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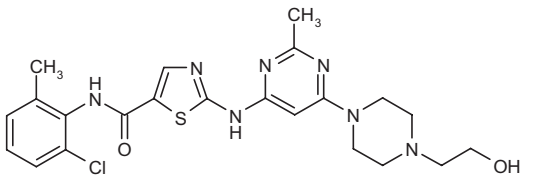
Dasatinib

Prop INN; USAN

Treatment of Leukemia
Treatment of Solid Tumors
Bcr-Abl and Src Kinase Inhibitor

BMS-354825

N-(2-Chloro-6-methylphenyl)-2-[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-ylamino]thiazole-5-carboxamide



$C_{22}H_{26}ClN_7O_2S$
Mol wt: 488.0065

CAS: 302962-49-8

EN: 365055

Abstract

Chronic myelogenous leukemia (CML) results from a single mutagenic event leading to acquisition of the Philadelphia chromosome abnormality and the *BCR-ABL* fusion gene. Improved understanding of the molecular mechanisms of resistance in CML led to the discovery of the small-molecule, dual Bcr-Abl and Src kinase inhibitor dasatinib (BMS-354825). Dasatinib has 325-fold increased potency relative to imatinib and has demonstrated activity against a wide range of clinically relevant isoforms resistant to imatinib. In a mouse model of imatinib-resistant, Bcr-Abl-dependent disease, dasatinib significantly prolonged survival. It also demonstrated potent activity against cell lines overexpressing Src and Kit tyrosine kinases. Preclinical studies have shown that dasatinib may also be active against solid malignancies, including breast and prostate carcinoma. In phase I and II studies in patients with chronic-phase, accelerated-phase and myeloid blast crisis CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) with hematological resistance or intolerance to imatinib, durable responses were obtained, as well as cytogenetic responses in patients with a wide spectrum of Bcr-Abl mutations. Regulatory submissions have been made in the U.S. and the E.U. for its use in CML and Ph+ ALL patients resistant or intolerant to prior therapy, and clinical studies are under way in several solid tumors.

Synthesis

Dasatinib can be prepared by three main synthetic pathways:

1) Reaction of *N*-(2-chloro-6-methylphenyl)-2-(6-chloro-2-methylpyrimidin-4-ylamino)-1,3-thiazole-5-carboxamide (I) with 1-(2-hydroxyethyl)piperazine (II) by heating the mixture at 80 °C (1, 2), refluxing in dioxane (3) or by means of DIEA in *tert*-butanol at 118 °C (4, 5). Scheme 1.

Intermediate (I) can be synthesized by several different procedures:

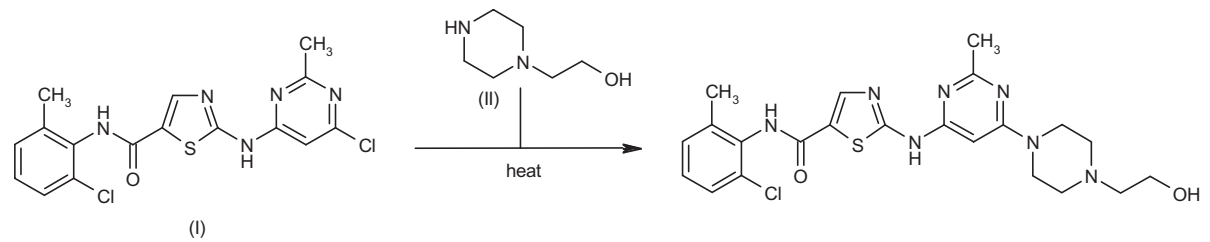
a) Protection of ethyl 2-aminothiazole-5-carboxylate (III) with Boc_2O and DMAP in THF gives intermediate (IV), which is hydrolyzed with NaOH in THF/MeOH to yield the corresponding carboxylic acid derivative (V). Reaction of compound (V) with oxalyl chloride in THF affords the acyl chloride (VI), which is condensed with 2-chloro-6-methylaniline (VII) by means of TEA in CH_2Cl_2 to provide the intermediate (VIII). Deprotection of compound (VIII) by means of TFA gives the 2-aminothiazole derivative (IX), which finally reacts with 4,6-dichloro-2-methylpyrimidine (X) by means of NaH in THF (1, 2). Scheme 2.

b) Reaction 2-chlorothiazole (XI) with 2-chloro-6-phenylisocyanate (XII) by means of BuLi in THF gives 2-chloro-*N*-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (XIII), which is *N*-protected with 4-methoxybenzyl chloride (XIV) and NaH in THF to yield compound (XV). Reaction of intermediate (XV) with 4-amino-6-chloro-2-methylpyrimidine (XVI) by means of NaH in THF affords the adduct (XVII), which is finally deprotected by means of TfOH/TFA in CH_2Cl_2 (3). Scheme 3.

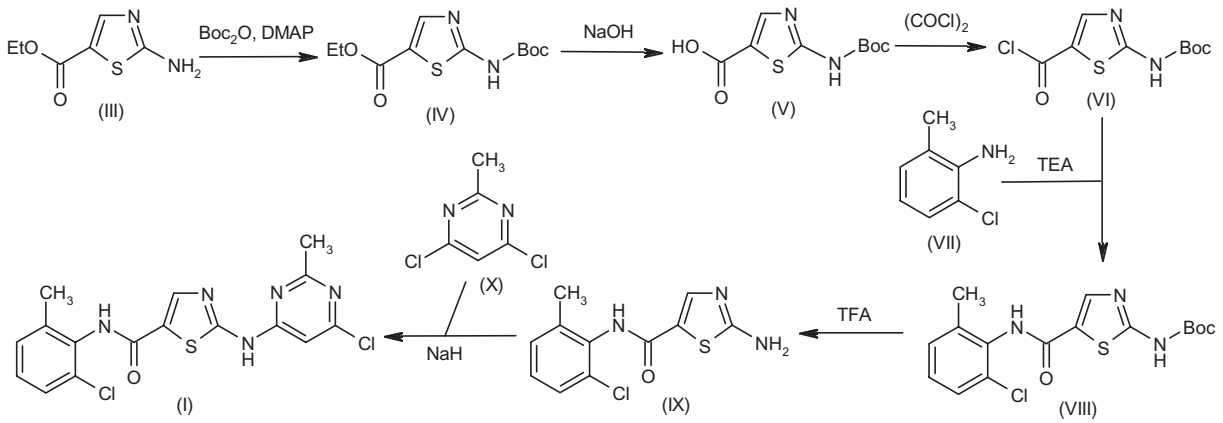
c) Condensation of 2-chloro-6-methylaniline (VII) with 2-ethoxyacryloyl chloride (XVIII) by means of pyridine in THF gives the corresponding acrylamide derivative (XIX), which is cyclized with thiourea (XX) by means of NBS in hot dioxane to yield 2-amino-*N*-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (IX). Finally, compound (IX) is condensed with 4,6-dichloro-2-methylpyrimidine (X) by means of *t*-BuONa in THF (4, 5). Scheme 4.

