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(54) Title: CYCLIC BOROPROLINE COMPOUNDS

(57) Abstract

Substantially pure preparations of cyclid boroProline compounds that bind, in cyclic or linear form, to CD26 are provided. Methods for using the cyclic compounds to stimulate the activation and/or proliferation of immune cells to achieve preselected normal in vivo levels of these cells also are provided. Evidence of the oral bioavailability and activity of a preferred cyclic compound, valine–prolineboronic acid (ValboroPro), also is provided.

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CYCLIC BOROPROLINE COMPOUNDS

Related Application

This application claims priority under Title 35 §119(e), of United States Provisional Application No. 60/088,540, filed June 5, 1998, and entitled "CYCLIC BOROPROLINE COMPOUNDS," the entire contents of which are incorporated herein by reference.

The Field Of The Invention

This invention relates to substantially pure forms of cyclic boroProline compounds that bind, in cyclic or linear form, to CD26. The invention also relates to methods for using these compounds to stimulate the activation and/or proliferation of CD26-bearing cells to mobilize hematopoietic progenitor cells to spleen and periphery.

Background Of The Invention

CD26, a type II transmembrane protein, is expressed on the cell surface of a number of cell types, including lymphocytes (Marguet, D. et al., *Advances in Neuroimmunol.* 3:209-215 (1993)), hematopoietic cells (Vivier, I. et al., *J. Immunol.* 147:447-454 (1991); Bristol, et al., *J. Immunol.* 149:367 (1992)), thymocytes (Dang, N.H. et al., *J. Immunol.* 147:2825-2832 (1991), Tanaka, T. et al., *J. Immunol.* 149:481-486 (1992), Darmoul, D. et al., *J. Biol. Chem.* 267:4824-4833 (1992)), intestinal brush border membrane, endothelial cells, fibroblasts, and stromal cells. Cell surface associated CD26 is a sialoglycoprotein, with most of its mass on the outside of the cell.

CD26 has been best characterized on peripheral T cells where it functions as a potent costimulatory signal for T cell activation. Its surface expression is up regulated upon T cell activation (Dong, R.P. et al., *Cell* 9:153-162 (1996), Torimoto, Y. et al., *J. Immunol.* 147:2514 (1991), Mittrucker, H-W. et al., Eur. *J. Immun.* 25:295-297 (1995), Hafler, D.A. et al, *J. Immunol.* 142:2590-2596 (1989), Dang, N.H. et al., *J. Immunol.* 144:409 (1990)). CD26 has also been identified in rodents as an important regulatory surface receptor in hematopoiesis and lymphoid development (Vivier, I. et al., *J. Immunol.* 147:447-454 (1991)). The primary structure of CD26 is highly conserved between species (Ogata, S. et al., *J. Biol. Chem.* 264:3596-3601 (1998)). In humans, CD26 reportedly is involved in the regulation of thymocyte activation, differentiation and maturation (Dang, N.H. et al., *J. Immunol.* 147:2825-2832 (1991); Kameoka, J. et al., *Blood* 85:1132-1137 (1995)).

CD26 has an enzymatic activity that is identical to that of Dipeptidyl Peptidase IV

(DPP-IV), a serine type exopeptidase with high substrate specificity. It cleaves N-terminal dipeptides from proteins if the penultimate amino acid is proline, or in some cases alanine (Fleischer, B. *Immunol. Today* 15:180 (1994)).

A class of low molecular weight synthetic monomeric molecules with high affinity for CD26 have previously been developed and characterized (G.R. Flentke, et al. Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function, *PNAS (USA)* 88, 1556-1559 (1991); W.G. Gutheil and W.W. Bachovchin. Separation of L-Pro-DL-boroPro into Its Component Diastereomers and Kinetic Analysis of Their Inhibition of Dipeptidyl Peptidase IV. A New Method for the Analysis of Slow, Tight-Binding Inhibition, *Biochemistry* 32, 8723-8731 (1993)). These molecules have been shown to be potent and specific synthetic inhibitors for CD26's associated DP IV proteinase activity.

