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(54) Title: CYCLIC CARBOXYLIC ACIDS AS INTEGRIN ANTAGONISTS

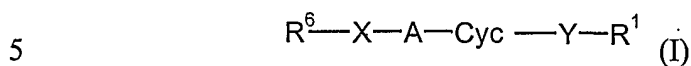
(57) Abstract: The present invention relates to compounds of the general formula (I), process for their preparation, pharmaceutical compositions containing them as well as their use for the production of pharmaceutical compositions for the treatment of inflammatory diseases.



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Cyclic carboxylic acids as integrin antagonists

The present invention relates to compounds of formula (I),



their preparation and use as pharmaceutical compositions as integrin antagonists, especially as $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ and/or $\alpha_9\beta_1$ integrin antagonists and in particular for the production of pharmaceutical compositions suitable for the inhibition or the prevention of cell adhesion and cell-adhesion mediated disorders. Examples are the treatment and the prophylaxis of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.

15 Adhesive interactions between the leukocytes and endothelial cells play a critical role in leukocyte trafficking to sites of inflammation. These events are essential for normal host defense against pathogens and repair of tissue damage, but can also contribute to the pathology of a variety of inflammatory and autoimmune disorders. Indeed, eosinophil and T cell infiltration into the tissue is known as a cardinal feature of allergic inflammation such as asthma.

20 The interaction of circulating leukocytes with adhesion molecules on the luminal surface of blood vessels appears to modulate leukocyte transmigration. These vascular cell adhesion molecules arrest circulating leukocytes, thereby serving as the first step in their recruitment to infected or inflamed tissue sites. Subsequently, the leukocytes reaching the extravascular space interact with connective tissue cells such as fibroblasts as well as extracellular matrix proteins such as fibronectin, laminin, and collagen. Adhesion molecules on the leukocytes and on the vascular endothelium are hence essential to leukocyte migration and attractive therapeutic targets for intervention in many inflammatory disorders.

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Leukocyte recruitment to sites of inflammation occurs in a stepwise fashion beginning with leukocyte tethering to the endothelial cells lining the blood vessels. This is followed by leukocyte rolling, activation, firm adhesion, and transmigration. A number of cell adhesion molecules involved in those four recruitment steps have been identified and characterized to date. Among them, the interaction between vascular cell adhesion molecule 1 (VCAM-1) and very late antigen 4 (VLA-4, $\alpha_4\beta_1$ integrin), as well as the interaction between mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and $\alpha_4\beta_7$ integrin, has been shown to mediate the tethering, rolling, and adhesion of lymphocytes and eosinophils, but not neutrophils, to endothelial cells under a physiologic flow condition. This suggests that the VCAM-1 / VLA-4 and/or MAdCAM-1 / $\alpha_4\beta_7$ integrin mediated interactions could predominantly mediate a selective recruitment of leukocyte subpopulations *in vivo*. The inhibition of this interaction is a point of departure for therapeutic intervention (A. J. Wardlaw, *J. Allergy Clin. Immunol.* **1999**, 104, 917-26).

VCAM-1 is a member of immunoglobulin (Ig) superfamily and is one of the key regulators of leukocyte trafficking to sites of inflammation. VCAM-1, along with intracellular adhesion molecule 1 (ICAM-1) and E-selectin, is expressed on inflamed endothelium activated by such cytokines as interleukin 1 (IL-1) and tumor necrosis factor α (TNF- α), as well as by lipopolysaccharide (LPS), via nuclear factor κ B (NF- κ B) dependent pathway. However, these molecules are not expressed on resting endothelium. Cell adhesion mediated by VCAM-1 may be involved in numerous physiological and pathological processes including myogenesis, hematopoiesis, inflammatory reactions, and the development of autoimmune disorders. Integrins VLA-4 and $\alpha_4\beta_7$ both function as leukocyte receptors for VCAM-1.

The integrin $\alpha_4\beta_1$ is a heterodimeric protein expressed in substantial levels on all circulating leukocytes except mature neutrophils. It regulates cell migration into tissues during inflammatory responses and normal lymphocyte trafficking. VLA-4 binds to different primary sequence determinants, such as a QIDSP motif of VCAM-

1 and an ILDVP sequence of the major cell type-specific adhesion site of the alternatively spliced type III connecting segment domain (CS-1) of fibronectin.

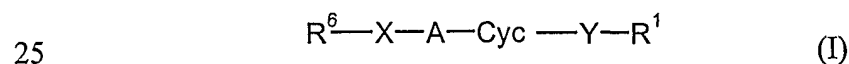
5 *In vivo* studies with neutralizing monoclonal antibodies and inhibitor peptides have demonstrated a critical role for α_4 integrins interaction in leukocyte-mediated inflammation. Blocking of VLA-4/ligand interactions, thus, holds promise for therapeutic intervention in a variety of inflammatory, autoimmune and immune diseases (Zimmerman, C.; *Exp. Opin. Ther. Patents* **1999**, *9*, 129-133).

10 Furthermore, compounds containing a bisarylurea moiety as a substituent were disclosed as $\alpha_4\beta_1$ integrin receptor antagonists: WO 96/22966, WO 97/03094, WO 99/33789, WO 99/37605. However, no aminobenzoic acids or aminocycloalkyl-carboxylic acids or homologues thereof or heterocyclic analogues thereof with $\alpha_4\beta_1$ integrin receptor antagonists activity have been described.

15 Further to their $\alpha_4\beta_1$ integrin antagonistic activity, the compounds of the present invention may also be used as $\alpha_4\beta_7$ or $\alpha_9\beta_1$ integrin antagonists.

20 An object of the present invention is to provide new, alternative, cyclic carboxylic acids or homologues thereof derived integrin antagonists for the treatment of inflammatory, autoimmune and immune diseases.

The present invention therefore relates to compounds of the general formula (I):



wherein

30 Cyc represents a 5- or 6-membered carbocycle,
which can optionally be substituted with up to two residues R^{cyc} ,

wherein the residues R^{cy} can independently be selected from the group consisting of halogen, trifluoromethyl, amino, nitro, cyano,

A represents an amide moiety of the structure

5

$-NR^{A-1}C(O)-$ or $-C(O)NR^{A-1}-$,

wherein R^{A-1} represents hydrogen or C_1 - C_{10} alkyl,

10

R^1 represents a 4- to 9-membered saturated, unsaturated or aromatic cyclic residue,

which can contain 0 to 3 heteroatoms selected independently from the group N, S and O,

15

and wherein R^1 is substituted by $-R^{1-1}-Z$,
wherein

20

R^{1-1} represents a bond, $-O-$, $-S-$, NR^{1-2} , C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, C_6 or C_{10} aryl, C_3 - C_7 cycloalkyl or a 4-9-membered saturated or unsaturated heterocyclic residue containing up to 3 heteroatoms selected from the group oxygen, nitrogen or sulfur,

25

wherein R^{1-1} can optionally be substituted by 1 to 2 substituents selected from the group R^{1-3} ,

wherein R^{1-2} can optionally be hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl or C_2 - C_{10} alkynyl, and

30

wherein R^{1-3} represents hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, C_6 or C_{10} aryl, C_3 - C_7 cycloalkyl or a 4-9-membered saturated or unsaturated heterocyclic residue containing up to 3 heteroatoms selected from the group oxygen, nitrogen or sulfur,

5

Z represents $-C(O)OR^{Z-1}$, $-C(O)NR^{Z-2}R^{Z-3}$, $-SO_2NR^{Z-2}R^{Z-3}$, $-SO(OR^{Z-1})$, $-SO_2(OR^{Z-1})$, $-P(O)R^{Z-1}(OR^{Z-3})$ or $-PO(OR^{Z-1})(OR^{Z-3})$,

10

wherein R^{Z-2} is hydrogen, C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl, $-C(O)R^{Z-4}$ or $-SO_2R^{Z-4}$,

wherein R^{Z-4} is C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl,

15

R^{Z-1} and R^{Z-3} are independently selected from the group hydrogen, C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl or benzyl,

20

wherein R^{Z-1} and R^{Z-3} can optionally be substituted by 1 to 3 substituents selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, halogen, nitro, cyano,

and wherein R^1 can optionally be substituted by 0 to 2 substituents R^{1-4} , halogen, nitro, amino, cyano and oxo,

25

wherein

R^{1-4} is selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, phenyl, phenoxy, phenylamino, C_3 - C_6 cycloalkyl,

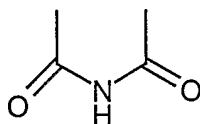
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R^6 represents phenyl or a 5- to 6-membered aromatic heterocyclic residue containing up to 3 heteroatoms independently selected from the group oxygen, nitrogen or sulfur,

5 which is substituted by $-NR^{6-2}C(O)NR^{6-3}R^{6-4}$ and can furthermore optionally be substituted by halogen,

wherein R^{6-2} and R^{6-3} are independently selected from the group hydrogen or C_1 - C_4 alkyl, or together form a group

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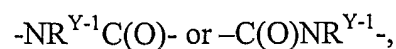
and wherein R^{6-4} represents phenyl,

15 wherein R^{6-4} can optionally be substituted by 1-2 substituents selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, halogen, nitro, trifluoromethyl, trifluoromethoxy or cyano,

20 X represents bond or $-CR^{X-1}R^{X-2}$,

wherein R^{X-1} and R^{X-2} can be independently selected from the group hydrogen, C_1 - C_4 alkyl, C_2 - C_4 alkenyl, C_2 - C_4 alkynyl,

25 Y represents an amide moiety of the structure



wherein R^{Y-1} represents hydrogen or C_1 - C_4 alkyl,

wherein the moiety A-Cyc-Y represents a γ -amino acid,
and pharmaceutically acceptable salts thereof.

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In a preferred embodiment, the present invention relates to compounds of general formula (I),

10

wherein Cyc represents a 5- membered carbocycle.

In another preferred embodiment, the present invention relates to compounds of general formula (I),

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wherein R^1 represents a 1,4-substituted phenyl ring.

