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Depen. cia 2º23 DIVISION DE NUEVAS CREACIONES

Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E S D

TRAMITE 02 - EVENTO 01 ACTUACION 329

Referencia Expediente No 00 034 117
Solicitante BOEHRINGER INGELHEIM PHARMA KG
Asunto Solicitud de patente para COMPUESTOS
ANTI TUMORALES ACTIVADOS POR PAF
Fecha de solicitud 11 de Mayo de 2000

1 Yo Emilio FERRERO obrando como apoderado de la solicitud en referencia atentamente presento con este escrito copia certificada de la solicitud de patente estadounidense numero 60/134 136 del 14 de Mayo de 1999 junto con la respectiva traducción de las reivindicaciones y certificaciones de la cual se reclama prioridad en esta solicitud de patente

2 Ruego a la Señora Jefe se sirva aceptar este escrito con sus anexos y se continúe con el trámite de la solicitud

Bogotá 10 AGO 2000
Señora Jefe



EMILIO FERRERO
T P A No 31 929

COLO 10903 801 039 2
RCB/mrm Id 256

NIT 860 041 367 3



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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 04, 2000

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/134,136

FILING DATE: May 14, 1999



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

W. Montgomery
W. MONTGOMERY
Certifying Officer

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (b)(2)

A/PROV 98

96

Docket: I/1087PV
Number

Type a plus sign
(+) inside this box

+

INVENTORS(S)/APPLICANT(S)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
PARK	John	E.	Biberach, Germany
RETTIG	Wolfgang	J.	Biberach, Germany

TITLE OF THE INVENTION (280 characters max)

FAP-Activated Anti-Tumour Compounds

CORRESPONDENCE ADDRESS

Dr. R.P. Raymond
Boehringer Ingelheim Corp.
900 Ridgebury Road, P.O. Box 368
Ridgefield

STATE Connecticut ZIP CODE 06877 COUNTRY USA

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of Pages	39	☐ Small Entity Statement
☒ Drawing(s)	Number of Sheets	5	☐ Other (specify)

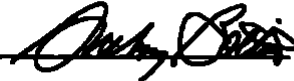
METHOD OF PAYMENT (check one)

☐ A check or money order is enclosed to cover the Provisional filing fee		PROVISIONAL FILING FEE AMOUNT (\$)	\$150.00
☒ The Commissioner is hereby authorized to charge the filing fee and credit Deposit Account Number A duplicate copy of this request is enclosed for the convenience of the Assistant Commissioner.	02-2955		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- ☒ No.
☐ Yes, the Name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE: 

Date:

May 14, 1999

TYPED or PRINTED NAME: Anthony P. Bottino

Registration No.: 41,629

(if appropriate)

- ☒ Additional inventors are being named on separately numbers sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

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ADDITIONAL INVENTORS (page 2 of 2)

INVENTORS(S)/APPLICANT(S)			
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
LESTER	Martin	A.	Ulm, Germany
PETERS	Stefan		Ingelheim/Rhein, Germany
GARIN-CHESA	Pilar		Biberach, Germany
FIRESTONE,	Raymond		Stamford, Connecticut

60134136-051499

FAP-activated anti-tumour compounds

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Field of the invention

The present invention relates to the field of tumour treatment by administration of a prodrug that is converted into a drug at the site of the tumour. In particular, the invention relates to prodrugs which may be converted into a drug by the catalytic action of FAP α , their manufacture and pharmaceutical use.

Background and prior art

The human fibroblast activation protein (FAP α) is a M_r 95,000 cell surface molecule originally identified with monoclonal antibody (mAb) F19 (Rettig *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85, 3110-3114; Rettig *et al.* (1993) *Cancer Res.* 53, 3327-3335). The FAP α cDNA codes for a type II integral membrane protein with a large extracellular domain, trans-membrane segment, and short cytoplasmic tail (Scanlan *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91, 5657-5661; WO 97/34927). FAP α shows 48 % amino acid sequence identity to the T-cell activation antigen CD26, also known as dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5), a membrane-bound protein with dipeptidyl peptidase activity (Scanlan *et al.*, *loc. cit.*). FAP α has enzymatic activity and is a member of the serine protease family, with serine 624 being critical for enzymatic function (WO 97/34927). Work using a membrane overlay assay revealed that FAP α dimers are able to cleave Ala-Pro-7-amino-4-trifluoromethyl coumarin, Gly-Pro-7-amino-4-trifluoromethyl coumarin, and Lys-Pro-7-amino-4-trifluoromethyl coumarin dipeptides (WO 97/34927).