Representative monomeric structures of these transition-state-analog-based inhibitors, Xaa-boroPro, include Pro-boroPro, Ala-boroPro, Val-boroPro, and Lys-boroPro. BoroPro refers to the analog of proline in which the carboxylate group (COOH) is replaced with a boronyl group [$B(OH)_2$]. Pro-boroPro, the most thoroughly characterized of these inhibitors has a Ki of 16 picomolar (pM) (W.G. Gutheil and W.W. Bachovchin. Separation of L-Pro-DL-boroPro into Its Component Diastereomers and Kinetic Analysis of Their Inhibition of Dipeptidyl Peptidase IV. A New Method for the Analysis of Slow, Tight-Binding Inhibition, *Biochemistry* 32, 8723-8731 (1993)). Val-boroPro has even a higher affinity, with a Ki of 1.6 pM (W.G. Gutheil and W.W. Bachovchin. Supra; R.J. Snow, et al. Studies on Proline boronic Acid Dipeptide Inhibitors of Dipeptidyl Peptidase IV: Identification of a Cyclic Species Containing a B-N Bond, *J. Am. Chem. Soc.* 116, 10860-10869 (1994)). Thus, these Xaa-boroPro inhibitors are about 10^{+6} fold more potent than the next best known inhibitors.

United States Patent Nos. 4,935,493 (Bachovchin '493) and 5,462,928 (Bachovchin '928), both of which are incorporated herein by reference, disclose protease inhibitors and transition state analogs (the '493 patent) and methods for treating transplant rejection in a patient, arthritis, or systemic lupus erythematosis (SLE) by administering a potent inhibitor of the catalytic activity of soluble amino peptidase activity of dipeptidyl peptidase type IV (DP-IV; (G.R. Flentke, et al. Inhibition of dipeptidyl aminopeptidase IV (DP-IV) by Xaa-boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function, *PNAS (USA)* 88, 1556-1559 (1991)).

PCT published application WO 98/00439 (Multivalent Compounds for Crosslinking Receptors and Uses Thereof) reports that in aqueous solution at all pH values, a boroProline-type CD26 inhibitor exists as a slowly equilibrating mixture of two conformations: an open chain structure which is inhibitory (active species), and a cyclic structure which is non-inhibitory (inactive species). The open, active, inhibitory chain species is favored at low pH while the cyclized structure is favored at high pH. In view of the foregoing, the WO 98/00439 proposes *preventing* peptide conformational changes, e.g., intermolecular cyclization, by constructing a bivalent or multivalent compound containing an olefin group to form novel CD26 inhibitors. According to WO 98/00439, "if cyclization can be blocked, the inventors predict that the bioavailability of the compounds taught herein can be increased by approximately 100 - 1000 fold".

Summary Of The Invention

The invention is based upon a variety of surprising and unexpected findings. It has been discovered, unexpectedly, that boro-Pro compounds of the type described in U.S. 4,935,493 (Bachovchin '493) *in cyclic form* can be orally administered to a subject for treating the same types of conditions for which the linear molecules are useful. It is believed that the cyclic boro-Pro compounds undergo a transformation reaction under acidic conditions in vivo (e.g., stomach) to form a linear reaction product that is capable of selectively binding to CD26 (DP-IV). Thus, according to this aspect, the methods and compositions of the invention are directed to a novel pharmaceutical prodrug, namely, cyclic boro-Proline compounds, for oral administration. Novel compositions containing the substantially pure cyclic boroProline compounds of the invention, in solution or dry form, also are provided.

It is believed that the cyclic compounds of the invention are biologically active in cyclic form, as well as in linear form. Accordingly, the invention also embraces methods and compositions in which the cyclic compounds are administered to a subject or otherwise used in vitro (e.g., screening assays for selection of competitive molecules) in which the cyclic compound is not first subjected to conditions to induce conversion to the linear form. Thus, the cyclic compounds can be administered in oral form (whereby they may or may not be substantially converted to a linear form in the acidic conditions of the stomach), as well as in parenteral form with, or without, prior treatment to convert to the linear form.

The agents useful according to the invention are the *cyclic* forms of the compounds of

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