In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein

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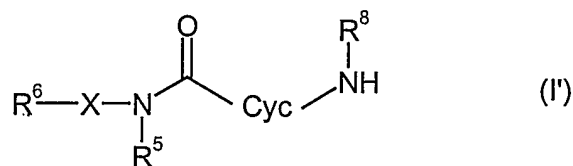
wherein R^{1-1} represents a bond and Z represents $COOR^{Z-1}$,
wherein R^{Z-1} has the meaning indicated above.

In another preferred embodiment, the present invention relates to compounds of general formula (I),

25

wherein R^6 represents phenyl, which is substituted by $-NHC(O)NHR^{6-4}$,
wherein R^{6-4} is substituted with methyl or trifluoromethoxy.

A preferred process for preparation of compounds of general formula (VII) has also been found, which comprises reaction of compounds of general formula (I')

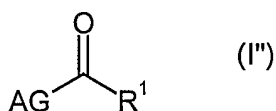


wherein

Cyc, X, R⁵, R⁶ and R⁸ have the abovementioned meaning,

5

with compounds of the general formula (I'')



wherein

10

R¹ has the abovementioned meaning and AG represents an activating group,

in inert solvents, which will be described in more detail in the descriptive part of the specification.

15

In the context of the present invention alkyl stands for a straight-chain or branched alkyl residue, such as methyl, ethyl, n-propyl, iso-propyl, n-pentyl. If not stated otherwise, preferred is C₁-C₁₀ alkyl, very preferred is C₁-C₆ alkyl.

20

Alkenyl and alkynyl stand for straight-chain or branched residues containing one or more double or triple bonds, e.g. vinyl, allyl, isopropinyl, ethinyl. If not stated otherwise, preferred is C₁-C₁₀ alkenyl or alkynyl, very preferred is C₁-C₆ alkenyl or alkynyl.

25

Cycloalkyl stands for a cyclic alkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl. Preferred is monocyclic C₃-C₇ cycloalkyl.

Halogen in the context of the present invention stands for fluorine, chlorine, bromine or iodine. If not specified otherwise, chlorine or fluorine are preferred.

5 A 4- to 9-membered saturated, unsaturated or aromatic cyclic residue stands for a monocyclic system containing 4 to 9 ring atoms and containing 0, 1 or more double bonds, which can be attached via a carbon atom or eventually via a heteroatom within the ring, for example phenyl, thiazolyl, pyridyl, cyclopentyl.

10 Aryl stands for a monocyclic Hückel-aromatic cyclic system containing 6 or 10 ring carbon atoms.

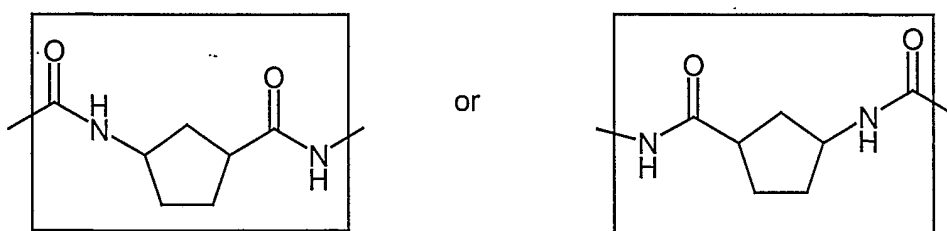
Heteroaryl or aromatic heterocyclic residue stands for a monocyclic heteroaromatic system containing 4 to 9 ring atoms, which can be attached via a carbon atom or eventually via a nitrogen atom within the ring, for example, furan-2-yl, furan-3-yl, 15 pyrrol-1-yl, pyrrol-2-yl, pyrrol-3-yl, thienyl, thiazolyl, oxazolyl, imidazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl or pyridazinyl.

20 A saturated or unsaturated heterocyclic residue stands for a heterocyclic system containing 4 to 9 ring atoms, which can contain one or more double bonds and which can be attached via a ring carbon atom or eventually via a nitrogen atom, e.g. tetrahydrofuran-2-yl, pyrrolidine-1-yl, piperidine-1-yl, piperidine-2-yl, piperidine-3-yl, piperidine-4-yl, piperazine-1-yl, piperazine-2-yl morpholine-1-yl, 1,4-diazepine-1-yl or 1,4-dihydropyridine-1-yl.

25 Carbocycle stands for a ring consisting of carbon atoms.

If not specified otherwise, in the context of the present invention heteroatom stands preferably for O, S, N or P.

30 In the context of the present invention, the moiety A-Cyc-Y represents a γ -amino acid. This group can therefore be represented as:



5 Surprisingly, the compounds of the present invention show good integrin antagonistic activity. They are therefore suitable for the treatment of diseases, especially as $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ and/or $\alpha_9\beta_1$ integrin antagonists and in the manufacture of a medicament for the treatment or the prevention of a condition mediated by integrins and in particular for the production of pharmaceutical compositions for the inhibition or the prevention of cell adhesion and cell-adhesion mediated disorders. Examples
10 are the treatment and the prophylaxis of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.

15 The integrin antagonists of the invention are useful not only for treatment of the physiological conditions discussed above, but are also useful in such activities as purification of integrins and testing for activity.

20 For the treatment of the above-mentioned diseases, the compounds according to the invention can exhibit non-systemic or systemic activity, wherein the latter is preferred. To obtain systemic activity the active compounds can be administered, among other things, orally or parenterally, wherein oral administration is preferred.

25 For parenteral administration, forms of administration to the mucous membranes (i.e. buccal, lingual, sublingual, rectal, nasal, pulmonary, conjunctival or intravaginal) or into the interior of the body are particularly suitable. Administration can be carried out by avoiding absorption (i.e. intracardiac, intra-arterial, intravenous, intraspinal or

intralumbar administration) or by including absorption (i.e. intracutaneous, subcutaneous, percutaneous, intramuscular or intraperitoneal administration).

5 For the above purpose the active compounds can be administered per se or in administration forms.

Suitable administration forms for oral administration are, inter alia, normal and enteric-coated tablets, capsules, coated tablets, pills, granules, pellets, powders, solid and liquid aerosols, syrups, emulsions, suspensions and solutions. Suitable administration forms for parenteral administration are injection and infusion solutions.

10 The active compound can be present in the administration forms in concentrations of from 0.001 - 100 % by weight; preferably the concentration of the active compound should be 0.5 - 90% by weight, i.e. quantities which are sufficient to allow the specified range of dosage.

15 The active compounds can be converted in the known manner into the abovementioned administration forms using inert non-toxic pharmaceutically suitable auxiliaries, such as for example excipients, solvents, vehicles, emulsifiers and/or dispersants.

20 The following auxiliaries can be mentioned as examples: water, solid excipients such as ground natural or synthetic minerals (e.g. talcum or silicates), sugar (e.g. lactose), non-toxic organic solvents such as paraffins, vegetable oils (e.g. sesame oil), alcohols (e.g. ethanol, glycerol), glycols (e.g. polyethylene glycol), emulsifying agents, dispersants (e.g. polyvinylpyrrolidone) and lubricants (e.g. magnesium sulphate).

25 In the case of oral administration tablets can of course also contain additives such as sodium citrate as well as additives such as starch, gelatin and the like. Flavour enhancers or colorants can also be added to aqueous preparations for oral administration.

30

For the obtainment of effective results in the case of parenteral administration it has generally proven advantageous to administer quantities of about 0.001 to 100 mg/kg, preferably about 0.01 to 1 mg/kg of body weight. In the case of oral administration
5 the quantity is about 0.01 to 100 mg/kg, preferably about 0.1 to 10 mg/kg of body weight.

It may nevertheless be necessary to use quantities other than those mentioned above, depending on the body weight concerned, the method of administration, the individual
10 response to the active compound, the type of preparation and the time or interval of administration.

Pharmaceutically acceptable salts of the compounds of the present invention that contain an acidic moiety include addition salts formed with organic or inorganic
15 bases. The salt forming ion derived from such bases can be metal ions, e.g., aluminum, alkali metal ions, such as sodium or potassium, alkaline earth metal ions such as calcium or magnesium, or an amine salt ion, of which a number are known for this purpose. Examples include ammonium salts, arylalkylamines such as dibenzylamine and *N,N*-dibenzylethylenediamine, lower alkylamines such as methylamine, *t*-
20 butylamine, procaine, lower alkylpiperidines such as *N*-ethylpiperidine, cycloalkylamines such as cyclohexylamine or dicyclohexylamine, 1-adamantylamine, benzathine, or salts derived from amino acids like arginine, lysine or the like. The physiologically acceptable salts such as the sodium or potassium salts and the amino acid salts can be used medicinally as described below and are preferred.

25 Pharmaceutically acceptable salts of the compounds of the present invention that contain a basic moiety include addition salts formed with organic or inorganic acids. The salt forming ion derived from such acids can be halide ions or ions of natural or unnatural carboxylic or sulfonic acids, of which a number are known for this
30 purpose. Examples include chlorides, acetates, trifluoroacetates, tartrates, or salts derived from amino acids like glycine or the like. The physiologically acceptable

salts such as the chloride salts, the trifluoroacetic acid salts and the amino acid salts can be used medicinally as described below and are preferred.

