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[54] **INHIBITORS OF DP-MEDIATED PROCESSES, COMPOSITIONS AND THERAPEUTIC METHODS THEREOF**

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[51] **Int. Cl.**[6] **A61K 38/05**

[52] **U.S. Cl.** **548/535**; 514/19; 548/400; 548/405

[58] **Field of Search** 514/19; 548/535, 548/400, 405

[56] **References Cited**

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Patents Abstracts of Japan, 1(120): 2929 C 77 (Oct. 12, 1977).
Lotti et al., European Journal of Pharmacology, 162: 273–280 (1989).

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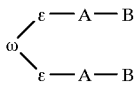
Assistant Examiner—David Lukton
Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

A-B (Groups I and II)

(1)



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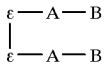
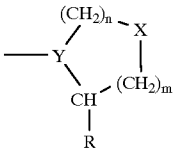
(3)



(Group III)

(4)



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.

8 Claims, No Drawings

INHIBITORS OF DP-MEDIATED PROCESSES, COMPOSITIONS AND THERAPEUTIC METHODS THEREOF

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, Oct. 25–27th 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 glycoprotein 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 (t½ 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 praline 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.

We now disclose highly potent competitive inhibitors of DP-IV (with K_i values in the 10^{-6}–10^{-10} range) which are also chemically stable (t½>24 h). They fall into three broad groups of compounds (Groups I, II and III).

GROUP I

These are molecules designed to bind tightly in the active site of DP-IV and to inhibit its proteolytic activity without interfering with attachment of any accessory ligands which may bind to the surface of DP-IV (i.e. not at its active site). Such Group I compounds could be useful as immunosuppressants; anti-HIV infectivity agents; agents to suppress release of certain cytokines (eg. IL-2, IL-6, γ-INF) from activated T-cells. The boronic acids and pyrrolidides referred to earlier also fall into this category.

GROUP II

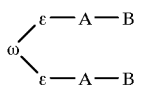
These are evolved from Group I compounds; however they contain long-chain extensions to the side-chains of the amino-acid defined as A in the general structure. The resulting compounds bind tightly to the active-site of DP-IV but the long-chain extensions protrude from the enzyme active site and serve to prevent the attachment of any other ligand which may bind to the surface of DP-IV. Such compounds could have the same uses as Group I compounds but in addition could block the interaction of DP-IV with (i) CD45 (ii) the gp 120 V3 loop of HIV-1 (iii) tumour cell surface fibronectin (iv) any other ligand important for T-cell activation, virus entry into T-cells or tumour cell adhesion.

GROUP III

This group comprises novel dimers in which two active-site directed inhibitors of DP-IV are linked via the side-chains of their amino-acid residues designated A in the general structure by a long chain. Such dimers can inhibit two molecules of DP-IV concurrently and also prevent accessory ligands binding to the surface of DP-IV. These dimers would have the same uses as Group II compounds but may be more effective.

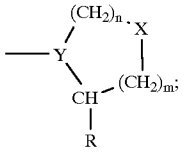
The invention provides inhibitors of DP-IV mediated processes, the inhibitors being of general formula:

A-B (Groups I and II) or



(Group II)

where B is



n=1 or 2;

m=0, 1 or 2;

X=CH_2, O, S, SO, SO_2,

NH or NR_1 where R_1=lower alkyl (C_1 to C_6);

A is attached to Y;

—Y=—N, —CH or =C (when the —CO group of A is replaced with CH=or CF=);

R=H, CN, CHO, $B(OH)_2$, C≡C—R_7, or CH=N—R_8;

R_7=H, F, lower alkyl (C_1 to C_6), CN, NO_2, OR_9, CO_2R_9 or COR_9;

R_8=Ph, OH, OR_9, $OCOR_9$, or OBn;

R_9=lower alkyl (C_1–C_6); and either ω or both ε's may be absent.

The structure of A is dependent on the nature of R in moiety B and on the nature of the group to which the resulting compound belongs.

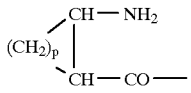
Group I Compounds

(a) R=H

A is an a-amino-acyl group derived from an α-amino-acid bearing a cycloaliphatic side-chain (e.g. C_4 to C_{10}, mono or bicyclic) whose ring may contain one or more heteroatoms e.g. L-cyclohexylglycine, L-cyclopentylglycine, L-decahydronaphthylglycine, L-piperidylglycine;

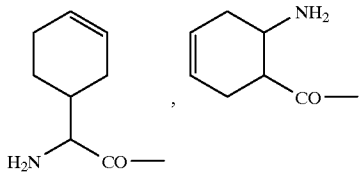
or

A is a β-amino-acyl group of general formula



where p=1–6 and the ring may also contain one or more heteroatoms replacing CH_2 unit(s).