J.A. McIntyre, J. Castañer, M. Bayés. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

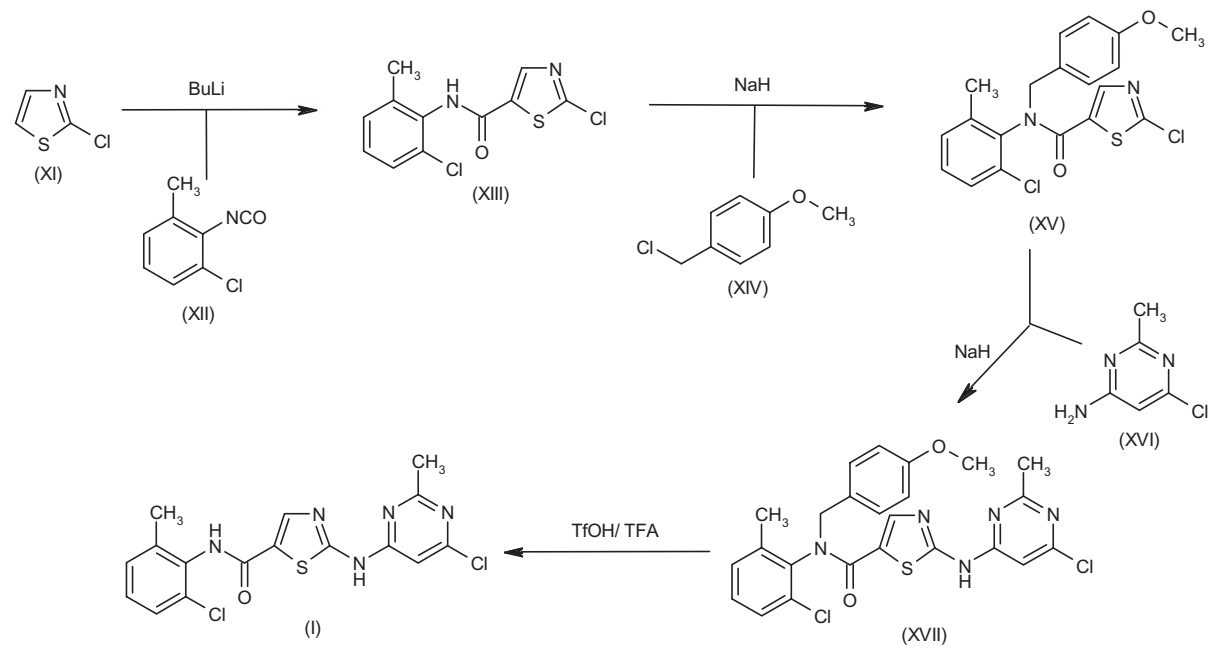
Scheme 1: Synthesis of Dasatinib



Scheme 2: Synthesis of Intermediate (I)



Scheme 3: Synthesis of Intermediate (I)

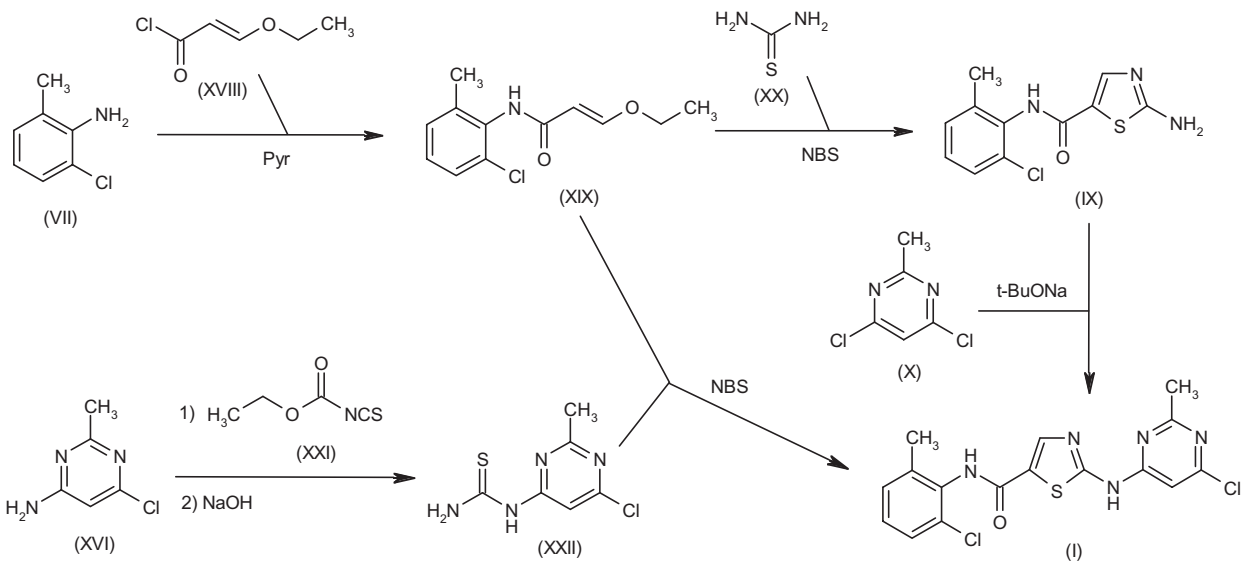


d) Reaction of 4-amino-6-chloro-2-methylpyrimidine (XVI) with ethyl isothiocyanatoformate (XXI) in THF at reflux followed by hydrolysis in 1M NaOH at 50 °C yields the thiourea derivative (XXII), which is finally cyclized with the acrylamide (XIX) by means of NBS in THF/H_2O (4, 5). Scheme 4.