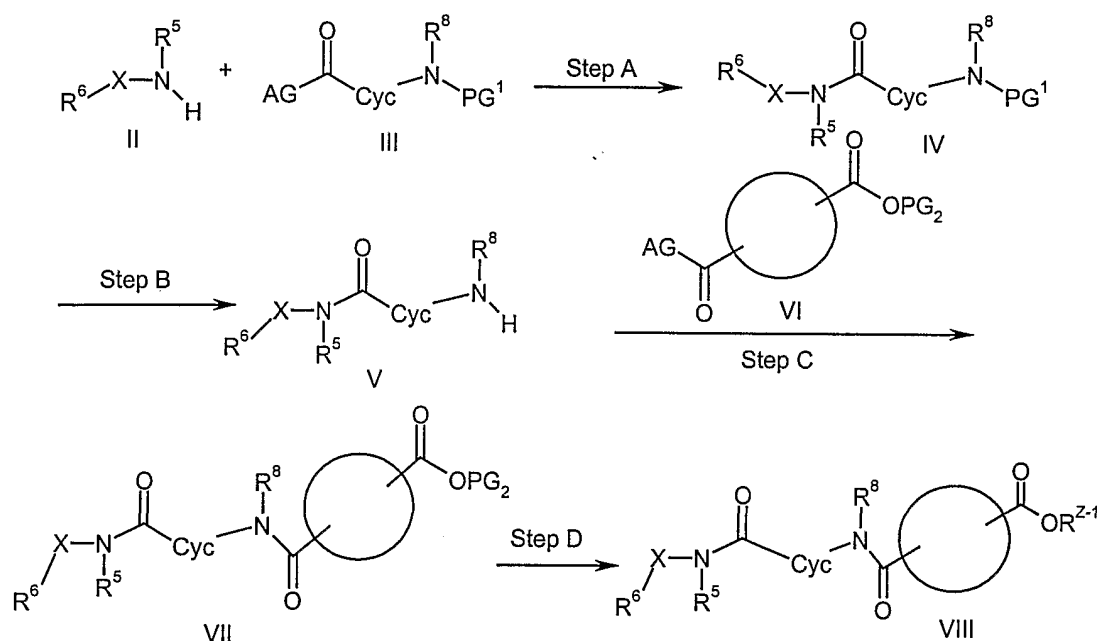
5 These and other salts which are not necessarily physiologically acceptable are useful in isolating or purifying a product acceptable for the purposes described below.

10 The compounds according to the invention can exist in different stereoisomeric forms, which relate to each other in an enantiomeric way (image and mirror image) or in a diastereomeric way (image different from mirror image). The invention relates to the enantiomers and the diastereomers as well as their mixtures. They can be separated according to customary methods.

15 The compounds according to the invention can exist in tautomeric forms. This is known to the artisan and such compounds are also object of the present invention.

General compound synthesis

The compounds according to the present invention are γ -amino acids. They can be prepared employing standard amide coupling procedures. In case A stands for $-\text{NR}^2\text{C}(\text{O})-$ and Y stands for $-\text{NR}^8\text{C}(\text{O})-$, the synthesis of compounds according to general formula (I) can be illustrated by the following scheme 1:



Scheme 1

By coupling of the amines **(II)** with the carboxylic acids or activated derivatives **(III)**, followed by removal of the protecting group PG^1 the amides **(V)** can be obtained. Coupling with the carboxylic acids or activated derivatives **(VI)** affords carboxylic acids of type **(VII)**. If an protecting group PG^2 is used to protect an carboxylic acid functionality on R^1 , removal of the protecting group PG^2 follows.

15

In the above scheme cyc in formulas **(III)** – **(V)** and **(VII)** as well as in scheme 2 represents a cyclic moiety. The depicted ring in formulas **(VI)** - **(VIII)** as well as in scheme 2 and 3 represents a cyclic moiety. AG stands for hydroxyl or a suitable activating group forming an activated carboxylic acid derivative. Activated carboxylic acids derivatives of this type are known to the person skilled in the art and are described in detail in standard textbooks such as, for example in (i) Houben-

20

Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart or (ii) Comprehensive Organic Synthesis, Ed. B. M. Trost, Pergamon Press, Oxford, 1991. The carboxylic acid is preferably activated as mixed anhydride, such as, for example, AG = *iso*-butyl-carbonate or by a coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylamino-propyl)carbodiimidexHCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Other activated carboxylic acid derivatives such as, for example symmetric anhydrides, halides, or activated esters e.g. succinyl, pentafluorophenyl or N-hydroxybenzotriazole esters may also be employed.

In the above scheme PG¹ stands for a suitable protecting group of the amino group that is stable under the respective reaction conditions. Protecting groups of this type are known to the person skilled in the art and are described in detail in T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley, New York, 1999. The amino group is preferably protected by carbamates, PG¹ being for example *tert*-butyloxycarbonyl (Boc), 9-fluorenylmethyloxycarbonyl (Fmoc) or benzyloxycarbonyl (Cbz- / Z-) or other oxycarbonyl derivatives.

PG² stands for a suitable protecting group of the carboxyl group or COOPG² stands for the carboxylic group attached to a polymeric resin suitable for solid phase synthesis. Protecting groups of this type are known to the person skilled in the art and are described in detail in T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley, New York, 1999. The carboxyl group is preferably esterified, PG² being C₁₋₆-alkyl such as, for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, a C₃₋₇-cycloalkyl such as, for example, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, cyclohexyl, an aryl such as, for example, phenyl, benzyl, tolyl or a substituted derivative thereof.

Step A

Formation of the amides (IV) can take place by reacting an activated form of the respective carboxylic acid (III), such as an *iso*-butylcarbonate or N-hydroxybenzotriazole ester - with the desired amine (II) or an acceptable salt thereof.

5

Iso-butylcarbonates can be prepared *in situ* by reaction of the N-protected amino acid (III) with *iso*-butylchloroformate as described below. Activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl, N-hydroxybenzotriazole or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate may also be employed.

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15

1-Hydroxy-1H-benzotriazol ester of (III) can be prepared, for example, by the reaction of the 1-hydroxy-1H-benzotriazol with the carboxylic acids (III) in presence of an coupling agents such as, for example, dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Further activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with may also be employed.

20

For example, amides of type (IV) can be prepared as follows:

25

1) Mixed anhydride procedure

A solution of the carboxylic acid derivative (III) and of N-methylmorpholine in an inert solvent was cooled to -15°C and *iso*-butyl chloroformate was added and stirred at 0°C. The amine (II) in an inert solvent was added at -15°C. The solution was stirred at 0°C, and at r.t. and was evaporated. The residue was redissolved in ethyl

30

acetate, washed with aqueous acid and base, dried and evaporated. If necessary the product was purified by trituration or by flash-chromatography or used without further purification.

5 **2) 1-Hydroxy-1H-benzotriazol ester procedure**

A solution of carboxylic acid, 1-hydroxy-1H-benzotriazol (HOBt) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI) in an inert solvent is stirred at r.t. After addition of the amine and a non-nucleophilic base such as ethyldiisopropyl-
10 amine or potassium carbonate stirring is continued at r.t. or elevated temperature. After evaporation, the residue was redissolved in ethyl acetate, washed with aqueous acid and base, dried and evaporated. If necessary the product was purified by trituration or by flash-chromatography or used without further purification.

15 The above reactions and their implementation are well known to the person skilled in the art and are described in detail in standard textbooks such as, for example, in (i) Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart or Stuttgart or (ii) Comprehensive Organic Synthesis, Ed. B. M. Trost, Pergamon Press, Oxford, 1991.

20 Compounds of general formula (II) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

In case R^{1-1} is a methylen group, the carbon chain can be elongated by Arndt-Eistert-
25 reaction and optionally be derivatized by common methods for α -derivatization of carboxylic acids such as nucleophilic substitution.

Step B

The removal of protecting group PG^1 can be performed, depending on the nature of
30 PG^1 , either by an acid such as trifluoroacetic acid for example in the case PG^1 is tert-butylloxycarbonyl (Boc), a base such as piperidine for example in the case PG^1 is 9-

fluorenylmethoxycarbonyl (FMOC) or by catalytic hydrogenation for example in the case PG¹ is benzyloxycarbonyl (Cbz- / Z-).

Step C

5

Formation of the amides (VII) can take place by reacting the respective carboxylic acids (VI) - activated by a coupling agent such as DCC and HOBt; EDCI and HOBt or HATU - with the desired amines (V) or an acceptable salt thereof. Activated derivatives of the acids (VI) such as anhydrides, halides, and esters e.g. succinyl or pentafluorophenyl esters may also be employed.

10

For example, amides (VII) can be prepared as follows:

A solution of carboxylic acid, HOBt and EDCI in an inert solvent is stirred at r.t.. After addition of the amine and a non-nucleophilic base such as ethyldiisopropylamine stirring is continued at r.t. or elevated temperature. The reaction mixture is poured into water and worked up by standard procedures.

15

Compounds of general formula (VI) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

20

Bisarylureas can be prepared by coupling of an amino phenyl acetic acid derivative and a phenylisocyanate.

Step D

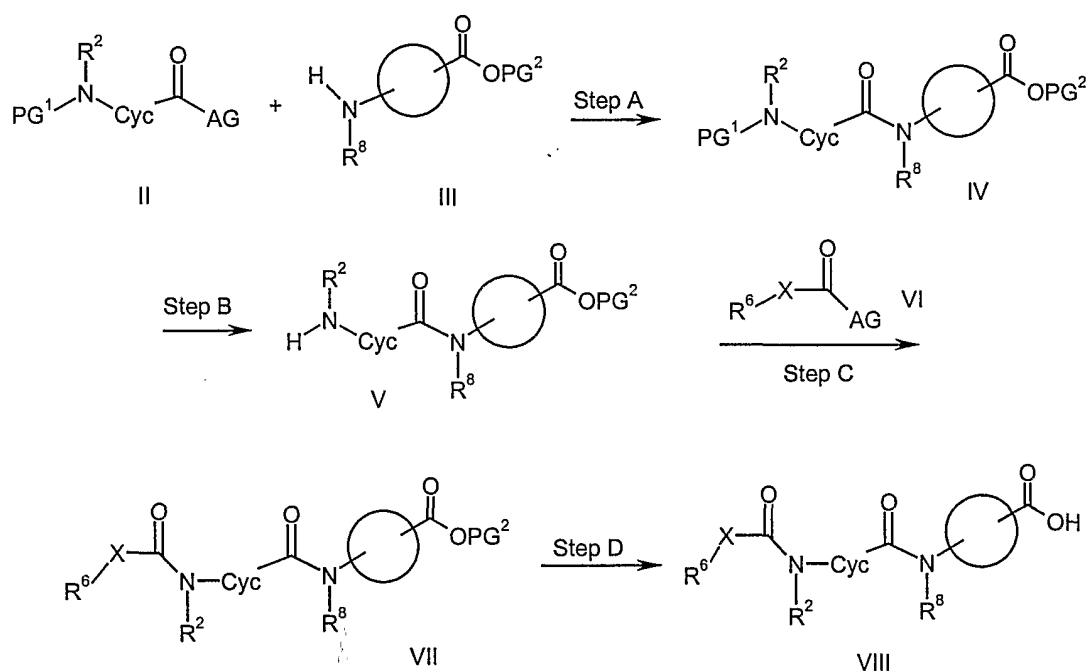
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If necessary, a removal of the protecting group PG² can be performed either by an acid such as trifluoroacetic acid or an base such as potassium hydroxide or lithium hydroxide, depending on the nature of PG². Reactions are carried out in aqueous, inert organic solvents such as alcohols e.g. methanol or ethanol, ethers e.g.

