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(54) Title: NEW EFFECTORS OF DIPEPTIDYL PEPTIDASE IV FOR TOPICAL USE

(54) Bezeichnung: NEUE EFFEKTOREN DER DIPEPTIDYL PEPTIDASE IV ZUR TOPISCHEN ANWENDUNG

A-C
|
B (I)

(57) **Abstract:** The invention relates to compounds of general formula (I), wherein A represents an amino acid with at least one functional group in the side chain; while B is a chemical compound covalently bound to a functional group of the side chain of A, namely oligopeptide with a chain length of up to 20 amino acids with the exception of glycine homopolymers which have up to 6 glycine monomers or polyethylene glycols with molar masses of up to 20000 g/mole; C represents a thiazolidine, pyrrolidine, cyanopyrrolidine, hydroxyproline, dehydroproline or piperidine group which is amide bonded. These compounds can be used to topically influence the activity of dipeptidyl Peptidase IV.

(57) **Zusammenfassung:** Die Erfindung betrifft Verbindungen der allgemeinen Formel (I), wobei A eine Aminosäure mit mindestens einer funktionellen Gruppe in der Seitenkette ist, B eine chemische Verbindung ist, die kovalent an eine funktionelle Gruppe der Seitenkette von A gebunden ist, nämlich Oligopeptide mit einer Kettenlänge von bis zu 20 Aminosäuren ausser Homopolymeren von Glycin aus bis zu 6 Glycinmonomeren oder Polyethylenglykole mit Molmassen von bis zu 20000 g/mol, C eine Thiazolidin-, Pyrrolidin-, Cyanopyrrolidin-, Hydroxyprolin-, Dehydroprolin- oder Piperidingruppe ist, die in Amidbindung mit A vorliegt. Diese Verbindungen können zur topischen Beeinflussung der Aktivität von Dipeptidyl Peptidase IV eingesetzt werden.

WO 01/14318 A3

NEW EFFECTORS OF DIPEPTIDYL PEPTIDASE IV FOR TOPICAL USE

The invention relates to new effectors of dipeptidyl peptidase IV. These effectors can be used for targeted influencing of locally limited pathophysiological and physiological processes (inflammation, chemotaxis, autoimmune diseases, wound healing), wherein the enzymatic activity and binding activities of dipeptidyl peptidase IV and of enzymes having comparable or identical activity and of proteins having a related primary structure (e.g. FAP, Fibroblast Activation Protein (Levy *et al.*, 1999)) are influenced by means of effectors (substrates, pseudo-substrates, inhibitors, antibodies, binding proteins, binding antagonists, binding agonists, *inter alia*).

In addition to proteases involved in non-specific proteolysis, which results, finally, in the breakdown of proteins into amino acids, regulatory proteases are known, which take part in the functionalisation (activation, deactivation, modification) of endogenous peptide active substances (Kirschke *et al.*, 1995) (Kräusslich and Wimmer, 1987). Especially in the context of immunological research and neuropeptide research, a number of such so-called convertases, signal peptidases or enkephalinases have been discovered (Gomez *et al.*, 1988) (Ansorge *et al.*, 1991).

Because of the frequency of the presence of the amino acid proline in a multiplicity of peptide hormones and because of the related structural properties of those peptides, a function analogous to the signal peptidases is being discussed for proline-specific peptidases (Yaron and Naider, 1993); (Vanhoof *et al.* 1995). As a result of its particular structure, proline in those peptides determines both the conformation and stability of those peptides, protecting them from breakdown by non-specific proteases (Kessler, 1982). Enzymes that, in contrast, act in highly specific, structure-modifying manner on proline-containing sequences (HIV-protease, cyclophilin, *inter alia*) are attractive targets for current active substance research. In particular, for the peptidases prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DP IV), which cleave after the proline, it has been possible to conclude that there probably are connections between modification of the biological activity of natural peptide substrates and selective cleavage thereof by those enzymes. It is accordingly postulated that PEP plays a part in learning and in the memory process and that DP IV is involved in signal transmission during the immune response (Ishiura *et al.*, 1989); (Hegen *et al.*, 1990).

DP IV activity and DP IV-analogous activity (for example, the lysosomal DP II has a substrate specificity that is almost identical to DP IV) is to be found in the bloodstream and in almost all organs, where it cleaves dipeptides from the N terminus of biologically active peptides with high specificity when their sequence contains proline or alanine as residues adjacent to the N-terminal amino acid. It is therefore assumed that this enzyme is involved in regulating the biological activity of polypeptides *in vivo* (Vanhoof *et al.*, 1995).

It has recently been shown that a series of chemokines (RANTES, SDF-1alpha, MDC, eotaxin, *inter alia*) are substrates of DP IV and that they are modulated in their function by DP IV (Proost *et al.*, 1998; Proost *et al.*, 1998; Proost *et al.*, 1999); (Shioda *et al.*, 1998). As a result of their chemotactic action, chemokines are substantially involved in the regulation of local immunological processes, such as autoimmune processes, inflammation and wound healing (Nelson and Krensky, 1998). In more recent work, we have been able to demonstrate that biologically active peptides having serine or threonine in the P_1-position (glucagon, VIP, PACAP) are also substrates of DP IV.

