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Danusertib

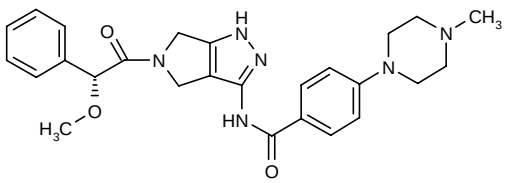
Prop INN

Aurora Kinase Inhibitor
Oncolytic

PHA-739358

N-[5-[2(*R*)-Methoxy-2-phenylacetyl]-1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazol-3-yl]-4-(4-methylpiperazin-1-yl)benzamide

InChI=1/C26H30N6O3/c1-30-12-14-31(15-13-30)20-10-8-19(9-11-20)25(33)27-24-21-16-32(17-22(21)28-29-24)26(34)23(35-2)18-6-4-3-5-7-18/h3-11,23H,12-17H2,1-2H3,(H2,27,28,29,33)/t23-/m1/s1



$C_{26}H_{30}N_6O_3$
Mol wt: 474.5548
CAS: 827318-97-8
EN: 394773

Abstract

The emergence of point mutations in the *BCR-ABL* gene is associated with the development of resistance to imatinib in patients with chronic myeloid leukemia (CML). Inhibitors of Aurora kinases, enzymes essential for the successful execution of cell division, or mitosis, represent a novel, alternative class of agents for the treatment of CML and the targeted therapy of imatinib-resistant patients. The Aurora kinase inhibitor danusertib simultaneously targets the Aurora kinases and BCR-ABL, including imatinib-resistant mutant BCR-ABL, and is effective in multiple preclinical tumor models. Phase I investigations have confirmed that danusertib is well tolerated, with few dose-limiting toxicities and evidence of clinically relevant disease stabilization. Phase II studies are ongoing and preliminary data support phase I outcomes and provide evidence for complete hematological responses in CML patients with T315I-mutated BCR-ABL.