30

tetrahydrofurane or dioxane or polar aprotic solvents e.g. dimethylformamide. If necessary, mixtures of the above solvents may be used.

In case A stands for $-\text{C}(\text{O})\text{NR}^2-$ and Y stands for $-\text{C}(\text{O})\text{NR}^8-$, the synthesis of compounds according to general formula (I) can be illustrated by the following scheme 2:



Scheme 2

- 10 By coupling of the carboxylic acids or activated derivatives (II) with the amines (III), followed by removal of the protecting group PG^1 , the amides (V) can be obtained. Coupling with the carboxylic acids or activated derivatives (VI) affords carboxylic acids of type (VII). If an protecting group PG^2 is used to protect an carboxylic acid functionality on R^1 , removal of the protecting group PG^2 follows.
- 15 In the above scheme the depicted ring in formulas (III) – (V), (VII) and (VIII) as well as in scheme 1 represents a cyclic moiety. AG stands for hydroxyl or a suitable activating group forming an activated carboxylic acid derivative.

Step A

Formation of the amides (IV) can take place by reacting an activated form of the respective carboxylic acid (III), such as an *iso*-butylcarbonate or N-hydroxybenzotriazole ester - with the desired amine (II) or an acceptable salt thereof.

5

Iso-butylcarbonates can be prepared *in situ* by reaction of the N-protected amino acid (III) with *iso*-butylchloroformate as described below. Activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl, N-hydroxybenzotriazole or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimid×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate may also be employed.

10

1-Hydroxy-1H-benzotriazol ester of (III) can be prepared, for example, by the reaction of the 1-hydroxy-1H-benzotriazol with the carboxylic acids (III) in presence of an coupling agents such as, for example, dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimid×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Further activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with may also be employed.

15

20

25

For example, amides of type (IV) can be prepared as follows:

1) Mixed anhydride procedure

A solution of the carboxylic acid derivative (III) and of N-methylmorpholine in an inert solvent was cooled to -15°C and *iso*-butyl chloroformate was added and stirred at 0°C. The amine (II) in an inert solvent was added at -15°C. The solution was stirred at 0°C, and at r.t. and was evaporated. The residue was redissolved in ethyl acetate, washed with aqueous acid and base, dried and evaporated. If necessary the

30

product was purified by trituration or by flash-chromatography or used without further purification.

2) 1-Hydroxy-1H-benzotriazol ester procedure

5 A solution of carboxylic acid, 1-hydroxy-1H-benzotriazol (HOBT) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI) in an inert solvent is stirred at r.t. After addition of the amine and a non-nucleophilic base such as ethyldiisopropylamine or potassium carbonate stirring is continued at r.t. or elevated temperature. After evaporation, the residue was redissolved in ethyl acetate, washed with aqueous
10 acid and base, dried and evaporated. If necessary the product was purified by trituration or by flash-chromatography or used without further purification.

Compounds of general formulas (II) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

15

Bisarylureas can be prepared by coupling of an amino phenyl acetic acid derivative and a phenylisocyanate. Bisarylamides can be prepared by coupling of an amino phenyl acetic acid and an activated benzoic acid derivative such as described under Step A. Bisarylcarbamates can be prepared by coupling of an isocyanato phenyl acetic acid ester and a phenol derivative followed by saponification as described in Step
20 D.

Step B

The removal of protecting group PG¹ can be performed, depending on the nature of
25 PG¹, either by an acid such as trifluoroacetic acid for example in the case PG¹ is *tert*-butyloxycarbonyl (Boc), a base such as piperidine for example in the case PG¹ is 9-fluorenylmethyloxycarbonyl (Fmoc) or by catalytic hydrogenation for example in the case PG¹ is benzyloxycarbonyl (Cbz- / Z-).

Step C

Formation of the amides (VII) can take place by reacting the respective carboxylic acids (VI) - activated by a coupling agent such as DCC and HOBt; EDCI and HOBt or HATU - with the desired amines (V) or an acceptable salt thereof. Activated derivatives of the acids (VI) such as anhydrides, halides, and esters e.g. succinyl or pentafluorophenyl esters may also be employed.

For example, amides (VII) can be prepared as follows:

A solution of carboxylic acid, HOBt and EDCI in an inert solvent is stirred at r.t.. After addition of the amine and a non-nucleophilic base such as ethyldiisopropylamine stirring is continued at r.t. or elevated temperature. The reaction mixture is poured into water and worked up by standard procedures.

Compounds of general formula (VI) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

Bisarylureas can be prepared by coupling of an amino phenyl acetic acid derivative and a phenylisocyanate. Bisarylamides can be prepared by coupling of an amino phenyl acetic acid and an activated benzoic acid derivative such as described under Step A. Bisarylcarbamates can be prepared by coupling of an isocyanato phenyl acetic acid ester and a phenol derivative followed by saponification as described in Step D.

Step D

The removal of the protecting group PG² can be performed either by an acid such as trifluoroacetic acid or an base such as potassium hydroxide or lithium hydroxide, depending on the nature of PG². Reactions are carried out in aqueous, inert organic solvents such as alcohols e.g. methanol or ethanol, ethers e.g. tetrahydrofurane or dioxane or polar aprotic solvents e.g. dimethylformamide. If necessary, mixtures of the above solvents may be used.

Examples**Abbreviations**

5	AcOH	acetic acid
	Boc	tert-butyloxycarbonyl
	DCC	dicyclohexylcarbodiimid
	DCM	dichloromethane
	DIPEA	diisopropylethylamine
10	EDCI	1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl
	eq.	equivalents
	EtOAc	ethyl acetate
	FC	flash chromatography
	GC	gas chromatography
15	HATU	2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluro- nium hexafluorophosphate
	HOBt	N-hydroxybenzotriazole monohydrate
	HPLC	high performance liquid chromatography
	ICAM-1	intracellular adhesion molecule 1
20	IL-1	interleukin 1
	LPS	lipopolysaccharide
	MAdCAM-1	mucosal addressin cell adhesion molecule 1
	MeOH	methanol
	MeCN	acetonitril
25	min.	minutes
	M.p.	melting point
	NF-κB	nuclear factor κB
	NMR	nuclear magnetic resonance
	n.d.	not determined
30	PE	light petroleum (b.p. 40-60 °C)
	r.t.	room temperature

	R _f	TLC: R _f value = distance spot traveled / distance solvent front traveled
	TFA	trifluoroacetic acid
	THF	tetrahydrofuran
	TLC	thin layer chromatography
5	TNF- α	tumor necrosis factor α
	t _R	retention time determined by HPLC
	VCAM-1	vascular cell adhesion molecule 1
	VLA-4	very late antigen 4 ($\alpha_4\beta_1$ integrin)

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General remarks

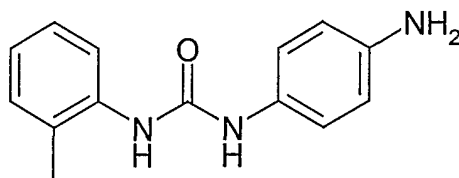
In the examples below, all quantitative data, if not stated otherwise, relate to percentages by weight.

15 Flash chromatography was carried out on silica gel 60, 40–63 μ m (E. Merck, Darmstadt, Germany).

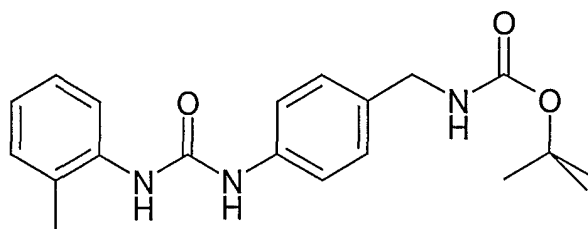
Thin layer chromatography was carried out, employing silica gel 60 F₂₅₄ coated aluminum sheets (E. Merck, Darmstadt, Germany) with the mobile phase indicated.

Melting points were determined in open capillaries and are not corrected.

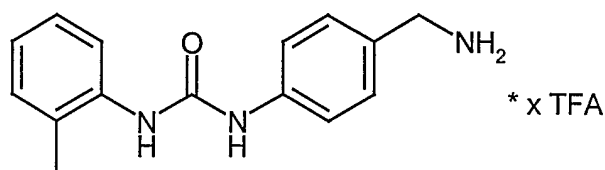
20 The mass determinations were carried out using the electron spray ionization (ESI) method employing loop injection or split injection via a HPLC system.

Precursor synthesis**Example I:** N-(4-aminophenyl)-N'-(2-methylphenyl)urea

5 2- Methylphenylisocyanate (24.6 g, 184.9 mmol) was added dropwise at 0 °C to a solution of 1,4-diamino benzene (20.00 g, 184.9 mmol) in 1000 mL EtOAc. After stirring for 2 h at r.t. the product was collected by filtration (42.7 g, 177.0 mmol). M.p. >300 °C; TLC (PE/EtOAc 1/4) R_f 0.32; $^1\text{H-NMR}$ (400 MHz, $\text{D}_6\text{-DMSO}$) δ 2.10 (s, 3H); 4.76 (s, 2H); 6.59 (mc, 2H); 6.89 (mc, 1H); 7.07-7.15 (m, 4H); 7.73 (s, 1H);
10 7.85 (mc, 2H); 8.50 (s, 1H).