A series of biologically active DP IV-substrates (substance P, somatostatin, VIP, PACAP, *inter alia*) are involved in the regulation of neuronal, immunological and vasoactive processes in the skin (Scholzen *et al.*, 1998); (Wallengren, 1997). Dipeptidyl peptidase IV accordingly represents an important control centre in regulating the activity of gastrointestinally, immunologically and neurologically active peptides and, consequently, is an interesting therapeutic target (Augustyns *et al.*, 1999). The precise details of the signal cascades have not, however, been clarified fully.

The role of DP IV in the regulation of blood sugar is known in greater detail. As a result of limited proteolysis, the incretins GIP_{1-4} and $GLP\text{-}1_{7-37}$ are inactivated. Inhibition of plasma-DP IV activity leads, by way of prolonged activity of the incretins and increased insulin release, to normalisation of the blood sugar level (Demuth *et al.*, 1996; Pauly *et al.*, 1999; Pauly *et al.*, 1996).

The role of DP IV in the immune system has not yet been fully clarified. It is an activation marker of T-lymphocytes and a receptor for adenosinedeaminase. The use of DP IV-inhibitors has immunosuppressant effects in cell culture and *in vivo* (Ansorge *et al.*, 1995; Reinhold *et al.*, 1997; Kubota *et al.*, 1992). Using monoclonal antibodies against CD26,

stimulatory effects on intracellular signal cascades (Ca^{2+} influx, kinase activations) have been obtained, in some cases independently of the enzymatic activity of the enzyme (Hegen *et al.*, 1993; Kameoka *et al.*, 1995; Tanaka *et al.*, 1993; Kähne *et al.*, 1995).

Lysyl-prolyl analogues derived from the N-terminal sequence of substance P have shown a wound-healing-promoting effect, which is attributed to the structural similarity to substance P. In contrast, irreversible DP IV-inhibitors used systemically have resulted in inhibition of wound healing (Buntrock *et al.*, 1988; Kohl *et al.*, 1991; Kohl *et al.*, 1989).

In addition to the use of DP IV-inhibitors for the normalisation of blood glucose, DP IV-inhibitors have hitherto been used systemically for treating arthritis in an animal model.

In arthritis patients and in animal arthritis models, a reduction in DP IV activity has been observed (Küllertz and Boigk, 1986 (Fujita *et al.*, 1992). In particular, as a result of oral or subcutaneous administration of systemically acting DP IV-inhibitors, suppression of alkyldiamine-induced arthritis has been achieved in an animal model (Tanaka *et al.*, 1997; Tanaka *et al.*, 1998).

In relation to other autoimmune diseases as well, an effect has been obtained using DP IV-inhibitors. For example, as a result of DP IV inhibition it has been possible to achieve suppression of the proliferation of myelin basic protein-specific T cell clones (Reinhold *et al.*, 1998).

In the case of various skin diseases (psoriasis, lichen planus) and cancerigenic diseases of the skin, it has been possible to demonstrate increased DP IV activity in keratinocytes and fibroblasts (Novelli *et al.*, 1996) (Raynaud *et al.*, 1992). Fibroblast activation protein, which is closely related to DP IV, having approximately 50 % sequence homology with respect to DP IV, and which is probably the same as the seprase described by Piñeiro-Sanchez *et al.*, 1997, is also expressed to an increased extent by inactivated fibroblasts of epithelial carcinomas and healing wounds (Niedermeyer *et al.*, 1998).

Because of the wide distribution of the protein in the body and the wide variety of mechanisms involving DP IV, DP IV activities and DP IV-related proteins, systemic therapy (enteral or parenteral administration) with DP IV-inhibitors can result in a series of

undesirable side-effects. For example, parenteral or enteral administration of DP IV-inhibitors will intervene in a regulating or deregulating manner in glucose metabolism.

It has now been possible to show that side chain-modified substrates of the enzyme dipeptidyl peptidase IV can be recognised by the enzyme and cleaved in the same way as unmodified substrates (DEMUTH, H.-U., HEINS, J., 1995).

For example, it has been possible to show that phosphorylated dipeptide-(B)-*p*-nitroanilides [KASPARI, A., *et al.*, 1996] are substrates of DP IV. DP IV-inhibitors such as, for example, Glu(Gly)-Thia or Lys(Z-NO_2)-Thia [REINHOLD, D., *et al.*, 1998] are transported completely.

The problem to be solved consisted in preparing compounds that can be used for targeted influencing of locally limited pathophysiological and physiological processes. The problem of the invention especially consists in obtaining locally limited inhibition of DP IV or DP IV-analogous activity for the purpose of targeted intervention in the regulation of the activity of locally active peptide hormones.

