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(54) Title: PROLINE PHOSPHONATE DERIVATIVES

(57) Abstract

Peptidyl derivatives of diesters of α-aminoalkylphosphonic acids, particularly those with proline or related structures, their use in inhibiting serine proteases with chymotrypsin-like, trypsin-like, elastase-like, and dipeptidyl peptidase IV specificity and their roles as anti-inflammatory agents, anticoagulants, anti-tumor agents, and anti-AIDS agents.

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PROLINE PHOSPHONATE DERIVATIVES

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under Grants No. HL34035 and HL29307 awarded by the National Heart, Lung and Blood Institute of the National Institutes of Health. The United States government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a novel class of peptidyl derivatives of aromatic diesters of α-aminoalkylphosphonic acids useful for selectively inhibiting elastase, selectively inhibiting chymotrypsin-like enzymes, selectively inhibiting trypsin-like enzymes and selectively inhibiting dipeptidyl peptidase IV (DPP-IV). The diesters of α-aminoalkylphosphonic acids are analogues of natural α-amino acids. This invention also relates to a method for controlling tumor invasion, treating inflammation and controlling blood coagulation in patients using the novel compounds of the present invention. We have found that peptidyl derivatives of aromatic diesters of α-aminoalkylphosphonic acids are potent inhibitors of chymotrypsin-like enzymes, elastases, blood coagulation enzymes, tryptases, kallikreins, and therefore they are useful as anti-tumor, anti-inflammatory and anticoagulant agents. We have also found that dipeptide proline phosphonates are inhibitors of dipeptidyl peptidase IV (DPP-IV, enzyme number EC 3.4.14.5, also known as CD26) and are thus useful in treatment of immune system disorders and acute respiratory distress syndrome (AIDS).

2. Description of the Related Art

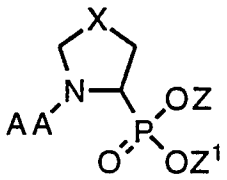
Serine proteases play critical roles in several physiological processes such as digestion, blood coagulation, complement activation, fibrinolysis, and reproduction. Serine proteases are not only a physiological necessity, but also a potential hazard if they are not controlled. Blood coagulation serine proteases are responsible for vascular clotting, cerebral infarction and coronary infarction. Chymotrypsin-like enzymes and plasmin are involved in tumor invasion, tissue remodeling, and clot dissociation. Uncontrolled proteolysis by other serine proteases such as elastase may cause pancreatitis, emphysema, rheumatoid arthritis, inflammation and adult respiratory distress syndrome. Accordingly, specific and selective inhibitors of these proteases should be potent anticoagulants, anti-inflammatory agents and anti-tumor agents useful in the treatment of protease-related diseases. *In vitro* proteolysis by trypsin, chymotrypsin or the elastase family is a serious problem in the production, purification, isolation, transport or storage of peptides and proteins.

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5, CD26) is a post-proline cleaving enzyme which will remove the dipeptides AA-Pro (AA = amino acid residue) from the N-

terminus of proteins or polypeptides. DPP-IV has been found in a variety of mammalian cells and tissues including kidney, placenta, blood plasma and on the surface of certain T-lymphocyte subsets. Despite extensive studies, the biological role of DPP-IV in mammalian systems has not been completely established, although a number of functions have been postulated. DPP-IV may participate in the metabolism and uptake of proline-containing peptides in the intestine and kidney and may be involved in fibronectin-mediated cell movement and adhesion. DPP-IV may also play a role in the metabolism or catabolism of collagen which has a high frequency of Gly-Pro sequences. DPP-IV in human plasma has been shown to cleave N-terminal Tyr-Ala from growth hormone-releasing factor and cause inactivation of this hormone. DPP-IV is also involved in T-cell activation and regulation of T-cell proliferation. Thus, inhibitors of DPP-IV may have therapeutic utility in the modulation of the rejection of transplanted tissue by the host organism. Recently DPP-IV or CD26 has been postulated to act as a cofactor for entry into HIV in $CD4^+$ cells (Callebaut, C., Krust, B., Jacotot, E., Hovanessian, A. G. T cell activation antigen, CD26, as a cofactor for entry of HIV in $CD4^+$ cells. *Science*. 1993, *262*, 2045-2050). Thus inhibitors of DPP-IV should have therapeutic utility in the treatment of AIDS.