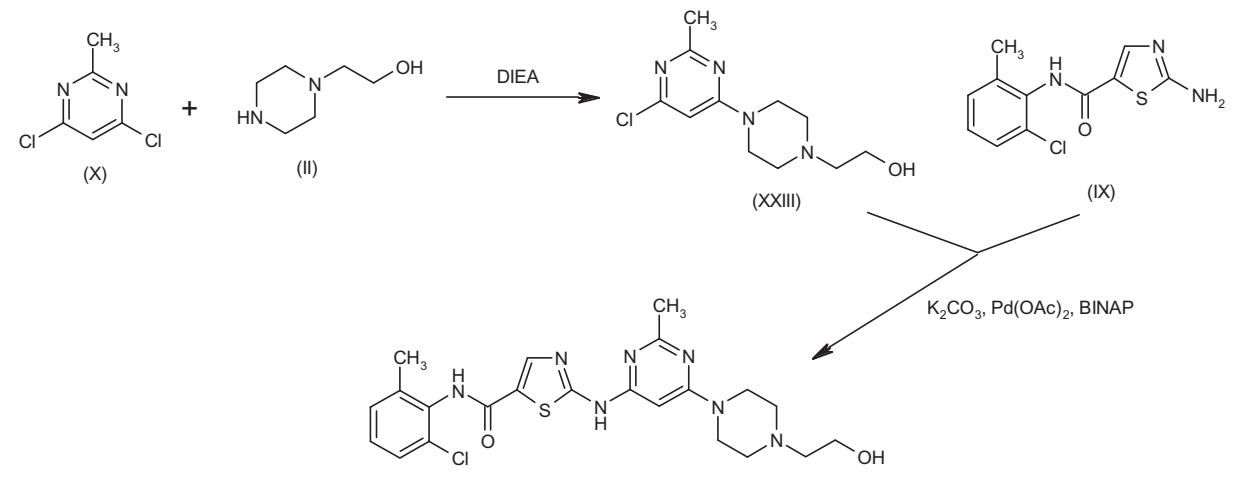
2) Reaction of 4,6-dichloro-2-methylpyrimidine (X) with 1-(2-hydroxyethyl)piperazine (II) by means of DIEA in CH_2Cl_2 affords the pyrimidine derivative (XXIII), which is condensed with the 2-aminothiazole derivative (IX) by means of K_2CO_3, $Pd(AcO)_2$ and BINAP in toluene at 100 °C (4, 5). Scheme 5.

3) Treatment of 4-amino-6-chloro-2-methylpyrimidine (XVI) with 1-(2-hydroxyethyl)piperazine (II) by means of DIEA in CH_2Cl_2 affords the pyrimidine derivative (XXIV), which after reaction with benzoyl isothiocyanate (XXV) in $CHCl_3$ followed by hydrolysis with aqueous NaOH in MeOH provides the thiourea intermediate (XXVI). Condensation of compound (XXVI) with *N,N*-dimethylformamide dimethyl acetal (XXVII) in EtOH at 73 °C yields compound (XXVIII) which is finally reacted with 2-chloro-*N*-(2-chloro-6-methylphenyl)acetamide (XXIX) – prepared by acylation of the aniline (VII) with chloroacetyl chloride (XXX) by means of NMM in acetone – in refluxing MeOH (6). Scheme 6.

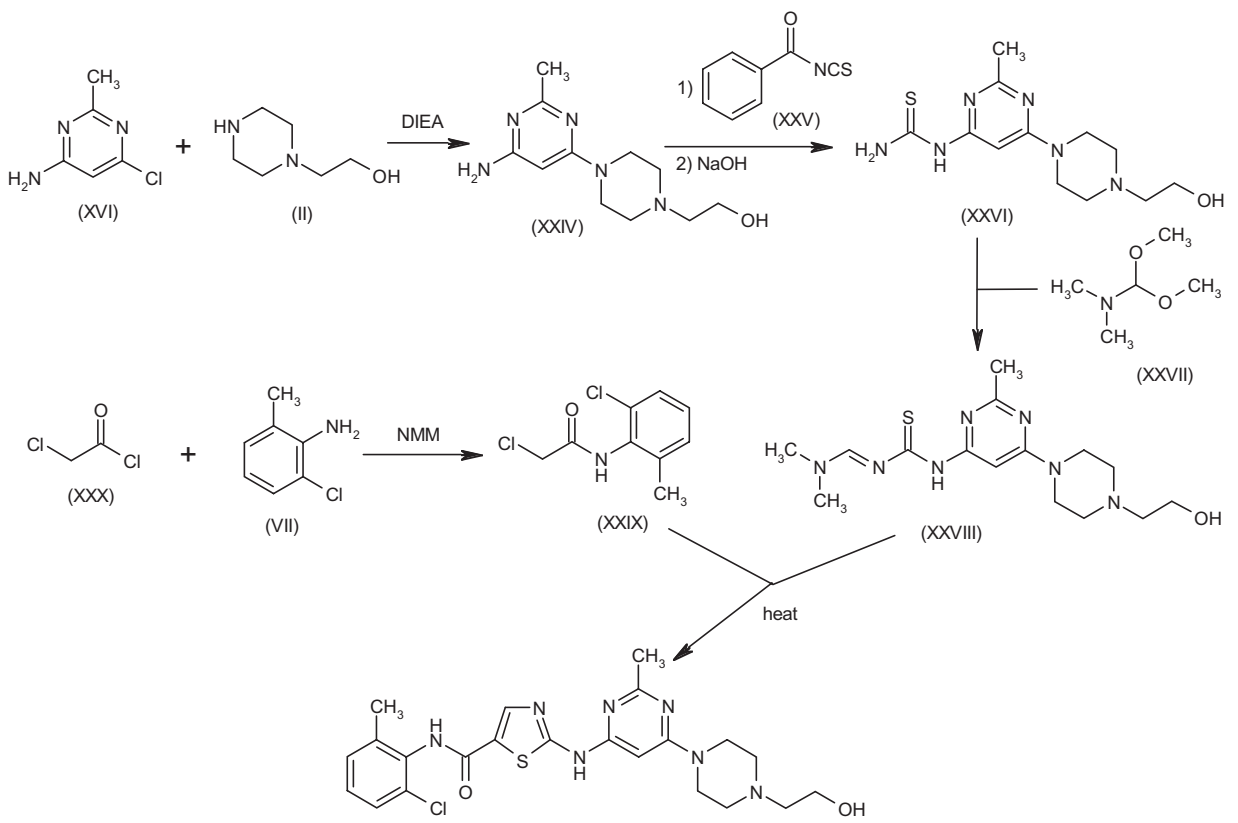
Scheme 4: Synthesis of Intermediate (I)



