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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07D 207/16, 295/18, C07C 211/25, 255/46, A61K 31/40

A1

(11) International Publication Number: **WO 95/15309**

(43) International Publication Date: 8 June 1995 (08.06.95)

(21) International Application Number: PCT/GB94/02615

(22) International Filing Date: 30 November 1994 (30.11.94)

(30) Priority Data:

9324803.7	3 December 1993 (03.12.93)	GB
9324981.1	6 December 1993 (06.12.93)	GB

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.

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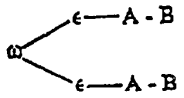
(54) Title: DP-IV-SERINE PROTEASE INHIBITORS

A-B (Groups I and II)



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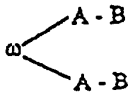

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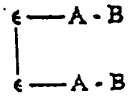

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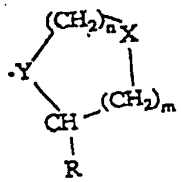
(Group III)



(4)

(57) Abstract

Compounds selected from those of general formula [A-B (Groups I and II)] and (group III), (1, 2 and 3) where B is (4) and A is selected from specified aminoacyl compounds are inhibitors of DP-IV mediated processes.

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DP-IV-SERINE PROTEASE INHIBITORS

Background

DP-IV (EC 3.4.14.5) is a membrane-bound serine protease first identified in rat kidney by its ability to cleave dipeptides from the N-terminus of certain peptides (Hopsu-Havu, V.K. and Glenner, G.G., *Histochemie*, **1966**, 7, 197). The dipeptides must be of the type X-Pro or X-Ala where X = any amino acid. X-Proline is more efficiently cleaved than X-Ala.

DP-IV is widely distributed in mammalian tissues and is found in great abundance in the kidney, intestinal epithelium and placenta (Yaron, A. and Naider, F., *Critical Reviews in Biochem. Mol. Biol.* **1993**, 28 (1), 31). In the human immune system the enzyme is expressed almost exclusively by activated T-lymphocytes of the $CD4^+$ type where the enzyme has been shown to be synonymous with the cell-surface antigen CD26.

The exact role of DP-IV in human physiology is not completely understood but recent research has shown that the enzyme clearly has a major role in human physiology and pathophysiology, eg.

(a) The immune response: DP-IV expression is increased in T-cells upon mitogenic or antigenic stimulation (Mattern, T. et al., *Scand. J. Immunol.* **1991**, 33, 737). It has been reported that inhibitors of DP-IV and antibodies to DP-IV suppress the proliferation of mitogen- and antigen-stimulated T-cells in a dose-dependant manner (Schön, E. et al., *Biol. Chem. Hoppe-Seyler*, **1991**, 372, 305 and refs. within).

Various other functions of T-lymphocytes such as cytokine production, IL-2 mediated cell proliferation and B-cell helper activity have been shown to be dependant on DP-IV activity (Schön, E. et al., *Scand. J. Immunol.* **1989**, 29, 127). Recently, DP-IV inhibitors based on boroproline where reported (Flentke, G.R. et al., *Proc. Natl. Acad. Sci. USA*, **1991**, 88, 1556) which, although unstable, were effective in inhibiting antigen-induced lymphocyte proliferation and IL-2 production in murine $CD4^+$ T-helper cells. Such boronic acid inhibitors have been shown to have an effect in vivo in mice causing suppression of antibody production induced by immune challenge (Kubota, T. et al., *Clin. Exp. Immunol.* **1992**, 89, 192). Other recent papers also provide evidence for the involvement of DP-IV in the immune response (eg. Tanaka, T. et al., *Proc. Natl. Acad. Sci. NY*, **1993**, 90, 4586; Hegen, M. et al., *Cell Immun.* **1993**, 146, 249; Subramanyan, M. et al., *J. Immunol.* **1993**, 150, 2544).

The importance of DP-IV is attributed by some investigators to its cell-surface association with the transmembrane phosphatase CD45 (Torimoto, Y. et al., *J. Immunol.* **1991**, 147, 2514). The CD45 - DP-IV association is possibly disrupted by DP-IV inhibitors or non-active site ligands. CD45 is known to be an integral component of T-cell signalling.