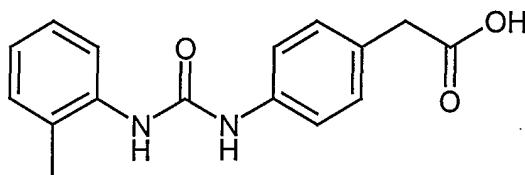
Example II: *tert*-Butyl 4-({[(2-methylphenyl)amino]carbonyl}amino)benzyl-carbamate

15 2-Methylphenylisocyanate (7.57 g, 59.83 mmol) was added dropwise at 0 °C to a solution of (4-amino-benzyl)-carbamic acid *tert*-butyl ester (Moloney, Gerard P.; Martin, Graeme R.; Mathews, Neil; Milne, Aynsley; Hobbs, Heather; et al.; *J. Med. Chem.* **1999**, *42*, 2504 - 2526; 13.30 g, 59.83 mmol) in 120 mL DCM. The reaction was heated under reflux for 16 h, cooled to r.t. and the precipitated product was
20 collected by filtration and dried in vacuum (19.20 g, 54.00 mmol). M.p. 200-202 °C; TLC (PE/EtOAc 1/1) R_f 0.65; $^1\text{H NMR}$ (400 MHz, $\text{D}_6\text{-DMSO}$) δ 1.39 (s, 9H); 2.24 (s, 3H); 4.06 (d, $J=6$ Hz, 2H); 6.93 (mc, 1H); 7.12-7.17 (m, 4); 7.32 (mc, 1H); 7.40 (mc, 2H); 7.85 (mc, 1H); 7.90 (s, 1H); 8.98 (s, 1H).

Example III: N-[4-(Aminomethyl)phenyl]-N'-(2-methylphenyl)urea

To a solution of *tert*-butyl 4-({[(2-methylphenyl)amino]carbonyl}amino)benzylcarb-
 5 amate (2.00 g, 5.63 mmol) in CH₂Cl₂ (120 mL) TFA (36 mL) was added at 0 °C and
 stirred for 2 h at r.t.. The reaction mixture was evaporated and the product was
 collected (2.72 g, TFA salt). M.p. 142-143 °C; TLC (PE/EtOAc 3/2) R_f 0.14; ¹H
 NMR (400 MHz, D₆-DMSO) δ 2.24 (s, 3H); 3.97 (q, J=5 Hz, 2H); 6.96 (mc, 1H);
 7.13-7.19 (m, 2); 7.36 (mc, 2H); 7.51 (mc, 2H); 7.81 (mc, 2H); 8.06 (s, 1H); 8.08 (s,
 3H); 9.23 (s, 1H).

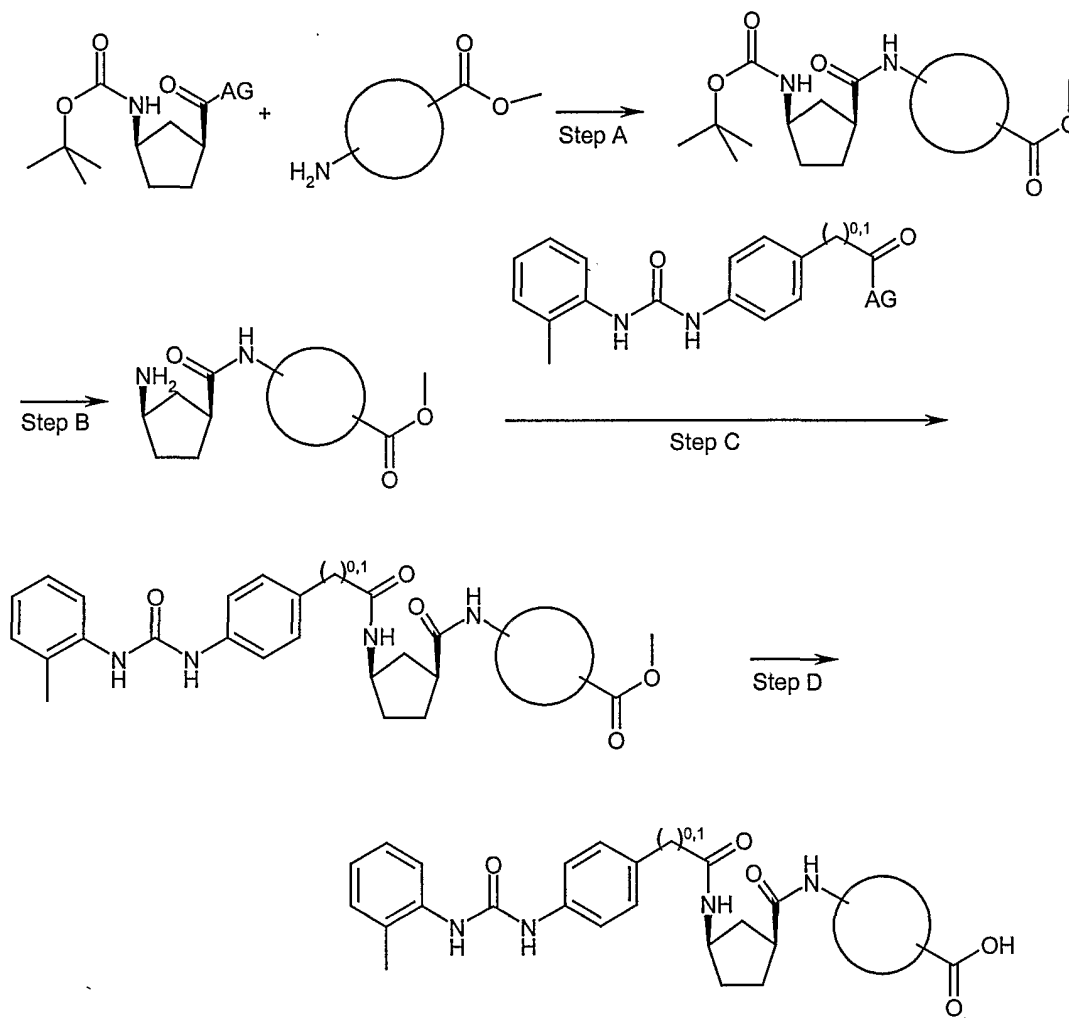
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Example IV: [4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]acetic acid

To a solution of 2-(4-aminophenyl)acetic acid (108.8 g, 0.72 mol) in CH₂Cl₂ (1.0 l)
 and triethylamine (120 ml) was added a solution of 2-methylphenyl isocyanate
 15 (90.5 ml, 0.72 mol) in CH₂Cl₂ (500 ml) dropwise at r.t.. After stirring for 18 h at r.t.,
 water (2.5 l) and CH₂Cl₂ (2.0 l) were added and the layers were separated. The or-
 ganic layer was extracted with water (3 × 400 ml). The combined aqueous layers
 were concentrated to 3.0 l and acidified to pH 2 by the addition of concentrated
 aqueous HCl. The precipitate was collected by filtration, washed with cold water and
 20 dried in an exsiccator over concentrated H₂SO₄ affording 166.5 g (82%) white solid.
 M.p. 205-206°C; TLC (CH₂Cl₂/MeOH 9:1): R_f 0.14. ¹H-NMR (400 MHz, D₆-
 DMSO): 12.21 (br s, 1H), 9.11 (s, 1H), 8.00 (s, 1H), 7.83 (d, 7.6 Hz, 1H), 7.40 (d,
 8.5 Hz, 2H), 7.17-7.12 (m, 4H), 6.96-6.92 (m, 1H), 3.48 (s, 2H), 2.24 (s, 3H).

Compound synthesis

Examples 1 and 2 were prepared by the following general procedure. Schemes 3 and 4 shall illustrate the process in an exemplaric way:

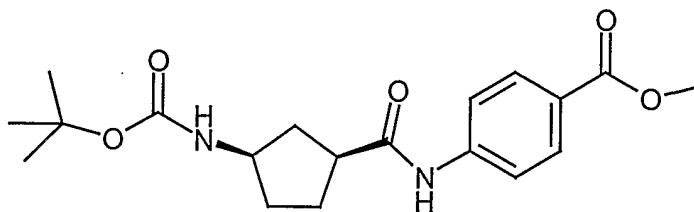


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Scheme 3

Step A:

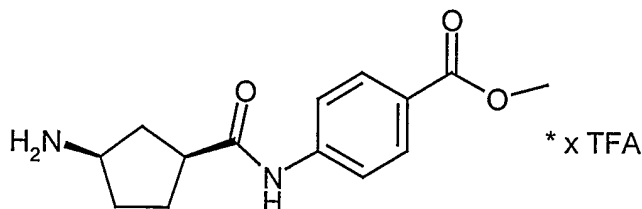
Example V: Methyl 4-[($(1S^*,3R^*)$ -3-[(tert-butoxycarbonyl)amino]cyclopentyl)-carbonyl]amino]benzoate



5 ($(1S^*,3R^*)$ -3-[(tert-Butoxycarbonyl)amino]cyclopentanecarboxylic acid (Lit.: Marco-Contelles, Jose; Bernabe, Manuel; *Tetrahedron Lett.* **1994**; 35, 6361-6364) (4.59 g, 20.00 mmol) was dissolved in THF (50 mL), N-methylmorpholin (2.04 g, 20.00 mmol) and isobutylchloroformate (2.73 g, 20.00 mmol) were added at $-15\text{ }^\circ\text{C}$. Stirring was continued for 45 min at $0\text{ }^\circ\text{C}$. 4-Aminomethylbenzoate (3.02 g, 20.00 mmol, dissolved in 10 mL THF) were added dropwise at $-15\text{ }^\circ\text{C}$. The reaction mixture was heated under reflux for 48 h. The solvent was evaporated, the residue was redissolved in EtOAc and washed with 1 N HCl, sat. soda, brine and dried (MgSO_4). FC (PE/EE 9:1) yielded methyl 4-[($(1S^*,3R^*)$ -3-[(tert-butoxycarbonyl)-amino]cyclopentyl)-carbonyl]amino]benzoate (5.95 g, 16.42 mmol). TLC (PE/EtOAc 6/4) R_f 0.26; $^1\text{H-NMR}$ (400 MHz, $\text{D}_6\text{-DMSO}$) δ 1.38 (s, 9H); 1.47-1.64 (m, 2H); 1.82 (mc, 3H); 2.08-2.15 (m, 1H); 2.80-2.88 (m, 1H); 3.82 (s, 4H); 6.82 (bs, 1H); 7.72-7.74 (m, 2H); 7.89-7.91 (m, 2H); 10.10 (bs, 1H).