The problem is solved according to the invention by providing compounds of the general formula A – C

B

wherein

A is an amino acid having at least one functional group in the side chain,

B is a chemical compound covalently bound to at least one functional group of the side chain of A, namely

- oligopeptides having a chain length of up to 20 amino acids, except for homopolymers of glycine consisting of up to 6 glycine monomers, or
- polyethylene glycols having molar masses of up to 20 000 g/mol, and

C is a thiazolidine, pyrrolidine, cyanopyrrolidine, hydroxyproline, dehydroproline or piperidine group amide-bonded to A.

In accordance with the invention, at least one pharmaceutical composition is especially provided which comprises

at least one compound of the general formula A – C
|
B

wherein
A is an amino acid, preferably an α-amino acid, especially a natural α-amino acid having at least one functional group in the side chain, preferably threonine, tyrosine, serine, arginine, lysine, aspartic acid, glutamic acid or cysteine,

B is a chemical compound covalently bound to at least one functional group in the side chain of A, namely oligopeptides having a chain length of up to 20 amino acids, polyethylene glycols having molar masses of up to 20 000 g/mol, optionally substituted organic amines, amides, alcohols, acids or aromatic compounds having from 8 to 50 C atoms,

C is a thiazolidine, pyrrolidine, cyanopyrrolidine, hydroxyproline, dehydroproline or piperidine group amide-bonded to A,

and

at least one customary adjuvant appropriate for the site of action.

Furthermore, such compounds or pharmaceutical compositions are used for influencing topically especially reducing the activity of dipeptidyl peptidase IV or analogous enzymes.

Throughout the description and the claims, the expression "alkyl" can denote a C_{1-50}alkyl group, preferably a C_{6-30}alkyl group, especially a C_{8-12}alkyl group; for example, an alkyl group may be a methyl, ethyl, propyl, isopropyl or butyl group;

the expression "alk", for example in the expression "alkoxy", and the expression "alkan", for example in the expression "alkanoyl", are defined as for "alkyl";

aromatic compounds are preferably substituted or optionally unsubstituted phenyl, benzyl, naphthyl, biphenyl or anthracene groups, which preferably have at least 8 C atoms;

the expression "alkenyl" can denote a C_{2-10}alkenyl group, preferably a C_{2-6}alkenyl group, which has the double bond(s) at any desired location and may be substituted or unsubstituted;

the expression "alkynyl" can denote a C_{2-10}alkynyl group, preferably a C_{2-6}alkynyl group, which has the triple bond(s) at any desired location and may be substituted or unsubstituted;

the expression "substituted" or substituent can denote any desired substitution by one or more, preferably one or two, alkyl, alkenyl, alkynyl, mono- or multi-valent acyl, alkanoyl, alkoxyalkanoyl or alkoxyalkyl groups; the afore-mentioned substituents may in turn have one or more (but preferably zero) alkyl, alkenyl, alkynyl, mono- or multi-valent acyl, alkanoyl, alkoxyalkanoyl or alkoxyalkyl groups as side groups;

organic amines, amides, alcohols or acids, each having from 8 to 50 C atoms, preferably from 10 to 20 C atoms, can have the formulae $(alkyl)_2N-$ or alkyl–NH–, $-CO-N(alkyl)_2$ or –CO–NH(alkyl), –alkyl–OH or –alkyl–COOH.

The term "oligopeptides" includes homopolymers, copolymers or block copolymers.

Despite an extended side chain function, the compounds according to the invention can still bind to the active centre of the enzyme dipeptidyl peptidase IV and analogous enzymes but are no longer actively transported by the peptide transporter PepT1. The resulting reduced or greatly restricted transportability of the compounds according to the invention leads, in ideal manner, to local, topical inhibition of DP IV and of analogous enzymes.

The compounds according to the invention or compounds used in accordance with the invention can be present or used, respectively, in the form of racemates or in the form of enantiomerically pure compounds, preferably in the L-threo or L-allo form with respect to A.

Local intervention in the regulation of peptide hormones by means of topical administration of specific DP IV-inhibitors accordingly makes it possible to avoid systemic side-effects caused by enteral or parenteral administration of DP IV-inhibitors, because only locally limited inhibition of DP IV activity takes place. Systemic regulation processes or regulation processes in other tissues remain unaffected to a very considerable extent because rapid systemic distribution of the compounds is avoided.

By extending/expanding the side chain modifications, for example beyond a number of seven carbon atoms, it is accordingly possible in accordance with the invention to obtain a dramatic reduction in transportability (Table 1). The Examples in Table 1 clearly show that, with increasing spatial size of the side chains, there is a reduction in the transportability of the substances. By spatially and sterically expanding the side chains, for example beyond the atom group size of a monosubstituted phenyl radical, hydroxylamine radical or amino acid residue, it is possible according to the invention to modify or suppress the transportability of the target substances.

It is accordingly possible to influence DP IV activity in the living body in discriminating manner.

By means of the invention, it is accordingly possible, on the one hand, to achieve effective action of the inhibitors in the tissue to be treated and, on the other hand, by virtue of locally limited, that is to say topical, administration of DP IV-inhibitors it is possible to avoid systemic actions of the inhibitors to a very considerable extent. It is accordingly possible to influence local physiological and pathophysiological processes (inflammation, psoriasis, arthritis, autoimmune diseases, allergies) effectively and with few side-effects.

