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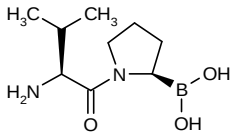
Talabostat

Oncolytic
Hematopoietic Agent
Dipeptidyl-Peptidase IV (CD26) Inhibitor
Fibroblast Activation Protein Inhibitor

ValboroPro
PT-100

L-Valyl-L-boroproline
[1-[2(*S*)-Amino-3-methylbutanoyl]pyrrolidin-2(*R*)-yl]boronic acid



$C_9H_{19}BN_2O_3$
Mol wt: 214.0711

CAS: 149682-77-9

CAS: 153737-95-2 (as monohydrochloride)

CAS: 150080-09-4 (as monomethanesufonate salt)

EN: 303902

Abstract

The process of maturation of blood cells via hematopoiesis involves cytokines and their regulation by the serine proteases CD26/dipeptidyl-peptidase IV (DPP-IV) and fibroblast activation protein (FAP). The amino boronic dipeptide talabostat (PT-100) has potent hematopoietic activity *in vitro* and *in vivo*. In preclinical studies, talabostat stimulated the survival and growth of primitive hematopoietic progenitors *in vitro* and rapidly increased levels of the cytokines G-CSF (granulocyte colony-stimulating factor), IL-6 and IL-11 above basal levels. The hematopoietic target of talabostat was identified as FAP. In mice, tumor growth was significantly suppressed following treatment with talabostat, and when given in combination with other chemotherapeutic agents, tumor rejection and immunity to secondary challenge were increased compared with single-agent treatment. A dose-escalation study in patients receiving myelosuppressive chemotherapy showed that a daily dose of 800 μg/day on days 2-8 of the chemotherapy cycle resulted in a 62% improvement in the duration and degree of severe neutropenia. Four phase II studies are ongoing in cancer patients, and talabostat is also under development for the treatment of hematopoietic disorders, such as neutropenia, anemia and thrombocytopenia.

Synthesis

The metalation of *N*-Boc-pyrrole (I) with lithium tetramethylpiperidine (LiTMP) in THF and TMEDA, followed by reaction with triethyl borate and acidic work-up gives *N*-Boc-pyrrole-2-boronic acid (II), which is hydrogenated with H_2 over Pd/C in EtOAc to yield racemic *N*-Boc-pyrrolidine-2-boronic acid (III) (1-3). Alternatively, boronic acid (III) can be obtained by reaction of pyrrolidine (IV) with Boc_2O to afford *N*-Boc-pyrrolidine (V), which is treated with *sec*-butyl lithium in diethyl ether and TMEDA followed by reaction with triethyl borate and acidic work-up (1-4). Esterification of racemic boronic acid (III) with (+)-pinanediol (VI) provides a diastereomeric mixture of cyclic esters, from which the desired isomer (VII) can be separated by chromatography. Removing the Boc-protecting group of ester (VII) with HCl gives the pyrrolidine-boronic ester (VIII) (1-5), which is coupled with *N*-Boc-L-valine (IX) by means of EDC, HOBT and NMM to produce amide (X). Treatment of amide (X) with HCl in diethyl ether removes the Boc-protecting group to yield the deprotected amine (XI) (1-4). Finally, the pinanediol moiety of amine (XI) is removed by transesterification with phenylboronic acid and HCl (1, 2, 4-6). Alternatively, the cleavage of the pinanediol moiety can also be performed by treatment of amine (XI) with $NaIO_4$ and HCl (6). Scheme 1.

An improved synthesis that includes the recovery of the costly chiral auxiliary (+)-pinanediol (VI) has been developed: The activation of *N*-trifluoroacetyl-L-valine (XII) with the Vilsmeier reagent gives the corresponding acyl chloride (XIII), which is coupled with the pyrrolidine-boronic ester (VIII) to yield amide (XIV). Removing the trifluoroacetyl group of (XIV) by alkaline hydrolysis affords amine (XI), which is finally deprotected with simultaneous recycling of the (+)-pinanediol auxiliary by transesterification with *N*-Boc-pyrrolidine-2-boronic acid (III) in the presence of MsOH, providing talabostat along with the recyclable pinanediol boronate (VII) (4) Scheme 2.