FAP α is selectively expressed in reactive stromal fibroblasts of many histological types of human epithelial cancers, granulation tissue of healing wounds, and malignant cells of certain bone and soft tissue sarcomas. Normal adult tissues are generally devoid of detectable FAP α , but some foetal mesenchymal tissues transiently express the molecule. In contrast, most of the common types of epithelial cancers, including >90% of breast, non-small-cell lung, and colorectal carcinomas, contain FAP α -reactive stromal fibroblasts (Scanlan *et al.*, *loc. cit.*). These FAP α^+ fibroblasts accompany newly formed tumour blood vessels, forming a distinct cellular compartment interposed between the tumour capillary endothelium and the basal aspect of malignant epithelial cell clusters (Welt *et al.* (1994) *J. Clin. Oncol.* 12(6), 1193-1203). While FAP α^+ stromal fibroblasts are found in both primary and metastatic carcinomas, the benign and premalignant epithelial lesions tested (Welt *et al.*, *loc.*

cit.), such as fibroadenomas of the breast and colorectal adenomas, only rarely contain FAP α ⁺ stromal cells. Based on the restricted distribution pattern of FAP α in normal tissues and its uniform expression in the supporting stroma of many malignant tumours, clinical trials with ¹³¹I-labeled mAb F19 have been initiated in patients with metastatic colon carcinomas (Welt *et al.*, *loc. cit.*).

For new cancer therapies based on cytotoxic or cytostatic drugs, a major consideration is to increase the therapeutic index by improving the efficacy of cancerous tissue killing and/or reducing the toxicity for normal tissue of the cytotoxic or cytostatic agents. To increase specificity of tumour tissue killing and reduce toxicity in normal tissues, trigger mechanisms can be designed so that the toxic agents synthesised in their prodrug or inactive forms are rendered active when and where required, notably in the cancerous tissues (Panchal (1998) *Biochem. Pharmacol.* 55, 247-252). Triggering mechanisms may include either exogenous factors such as light or chemicals or endogenous cellular factors, such as enzymes with restricted expression in cancer tissues. Another concept, that has been further elaborated, is called 'antibody-directed enzyme prodrug therapy' (ADEPT) or 'antibody-directed catalysis' (ADC) (Huennkens (1994) *Trends Biotechnol.* 12, 234-239; Bagshawe (1994) *Clin. Pharmacokinet.* 27, 368-376; Wang *et al.* (1992) *Cancer Res.* 52, 4484-4491; Sperker *et al.* (1997) *Clin. Pharmacokinet.* 33(1), 18-31). In ADEPT, an antibody directed at a tumour-associated antigen is used to target a specific enzyme to the tumour site. The tumour-located enzyme converts a subsequently administered prodrug into an active cytotoxic agent. The antibody-enzyme conjugate (AEC) binds to a target antigen on cell membranes or to free antigen in extracellular fluid (ECF). A time interval between giving the AEC and prodrug allows for the AEC to be cleared from normal tissues so that the prodrug is not activated in the normal tissues or blood. However, some disadvantages of ADEPT are related to the properties of the AEC (Bagshawe, *loc. cit.*). For example, in humans, only a small fraction of the administered dose of the targeting AEC binds to tumour tissue and the remainder is distributed through body fluids from which it is cleared with significant time delays. Even very low concentrations of targeted enzyme can catalyse enough prodrug to have toxic effects because plasma and normal ECF volumes are much greater than those of tumour ECF. The AEC may also be immunogenic, thus preventing repeat administration, in many instances.

The problem underlying the present invention was to provide methods and means for improving normal tissue tolerability of cytotoxic or cytostatic agents with known efficacy against tumour tissues.

Disclosure of the invention

The present invention relates to enzyme-activated anti-tumour compounds. In particular, the invention provides prodrugs that are capable of being converted into drugs by the catalytic action of endogenous fibroblast activating protein alpha (FAP α) shown to reside in human cancer tissues. Preferably, a prodrug of the present invention is capable of being converted into a drug by the catalytic action of FAP α , said prodrug having a cleavage site which is recognised by FAP α , and said drug being cytotoxic or cytostatic against cancer cells under physiological conditions.

In the context of this invention, a "drug" shall mean a chemical compound that may be administered to humans or animals as an aid in the treatment of disease. In particular, a drug is an active pharmacological agent.

The term "cytotoxic compound" shall mean a chemical compound which is toxic to living cells, in particular a drug that destroys or kills cells. The term "cytostatic compound" shall mean a compound that suppresses cell growth and multiplication and thus inhibits the proliferation of cells. Examples for cytotoxic or cytostatic compounds suitable for the present invention are doxorubicin, methotrexate, melphalan, and analogs of other chemotherapeutic agents such as 9-aminocamptothecin.

A "prodrug" shall mean a compound that, on administration, must undergo chemical conversion by metabolic processes before becoming an active pharmacological agent. In particular, a prodrug is a precursor of a drug. In the context of the present invention, the prodrug is significantly less cytotoxic or cytostatic than the drug it is converted into upon the catalytic action of FAP α . The expert knows methods of determining cytotoxicity of a compound, see e.g. example 6 herein, or Mosmann ((1983) *J. Immun. Meth.* 65, 55-63). Preferably, the prodrug is at least three times less cytotoxic as compared to the drug in an *in vitro* assay.