Synthesis

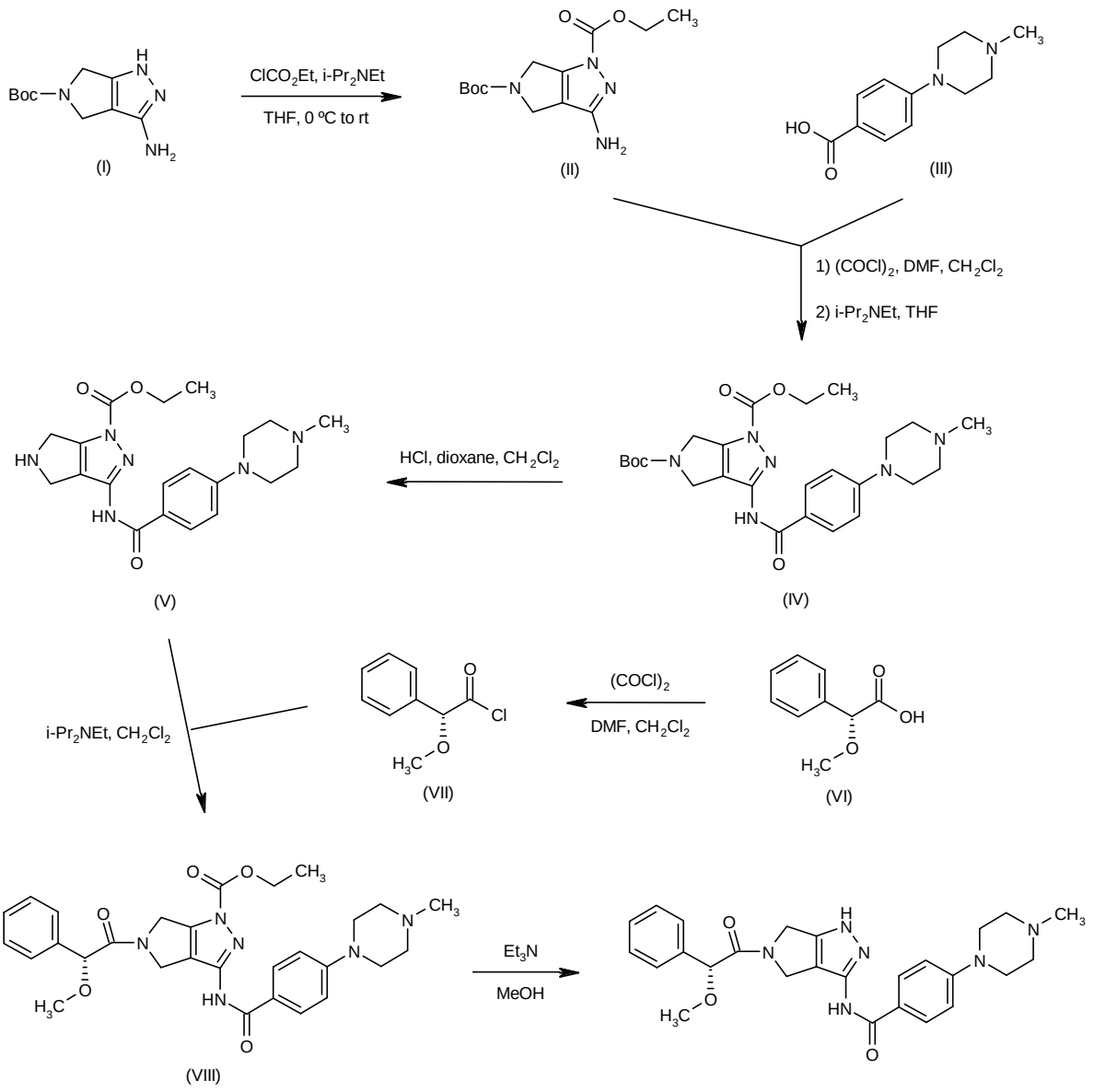
Danusertib can be synthesized as follows. Protection of 3-amino-5-Boc-1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole (I) by treatment with ethyl chloroformate and DIEA gives the 1-ethoxycarbonyl derivative (II). After conversion of 4-(4-methylpiperazinyl)benzoic acid (III) to the corresponding acid chloride by means of oxalyl chloride and DMF, coupling with the amino heterocycle (II) furnishes the amide (IV). The *N*-Boc group of (IV) is then selectively deprotected under acidic conditions to give (V). Activation of (*R*)-α-methoxyphenylacetic acid (VI) with oxalyl choride and DMF gives the acid chloride (VII), which is coupled with the deprotected pyrrolopyrazole (V) to yield amide (VIII). Finally, the ethoxycarbonyl group of (VIII) is removed by treatment with Et_3N in MeOH to provide the title compound (1, 2). Scheme 1.

Background

One of the most common types of leukemia is chronic myeloid leukemia (CML). CML arises in bone marrow stem cells and affects the myeloid lineage that produces granulocytes and macrophages. As the name suggests, the disease often exists for years with only moderately elevated numbers of leukemic cells and few symptoms. At some point, however, the CML patient will go through a "blast crisis" when the leukemic granulocyte–macrophage progenitors begin to divide by themselves, increasing their numbers enormously while failing to continue their differentiation. In most cases of CML, the leukemic cells share a unique chromosome abnormality, *i.e.,* reciprocal translocation between one chromosome 9 and one chromosome 22, designated t(9;22). It results in one chromosome 9 longer than normal and one chromosome 22 shorter than normal (the Philadelphia chromosome, or Ph). The DNA removed from chromosome 9 contains most of the c-*ABL* proto-oncogene. The break in chromosome 22 occurs in the middle of a gene designated *BCR*. The resulting Philadelphia chromosome has the 5' section of *BCR* fused with most of c-*ABL* (*BCR-ABL*) (3). Treatment with the c-ABL kinase inhibitor imatinib mesilate has been shown to greatly improve the prognosis of patients with CML, although the emergence of point mutations in the *BCR-ABL* gene is associated with the development of resistance to imatinib and this represents a new clinical challenge in the treatment of CML.

C. Dulsat, N. Mealy, R. Castañer. Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1: Synthesis of Danusertib



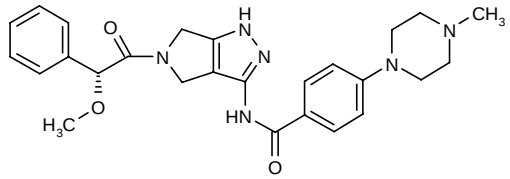
Furthermore, second-generation BCR-ABL inhibitors, while able to overcome most imatinib-resistant mutants, struggle against the common T315I substitution (4).