Step B:

20 **Example VI:** Methyl 4-[($(1S^*,3R^*)$ -3-aminocyclopentyl]carbonyl]amino]benzoate

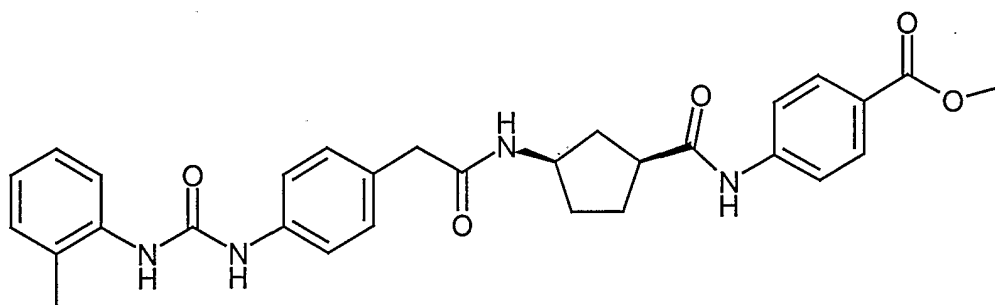


To a solution of methyl 4-[($(1S^*,3R^*)$ -3-[(tert-butoxycarbonyl)amino]cyclopentyl)-carbonyl]amino]benzoate (2.70 g, 7.45 mmol) in CH_2Cl_2 (50 mL) TFA (10 mL) was added at $0\text{ }^\circ\text{C}$ and stirred for 2 h at r.t.. The reaction mixture was evaporated and the product was collected (4.10 g, TFA salt). $^1\text{H-NMR}$ (400 MHz, $\text{D}_6\text{-DMSO}$) δ 1.70

(mc, 1H); 1.80-2.03 (m, 4H); 2.25 (mc, 1H); 2.98 (mc, 1H); 3.60 (mc, 1H); 3.83 (s, 3H); 7.73-7.76 (m, 2H); 7.88-7.96 (m, 5H); 10.16 (s, 1H).

Step C:

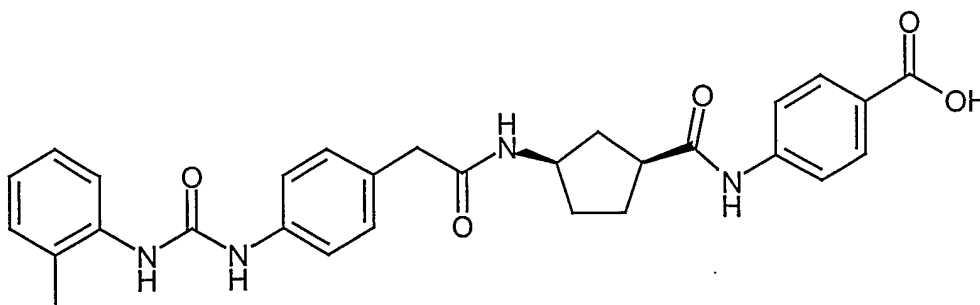
5 **Example 1:** Methyl 4-({[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}-amino)phenyl]acetyl}amino)cyclopentyl]carbonyl}amino)benzoate



[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]acetic acid (1.48 g, 5.21 mmol) was dissolved in DMF (10 mL), HOBT (880 mg, 5.73 mmol), EDCI (1.10 g, 5.73 mmol), DIPEA (6.06 g, 46.89 mmol) was added and stirring was continued for 2 h at r.t. Methyl 4-({[(1*S**,3*R**)-3-aminocyclopentyl]carbonyl}amino)benzoate (1.96 g, 5.21 mmol dissolved in 8 mL DMF) was added at r.t. After stirring for 48 h, water (80 mL) was added and the precipitate was collected, yielding methyl 4-({[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]acetyl}-amino)cyclopentyl]carbonyl}amino)benzoate (2.10 g, 3.97 mmol). M.p. 208-210 °C. ESI-MS: 529 [M+H]⁺

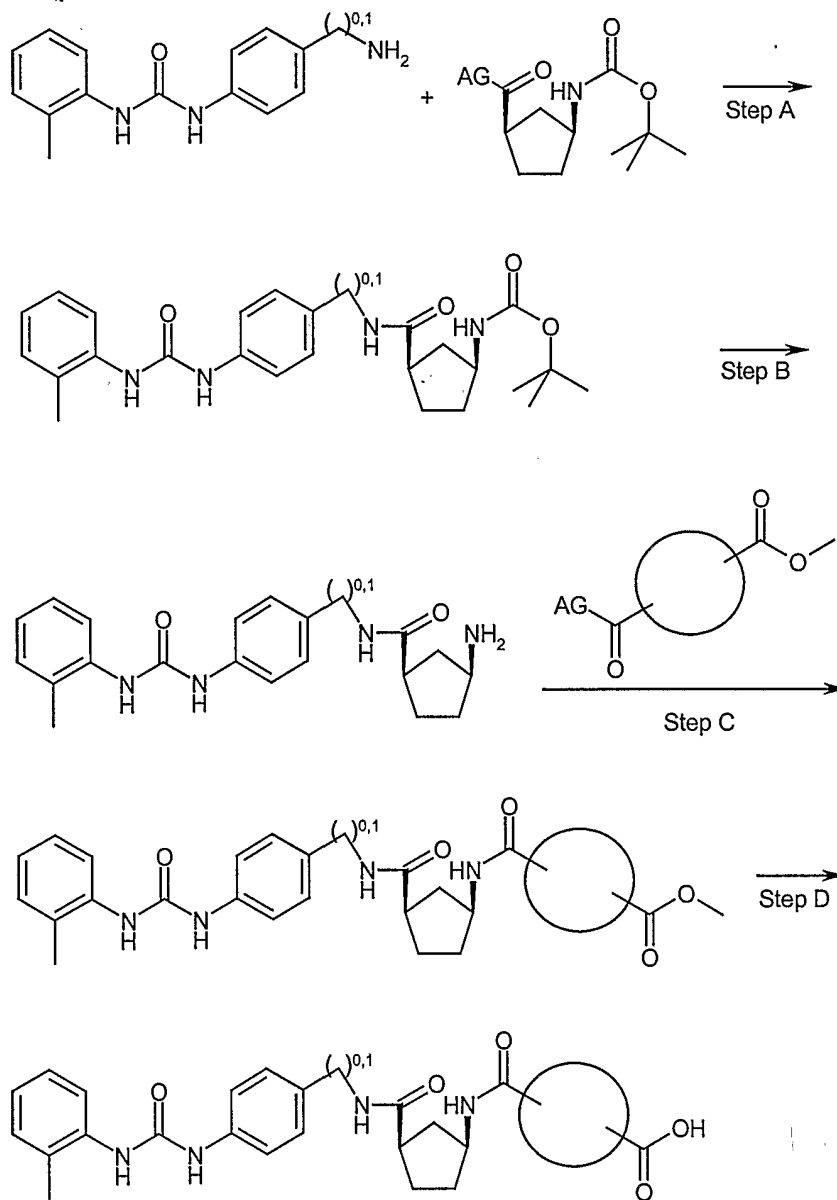
Step D:

20 **Example 2:** 4-({[(1*S**,3*R**)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]acetyl}amino)cyclopentyl]carbonyl}amino)benzoic acid



Methyl 4-({[(1*S*^{*},3*R*^{*})-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]-acetyl}amino)cyclopentyl]carbonyl}amino)benzoate (1.39 g, 2.63 mmol) was dissolved in MeOH/water (1/1), potassium hydroxide (160 mg, 2.89 mmol) was added and the reaction mixture was stirred at 30 °C for 12 h. MTBE was added, the aqueous
5 layer was extracted once with MTBE, HCl was added to the organic layer (pH 2) and 4-({[(1*S*^{*},3*R*^{*})-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]acetyl}-amino)cyclopentyl]carbonyl}amino)benzoic acid (1.18 g, 2.29 mmol). M.p. 270-273 °C. ESI-MS: 513 [M-H]⁺

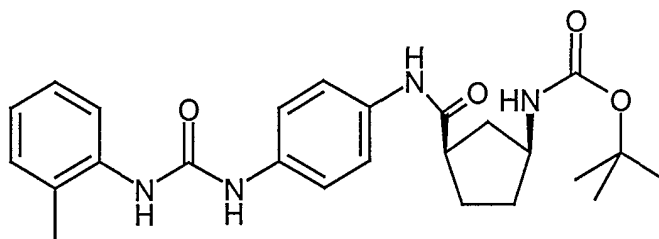
Examples 3-10 were prepared by the following general procedure.