Scheme 5: Synthesis of Dasatinib



Scheme 6: Synthesis of Dasatinib



Introduction

Leukemia is a malignant disease of the bone marrow and blood. Myelogenous leukemia involves myeloid precursor cells, and lymphocytic leukemia involves B-lymphocytes. Leukemia is further classified according to the progress of the disease as either acute or chronic. Acute myelogenous leukemia (AML) is the most common form of leukemia in adults, while the chronic form accounts for approximately 20% of all leukemia cases (7).

All four major forms of leukemia result from acquired genetic damage to the DNA of specific cells in the bone marrow. Chronic myelogenous leukemia (CML) results from a single mutagenic event leading to acquisition of the Philadelphia chromosome abnormality and the *BCR-ABL* fusion gene. The fusion protein has constitutive tyrosine kinase activity, with the resulting deregulation of signal transduction pathways causing abnormal cell cycling, inhibition of apoptosis and increased cell proliferation (7, 8). The Philadelphia chromosome abnormality also occurs in some patients with acute lymphocytic leukemia (ALL).

The unique role of the *BCR-ABL* fusion gene product in the pathogenesis of CML has provided a focus for investigation in the development of targeted therapies against CML (9). This led to the development of imatinib mesilate (Gleevec®, Glivec®), which, following its approval by the U.S. FDA in 2001, became the new gold standard for the treatment of this disease (10). Imatinib is a specific small-molecule inhibitor of Bcr-Abl, and estimated rates of complete hematological response of 98% have been achieved in newly diagnosed chronic-phase patients (11). However, despite unprecedented rates of complete cytogenetic response (up to 86%), molecular remission is achieved in very few patients, suggesting the persistence of leukemic stem cells (11). In approximately 15% of chronic-phase cases, primary cytogenetic resistance is also encountered. In addition, a subset of patients lose their best response despite continuing treatment (secondary or “acquired” resistance), and some of these patients progress to accelerated- or blast-phase CML (12). Imatinib is also much less effective in advanced-phase CML. Studies in blast-phase patients showed that there was a reactivation of Bcr-Abl signaling at the time of relapse. In the majority of patients with acquired resistance, there is evidence of either increased expression of Bcr-Abl, or more often, mutations in the kinase domain of Bcr-Abl that interfere with drug binding (50-90% of cases) (11, 12). Indeed, more than 40 different mutations have been associated with clinical resistance to imatinib (12).

Recent advances in the understanding of the molecular mechanisms of resistance in CML have led to the discovery of the small-molecule, dual Bcr-Abl and Src kinase inhibitor dasatinib (BMS-354825) (3, 13). Dasatinib was selected from a series of substituted thiazolecarboxamides based on its robust *in vivo* activity in a murine xenograft model of CML and favorable pharmacokinetic profile (3). It is undergoing regulatory review in the U.S. and the E.U. for the treatment of CML and Philadelphia chromosome-positive (Ph+) ALL. The NDA has been granted priority review by the FDA (14-16).

Preclinical Pharmacology

Dasatinib is a highly potent, ATP-competitive inhibitor of both Src and Bcr-Abl, with measured K_i values in a kinase selectivity panel of 16 ± 1.0 and 30 ± 22 pM, respectively. Dasatinib also potently inhibited other Src family members and demonstrated significant activity against c-Kit and platelet-derived growth factor receptor β (PDGFRβ). In a human chronic myelogenous leukemia K-562 xenograft model in nude mice, once-daily doses of dasatinib of 5 or 50 mg/kg for 5 days resulted in partial tumor regressions after 1 cycle and complete disappearance of the tumor at the end of treatment. No toxicity was observed at these dose levels (3, 17, 18).

In biochemical assays using purified, dephosphorylated, wild-type glutathione *S*-transferase (GST)-Abl kinase, dasatinib had 325-fold increased potency relative to imatinib (IC_{50} = 0.6 nmol/l vs. 280 nmol/l) (19).

The activity of dasatinib against imatinib-resistant Bcr-Abl mutants was assessed in Ba/F3 cells expressing various imatinib-resistant isoforms. The kinase activity of 14 of 15 clinically relevant isoforms was inhibited after 2 h of incubation with low nanomolar concentrations of dasatinib. The growth of Ba/F3 cells expressing each of the isoforms was similarly inhibited. However, the T315I mutant was resistant to dasatinib even in the presence of micromolar concentrations of the drug (20, 21). Similar findings were observed in cellular and biochemical assays against a panel of 16 kinase domain mutants representing more than 90% of clinical isolates (19).