(b) Recently, a press release from the Pasteur Institute in Paris (and subsequently a presentation by A.G. Hovanessian at the 8th Cent. Gardes Meeting, Paris, 25-27th October 1993) reported that DP-IV was essential for the penetration and infectivity of HIV-1 and HIV-2 viruses in $CD4^+$ T-cells. The French group claimed that DP-IV interacted with and may have cleaved the V3 loop of the gp120 envelope glyco-protein of the virus. They also reported that inhibitors or antibodies to DP-IV successfully prevented entry of the virus into cells. It was known previously that there is a selective decrease of CD26 expression in T-cells from HIV-1 infected individuals (Valle-Blazquez, M. et al., *J. Immunol.* **1992**, 149, 3073), and that HIV-1 Tat protein binds to DP-IV (Subramanyam, M. et al., *J. Immunol.* **1993**, 150, 2544).

(c) It has been shown recently that lung endothelial DP-IV is an adhesion molecule for lung-metastatic rat breast and prostate carcinoma cells (Johnson, R.C. et al., *J. Cell. Biol.* **1993**, 121, 1423). DP-IV is known to bind to fibronectin and some metastatic tumour cells are known to carry large amounts of fibronectin on their surface.

(d) DP-IV has been shown to associate with the enzyme adenosine deaminase (ADA) on the surface of T-cells (Kameoka, J. et al., *Science*, **1993**, 261, 466). ADA deficiency causes severe combined immunodeficiency disease (SCID) in humans. This ADA-CD26 interaction may provide clues to the pathophysiology of SCID.

(e) High levels of DP-IV expression have been found in human skin fibroblast cells from patients with psoriasis, rheumatoid arthritis (RA) and lichen planus (Raynaud, F. et al., *J. Cell. Physiol.* **1992**, 151, 378).

(f) High DP-IV activity has been found in tissue homogenates from patients with benign prostate hypertrophy and in prostatosomes. These are prostate derived organelles important for the enhancement of sperm forward motility (Vanhoof, G. et al., *Eur. J. Clin. Chem. Clin. Biochem.* **1992**, 30, 333).

(g) DP-IV has been shown to be responsible for the degradation and inactivation of circulating peptides with penultimate proline or alanine at the N-terminus, eg. substance P, growth hormone releasing factor and members of the glucagon/vasoactive intestinal peptide family (Menthein, R. et al., *Eur. J. Biochem.* **1993**, 214, 829).

(h) Raised levels of DP-IV have been observed in the gingiva of patients with periodontitis (Cox, S.W. et al., *Arch. Oral. Biol.* **1992**, 37, 167).

(i) There are also a number of other reports of raised (or sometimes lowered) levels of DP-IV in various pathological conditions.

It follows from the above that potent inhibitors of DP-IV may be useful as drugs for the treatment of human disease. Such inhibitors could be useful as:

(a) Immunosuppressants, eg. in organ transplantation; cytokine release suppressants eg. in various autoimmune diseases such as inflammatory bowel disease, multiple sclerosis, RA.

(b) Drugs for the prevention of HIV entry into T-cells and therefore useful in the prophylaxis and treatment of AIDS.

(c) Drugs for the prevention of metastases, particularly of breast and prostate tumours to the lungs.

(d) Agents to treat dermatological diseases, eg. psoriasis, lichen planus.

(e) Drugs to suppress sperm motility and therefore act as male contraceptive agents.

(f) Agents beneficial in benign prostate hypertrophy.

Inhibitors of DP-IV

The only competitive inhibitors of DP-IV enzyme activity reported so far are the unstable boronic acids ($t_{\frac{1}{2}}$ 30 - 90 min at pH 7) mentioned above. (Bachovchin et al., WO 91/16339, October 1991) having K_i values in the nanomolar range for DP-IV, and simple amino-acid pyrrolidides or thiazolides (Neubert et al., DD 296 075 A5, November 1991) which have only modest potency ($K_i > 0.1$ μM). Amino-acyl proline aldehydes claimed in the same German patent cannot be synthesised due to a facile intramolecular condensation of the N-terminal amino group with the aldehyde function.

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