leukemic cells which expressed not only wild-type but also mutated BCR/ABL in the murine CNS. Furthermore, ciclosporin, a P-glycoprotein inhibitor, enhanced the in vivo activity of bafetinib against CNS Ph$^+$ leukemia. These findings indicate that bafetinib is a promising agent for the treatment of CNS Ph$^+$ leukemia (36). Thus, the efficacy of bafetinib in the mouse leukemia models mirrors its in vitro activity, a result which suggests that bafetinib will be clinically effective (31).

PHARMACOKINETICS AND METABOLISM

When BALB/c mice were given bafetinib orally at a dose of 30 mg/kg, the pharmacokinetic parameters were as follows: time to peak plasma concentration (t_{max}) 2 h; peak plasma concentration (C_{max}) 661 ng/mL; area under the curve $(AUC)_{0-\infty}$ 2294 ng·h/mL; half-life ($t_{1/2}$) 1.0 h; and bioavailability 32%. The maximum tolerated dose (MTD) of bafetinib in BALB/c or BALB/c *nu/nu* mice was 200 mg/kg/day (100 mg/kg b.i.d.). C_{max} for bafetinib was estimated at 2226 ng/mL (4.0 μM) when mice were treated with a dose of 100 mg/kg p.o. b.i.d. (25).

CLINICAL STUDIES

Based on these preclinical studies, a phase I study on bafetinib commenced in 2006 and the final results were reported at the 2007 Annual Meeting of American Society of Hematology (37). In this dose-finding study, patients with imatinib-resistant or -intolerant Ph$^+$ leukemias were eligible for oral treatment with bafetinib at doses ranging from 30 mg once daily to 480 mg b.i.d. Bafetinib was administered to 49 patients and was generally well tolerated. This study showed no grade 3/4 pleural effusions, peripheral edema or

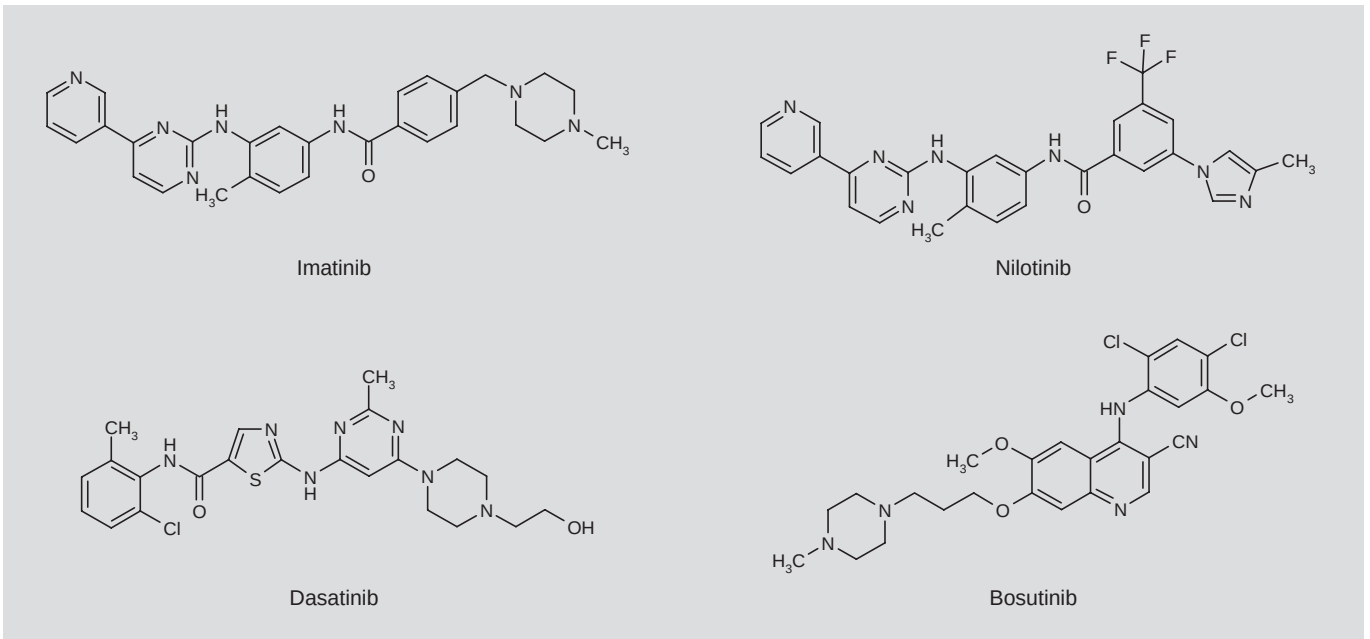


Figure 5. Chemical structures of ABL tyrosine kinase inhibitors imatinib, dasatinib, nilotinib and bosutinib.

Table II. *Comparison of ABL tyrosine kinase inhibitors.*

	2-Phenylaminopyrimidine-based inhibitors		Src kinase inhibitors		
	Imatinib	Nilotinib	Bafetinib	Dasatinib	Bosutinib
ABL	X 1	X 30	X 55	X 325	X 30
Active	(–)	(–)	(+)	(+++)	(+++)
Inactive	(+)	(++)	(++)	(+++)	(+++)
PDGF-R	(+)	(+)	(+)	(+++)	(–)
Kit	(+)	(+)	(+/–)	(+++)	(–)
Src	(–)	(–)	(–)	(+++)	(++++)
Lyn	(–)	(–)	(+)	(++)	(+++)
Most mutations	(–)	(+)	(+)	(+)	(+)
T315I	(–)	(–)	(–)	(–)	(–)
T315A	(–)	(+)	(++)	(–)	NA
F317L	(–)	(+)	(+++)	(–)	NA
F317V	(+/–)	(++)	(++)	(–)	NA
Adverse effects		Lipase↑	ALT/AST↑	Pleural effusion	Diarrhea

pericardial effusions, a low rate of hematological toxicity and a minimal Q-T_c effect. Bafetinib demonstrated activity in heavily pretreated patients with Ph^+ leukemias who were intolerant of or resistant to imatinib and multiple second-generation TKIs.

CONCLUSIONS

Using x-ray crystallographic information and computer modeling, bafetinib was developed as a highly potent and selective dual ABL/Lyn TKI. Based on preclinical studies, bafetinib was expected to be useful not only for imatinib- but also other ABL TKI-resistant and -intolerant patients. According to phase I clinical trial results, bafetinib was well tolerated in patients, with clinical activity demonstrated across a range of doses. Responses occurred even in the setting of a heavily pretreated population, thus making bafetinib a viable option for CML therapy.

DISCLOSURE

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SOURCES

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