BRIEF SUMMARY OF THE INVENTION

The proline phosphonates derivatives of this invention have the following general structure:



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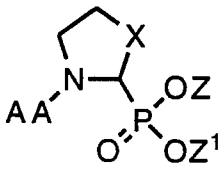

or a pharmaceutically acceptable salt thereof, wherein Z and Z^1 are the same or different and are selected from the group consisting of C_{1-6} perfluoroalkyl, phenyl, phenyl substituted with J, phenyl disubstituted with J, phenyl trisubstituted with J, and pentafluorophenyl; J is selected from the group consisting of halogen, C_{1-6} alkyl, C_{1-6} perfluoroalkyl, C_{1-6} alkoxy, NO_2, CN, OH, CO_2H, amino, C_{1-6} alkylamino, C_{2-12} dialkylamino, C_{1-6} acyl, and C_{1-6} alkoxy-CO-, and C_{1-6} alkyl-S-; X is selected from the group consisting of (a) a single bond, (b)-CH_2-, (c) -CH_2CH_2-, (d) -$CH_2CH_2CH_2$-, (e) -$CH_2CH_2CH_2CH_2$-, (f) -Y-, (g) -CH_2-Y-, (h) -Y-CH_2-, and (i) -H, H-, wherein Y is O or S; and AA is selected from the group consisting of (a) the structure NH_2-CHR-CO- where R is selected from the group consisting of C_{1-6} alkyl and C_{1-6} fluorinated alkyl, (b) a side chain blocked or unblocked alpha amino acid residue with the L, D or DL configuration at the α-carbon atom selected from the group consisting of alanine, valine, leucine, isoleucine, proline, methionine, methionine sulfoxide, phenylalanine, tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, phenylglycine, norleucine, norvaline, alpha-aminobutyric acid,

citrulline, hydroxyproline, ornithine, homoarginine, O-methylserine, O-ethylserine, S-methylcysteine, S-ethylcysteine, S-benzylcysteine, NH_2-CH(CH_2CHEt_2)-COOH, alpha-aminoheptanoic acid, NH_2-CH(CH_2-1-napthyl)-COOH, NH_2-CH(CH_2-2-napthyl)-COOH, NH_2-CH(CH_2-cyclohexyl)-COOH, NH_2-CH(CH_2-cyclopentyl)-COOH, NH_2-CH(CH_2-cyclobutyl)-COOH, NH_2-CH(CH_2-cyclopropyl)-COOH, 5,5,5-trifluoroleucine, and hexafluoroleucine, (c) an amino acid residue selected from the group consisting of beta-alanine, glycine, epsilon-aminocaproic acid, and sarcosine, (d) H, and (e) $C_6H_5CH_2OCO$-.

A therapeutically effective amount of these compounds can be used to inhibit dipeptidyl peptidase IV in mammals.

A therapeutically effective amount of these compounds can be used to treati AIDS in mammals.

A therapeutically effective amount of these compounds can be used to prevent tissue transplant rejection in mammals.

It is an object of this invention to define a novel group of specific inhibitors for trypsin, elastase, chymotrypsin and other serine proteases. Inhibitors are compounds that can reduce or eliminate the catalytic activity of the enzyme. Trypsin and trypsin-like enzymes normally cleave peptide bonds in proteins and peptides where the amino acid residue on the carbonyl side of the split bond (P_1 residue) is Lys or Arg. Elastase and elastase-like enzymes cleave peptide bonds where the P_1 amino acid is Ala, Val, Ser, Leu and other similar amino acids. Chymotrypsin and chymotrypsin-like enzymes hydrolyze peptide bonds where the P_1 amino acid is Trp, Tyr, Phe, Met, Leu or other amino acid residue which contain an aromatic or large alkyl side chain. All of the above enzymes have extensive secondary specificity and recognize amino acid residues removed from the P_1 residue.

It is a further object of this invention to define new protease inhibitors, especially inhibitors for chymotrypsin and chymotrypsin-like enzymes, elastase inhibitors, blood coagulation enzyme inhibitors and tryptase inhibitors. These inhibitors are useful for controlling tumor invasion, blood coagulation and various inflammatory conditions mediated by serine proteases. The inhibitors of this invention are useful for treating diseases such as vascular clotting, inflammations, tumor invasion, pancreatitis, emphysema or infantile and adult respiratory distress syndrome. The inhibitors of this invention are also useful for controlling hormone processing by serine proteases and for treating diseases related to tryptases such as inflammation and skin blistering.

It is yet another object of this invention to define a novel group of specific inhibitors useful *in vitro* for inhibiting trypsin, elastase, chymotrypsin and other serine proteases of similar specificity. Such inhibitors could be used to identify new proteolytic enzymes encountered in research. They can be used in research and industrially to prevent undesired proteolysis that occurs during the production, isolation, purification, transport and storage

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