Dasatinib was evaluated in a mouse model of imatinib-resistant, Bcr-Abl-dependent disease. Severe combined immunodeficient (SCID) mice were injected i.v. with Ba/F3 cells expressing different Bcr-Abl isoforms, as well as the firefly luciferase gene. In mice treated with dasatinib 10 mg/kg twice daily by gavage for 2 weeks beginning 3 days after injection of Ba/F3 cells, levels of bioluminescent activity were > 1 log lower than in vehicle-treated controls. Dasatinib-treated mice appeared healthy, with no evidence of weight loss, lethargy or ruffled fur. Survival was also significantly prolonged in these mice. Mice harboring T315I tumors did not significantly respond to treatment (20, 21).

In *in vitro* colony-forming unit (CFU) assays, dasatinib 5 nM inhibited the growth of bone marrow progenitors isolated from CML patients by 60-80%, but did not inhibit the growth of bone marrow progenitors isolated from healthy volunteers (20).

The development of resistance to imatinib is mainly due to mutations at different amino acid positions within the Bcr-Abl kinase domain, preventing the ability of the kinase to adopt the specific closed conformation to which imatinib binds (20). X-ray crystallography studies were used to investigate the 3-dimensional structure of the kinase domain complexed with dasatinib. The activation loop of the ATP-binding site was shown to be in the active conformation in the presence of bound dasatinib, in contrast to bound imatinib. The increased binding affinity of dasatinib is at least partially due to its ability to recognize multiple states of the enzyme (22). A saturation mutagenesis screen of Bcr-Abl showed that the spectrum of mutations allowing for dasatinib resistance is reduced compared with that of imatinib. Eight of 10 mutations capable of conferring resistance to dasatinib occurred at drug contact residues, and overlapping mutations with imatinib were observed (23, 24). In a further accelerated, cell-based mutagenesis screen, only the T315I mutation was found at a concentration of 25 nM dasatinib, out of 18 different kinase domain mutations recovered in the assays (25).

Src family kinases (including Blk, Hck, Lck, Fyn and Lyn) are involved in Bcr-Abl-mediated leukemogenesis and have also been shown to be upregulated in some cases of imatinib resistance. In particular, Hck and Lyn are activated in CML blast-crisis patients and their upregulation correlates with disease progression (13). The ability of dasatinib to overcome multiple mechanisms of imatinib resistance in CML cell lines and *in vivo* models, including Src overexpression, has been demonstrated in a number of studies (25-28). Dasatinib induced apoptosis in K-562 cells overexpressing Lyn and effectively reduced tumor growth in nude mice. This activity correlated with inhibition of Lyn activation and Bcr-Abl signaling (CrkL and Stat5). Dasatinib completely suppressed Lyn/Hck phosphorylation and demonstrated potent antitumor activity in clinical specimens from imatinib-resistant CML patients, as well as overcoming imatinib resistance in Cos cells co-expressing Bcr-Abl and Lyn kinase (26-28). Dasatinib was highly effective against three human imatinib-resistant CML cell lines (K-562/IM, MEG-01/IM and SUP-B15/IM). Of these, K-562/IM overexpresses the Src family member Fyn. Dasatinib was also curative against doxorubicin-resistant K-562/ADM xenografts in mice overexpressing the P-glycoprotein (PGP) efflux pump (29).

Dasatinib was also evaluated in human myeloid cell lines expressing an internal tandem duplication of Flt3 (Flt3-ITD), granulocyte-macrophage colony-stimulating factor (GM-CSF) dependency, or granulocyte colony-stimulating factor (G-CSF) responsiveness. Dasatinib potently inhibited growth in these cell lines in which Lyn is the predominant Src kinase, thus providing further evidence for a mechanism of action independent of Bcr-Abl (30).

The activity of dasatinib was investigated in cells and in mice with Ph+ ALL using the Bcr-Abl mutant p210-

T315I, resistant to both imatinib and dasatinib. In p210-T315I-expressing B-cells, dasatinib inhibited phosphorylation of Src kinases at concentrations of 25 nM, although it did not reduce phosphorylation of p210-T315I. However, apoptosis of the cells was induced. In mice with p210-T315I-induced ALL, all animals remained alive 30 days after transplantation when treatment with dasatinib 30 mg/kg was initiated 8 days after transplantation. This compared with 50% of placebo-treated and 31% of imatinib-treated mice. Complete eradication of leukemic cells induced by wild-type Bcr-Abl was achieved in mice treated with dasatinib. In mice deficient for Lyn, Hck and Fgr, the activation of Akt and Stat5 was downregulated (31).

The efficacy of dasatinib was assessed in a model of established intracranial CML growth. SCID mice bearing K-562 tumors were treated with dasatinib 5 or 15 mg/kg twice daily for up to 40 days. The life span of the mice was increased by 268% and 450% at the doses of 5 and 15 mg/kg, respectively, compared with vehicle-treated controls. Bioluminescent imaging techniques demonstrated that dasatinib 15 mg/kg resulted in tumor regressions and complete stasis of tumor growth during therapy (32).

Dasatinib significantly inhibited the growth of 6 of 13 (46%) solid tumors grown in mice from a random panel of tumors. Responsive tumor types included breast, prostate, colon, pancreatic and small cell lung cancers and sarcoma (18). Further studies have demonstrated the potential efficacy of dasatinib against solid malignancies. Dasatinib suppressed cell adhesion, migration and invasion of human prostate cancer cells (DU 145 and LNCaP) at low nanomolar concentrations, with corresponding inhibition of the kinase activities of Lyn and Src (33). In head and neck squamous cell carcinoma and non-small cell lung cancer cell lines, dasatinib inhibited migration and induced cell cycle arrest and subsequent apoptosis. These effects correlated with inhibition of Src and Akt (34). Furthermore, single oral doses of dasatinib (5, 15 and 30 mg/kg) and multiple doses suppressed serum levels of calcium in a rat bone resorption model, with single doses of 15 and 30 mg/kg providing more effective suppression than calcitonin 5 IU. This potent antiosteoclastic activity of dasatinib indicates a role for dasatinib in malignancy-related bone resorption and the treatment of bone metastases (35). Dasatinib, at clinically achievable submicromolar concentrations, also significantly inhibited the viability of multiple myeloma cell lines and primary tumor specimens from multidrug-resistant multiple myeloma patients. The degree of responsiveness to dasatinib correlated with increased baseline expression of diverse antiproliferative and antiapoptotic genes (36).