The invention is supported by the following facts:

- DP IV-inhibitors administered enterally and parenterally, that is to say orally and intravenously or subcutaneously, are distributed systemically and inhibit DP IV and analogous activities throughout the body.

- A series of bioactive peptide substrates of DP IV are, however, involved in the regulation of local signal cascades (chemotaxis, inflammation, neurotransmission).

- The side chain-modified DP IV-inhibitors according to the invention exhibit, surprisingly, high inhibitory potency, but are absorbed and transported hardly at all or not at all and consequently do not result in demonstrable systemic effects.

The invention therefore makes available new DP IV-inhibitors and a novel approach for the use of DP IV-inhibitors *in vivo*. Such inhibitors can be matched to the type of use by means

of chemical modifications and/or formulations. For example, systemic distribution is made difficult or prevented by means of voluminous hydrophilic substitutions on the side chain.

The inhibitors can be administered in pharmaceutical and cosmetic preparations.

Topical use encompasses local use of the inhibitors by direct application to the tissue to be treated (e.g. skin, wounds, tumours) by means of ointments, creams or cosmetics, and indirect application by means of effector-containing patches, dressings or the like, by application in parts of the body (mouth, nose, ears, eyes, lungs) in the form of drops, sprays, inhalations or the like, by direct injection into or around the tissue to be treated and by implantation of effector-containing materials. Topical use further encompasses oral or anal administration of non-absorbable or not readily absorbable effectors of dipeptidyl peptidase IV or of DP IV-analogous sequences for the purpose of selectively influencing gastrointestinal DP IV.

In accordance with the invention, there are especially used compounds wherein the oligopeptides have chain lengths of from 3 to 15, especially from 4 to 10, amino acids, and/or the polyethylene glycols have molar masses of at least 250 g/mol, preferably of at least 1500 g/mol and up to 15 000 g/mol, and/or the optionally substituted organic amines, amides, alcohols, acids or aromatic compounds have at least 12 C atoms and preferably up to 30 C atoms. Furthermore, there are disclosed pharmaceutical and cosmetic compositions that comprise at least one compound according to the invention, optionally in combination with carriers or adjuvants customary *per se*.

The compounds or pharmaceutical or cosmetic compositions according to the invention can be used for topically influencing the activity of dipeptidyl peptidase IV or of analogous enzymes, especially for the prophylaxis or therapy of diseases of the skin or mucosa, autoimmune diseases and inflammation such as, for example, psoriasis, allergies, arthritis, tumours or autoimmune diseases.

The compounds and pharmaceutical or cosmetic compositions can be formulated and used in the form of an ointment, cream, cosmetic, patch, dressing, drops, spray, inhalation, implant or injection solution.

The adjuvants used in accordance with the invention are known *per se*.

The invention accordingly relates to the topical use of effectors of dipeptidyl peptidase IV and of DP IV-analogous enzyme activities and of DP IV-like proteins. Topical use allows local modification of the activities of the afore-mentioned highly specific enzymes which are crucially involved in inactivation and activation of biologically active peptides (chemokines, substance P, VIP, PHM, PACAP, growth factors, *inter alia*).

Targeted intervention in local immunological processes (chemotaxis, inflammatory processes, autoimmune diseases) is accordingly possible, as well as effective and targeted treatment of pathophysiological and physiological processes related thereto (psoriasis, periodontitis, arthritis, allergies, inflammation). The invention makes it possible for the inhibitors to be used simply and in high local concentrations.

As a result of low systemic loading with the corresponding effectors, an influence on the incretin system or systemic immune response is avoided.

Implementation Examples

Example 1: Action of side chain-modified glutamylthiazolidines as non-readily-transportable DP IV-inhibitors

Side chain-modified glutamylthiazolidines having a structure H-Glu(X)-Thia were synthesised, with polyethylene glycol or glycine oligomers of various chain lengths being used as X (see Method A for description of synthesis). The binding characteristics of those derivatives and their transportability by the peptide transporter PepT1 were investigated and the K_i values with respect to DP IV were determined (Table 1).

It was found, surprisingly, that the side chain modifications modify the binding characteristics to the compound only to a slight extent. In contrast, the ability of the inhibitors to be transported by the peptide transporter is dramatically diminished by the side chain modification.

The said DP IV-inhibitors are therefore excellently suited to achieving locally limited (topical) inhibition of DP IV in the body.

Table 1: Transportability and inhibitor constants of selected DP IV-inhibitors.