Scheme 4

Step A:

Example VII: *tert*-Butyl-(1*S*^{*},3*R*^{*})-3-({[4-({[(2-methylphenyl)amino]carbonyl}-amino)phenyl]amino}carbonyl)cyclopentylcarbamate



5 (1*S*^{*},3*R*^{*})-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid (Lit.: Marco-Contelles, Jose; Bernabe, Manuel; *Tetrahedron Lett.* **1994**, 35, 6361-6364) (2.00 g, 8.72 mmol) was dissolved in DMF (15 mL), HOBT (1.47 g, 9.60 mmol), EDCI (1.84 g, 9.60 mmol) and DIPEA (3.38 g, 26.17 mmol) were added at r.t. and stirred for 2 h. N-(4-Aminophenyl)-N'-(2-methylphenyl)urea (2.18 g, 9.60 mmol dissolved
10 in 25 mL DMSO) was added and stirring was continued for 12 h. The reaction mixture was hydrolysed with ice, *tert*-butyl-(1*S*^{*},3*R*^{*})-3-({[4-({[(2-methylphenyl)amino]carbonyl}-amino)phenyl]amino}carbonyl)cyclopentylcarbamate (3.32 g, 7.34 mmol) was collected by filtration, washed with water and isolated. M.p. 188-190 °C. ESI-MS: 453 [M+H]⁺

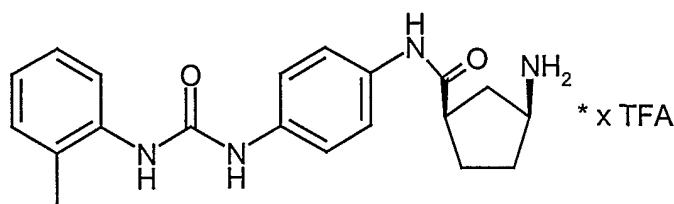
15

Table 1: The following compound was synthesized according to the same protocol

No	Structure	Name	M.p. (°C)
VIII		<i>tert</i> -Butyl (1 <i>S</i> [*] ,3 <i>R</i> [*])-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)benzyl]-amino}carbonyl)cyclopentylcarbamate	180-182

Step B:

20 **Example IX:** (1*R*^{*},3*S*^{*})-3-amino-N-[4-({[(2-methylphenyl)amino]carbonyl}amino)-phenyl]cyclopentanecarboxamid



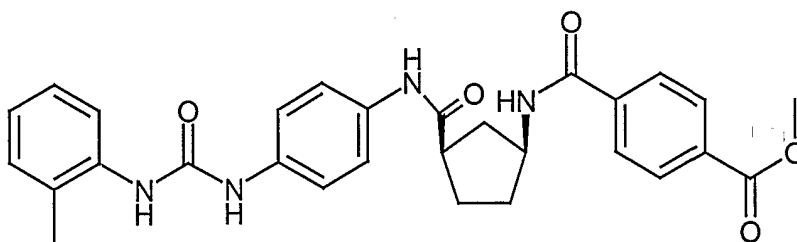
5 *tert*-Butyl-(1*S*^{*},3*R*^{*})-3-((4-(((2-methylphenyl)amino)carbonyl)amino)phenyl)-3-amino-N-cyclopentanecarboxamid (23.87 g, TFA salt). ESI-MS: 353 [M+H]⁺

Table 2: The following compound was synthesized according to the same protocol

No	Structure	Name
X		(1 <i>R</i> [*] ,3 <i>S</i> [*])-3-Amino-N-[4-(((2-methylphenyl)amino)carbonyl)amino]benzyl]-cyclopentanecarboxamide

10 **Step C:**

Example 3: Methyl 4-(((1*S*^{*},3*R*^{*})-3-((4-(((2-methylphenyl)amino)carbonyl)amino)phenyl)amino)carbonyl)cyclopentyl]amino)carbonyl)benzoate

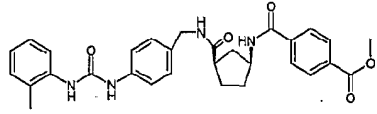
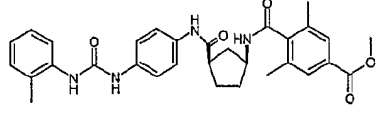
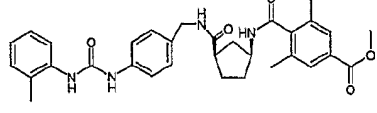


15 Monomethylterephthate (540 mg, 3.00 mmol) was dissolved in DMF (15 mL), HOBT (510 mg, 3.30 mmol), EDCI (630 mg, 3.30 mmol) and DIPEA (3.59 g, 27.00 mmol) were added. After stirring for 2 h at r.t., (1*R*^{*},3*S*^{*})-3-amino-N-[4-(((2-methylphenyl)amino)carbonyl)amino]phenyl]cyclopentanecarboxamid (2.33 g, TFA salt dissolved in 2 mL DMF) was added and stirring was continued for 12 h. The reaction mixture was hydrolysed with ice, methyl 4-(((1*S*^{*},3*R*^{*})-3-((4-(((2-methylphenyl)amino)carbonyl)amino)phenyl)amino)carbonyl)cyclopentyl]amino)carbonyl)benzoate (1.10 g,

20

2.14 mmol) was collected by filtration, washed with water and isolated. M.p. 314-316 °C. ESI-MS: 515 [M+H]⁺

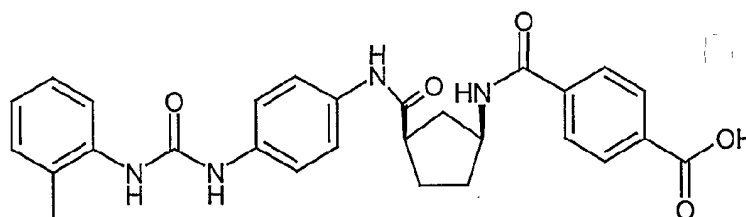
Table 3: The following examples were prepared according to the general procedure

No	Structure	Name	M.p.(°C)	ESI-MS
4		Methyl 4-(((1 <i>R</i> [*] ,3 <i>S</i> [*])-3-({[4-({[(2-methylphenyl)amino]carbonyl)-amino]benzyl}amino)carbonyl)cyclopentyl]amino)carbonyl)benzoate	217-219	
5		Methyl 3,5-dimethyl-4-(((1 <i>R</i> [*] ,3 <i>S</i> [*])-3-({[4-({[(2-methylphenyl)-amino]carbonyl)amino]phenyl}-amino)carbonyl)cyclopentyl]-amino)carbonyl)benzoate	269	
6		Methyl 3,5-dimethyl-4-(((1 <i>R</i> [*] ,3 <i>S</i> [*])-3-({[4-({[(2-methylphenyl)-amino]carbonyl)amino]benzyl}-amino)carbonyl)cyclopentyl]-amino)carbonyl)benzoate	n.d.	557 [M+H] ⁺

5

Step D:

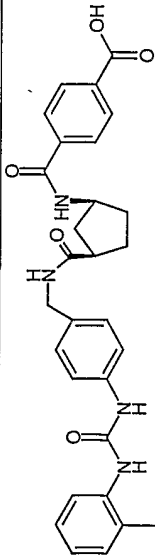
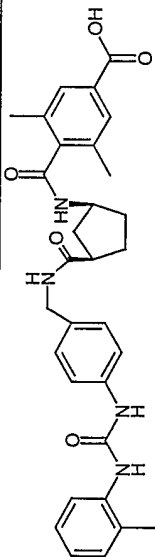
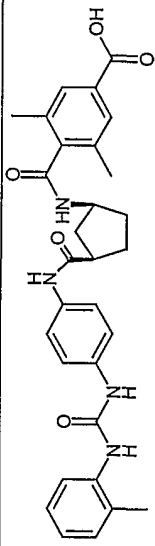
Example 7: 4-(((1*S*^{*},3*R*^{*})-3-({[4-({[(2-Methylphenyl)amino]carbonyl)amino]phenyl}amino)carbonyl)cyclopentyl]amino)carbonyl)benzoic acid



10 Methyl 4-(((1*S*^{*},3*R*^{*})-3-({[4-({[(2-methylphenyl)amino]carbonyl)amino]phenyl}amino)carbonyl)cyclopentyl]amino)carbonyl)benzoate (800 mg, 1.56 mmol) was dissolved in water/methanol/dioxane (1/1/4) and potassium hydroxide (870 mg, 15.55 mmol) was added at r.t. After stirring at 50 °C for 5 h, MTBE was added, the aqueous phase was acidified (pH 2), 4-(((1*S*^{*},3*R*^{*})-3-({[4-({[(2-methylphenyl)-

amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]amino}carbonyl)benzoic acid (564 mg, 1.13 mmol) was collected by filtration, washed with water and isolated. M.p. >270 °C. ESI-MS: 501 [M+H]⁺

Table 4: The following examples were prepared according to the general procedure

No	Structure	Name	ESI-MS	M.p. (°C)
8		4-({[(1 <i>R</i> *, 3 <i>S</i> *)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}-amino)benzyl]amino}carbonyl)cyclopentyl]amino}carbonyl)benzoic acid		258-262
9		3,5-Dimethyl-4-({[(1 <i>R</i> *, 3 <i>S</i> *)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)benzyl]amino}carbonyl)cyclopentyl]-amino}carbonyl)benzoic acid	543	n.d.
10		3,5-Dimethyl-4-({[(1 <i>S</i> , 3 <i>R</i>)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]amino}carbonyl)benzoic acid		>269

In vitro assay: adhesion of Jurkat cells to immobilized VCAM-1 (domains 1-3)**Preparation of VCAM-1 (extracellular domains 1-3)**

Complementary DNA (cDNA) encoding 7-domain form of VCAM-1 (GenBank accession #M60335) was obtained using Rapid-Screen™ cDNA library panels (OriGene Technologies, Inc) at Takara Gene Analysis Center (Shiga, Japan). The primers used were 5'-CCA AGG CAG AGT ACG CAA AC-3' (sense) and 5'-TGG CAG GTA TTA TTA AGG AG-3' (antisense). PCR amplification of the 3-domain VCAM-1 cDNA was performed using *Pfu* DNA polymerase (Stratagene) with the following sets of primers: (U-VCAMd1-3) 5'-CCA TAT GGT ACC TGA TCA ATT TAA AAT CGA GAC CAC CCC AGA A-3'; (L-VCAMd1-3) 5'-CCA TAT AGC AAT CCT AGG TCC AGG GGA GAT CTC AAC AGT AAA-3'. PCR cycle was 94 °C for 45 sec, 55 °C for 45 sec, 72 °C for 2 min, repeating 15 cycles. After the purification of the PCR product, the fragment was digested with KpnI-AvrII. The digested fragment was ligated into pBluescript IISK(-) (Stratagene), which was linearized by digesting with KpnI-XhoI. The ligation was followed by transformation to a Dam/Dcm methylase-free *E. coli* strain SCS110 (Stratagene) to create the donor plasmid pHH7. To direct VCAM-1 molecule into the insect cell secretory pathway, the VCAM-1 coding sequence was fused to signal peptide sequence of honeybee melittin. The resulting melittin-VCAM fusion was placed in correct orientation to the baculovirus polyhedrin promoter. Baculovirus transfer vector containing first 3-domain form VCAM-1 (pH10) was constructed by ligation of 0.9 kb fragment from AvrII/Klenow/BclI digests of pH7 into SalI/Klenow/BamHI digests of pMelBacB (Invitrogen). Recombinant baculovirus was generated by using Bac-N-Blue™ Transfection kit (Invitrogen) according to the manufacturer's instruction. The recombinant virus was amplified by infection to High-Five™ insect cells for 5 – 6 days, and virus titer was determined by plaque assay.