Evaluation of the activity of dasatinib has also focused on inhibition of wild-type Kit and certain mutant Kit isoforms that are associated with human neoplasms, including systemic mast cell disorders, seminoma and gastrointestinal stromal tumors (GIST). Dasatinib potently inhibited the ligand-dependent autophosphorylation of wild-type Kit kinase in the human myeloid leukemia cell line M-07e (IC_{50} = 1-10 nmol/l). In the human mast cell leukemia cell line HMC-1.1, which expresses the Kit V560G juxtamembrane domain mutation, dasatinib was at least as effective as imatinib in inhibiting cell proliferation. Moreover, in Ba/F3 cell lines expressing systemic mastocytosis-associated codon 816 mutations, dasatinib inhibited the autophosphorylation of human Kit D816V and D816F (IC_{50} approx. 100 nmol/l), which were not sensitive to imatinib. Consistent with these findings, dasatinib inhibited the proliferation of these cells and potently induced apoptosis, while imatinib had no significant inhibitory effect on the growth of the three cell lines. Dasatinib had an additive to synergistic effect with the mTOR inhibitor rapamycin in cells expressing Kit D816V, and a synergistic effect with cisplatin in Kit D816V/H cells. Dasatinib potently inhibited the Kit-dependent downstream pathways, such as Ras/mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3-kinase)/Akt (37-40).

In human mastocytosis HMC-1 cell lines carrying Kit mutations V560G and D816V, treatment with dasatinib (1 μM) resulted in almost complete growth inhibition (IC_{50} = 0.1-1.0 μM). In a flow cytometry-based assay, dasatinib (0.1 μM) demonstrated preferential cytotoxicity against primary human neoplastic mast cells carrying the D816V mutation, while sparing other hematopoietic cells (41, 42).

Potential biomarkers for predicting sensitivity to dasatinib have been identified. These include decreases in phospho-Stat5 levels (43), a decrease in phosphorylation of Src substrates (44), inhibition of phospho-Src in human prostate cancer PC-3 cells (45), and gene expression profiling in breast and lung cancer cell lines (46, 47). In more than 40 human breast cancer cell lines, the majority of dasatinib-sensitive cell lines were the nonluminal subtype (basal and mesenchymal). These mainly represent the triple negative subset of cancers that are more resistant to treatment (47).

In a flow cytometry assay of the downstream substrate CrKL, dasatinib inhibited CrKL phosphorylation and reduced total cell numbers in $CD34^+38^-$ cells at a clinically achievable concentration. However, dasatinib did not target the quiescent $CD34^+$ stem cell population, indicating that Bcr-Abl and Src may not be targets in these primitive cell populations. Persistence in these cell populations accounts for the small percentage of patients achieving complete molecular remission in CML (11, 48, 49).

Pharmacokinetics and Metabolism

In mice transplanted with K-562 xenografts, dasatinib was curative over a wide range of doses (1.25-50 mg/kg/dose). Pharmacokinetics were dose-dependent. The time course of tumoral Bcr-Abl and CrKL inhibition and recovery directly correlated with plasma levels of dasatinib. At the minimum effective dose of 1.25 mg/kg, maximum inhibition of Bcr-Abl was observed at approximately 3 h postdose. A pharmacokinetic/pharmacodynamic model predicted that a plasma concentration of 20 nM would be required to effectively inhibit Bcr-Abl in K-562 cells. The model also supported twice-daily dosing, which was confirmed by *in vivo* efficacy studies (50).

Clinical Studies

A phase I dose-escalation study was performed in chronic-phase CML patients with hematological resistance or intolerance to imatinib. Total daily doses of dasatinib ranged from 15 to 180 mg given as once- or twice-daily doses, and intrapatient dose escalation was permitted. In this study, 35 of 40 patients (88%) with a median duration of CML of 8 years had a complete hematological response. Major cytogenetic responses were obtained in 16 of 40 (40%) patients, with complete cytogenetic response in 13 of 40 (33%) patients. Responses were durable, with 36 (90%) patients remaining in response after a median of 13 months of treatment. Serum levels well above the concentration required to block CML cell proliferation *in vitro* were achieved. In blood samples obtained from 14 patients, determination of T-cell cytokine production showed that complete hematological response was achieved without any change in the distribution of T-cell subsets. Mutations associated with imatinib resistance were identified in 27 patients, but cytogenetic responses were observed in patients with a wide spectrum of Bcr-Abl mutations. Dasatinib was well tolerated. Grade 3 and 4 myelosuppression (thrombocytopenia and neutropenia) occurred in 38% of patients, but the events were reversible and easily managed with dose modification. Two patients developed unexplained pleural effusions, and mild Q-T_c prolongation was also observed. No patients discontinued treatment due to toxicity (51-54). The results from this and several of the following studies are summarized in Table I.

The phase I study was extended to include patients with accelerated-phase (n=10) or myeloid blast crisis (n=23) CML, or lymphoid blast crisis and Ph+ ALL (n=11) patients. A total of 44 patients were treated with dasatinib 70-240 mg/day for a median of 3-7 months. The rates of major hematological response (bone marrow blasts < 5%) were 80% (8/10) in patients with accelerated-phase, 77% (17/22) in patients with myeloid blast crisis and 60% (6/10) in patients with lymphoid blast crisis and Ph+ ALL. The corresponding rates of complete hematological response were 50%, 18% and 50%. The overall rates of major cytogenetic response and complete cytogenetic response in advanced disease were 36% and 21%, respectively. Consistent, rapid and sustained inhibition of Lyn kinase was demonstrated. Responses were durable in 80% of accelerated-phase patients, with a median duration of 7 months, but relapses occurred in the myeloid blast crisis and lymphoid blast crisis/Ph+ ALL cohorts, often due to dasatinib-resistant Bcr-Abl mutations. Reversible grade 3 and 4 myelosuppression was observed in all cohorts, and 6 patients developed pleural effusions. No patients discontinued treatment due to toxicity (51, 55, 56).