Compound *amino acid thiazolidides*	EC50 (mM) [1]	I_{max} (nA) [2]	K_i (mol/l) [3]
H-Ile-Thia	0.98	25 ± 8	1.3e-7 ± 11.1 %
H-Glu-Thia	1.1	35 ± 13	6.1e-7 ± 11.4 %
side chain-modified glutamylthiazolidines			
H-Gly(NHOH)-Thia	3.18	42 ± 11	1.7e-6 ± 8.6 %
H-Glu(Gly_3)-Thia	8.54	n.d. [4]	1.92e-7 ± 8.4 %
H-Glu(Gly_5)-Thia	> 10	n.d. [4]	9.93e-8 ± 11.4 %
H-Glu(PEG)-Thia	> 10	n.d. [4]	3.11e-6 ± 9.8 %

1 Effective concentrations of the compounds inhibiting the binding of ^{3}H-D-Phe-Ala (80mM) to PepT1-expressing *P. pastoris* cells by 50 % (EC_{50} values)

2 Transport characteristics at PepT1-expressing oocytes of X. leavis – by means of two-electrode voltage clamp method, I = inward currents generated by the transport

3 Inhibitor constants for competitive inhibition of purified kidney-DP IV by compounds of the Examples

4 Not detectable

Example 2: Effect of orally administered DP IV-inhibitors on activity of serum-DP IV

The inhibition of plasma-DP IV (systemic action) was investigated following oral administration of side chain-modified DP IV-inhibitors (5µM/300 mg rat) compared with unmodified inhibitors in healthy Wistar rats.

Although the inhibitors have approximately equal K_i values to DP IV (Table 1), plasma-DP IV is inhibited by the novel side chain-modified inhibitors much more slowly and to a much lesser extent overall. This means that the inhibitors are absorbed from the intestine much less readily or not at all. In the case of Glu$(Gly)_5$-Thia, especially, no systemic action of the orally administered active ingredient is detectable.

Those inhibitors may consequently act as basic structures for the synthesis of novel topically administrable DP IV-inhibitors without systemic action.

Example 3: Synthesis of side chain-modified inhibitors of DP IV

3.1 Synthesis of Boc-Glu-Thia

Reaction of Boc-Glu(OMe)-OH with Thia*HCl according to Method B (see section 3.4 for methods), hydrolysis of Boc-Glu(OMe)-Thia according to Method G

Table 2 Analytical data for Boc-Glu-Thia

Compound	Empirical formula M_r Synthesis method Yield	MS $[M+H]^+$ TLC: R_f/system m.p.	$[\alpha]^{20}D$ Concentration Solvent	Elemental analysis (calc./ found) %	HPLC R_t [min]/system
Boc-Glu-Thia	$C_{13}H_{22}N_2O_5S$ 318.38 B+G 62 %	319.5 0.52 / A[1] 0.42 / B[1] 115-118°C	-3.1 c = 1 methanol	C:49.04/48.89 H:6.96/6.82 N:8.80/8.59	13.93 / A[2]

[1] Thin-layer chromatography
System A: chloroform/methanol 90:10
System B: benzene/acetone/acetic acid 25:10:0.5
System C: n-butanol/EA/acetic acid/H_2O 1:1:1:1

[2] HPLC separation conditions
Column: Nucleosil C-18, 7µ, 250 mm x 21 mm
Eluant: isocratic, 40 % ACN/water/0.1 % TFA
Flow rate: 6 ml/min
λ = 220 nm

3.2 Side chain-modified Boc-glutamylthiazolidines

Boc-Glu-Thia was modified at the γ-carboxylic acid function by introducing radicals of varying size. The radicals were coupled by way of their amino group by forming an amide bond to the γ-carboxylic acid function, with a variety of coupling methods being used depending on the radical.

The following amino components were attached to Boc-Glu-Thia using the method stated:

Amino component	Coupling methods (see section 3.4)	Yields
Polyethylene glycol amine ($M_r \approx 8000$)	C	93 %
H-Gly-Gly-Gly-OH	D + E	49 %
H-Gly-Gly-Gly-Gly-Gly-OH	D + E	86 %

In 2 cases, purification of the reaction products differs from the general description of synthesis.

Boc-Glu(Gly$_5$)-Thia

The product already precipitates out from the mixture on stirring overnight; it is subsequently filtered off and washed with 0.1N HCl and copious amounts of water. It is then dried over P_4O_{10} *in vacuo*.

Boc-Glu(PEG)-Thia

In contrast to the general procedure, the starting materials for the synthesis are dissolved in a 500-fold excess of DMF. After the reaction is complete, the DMF is completely removed *in vacuo* and the residue is dissolved in a large amount of methanol. After ether is poured on, to form an upper layer, the product precipitates out together with the unreacted PEG. Fine purification was carried out by preparative HPLC separation on a gel filtration column (Pharmazia, Sephadex G-25, 90 µm, 260 mm – 100 mm).

Separating conditions: eluant: water; flow rate: 5 ml/min; λ = 220 nm

Table 3 Synthesis data for side chain-modified Boc-glutamylthiazolidines

Compound	Empirical formula M_r Yield	MS $[M+H]^+$ TLC/R_f/ system m.p.	$[\alpha]^{20}D$ Concentration Solvent	Elemental analysis (calc./ found) %	HPLC R_t [min]/system
Boc-Glu(Gly$_3$)-Thia	$C_{19}H_{31}N_5O_8S$ 489.54 49 %	490.5		C:46.62 H:6.38 N:14.31	
Boc-Glu(Gly$_5$)-Thia	$C_{23}H_{37}N_7O_{10}S$ 603.64 86 %	604.5 0.09 / C decomp. from 202°C	n.dm.	C:45.76/45.60 H:6.18/6.11 N:16.24/16.56	11.93 / A[2]
Boc-Glu(PEG)-Thia	93 %	≈ 8000 (mass emphasis) 52-53°C	n.dm.	n.dm.	n.dm.