Aurora kinase inhibitors represent a novel, alternative class of agents for the treatment of CML and the targeted therapy of imatinib-resistant patients. Aurora kinases are serine/threonine kinases that are essential for the successful execution of cell division, or mitosis. Their activity is delicately regulated, mainly by phosphorylation and degradation, and deregulation of Aurora kinase activity can result in mitotic abnormality and genetic instability, leading to defects in centrosome function, spindle assembly, chromosome alignment and cytokinesis. Both the expression level and the kinase activity of Aurora kinases are found to be upregulated in many human cancers, correlating with chromosomal instability and clinically aggressive disease in some instances (5-10), indicating that these kinases might serve as useful targets for the development of anticancer drugs. Table I summarizes the current clinical development status of small-molecule inhibitors of Aurora kinases.

Three Aurora kinases have been identified in mammalian cells to date: Aurora-A is involved in the prophase of mitosis and is required for the correct function of the centrosomes (microtubule-organizing centers in eukaryotic cells); Aurora-B functions in the attachment of

Table I: Small-molecule Aurora kinase inhibitors in clinical evaluation for cancer (from Prous Science Integrity®).

Compound	Source	Phase
1. Danusertib	Nerviano Medical Sciences	II
2. TSU-68	Taiho	II
3. AT-9283*	Astex	I/II
4. AZD-1152	AstraZeneca	I/II
5. AS-703569 (R-763)*	Merck Serono/Rigel	I
6. CYC-116	Cyclacel	I
7. ENMD-2076	EntreMed	I
8. KW-2449*	Kyowa Hakko	I
9. MK-5108 (VX-689)*	Merck & Co./Vertex	I
10. MLN-8054	Millennium Pharmaceuticals	I
11. MLN-8237	Millennium Pharmaceuticals	I
12. PF-3814735	Pfizer	I
13. SNS-314	Sunesis	I



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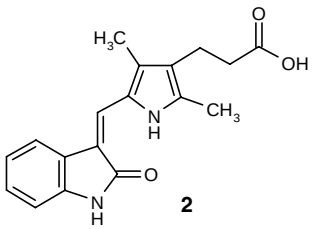

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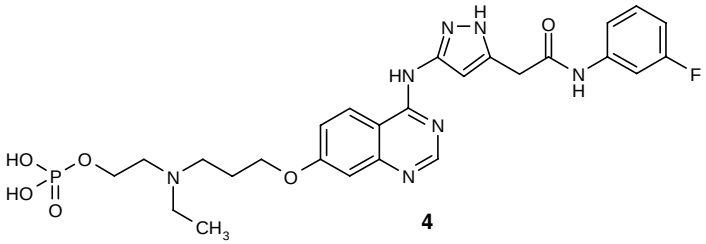

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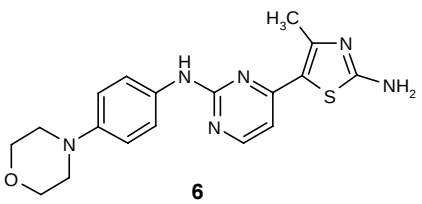

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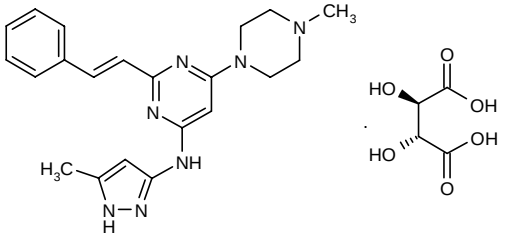

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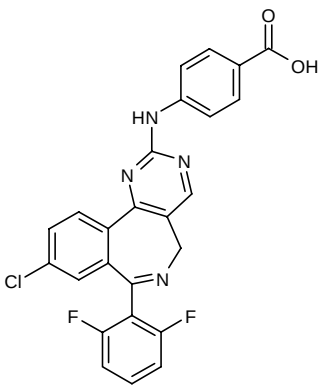