More recent data have been presented. Normalization of blood counts was reported in 93% of chronic-phase patients (n=40) and the major cytogenetic response rate was 45%. In the case of patients with advanced disease (n=44), including those in the accelerated phase, myeloid blast crisis, lymphoid blast crisis and Ph+ ALL patients, 70% showed hematological remission and 25% had a major cytogenetic response. Responses were durable (median follow-up > 12 and 8 months for chronic and acute patients, respectively), except for 1 Ph+ ALL patient and another in lymphoid blast crisis, both of whom relapsed in 6 months (57).

Mutation status was correlated with response by analysis of blood samples from 63 patients in the phase I

Table I: Clinical studies of dasatinib (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Leukemia, acute lymphocytic, Leukemia, chronic myeloid	Open	Dasatinib, 15-180 mg/d x 13 [median] mo (n=40) Dasatinib, 70-240 mg/d b.i.d. x 3-7 [median] mo (n=44)	84	Dasatinib was well tolerated and associated with durable responses in imatinib-resistant or -intolerant patients with chronic- and accelerated-phase chronic myeloid leukemia	51-56
Cancer, gastrointestinal (stromal)	Open	Dasatinib, 35 mg p.o. b.i.d. x 5 d 1x/wk Dasatinib, 50 mg p.o. b.i.d. x 5 d 1x/wk Dasatinib, 70 mg p.o. b.i.d. x 5 d 1x/wk	14	Dasatinib was safe in patients with gastrointestinal stromal or other solid tumors. Some patients with resistant gastrointestinal stromal tumors showed clinical benefit	62
Leukemia, chronic myeloid	Open Multicenter	Dasatinib, 70 mg p.o. b.i.d.	35	Preliminary results revealed hematological and cytogenetic activity for dasatinib in patients with imatinib-resistant or -intolerant chronic myeloid leukemia	63
Leukemia, chronic myeloid	Open Multicenter	Dasatinib, 70 mg p.o. b.i.d.	34	Dasatinib produced a major hematological response in 16 patients and a cytogenetic response in 13 patients with chronic myeloid leukemia in myeloid blast crisis resistant to or intolerant of imatinib; tolerability was acceptable	64

continuation

Table I: Clinical studies of dasatinib (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Leukemia, chronic myeloid	Open Multicenter	Dasatinib, 70 mg b.i.d.	30	Dasatinib was associated with cytogenetic responses in 7 of 16 evaluable patients with chronic myeloid leukemia resistant to or intolerant of imatinib	65
Leukemia, acute lymphocytic, Leukemia, chronic myeloid	Open Multicenter	Dasatinib, 70 mg p.o. b.i.d.	28	A major hematological response was seen in 13 patients with chronic myeloid leukemia in lymphoid blast crisis or Philadelphia chromosome-positive acute lymphoblastic leukemia treated with dasatinib	66
Cancer	Open	Dasatinib, 20 mg + Ketoconazole	60	Begun in August 2005, this phase I study in advanced cancer patients is designed to assess the effects of ketoconazole on the pharmacokinetics of dasatininb and the effects of dasatinib on tumor growth	69
Myeloproliferative disorder	Open	Dasatinib	120	Initiated in November 2005, this phase II clinical trial will evaluate the tolerability and efficacy of dasatinib in patients with myeloproliferative disorders	70
Leukemia, chronic myeloid	Open	Dasatinib p.o.	100	The safety and efficacy of dasatinib in controlling disease in patients with chronic myelogenous leukemia will be determined in this phase II study which began in November 2005	72
Leukemia, chronic myeloid	Open Multicenter	Dasatinib p.o. o.d. on d 1-5 1x/wk x 3 mo	50	In this phase I study, the maximum tolerated dose and overall safety profile of dasatinib will be determined in patients with imatinib-resistant chronic-phase chronic myelogenous leukemia	74
Leukemia, chronic myeloid	Open Multicenter	Dasatinib b.i.d. on d 1-28	60	This phase II study will assess hematological responses to dasatinib therapy in patients with accelerated-phase chronic myelogenous leukemia resistant to or intolerant of imatinib	75
Leukemia, chronic myeloid	Open Multicenter	Dasatinib p.o. b.i.d.	60	The ability of dasatinib to induce hematological responses in patients with blast-phase chronic myelogenous leukemia resistant to or intolerant of imatinib will be evaluated in this phase II study	76
Leukemia, acute lymphocytic, Leukemia, chronic myeloid	Open Multicenter	Dasatinib	60	Hematological responses to dasatinib therapy will be assessed in this phase II study in patients with blast-phase chronic myelogenous leukemia or acute lymphoblastic leukemia resistant to or intolerant of imatinib	77
Leukemia, chronic myeloid, Leukemia, myeloid	Randomized Open	Dasatinib Imatinib	150	This phase II study will compare major cytogenetic responses at week 12 in patients with imatinib-resistant chronic-phase chronic myelogenous leukemia treated with dasatinib or imatinib	78
Leukemia, chronic myeloid, Leukemia, myeloid	Open Multicenter	Dasatinib p.o. b.i.d.	100	The major cytogenetic response rate will be measured in this phase II study of dasatinib in patients with chronic-phase chronic myelogenous leukemia resistant to imatinib	79

study. A total of 17 different imatinib-resistant point mutations were identified in the Bcr-Abl kinase domain in 67% of the patients prior to treatment. Complete hematological remission was obtained in patients harboring each of these mutations, with the exception of T315I, F317L and D276G. Patients with the latter two mutations had partial responses. Of 9 patients with disease progression, 3 had the T315I mutation detected prior to treatment and in 2 patients it was detected at the time of progression. Preliminary analysis indicated a strong correlation between a lack of response and the presence of the T315I mutation (58, 59).