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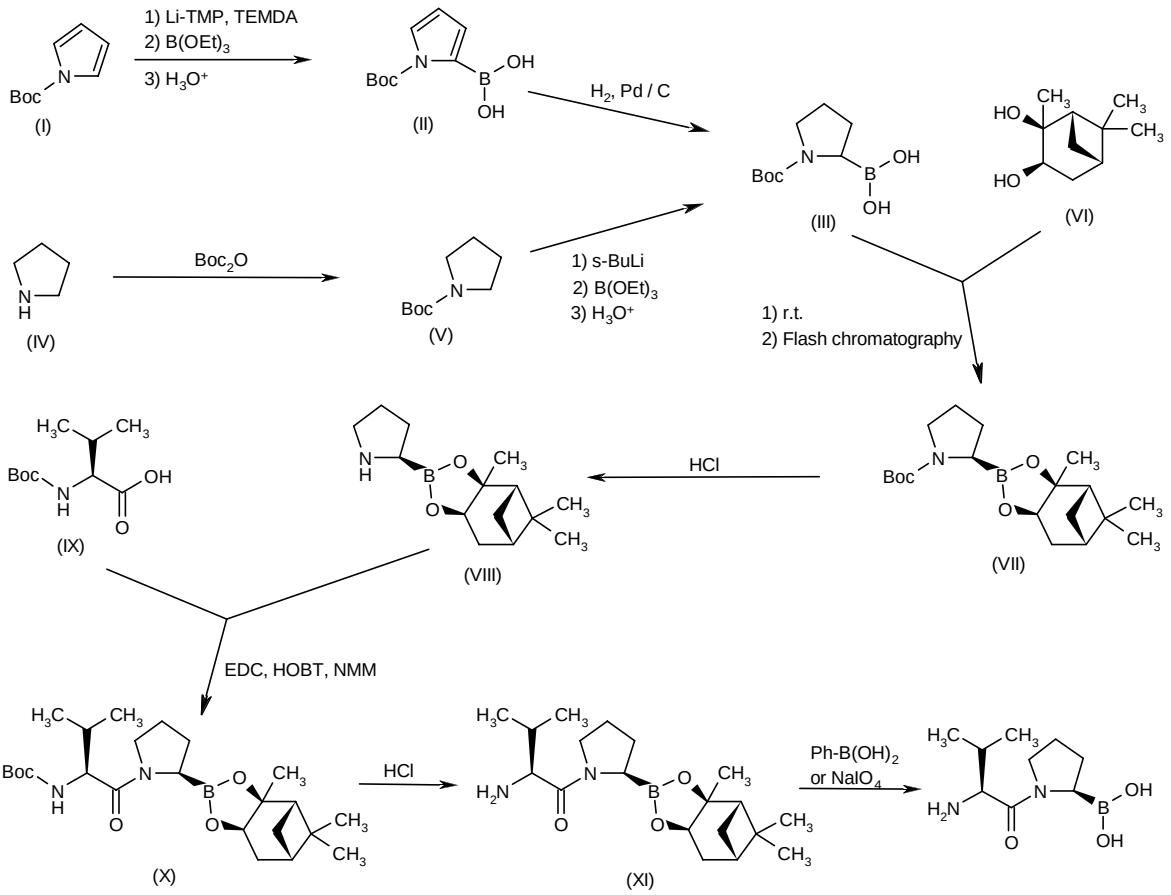
Scheme 1: Synthesis of Talabostat