[2] HPLC separation conditions
Column: Nucleosil C-18, 7µ, 250 mm x 21 mm
Eluant: isocratic, 40 % ACN/water/0.1 % TFA
Flow rate: 6 ml/min
λ = 220 nm

3.3 Side chain-modified glutamylthiazolidines

The N-terminal Boc protecting groups were cleaved off the compounds described in Table 3 using method F. The substances modified with Gly derivatives were purified by preparative HPLC separation and are present as trifluoroacetates. The H-Glu(PEG)-Thia was purified on a gel filtration column in the same manner as the Boc-protected precursor.

Table 4 Synthesis data for side chain-modified glutamylthiazolidines

Compound	Empirical formula M_r Yield	MS $[M+H]^+$ TLC/R_f/ system m.p.	$[\alpha]^{20}D$ Concentration Solvent	Elemental analysis (calc./ found) %	HPLC R_t [min]/ system
H-Glu(Gly_3)-Thia *TFA	$C_{16}H_{24}N_5O_8SF_3$ 503.45 94 %	503.45 0.32 / C 91-94°C	= 4.1 c = 1 methanol	C:38.17/37.56 H:4.80/4.78 N:13.91/13.43	7.84 / C[3]
H-Glu(Gly_5)-Thia *TFA	$C_{20}H_{30}N_7O_{10}SF_3$ 617.55 98 %	617.55 0.25 / C 105-107°C	n.dm.	C:38.90/38.82 H:4.90/4.79 N:15.88/15.39	8.22 / C[3]
H-Glu(PEG)-Thia *HCl	 92 %	≈ 8000 (mass emphasis)	n.dm.	n.dm.	n.dm.

[3] HPLC separation conditions
Column: Nucleosil C-18, 7µ, 250 mm x 21 mm
Eluant: ACN/water/0.1 % TFA
Gradient: 20 % ACN → 90 % ACN over 30 min
Flow rate: 6 ml/min
λ = 220 nm

n.dm. – not determined *or* not determinable

3.4 General synthesis procedures

Method A: Peptide bond attachment by the mixed anhydride method using CFIBE as activation reagent

10 mmol of N-terminally protected amino acid or peptide are dissolved in 20 ml of absolute THF. The solution is cooled to -15°C ± 2°C. With stirring in each case, 10 mmol of N-MM and 10 mmol of chloroformic acid isobutyl ester are added in succession, the stated temperature range being strictly adhered to. After approximately 6 min, 10 mmol of the amino component are added. When the amino component is a salt, a further 10 mmol of N-MM are then added to the reaction mixture. The reaction mixture is then stirred for 2 h in the cold state and overnight at room temperature.

The reaction mixture is concentrated using a rotary evaporator, taken up in EA, washed with 5 % KH_2SO_4 solution, saturated $NaHCO_3$ solution and saturated NaCl solution and dried over

$NaSO_4$. After removal of the solvent *in vacuo*, the compound is recrystallised from EA/pentane.

Method B: ***Peptide bond attachment by the mixed anhydride method using pivalic acid chloride as activation reagent***

10 mmol of N-terminally protected amino acid or peptide are dissolved in 20 ml of absolute THF. The solution is cooled to 0°C. With stirring in each case, 10 mmol of N-MM and 10 mmol of pivalic acid chloride are added in succession, the stated temperature range being strictly adhered to. After approximately 6 min, the mixture is cooled to -15°C and, once the lower temperature has been reached, 10 mmol of the amino component are added. When the amino component is a salt, a further 10 mmol of N-MM are then added to the reaction mixture. The reaction mixture is then stirred for 2 h in the cold state and overnight at room temperature.

Further working up is carried out as in *Method A*.

Method C: ***Peptide bond attachment using TBTU as activation reagent***

10 mmol of the N-terminally protected amino acid or peptide and 10 mmol of the C-terminally protected amino component are dissolved in 20 ml of absolute DMF. The solution is cooled to 0°C. With stirring in each case, 10 mmol of DIPEA and 10 mmol of TBTU are added in succession. The reaction mixture is stirred for one hour at 0°C and then overnight at room temperature. The DMF is completely removed *in vacuo* and the product is worked up as described in *Method A*.

Method D: ***Synthesis of an active ester (N-hydroxysuccinimide ester)***

10 mmol of N-terminally protected amino acid or peptide and 10 mmol of N-hydroxy-succinimide are dissolved in 20 ml of absolute THF. The solution is cooled to 0°C and 10 mmol of dicyclohexylcarbodiimide are added, with stirring. The reaction mixture is stirred for a further 2 h at 0°C and then overnight at room temperature. The resulting N,N'-dicyclohexylurea is filtered off, the solvent is removed *in vacuo* and the remaining product is recrystallised from EA/pentane.