Both α and β-amino acyl groups in (a) above may contain unsaturation in their rings e.g.



also may contain one or more heteroatoms.

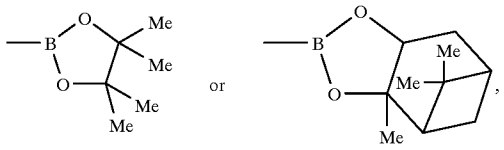
(b) R=CN: C≡C—R_7 or CH=N—R_8

A is as defined in (a) above but in addition may be derived from any L-α-amino acid bearing a lipophilic side-chain, eg. Ile.

(c) R=CHO or $B(OH)_2$

A is a β-amino-acyl group as defined in (a) above. The resulting A-B compounds are stable, unlike α-aminoacyl

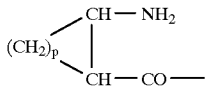
derivatives of the same type which undergo a facile intramolecular cyclisation. In compounds (c) $B(OH)_2$ may be present as a boronate ester eg.



these being labile in water giving the free boronic acids.

In a preferred embodiment, A is selected from the group consisting of T1, T2 and T3, wherein

T1 is a β-amino-acyl group of the formula



wherein p is an integer of 1–6, and wherein the ring present in T1 optionally contains one or more heteroatoms, and wherein the ring present in T1 optionally has one or two sites of unsaturation and wherein the carbonyl group of T1 is optionally replaced with CH═ or CF═;

T2 is an α-amino acyl group bearing a cycloaliphatic side chain, wherein the ring present in T2 optionally contains one or more heteroatoms, and wherein the ring present in T2 optionally has one or two sites of unsaturation, and wherein the carbonyl group of T2 is optionally replaced with CH═ or CF═;

T3 is an L-α-amino acid bearing a lipophilic side chain, wherein the carbonyl group of T3 is optionally replaced with CH═ or CF═;

with the provisos that,

(a) A is T1 only if R is H, —CHO or —B(OH)2;

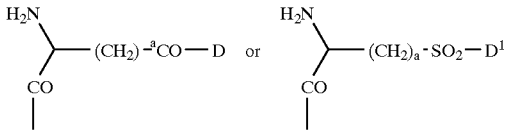
(b) A is T2 only if R is H; and

(c) A is T3 only if R is CN, C≡C—R_7, or CH═N—R_8.

Group II Compounds

Where R=H, CN, C≡C—R_7 or CH═N—R_8, A is an α-amino acid derivative whose side-chain carries a functional group which is derivatised to produce a long chain terminating in various groups R_3. A may be of the following three types of structure:

(i)



where a=1–5; D=G—$(CH_2)_b$—$(R_4)_q$—R_3; G=O, NH, or NMe;

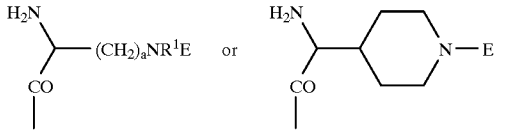
b=0–12; q=0–5;

D^1=D with G ≠O;

R_4=Z—NH—$(CH_2)_c$— or NH—Z—$(CH_2)_c$— where c=1–12 and Z=CO, CH_2 or SO_2; and

R_3=CO_2H or ester [e.g. any lower alkyl, fluoroalkyl or cycloalkyl (C_1 to C_8), or aromatic or heteroaromatic (5 or 6-membered rings, mono- or bicylic) ester] thereof; $CONH_2$; $CONHNH_2$; $CONR_5R_6$; $CONNR_5R_6$; PO_3H (or ester thereof e.g. as defined under CO_2H); SO_3H; SO_2NH_2; $SO_2NR_5R_6$; OH; OR_5; aryl or heteroaryl (e.g. 5 or 6-membered rings, monocyclic or bicyclic) [including substituted aryl or heteroaryl with substituents preferably chosen from F, Cl, I, Br, OH, OR_5, NO_2, SO_3H, SO_2NH_2, $SO_2NR_5R_6$, NH_2, NR_5R_6, CO_2R_5, CF_3, CN, $CONH_2$, $CONR_5R_6$, $NHCO_2R_5$, CH(:NR_5)NR_5R_6, NH—CH(:NR_5)NR_5R_6 and R_5]; NH_2; NR_5R_6; $NHCO_2R_5$; $NHSO_2NR_5R_6$; $NHCOR_5$; NH—SO_2R_5; NH—CH(:NR_5)NR_5R_6; $NHCONR_5R_6$; sugar (which may be attached via an ether or a glycosidic bond); CO-aminosugar (attached via the —NH_2) eg. glucosamine or galactosamine; NHCO-aminosugar, or NHCS-aminosugar. In the above definition of R_3 "sugar" refers to any carbohydrate or oligosaccharide, and R_5 and R_6 are independently selected from H and alkyl, fluoroalkyl and cycloalkyl groups (of up to 8 atoms), aryl, heteroaryl and alkylheteroaryl groups (of up to 11 atoms) or R_5 and R_6 together comprise a chain and (C_3 to C_8).