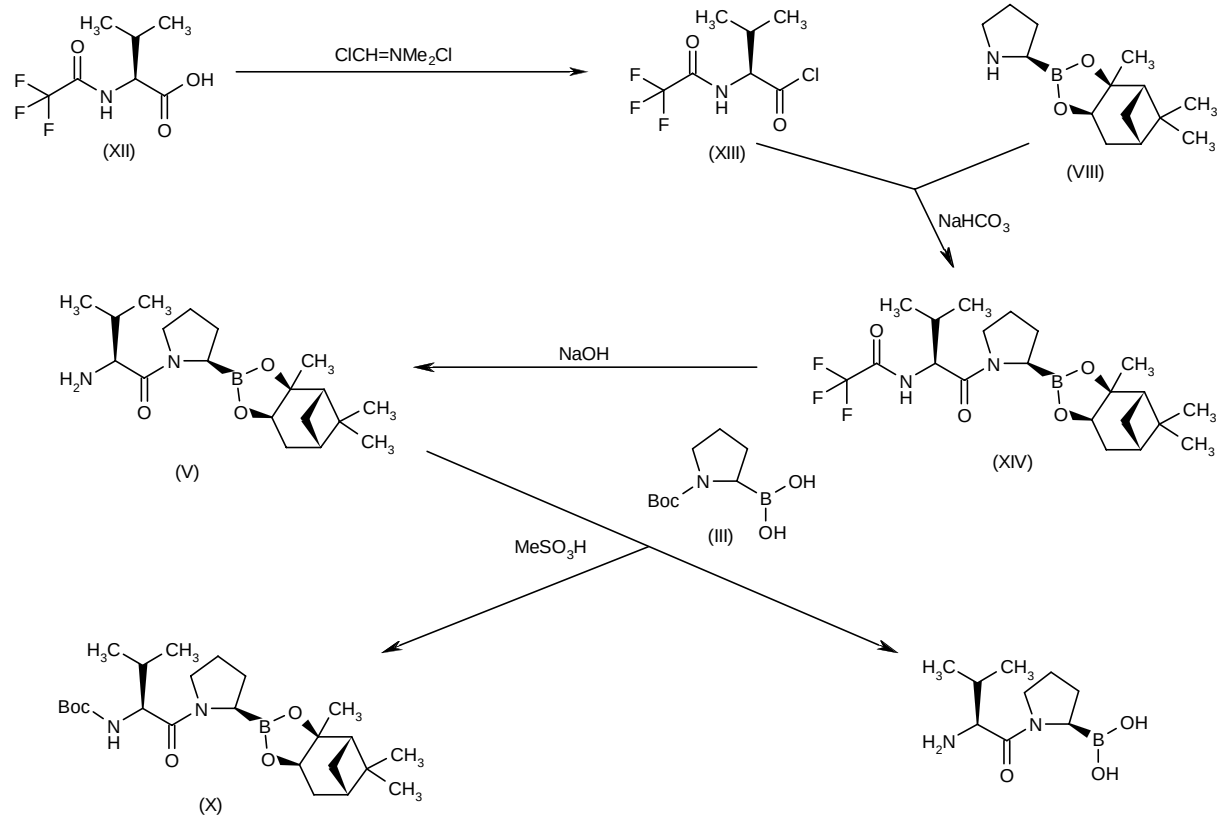
Introduction

Hematopoiesis is the formation of blood cells and involves both proliferation and differentiation from stem cells. Mature blood cells are produced by the progressive differentiation of intermediate progenitor cells, which is modulated by cytokines. Serine proteases that may play a role in regulating the hematopoietic activity of cytokines or other polypeptides involved in intercellular signaling include CD26/dipeptidyl-peptidase IV (CD26/DPP-IV) and the closely related fibroblast activation protein (FAP). The amino boronic dipeptide class of compounds are high-affinity transition-state analogues for the catalytic site of several serine proteases and have a potential role in hematopoietic stimulation and regulation of cytokine production via their ability to inhibit dipeptidyl-peptidase activity (1, 7-11). Talabostat (PT-100) is an orally active small molecule that stimulates the growth of primitive hematopoietic progenitor cells by increasing the production of a series of growth factors required for hematopoiesis (11). Talabostat therefore has potential in cancer patients and the treatment of hematopoietic disorders, such as neutropenia, anemia and thrombocytopenia. Phase II trials are in progress for the treatment of non-small cell lung cancer, lymphocytic leukemia and metastatic melanoma.

Pharmacological Actions

In vitro studies were performed using CD34+ cells propagated on supportive layers of X-irradiated stromal cells. The addition of talabostat stimulated the survival and growth of primitive hematopoietic progenitors and rapidly increased the supernatant levels of granulocyte colony-stimulating factor (G-CSF) and the interleukins IL-6 and IL-11 above basal levels. The effect of talabostat on hematopoiesis *in vivo* was studied in mice. Administration of talabostat (5 μg orally twice daily for 5 consecutive days) significantly increased the levels of erythroid and myeloid progenitor cells. These studies demonstrated that talabostat can stimulate hematopoiesis *in vitro*

Scheme 2: Synthesis of Talabostat



and *in vivo* by interacting with stromal cells to increase the production of cytokines known to stimulate the growth and survival of hematopoietic progenitor cells. Talabostat also stimulated neutrophil regeneration after myeloablative chemotherapy (cyclophosphamide), and enhanced erythropoiesis in response to anemia (11).

The molecular targets of talabostat involved in the stimulation of cytokine production in cultured human bone marrow stromal cells were identified as CD26 and FAP. However, further studies in CD26-deficient mice showed that talabostat accelerated neutrophil recovery at least as effectively in these mice as in nondeficient mice. These studies demonstrated that FAP, rather than CD26, was likely to be the hematopoietic target of talabostat (11).