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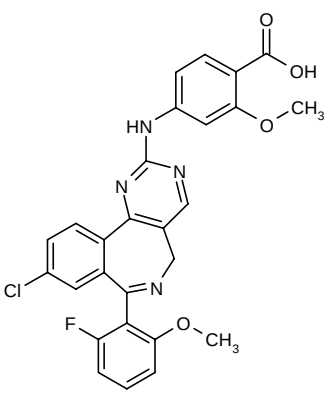

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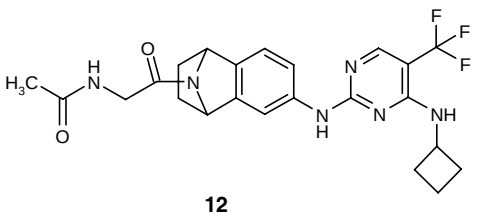

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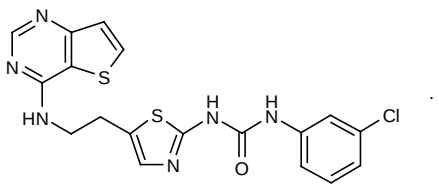

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*Structure not available.

the mitotic spindle to the centromere; and Aurora-C works in germ-line cells and, as yet, little is known about its function (11-13). Overexpression of Aurora-A has been shown to induce oncogenic transformation in cells (14) and elevated ectopic activity of Aurora-B has been shown to promote Ras-mediated enhancement of oncogenic signaling (15). Danusertib (PHA-739358), a pyrrolopyrazole (2) that inhibits Aurora kinases, is currently in phase II clinical development at the National Cancer Institute and Nerviano Medical Sciences for the treatment of patients with CML that has relapsed after imatinib mesilate or c-ABL therapy (16).

Preclinical Pharmacology

Biochemical cellular kinase inhibition assays have revealed that danusertib acts as a spectrum-selective kinase inhibitor for cancer-related kinases. Danusertib inhibits Aurora-A, -B and -C kinases with IC_{50} values of 13, 79 and 61 nmol/l, respectively. High activity was also observed against other kinases known to be mutated or overexpressed in different cancers, such as C-ret, Trk-A, c-ABL (approximately 2-fold higher IC_{50} *versus* Aurora-A) and FGFR-1 (4-fold higher IC_{50} compared with Aurora-A). Treatment of several human tumor cell lines, including colon carcinoma HCT 116, breast carcinoma MCF7, ovarian carcinoma A2780 and cervical adenocarcinoma HeLa cells, with danusertib significantly inhibited proliferation with IC_{50} values of 28-140 nM, and also led to an increase in levels of the tumor suppressor protein p53 and its downstream target p21. In tumor cells, danusertib induced a morphological phenotype typical of Aurora-B inhibition and molecular markers for Aurora-A and -B were also shown to be modulated (2, 17, 18).

In vitro investigations have confirmed that danusertib simultaneously targets the Aurora kinases and BCR-ABL, including imatinib-resistant mutant BCR-ABL. In human *BCR-ABL*-positive and -negative cell lines and murine Ba/F3 cells ectopically expressing wild-type or imatinib-resistant *BCR-ABL* mutants, including T315I, danusertib was shown to significantly decrease the phosphorylation of histone H3, a marker of Aurora-B activity, and of CRKL, a downstream target of BCR-ABL. This correlated with inhibition of leukemia cell proliferation. Furthermore, danusertib demonstrated strong antiproliferative effects and induced apoptosis in $CD34^+$ cells derived from untreated CML patients and from imatinib-resistant individuals in chronic phase or blast crisis, including those harboring the T315I mutation (19-21).

In vitro inhibition assays have also demonstrated that danusertib binds all forms of ABL with higher affinity than MK-0457, another Aurora kinase inhibitor. Furthermore, danusertib showed higher affinity for the T315I mutant (0.005 μmol/l) (22). Analysis of the crystallographic structure of the active phosphorylated T315I mutant kinase domain complexed with danusertib indicated that danusertib accommodates the ATP-binding pocket, avoiding the hydrophobic pocket. Furthermore, a favorable hydrophobic interaction between the inhibitor and the isoleucine side-chain has been suggested to account for the greater degree of activity against the T315I mutant *versus* the wild-type protein and other mutants (19, 22).