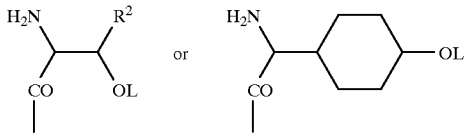
(ii)



where R^1=H, Me; the ring may also contain more heteroatoms;

E=J—$(CH_2)_b$—$(R_4)_q$—R_3; J=CO, CH_2 or SO_2; and a, b, q, R_3 and R_4 as defined under (i)

(iii)

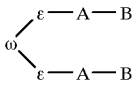


where R^2=H or Me; the ring may also contain one or more heteroatoms;

L=$(CH_2)_d$—[CO]$_r$—$(CH_2)_b$—$(R_4)_q$—R_3 or $(CH_2)_e$—NR^1—$(CH_2)_b$—$(R_4)_q$—R_3; r=0 or 1; d=0–4; e=2–4; and b, q, R_3 and R_4 as defined under (i).

Group III

Group m compounds are defined by the general formula:

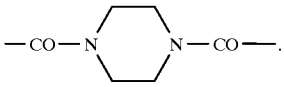


where ω=CH_2, O, NH, CO, S, SO_2, Ph or NMe and, independently, ϵ=CH_2, O, NH, CO, S, SO_2, Ph or NMe.

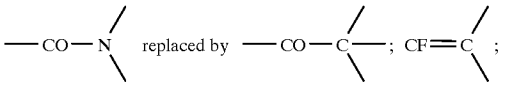
These compounds are symmetrical dimers. They may have any B structure as defined previously. A may be chosen from any group II structure [(i), (ii) or (iii)], but in this case the terminal group R_3 in each A residue is deleted and replaced with a shared symmetrical group [ϵ-•-ϵ] which connects the two halves of the dimer, • may be absent, in which case both ϵ's are joined together to constitute the chain linking the two A-B moieties; alternatively both ϵ's may be absent in which case • solely joins the two A-B moieties.

The structure of ϵ-•-ϵ must of course be chemically feasible eg. NH—CO—NH, CO—NH—CO—, SO_2—NMe—SO_2; it will be obvious to those skilled in the art which structures are not feasible, eg. —NH—NH—NH—. A specific possible example is shown in Table 7.

In such compounds as described under Groups II and III certain —CH_2— groups present in the long chains could be replaced with known bioisosteres eg. —O— without affecting inhibitory or binding activity towards DP-IV. Also such groupings as —$CONHCH_2CH_2NHCO$ if they occur could be replaced by eg.



Further, for compounds in Groups I, II and III any amide bond connecting A and B or any amide in the side-chains of A (in Groups II and III) may be replaced by known bioisosteres of amides eg.



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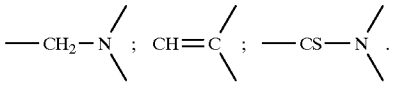


See Table 8 for examples of such replacements.

Biochemistry

All compounds were tested in vitro against pure human DP-IV (purchased from M & E, Copenhagen, Denmark). Inhibition of DP-IV was determined using the fluorescent substrate Ala-Pro-AFC (K_m 0.8 μM) at three concentrations for each inhibitor. A typical assay (total volume 0.4 ml) comprised sodium Hepes 83.3 mM, EDTA 1.67 mM, BSA 1.5 mg ml^{-1} pH 7.8, DP-IV 25 μU ml^{-1}, inhibitor (in 10 mM acetate pH 4.0). The reaction was started by the addition of substrate and readings taken every 30 s for 7.5 min, excitation at 395 nm, emission 450 nm. K_i values were determined using Dixon plots.

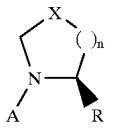
Chemistry

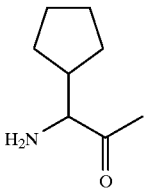
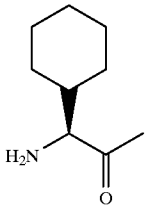
152 Examples of compounds synthesised are shown in Tables 1–8 followed by schemes and experimental details for the preparation of different structural types. All final products were characterised by FAB mass spectrometry and purity assessed by reverse phase hplc; all intermediates were characterised by ^1H NMR.

Table 9 shows selected K_i values against DP-IV determined for inhibitors of different structural types.

TABLE 1

Examples of Group I (a)



No.	A	X	R	n	Formula	Calculated Mol. Wt.	FAB Mass spec. $[M + H]^+$
1		CH_2	H	1	$C_{11}H_{20}N_2O$	196.2	197.2
2		CH_2	H	1	$C_{12}H_{22}N_2O$	210.2	211.2

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