Method E: ***Amide bond attachment using N-hydroxysuccinimide esters***

10 mmol of the C-terminally unprotected amino component are introduced into an $NaHCO_3$ solution (20 mmol in 20 ml of water). At room temperature and with stirring, 10 mmol of the

N–terminally protected N–hydroxysuccinimide ester dissolved in 10 ml of dioxane are slowly added dropwise. Stirring of the reaction mixture is continued overnight and the solvent is then removed *in vacuo*.

Further working up is carried out as in *Method A*.

Method F: Cleavage of the Boc protecting group

3 ml of 1.1N HCl/glacial acetic acid (*Method F1*) or 3 ml of 1.1N HCl/dioxane (*Method F2*) or 3 ml of 50% TFA in DCM (*Method F3*) are added to 1 mmol of Boc-protected amino acid pyrrolidide, thiazolidide or peptide. The cleavage at RT is monitored by means of TLC. After the reaction is complete (approximately 2 h), the compound is precipitated out in the form of the hydrochloride using absolute diethyl ether, is isolated with suction and dried over P_4O_{10} *in vacuo*. Using methanol/ether, the product is recrystallised or reprecipitated.

Method F: Hydrolysis

1 mmol of peptide methyl ester is dissolved in 10 ml of acetone and 11 ml of 0.1M NaOH solution and stirred at RT. The course of the hydrolysis is monitored by means of TLC. After the reaction is complete, the acetone is removed *in vacuo*. The remaining aqueous solution is acidified, using concentrated KH_2SO_4 solution, until a pH of 2-3 is reached. The product is then extracted several times using EA; the combined ethyl acetate fractions are washed with saturated NaCl solution and dried over NaSO4, and the solvent is removed *in vacuo*. Crystallisation from EA/pentane is carried out.

FIGURE 1

The course, over time, of the percentage inhibition of plasma-DP IV activity following oral administration of 5 µmol of inhibitor per 300 g of rat (n = 2) is shown.

The term "comprises", and grammatical variations thereof such as "comprising" when used in the description and claims does not preclude the presence of additional features, integers, steps or components; or groups thereof.

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Patent claims

1. Compound of the general formula A – C
|
B

wherein
A is an amino acid having at least one functional group in the side chain,

B is a chemical compound covalently bound to at least one functional group of the side chain of A, namely

- oligopeptides having a chain length of up to 20 amino acids, except for homopolymers of glycine consisting of up to 6 glycine monomers, or
- polyethylene glycols having molar masses of up to 20 000 g/mol,

C is a thiazolidine, pyrrolidine, cyanopyrrolidine, hydroxyproline, dehydroproline or piperidine group amide-bonded to A.

2. Compound according to claim 1, characterised in that A is an α-amino acid.

3. Compound according to one of the preceding claims, characterised in that A is a natural α-amino acid.

4. Compound according to one of the preceding claims, characterised in that the amino acid is threonine, tyrosine, serine, arginine, lysine, aspartic acid, glutamic acid or cysteine.

5. Compound according to one of the preceding claims, characterised in that the oligopeptides have chain lengths of from 3 to 15 amino acids.

6. Compound according to one of the preceding claims, characterised in that the oligopeptides are homopolymers, copolymers or block copolymers.

7. Compound according to one of the preceding claims, characterised in that the polyethylene glycols have molar masses of at least 250 g/mol.

8. Compound according to one of the preceding claims, characterised in that C is a thiazolidine, pyrrolidine or cyanopyrrolidine group.

9. Pharmaceutical composition that comprises a compound according to one of the preceding claims, optionally in combination with carriers or adjuvants customary *per se*.

10. Cosmetic composition that comprises a compound according to one of claims 1 to 8, optionally in combination with carriers or adjuvants customary *per se*.

11. Use of at least one compound or pharmaceutical or cosmetic composition according to one of the preceding claims for topically influencing the activity of dipeptidyl peptidase IV or of analogous enzymes.

12. Use of at least one compound or pharmaceutical or cosmetic composition according to one of claims 1 to 10 for prophylaxis or therapy of diseases of the skin or mucosa, autoimmune diseases and inflammation.

13. Use of at least one compound or pharmaceutical or cosmetic composition according to one of claims 1 to 10 for prophylaxis or therapy of inflammation, psoriasis, allergies, arthritis, tumours or autoimmune diseases.

14. Use of at least one compound or pharmaceutical or cosmetic composition according to one of claims 1 to 10 in the form of an ointment, cream, cosmetic, patch, dressing, drops, spray, inhalation, implant or injection solution.