A molecular analysis of response in the phase I study was performed using quantitative polymerase chain reaction (RQ-PCR) analysis in 14 patients with accelerated-phase or blast crisis CML, or Ph+ ALL, and 19 late-chronic-phase CML patients. At least a 2-log reduction of Bcr-Abl below the standardized baseline (approximating a complete cytogenetic response) was achieved in 6 of 14 (43%) patients with accelerated-phase or blast crisis CML, or Ph+ ALL, and in 7 of 19 (37%) patients with late-chronic-phase CML. The response was maintained in the majority of patients with late-chronic-phase CML. A 3-log reduction defined as a major molecular response was achieved in 4 (29%) and 4 (21%) patients, respectively. Baseline mutations were detected in 23 patients, all of whom were resistant. At the last recorded analysis, the same mutation that was present at baseline was present in 21 patients and 5 of these patients had an additional mutation. The other 2 patients had different mutations, one of which was F317I. Mutations were present in all 8 patients who progressed, and 6 of these patients had T315I that was detected at baseline or evolved during therapy. Overall, this mutation evolved in 6 patients during therapy and this was associated with significant increases in Bcr-Abl of 2.5-185-fold (60, 61).

Another phase I dose-escalation study was performed to evaluate the safety, tolerability and pharmacological profile of dasatinib in patients with treatment-resistant GIST and other refractory solid tumors. Fourteen patients were treated with 35, 50 or 70 mg dasatinib twice daily for 5 days every week. No dose-limiting toxicity was observed. Following a dose of 35 mg twice daily, the pharmacokinetic profile in fasting patients was similar to that of patients with hematological malignancies. The half-life increased (from 1.3 to 4.6 h) in the presence of a high-fat meal. No objective responses were observed on computed tomography scanning, but resolution of GIST-associated ascites was seen in 1 patient and 2 further patients continued on treatment for at least 3 months (62).

In a multicenter phase II study, 107 patients with heavily pretreated, accelerated-phase CML resistant or intolerant to imatinib were treated with dasatinib 70 mg twice daily (Src-Abl Tyrosine kinase inhibition Activity: Research Trials of dasatinib - “START-A” study). Preliminary results from 35 patients with a median time from diagnosis of 91 months showed that 30 (86%) patients had achieved a complete hematological response on prior imatinib, and 9 (26%) had a major cytogenetic response. The median duration on study was 2 months. Twenty-three patients (66%) achieved a major hematological response and cytogenetic responses were observed in 13 of 24 (54%) patients. Responses were seen in 3 patients who had never responded to imatinib. Profound myelosuppression was observed in 20 patients, and nonhematological toxicities included grades 1 and 2 diarrhea, nausea, headache, peripheral edema and pleural effusion (63).

In the START-B study, 74 patients with myeloid blast crisis who were resistant or intolerant to imatinib were treated with dasatinib 70 mg twice daily on a continuous daily dosing schedule. Preliminary data on the first 34 patients indicated best responses to imatinib of complete hematological response in 82% of patients and a major cytogenetic response in 39% of patients. Their median duration of disease was 49 months. Major hematological responses were obtained in 16 of 29 (55%) patients, 7 of which were documented as complete. Cytogenetic responses occurred in 13 (45%) patients. Myelosuppression occurred in approximately 60% of patients. Nonhematological toxicities were uncommon, and included diarrhea, rash, nausea, peripheral edema and pleural effusion (64).

A total of 186 patients with chronic-phase CML resistant or intolerant to imatinib were enrolled in the START-C phase II study. Dasatinib was administered at a dose of 70 mg twice daily. Preliminary data were available from the first 30 patients, with a median time from diagnosis of 71 months. The best response to prior imatinib therapy was a complete hematological response in 83% of patients, and complete and partial cytogenetic responses in 17% and 13% of patients, respectively. Following dasatinib administration, hematological responses were observed in 21 of 24 patients, and cytogenetic responses were observed at 3 months in 7 of 16 patients, including a complete cytogenetic response in 4 patients. Grades 3 and 4 neutropenia and thrombocytopenia were reported in 6 patients each. Nonhematological toxicities were similar to those reported in START-A and START-B (65).

Patients with CML in lymphoid blast crisis or Ph+ ALL who were resistant or intolerant to imatinib were enrolled in the START-L study. Of 77 patients enrolled, preliminary results were presented for 28 patients with median time from diagnosis of 17 months. The responses to prior imatinib therapy were a complete hematological response in 19 (68%) patients and a major cytogenetic response in 11 (39%) patients. On dasatinib therapy, 13 patients had a major hematological response, including 7 with a complete hematological response, and 12 patients had a cytogenetic response within 1-3 months. Response was maintained in 9 of 13 (69%) patients after a median follow-up of 14 weeks. Grade 3 or 4 myelosuppression, present at baseline in the majority of patients, persisted during treatment. Peripheral and facial edema was also observed (66).

The correlation of clinical response to dasatinib with Bcr-Abl mutation status was evaluated in 25 CML

patients in chronic phase (n=10), accelerated phase (n=11) or blast phase (n=4), and 1 patient with ALL. Thirteen different imatinib-resistant point mutations in the Bcr-Abl kinase domain were identified. The most common mutations were G250E/A, T315I, F317L and E355G/A. Twenty (77%) patients responded to therapy. Six patients did not respond (3 with T315I) and 3 patients lost their response after a median of 3 months without developing any detectable new mutations. Four patients (1 each with F486S and E255V, and 2 with G250E) had a sustained response beyond 6 months. Among 12 patients with P-loop mutations, 11 responded to therapy and their median survival was longer than 5 months. The results demonstrate that dasatinib is active in patients with a wide range of imatinib-resistant mutations, with the exception of T315I (67).

The T315I mutation was detected in patients in both the phase I and phase II studies with dasatinib, including 7 of 9 patients with acquired resistance to dasatinib. A novel Bcl-Abr mutation was also identified, T315A, which retained sensitivity to imatinib. Such findings support the use of combination kinase inhibitor therapy in CML to prevent the emergence of drug-resistant clones, and a phase I trial to evaluate the safety of combining imatinib with dasatinib is planned (68).

A number of phase I and II clinical trials are under way in patients with leukemia, solid tumors and myeloproliferative disorders (69-79).

Source

Bristol-Myers Squibb Co. (US).

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