High-Five™ insect cells were pelleted in a 225 ml conical tube by centrifugation at 1000 rpm for 5 min. After discarding the supernatant, the pellet was resuspended in 1.5×10^9 pfu (MOI = 5) of high-titer virus solution, followed by incubation for 1.5

hours at room temperature. The cells were pelleted again and washed once in fresh Express Five™ serum free medium. The cells were pelleted again and finally, resuspended in 200 ml of fresh Express Five™ medium, transferred to a 1,000 ml shaker flask, and incubated in a shaker at 27 °C, 130 rpm, for 48 hours before the culture supernatant was collected. The purification of 3-domain form of VCAM-1 from the culture supernatant was performed by one-step anion exchange chromatography. Protein concentration was determined by using Coomassie protein assay reagent (Pierce) according to the manufacture's instruction.

10 **Preparation of VCAM-1 coated microtiter plates**

Recombinant human VCAM-1 (extracellular domains 1-3) was dissolved at 1.0 µg/ml in PBS. Each well of the microtiter plates (Nalge Nunc International, Fluoronunc Cert, 437958) was coated with 100 µl of substrate or for background control with buffer alone for 15 hours at 4 °C. After discarding the substrate solution, the wells were blocked using 150 µl per well of block solution (Kirkegaard Perry Laboratories, 50-61-01) for 90 minutes. The plate was washed with wash buffer containing 24 mM Tris-HCl (pH 7.4), 137 mM NaCl, 27 mM KCl and 2 mM MnCl₂ just before addition of the assay.

20

In vitro assay using Jurkat cells

Preparation of fluorescence labeled Jurkat cells:

Jurkat cells (American Type Culture Collection, Clone E6-1, ATCC TIB-152) were cultured in RPMI 1640 medium (Nikken Bio Medical Laboratory, CM1101) supplemented with 10% fetal bovine serum (Hyclone, A-1119-L), 100 U/ml penicillin (Gibco BRL, 15140-122) and 100 µg/ml streptomycin (Gibco BRL, 15140-122) in a humidified incubator at 37 °C with 5% CO₂.

30 Jurkat cells were incubated with phosphate balanced solution (PBS, Nissui, 05913) containing 25 µM of 5(-and -6)-carboxyfluorescein diacetate, succinimidyle ester

(CFSE, Dojindo Laboratories, 345-06441) for 20 min at room temperature while gently swirling every 5 min. After centrifugation at 1000 rpm for 5 min, the cell pellet was resuspended with adhesion assay buffer at a cell density of 4×10^6 cells/ml. The adhesion assay buffer was composed of 24 mM Tris-HCl (pH 7.4), 137 mM NaCl, 27 mM KCl, 4 mM glucose, 0.1 % bovine serum albumin (BSA, Sigma, A9647) and 2 mM $MnCl_2$.

Assay procedure (Jurkat cells)

10 The assay solution containing each test compounds was transferred to the VCAM-1 coated plates. The final concentration of each test compounds was 5 μM , 10 μM or various concentrations ranging from 0.0001 μM to 10 μM using a standard 5-point serial dilution. The assay solution containing the labeled Jurkat cells was transferred to the VCAM-1 coated plates at a cell density of 2×10^5 cells per well and incubated for 1 hour at 37 °C. The non-adherent cells were removed by washing the plates 3 times with wash buffer. The adherent cells were broken by addition of 1 % Triton X-100 (Nacalai Tesque, 355-01). Released CFSC was quantified fluorescence measurement in a fluorometer (Wallac, ARVO 1420 multilabel counter).

20 The adhesion of Jurkat cells to VCAM-1 was analyzed by percent binding calculated by the formula:

25 $100 \times (FTS - FBG) / (FTB - FBG) = \% \text{ binding}$, where FTB is the total fluorescent intensity from VCAM-1 coated wells without test compound; FBG is the fluorescent intensity from wells lacking VCAM-1 and FTS is the fluorescent intensity from wells containing the test compound of this invention.

In vitro activity:

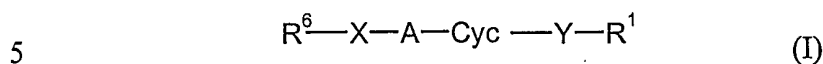
In the Jurkat - VCAM-1 assay the observed IC_{50} value ranges are indicated in Table 5 according to the scheme $B > 10 \mu M \geq A$.

Table 5.

Example No	IC_{50}
2	A
7	A
8	A
9	B
10	B

Claims

1. Compounds of the general formula (I),

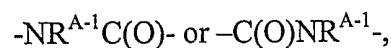


wherein

10 Cyc represents a 5- or 6-membered carbocycle,
which can optionally be substituted with up to two residues R^{cyc} ,

wherein the residues R^{cyc} can independently be selected from the group consisting of halogen, trifluoromethyl, amino, nitro, cyano,

15 A represents an amide moiety of the structure



20 wherein R^{A-1} represents hydrogen or C_1 - C_{10} alkyl,

R^1 represents a 4- to 9-membered saturated, unsaturated or aromatic cyclic residue,

25 which can contain 0 to 3 heteroatoms selected independently from the group N, S and O,

and wherein R^1 is substituted by $-R^{1-1}-Z$,
wherein

30

R^{1-1} represents a bond, -O-, -S-, NR^{1-2} , C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, C_6 or C_{10} aryl, C_3 - C_7 cycloalkyl or a 4-9-membered saturated or unsaturated heterocyclic residue containing up to 3 heteroatoms selected from the group oxygen, nitrogen or sulfur,

5

wherein R^{1-1} can optionally be substituted by 1 to 2 substituents selected from the group R^{1-3} ,

10

wherein R^{1-2} can optionally be hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl or C_2 - C_{10} alkynyl, and

15

wherein R^{1-3} represents hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, C_6 or C_{10} aryl, C_3 - C_7 cycloalkyl or a 4-9-membered saturated or unsaturated heterocyclic residue containing up to 3 heteroatoms selected from the group oxygen, nitrogen or sulfur,

Z represents $-C(O)OR^{Z-1}$, $-C(O)NR^{Z-2}R^{Z-3}$, $-SO_2NR^{Z-2}R^{Z-3}$, $-SO(OR^{Z-1})$, $-SO_2(OR^{Z-1})$, $-P(O)R^{Z-1}(OR^{Z-3})$ or $-PO(OR^{Z-1})(OR^{Z-3})$,

20

wherein R^{Z-2} is hydrogen, C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl, $-C(O)R^{Z-4}$ or $-SO_2R^{Z-4}$,

wherein R^{Z-4} is C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl,

25

R^{Z-1} and R^{Z-3} are independently selected from the group hydrogen, C_1 - C_4 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_6 cycloalkyl, C_6 or C_{10} aryl or benzyl,

wherein R^{Z-1} and R^{Z-3} can optionally be substituted by 1 to 3 substituents selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, halogen, nitro, cyano,

5 and wherein R^1 can optionally be substituted by 0 to 2 substituents R^{1-4} , halogen, nitro, amino, cyano and oxo,

wherein

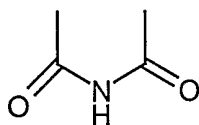
10 R^{1-4} is selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, phenyl, phenoxy, phenylamino, C_3 - C_6 cycloalkyl,

15 R^6 represents phenyl or a 5- to 6-membered aromatic heterocyclic residue containing up to 3 heteroatoms independently selected from the group oxygen, nitrogen or sulfur,

which is substituted by $-NR^{6-2}C(O)NR^{6-3}R^{6-4}$ and can furthermore optionally be substituted by halogen,

20

wherein R^{6-2} and R^{6-3} are independently selected from the group hydrogen or C_1 - C_4 alkyl, or together form a group



25

and wherein R^{6-4} represents phenyl,

wherein R^{6-4} can optionally be substituted by 1-2 substituents selected from the group C_1 - C_4 alkyl, C_1 - C_4 alkyloxy, halogen, nitro, trifluoromethyl, trifluoromethoxy or cyano,

5

X represents bond or $-CR^{X-1}R^{X-2}-$,

wherein R^{X-1} and R^{X-2} can be independently selected from the group hydrogen, C_1 - C_4 alkyl, C_2 - C_4 alkenyl, C_2 - C_4 alkynyl,

10

Y represents an amide moiety of the structure

$-NR^{Y-1}C(O)-$ or $-C(O)NR^{Y-1}-$,

15

wherein R^{Y-1} represents hydrogen or C_1 - C_4 alkyl,

wherein the moiety A-Cyc-Y represents a γ -amino acid,

and pharmaceutically acceptable salts thereof.

20

2. Compounds of general formula (I) according to claim 1,

wherein Cyc represents a 5-membered carbocycle.

25

3. Compounds of general formula (I) according to any one of claims 1 or 2,

wherein R^1 represents a 1,4-substituted phenyl ring.

30

7. Compounds according to any one of claims 1 to 5 for the treatment of diseases.
- 5 8. The use of a compound according to any one of claims 1 to 5 in the manufacture of a medicament for the treatment or the prevention of a condition mediated by integrins.
- 10 9. The use according to claim 8. in the manufacture of a medicament for the treatment or the prevention of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.
- 15 10. Pharmaceutical composition, comprising compounds according to any one of claims 1 to 5 and a pharmaceutically acceptable carrier.