15. Pharmaceutical composition which comprises
at least one compound of the general formula A – C

|

B

wherein
A is an amino acid having at least one functional group in the side chain,

B is a chemical compound covalently bound to at least one functional group in the side chain of A, namely oligopeptides having a chain length of up to 20 amino acids, polyethylene

glycols having molar masses of up to 20 000 g/mol, optionally substituted organic amines, amides, alcohols, acids or aromatic compounds having from 8 to 50 C atoms,

C is a thiazolidine, pyrrolidine, cyanopyrrolidine, hydroxyproline, dehydroproline or piperidine group amide-bonded to A,

with H-Glu[$NH(CH_2)_7CONH(CH_2)_3NHZ$]pyrrolidide and
H-Lys[$CO(CH_2)_3NHSO_2Pfp$]pyrrolidide being excluded,

and

at least one customary adjuvant appropriate for the site of action.

16. Pharmaceutical composition according to claim 15, characterised in that A is an α-amino acid.

17. Pharmaceutical composition according to one of claims 15 or 16, characterised in that A is a natural α-amino acid.

18. Pharmaceutical composition according to one of claims 15 to 17, characterised in that the amino acid is threonine, tyrosine, serine, arginine, lysine, aspartic acid, glutamic acid or cysteine.

19. Pharmaceutical composition according to one of claims 15 to 18, characterised in that the oligopeptides have chain lengths of from 3 to 15 amino acids.

20. Pharmaceutical composition according to one of claims 15 to 19, characterised in that the oligopeptides are homopolymers, copolymers or block copolymers.

21. Pharmaceutical composition according to one of claims 15 to 20, characterised in that the polyethylene glycols have molar masses of at least 250 g/mol.

22. Pharmaceutical composition according to one of claims 15 to 21, characterised in that C is a thiazolidine, pyrrolidine or cyanopyrrolidine group.

23. Pharmaceutical composition according to one of claims 15 to 22, characterised in that it is used in the form of an ointment, cream, cosmetic, patch, dressing, drops, spray, inhalation, implant or injection solution.

24. Pharmaceutical composition according to one of claims 15 to 23, characterised in that it is used in combination with carriers customary *per se*.

25. Use of a pharmaceutical composition according to one of claims 15 to 24 for topically influencing the activity of dipeptidyl peptidase IV or of analogous enzymes.

26. Use of a pharmaceutical composition according to one of claims 15 to 24 for prophylaxis or therapy of diseases of the skin or mucosa, autoimmune diseases and inflammation.

27. Use of a pharmaceutical composition according to one of claims 15 to 24 for prophylaxis or therapy of inflammation, psoriasis, periodontitis, allergies, arthritis, tumours or autoimmune diseases.

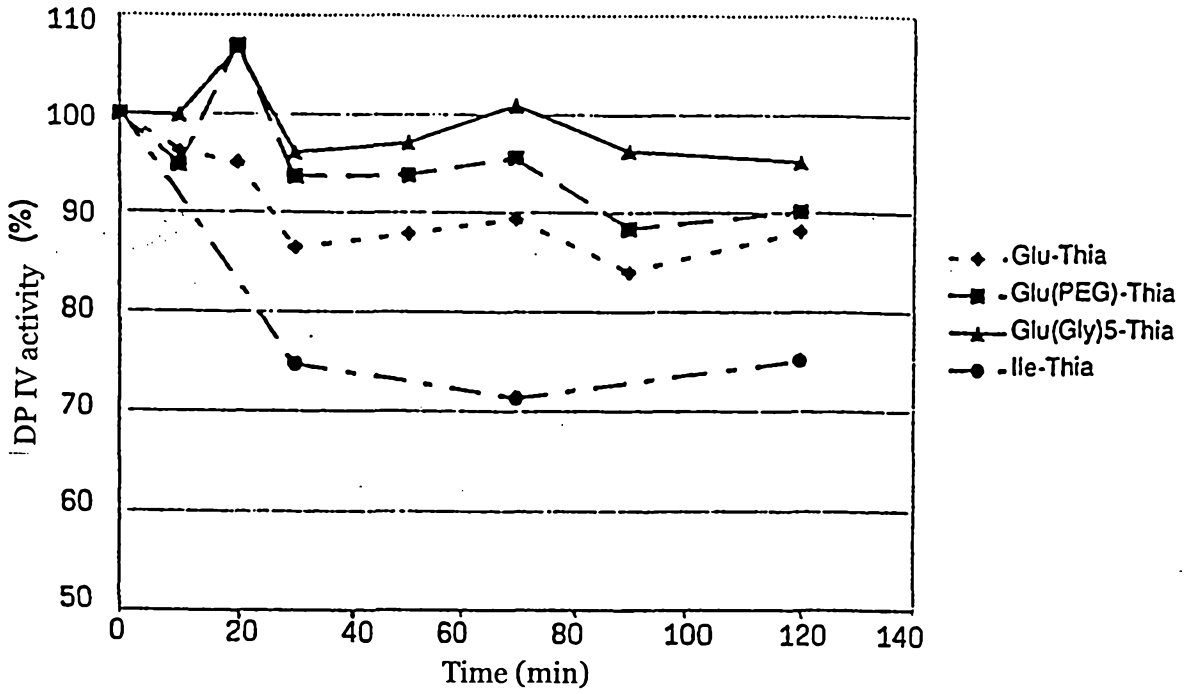

Fig. 1: Systemic action of orally administered DP IV-inhibitors in healthy Wistar rats.