As FAP is known to be present on the stroma of lymphoid tissue and tumors, the effect of talabostat on tumor growth was also studied *in vivo*. The expression of chemokines and cytokines that promote antitumor immune responses was increased in tumor-bearing mice treated with talabostat, and tumor growth was significantly suppressed. Talabostat inhibited the growth of human tumor xenografts by 50-60% in Rag-knockout and SCID (immunodeficient) mice, indicating that T-cell-independent activities contribute to the effect of the compound in addition to the recognized T-cell activity. The ability of talabostat to augment the antitumor activity of the anti-CD20 monoclonal antibody rituximab (Rituxan®) was also demonstrated in a human B-lymphoma xenograft model (12).

The treatment of established tumors in mice with talabostat was investigated in combination with other chemotherapeutic agents. Mice were treated with 30, 60 or 120 mg/kg gemcitabine 7 days after injection of WEHI 164 fibrosarcoma cells. Talabostat was administered twice daily from day 8 and tumor volumes were recorded on day 25. The antitumor effect of the combination was significantly greater than treatment with either agent alone. Combination therapy led to rejection of tumors in 40-60% of mice, with no relapses observed over a further 30 days. No tumor rejection was observed in mice treated with talabostat alone. A similar increase in rejection frequencies was observed when talabostat was administered in combination with cisplatin. Tumor immunity to rechallenge with WEHI 164 cells was also stimulated. These studies clearly demonstrated the potential of talabostat in combination chemotherapy (13).

Further studies in mice have demonstrated the ability of talabostat to cause tumor rejection when treatment was initiated earlier than day 8 following tumor inoculation. When treatment of mice bearing EL4 lymphoma and

WEHI 164 tumors was initiated 2 days after their inoculation, the tumors were rejected in 50-70% of mice. A long-lasting and specific immunity to rechallenge was also achieved in mice that rejected their tumors. Talabostat treatment increased expression of the cytokines IL-1, IL-6, G-CSF and interferon beta in the tumors and draining lymph node tissue. Chemokines that promote antitumor responses were also upregulated. Combination therapy with paclitaxel or cisplatin in mice bearing established WEHI 164 tumors resulted in tumor rejection in up to 50% of the mice (14).

In mice inoculated with syngeneic tumor cell lines derived from fibrosarcoma, lymphoma or melanoma, talabostat had a potent antitumor effect – it significantly reduced tumor growth and rejection in mice inoculated with B16F10, WEHI 164 or EL4 tumor cells. Talabostat also stimulated a tumor-specific cytotoxic lymphocyte response. In WEHI 164-inoculated mice, talabostat increased cytokine and chemokine mRNA expression. These effects of talabostat did not appear to involve CD26/DPP-IV. Talabostat enhanced the activity of tumor-specific antibodies against human tumor xenografts. The authors suggest that talabostat represents a novel oncology agent, either as monotherapy or combination therapy (15).

Preclinical studies have also demonstrated that talabostat may have a role as an adjuvant to enhance the efficacy of certain vaccines. In preclinical models of peptide vaccination, talabostat induced robust and dose-dependent CD4+ and CD8+ T-cell responses when coadministered with a cytotoxic T-lymphocyte peptide antigen and a T-cell helper peptide. Talabostat also enhanced the recall of memory T-cells at least 9 weeks after immunization (16).

Clinical Studies

A dose-escalation study was conducted to assess the safety of talabostat and its effects on neutrophil recovery in 29 patients receiving myelosuppressive chemotherapy. Patients received 2 cycles of chemotherapy and those with grade 3 or 4 neutropenia in cycle 1 received talabostat as a total daily dose of 200, 400, 800 or 1200 μg twice daily for 7 days in cycle 2. An optimal response was observed in patients who received 800 μg talabostat daily, with 5 of 13 patients having at least a 2-day improvement in their neutropenia. There was a 62% improvement in the duration and degree of severe neutropenia in cycle 2 compared with cycle 1 in the group of 7 patients who received 800 μg talabostat daily from day 2 to day 8 of the chemotherapy cycle. There was a corresponding upregulation of G-CSF, IL-6 and IL-8 in most patients. Talabostat was generally well tolerated; the most common drug-related nonhematological adverse events were edema and peripheral swelling, hypotension and hypovolemia. A maximum tolerated dose was not reached (17).

Ongoing phase II studies are evaluating talabostat as a single agent in advanced metastatic melanoma, in combination with docetaxel in stage IIIB/IV non-small cell lung cancer, in combination with cisplatin in advanced melanoma, and in combination with rituximab in chronic lymphocytic leukemia. Talabostat is also under development for the treatment of hematopoietic disorders caused by chemotherapeutic treatments, and in combination with rituximab for the treatment of non-Hodgkin's lymphoma (16, 18).

Source

Point Therapeutics, Inc. (US).

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