Danusertib has been shown to be effective in different tumor models, including DMBA-induced and MMTV-RAS transgenic mammary carcinomas in rats and mice, respectively, A2780 human ovarian carcinoma xenografts in nude mice, HCT 116 human colon carcinoma xenografts in nude mice, HL-60 human acute myelogenous leukemia xenografts in SCID mice and TRAMP transgenic prostate carcinoma in mice. Significant tumor growth inhibition, ranging from 66% to 98%, and a good safety profile were observed when danusertib was administered i.v. at the maximum tolerated dose of 60 mg/kg/day for 5 days or for 10 days at 30 and 45 mg/kg/day. Further examination of A2780 tumor xenografts indicated that suppression of tumor growth correlated with inhibition of Aurora kinase signaling, as demonstrated by inhibition of histone H3 phosphorylation and accumulation of p53 and its downstream target p21 (2, 17, 18, 23).

Pharmacokinetics and Metabolism

Pharmacokinetic investigations in mice bearing A2780 xenografts confirmed biexponential activity for danusertib when given at 45 mg/kg once daily over 10 days. Terminal half-life was recorded as approximately 3.5 h, with a low clearance (1.7 l/h/kg) and a high steady-state volume of distribution (V_{ss} = 2.7 l/kg) (18).

Clinical Studies

A phase I study of 6- and 3-h i.v. infusions (45, 90, 135, 190, 250, 330 and 400 mg/m^2) administered on days 1, 8 and 15 every 4 weeks in 42 patients with advanced solid tumors demonstrated that danusertib was well tolerated. Neutropenia was identified as a dose-limiting toxicity; however, it was of short duration and without complications. Nonhematological toxicities were mild or moderate. As a result, the study identified a recommended dose for phase II studies of 330 mg/m^2 for the 6-h schedule. Seven of 30 evaluable patients achieved clinically relevant stable disease, which lasted for over 6 months in 4 of these patients (24, 25).

Another phase I investigation of 14-day cycles of 24-h danusertib i.v. infusions (45-1000 mg/m^2) with or without granulocyte colony-stimulating factor (G-CSF) in patients with advanced tumors (N = 56) demonstrated that danusertib was well tolerated, with dose-limiting toxicity of brief grade 4 neutropenia, which could be prevented by coadministration of G-CSF. Nonhematological toxicities were mostly mild or moderate (grade 1-2) and grade 2 creatinine increase at 1000 mg/m^2 was also reported. The recommended dose for phase II studies was 500 and 750 mg/m^2 with and without G-CSF, respectively. A partial response was reported in 1 patient with small cell lung cancer, along with clinically relevant disease stabilization lasting over 3 months in 9 patients.

Biomarker modulation (decrease in histone H3 phosphorylation) was observed in skin biopsies starting at 500 mg/m^2, with low interpatient variability (26, 27).

A multicenter phase II study was conducted in patients with CML (N = 7) relapsing on imatinib or other c-ABL therapy administered danusertib 250 or 330 mg/m^2/day via once-weekly 6-h infusion for 3 consecutive weeks every 4 weeks. Two patients with T315I mutated *BCR-ABL* achieved a complete hematological response. One of these patients treated in the accelerated phase achieved a complete cytogenetic response and a complete molecular response after 3 months on a dose of 330 mg/m^2. The complete cytogenetic response was also ongoing after 6 months of treatment. The second patient who achieved a complete hematological response initiated therapy in the chronic phase. No grade 3/4 nonhematological toxicities were reported, with the exception of uncomplicated grade 4 neutropenia in 1 patient. Average exposure, $AUC_{0\text{-}t(168h)}$, at 330 mg/m^2/day was approximately 45 μM/h, with C_{max} values of approximately 4-6 μM/h. Pharmacodynamic analyses demonstrated treatment-associated decreases in phosphorylation of the oncogene *CRKL* in 6 of 7 patients, including both responders. Modulation of histone H3 phosphorylation was observed in 3 of 5 evaluable patients (28).

A phase II trial is under way in patients with CML relapsing after imatinib or c-ABL therapy (16).

Source

Nerviano Medical Sciences (IT).

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