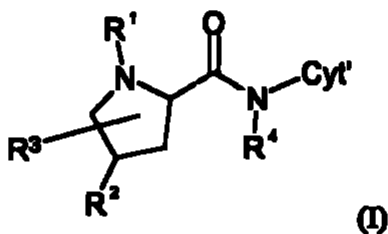
A "drug being cytostatic or cytotoxic under physiological conditions" shall mean a chemical compound which is cytostatic or cytotoxic in a living human or animal body, in particular a compound that kills cells or inhibits proliferation of cells within a living human or animal body.

A "prodrug having a cleavage site which is recognised by FAP α " shall mean a prodrug which can act as a substrate for the enzymatic activity of FAP α . In particular, the enzymatic

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- activity of FAP α can catalyse cleavage of a covalent bond of the prodrug under physiological conditions. By cleavage of this covalent bond, the prodrug is converted into the drug, either directly or indirectly. Indirect activation would be the case if the cleavage product of the FAP α catalysed step is not the pharmacologically active agent itself but undergoes a further reaction step, e.g. hydrolysis, to become active. More preferably, the cleavage site of the prodrug is specifically recognised by FAP α , but not by other proteolytic enzymes present in the human or animal body. Also preferably, the cleavage site is specifically recognised by FAP α , but not by proteolytic enzymes present in human or animal body fluids, especially plasma. In a particularly preferred embodiment, the prodrug is stable in plasma, other body fluids, or tissues, in which biologically active FAP α is not present or detectable. Preferably, in an in vitro assay as carried out in Example 7 herein, more than 50%, more preferably more than 80%, more preferably more than 90% of the prodrug are still present in a solution containing 10% (v/v) of plasma after 24 h at 37°C. The cleavage site should most preferably be specific for FAP α . In a preferred embodiment, the cleavage site comprises a L-proline residue which is linked to a cytotoxic or cytostatic drug via an amide bond. An example of this class is a doxorubicin-peptide conjugate. FAP α may catalyse the cleavage of a peptidic bond between the C-terminal amino acid residue of the peptide, which is preferably L-proline, and the cytotoxic or cytostatic compound.
- Preferred compounds show at least 10% conversion to free drug, under standard conditions listed below. More preferred are compounds that show at least 20% conversion to free drug, under standard conditions. Even more preferred are compounds that show at least 50% conversion to free drug, under standard conditions. In this context, standard conditions are defined as follows: Each compound is dissolved in 50 mM Hepes buffer, 150 mM NaCl, pH 7.2, at a final concentration of 20 μ M and incubated with 100 ng CD8FAP α (see example 4) for 16 hours at 37 °C. Release of free drug by CD8FAP α is determined as described in example 5.

Preferably, the present invention relates to a compound of the general formula (I)

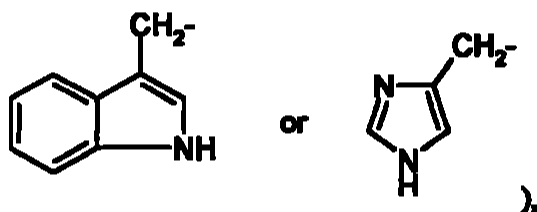


or the pharmaceutically acceptable salts thereof,

wherein R^1 is a residue of formula Cg-A, Cg-B-A, or Cg-D-B-A, wherein

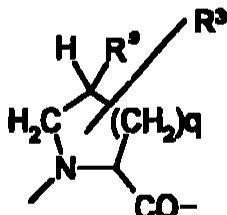
Cg is H, or a capping group with the structure R^2 -CO, R^2 -O-CO-, R^2 -NH-CO-, R^2 -SO₂- or R^2 -, wherein R^2 is an C₁-C₆-alkyl, C₃-C₆-cycloalkyl, aryl, or heteroaryl substituent; preferably Cg is an acetyl, benzyl, D-alanyl, (R)-H₂NCH(CH₃)-, or H₂NCOCH₂CH₂- substituent or another capping group for the protection of the N-terminal amino function;

and wherein A and B are units derived from amino carboxylic acids with the formula $[-NR^6-(X)_p-CO]-$ wherein X is CR⁷R⁸ and wherein R⁶, R⁷ and R⁸ are the same or different, and are H, C₁-C₆-alkyl (preferably CH₃-, CH₃CH₂-, CH₃CH₂CH₂-, (CH₃)₂CH-, CH₃CH₂CH₂CH₂-, (CH₃)₂CHCH₂-, CH₃CH₂CH(CH₃)-, (CH₃)₃C-, HOCH₂-, CH₃CH(OH)-, CH₃CH(OH)CH₂CH₂-, HOCH₂CH₂CH₂CH₂-, H₂NCH₂CH₂CH₂-, H₂NCH₂CH₂CH₂CH₂-, H₂NCH₂CH(OH)CH₂CH₂-, H₂NC(=NH)NHCH₂CH₂CH₂-, HSCH₂-, CH₃SCH₂CH₂-, HOOCCH₂-, HOOCCH₂CH₂-, H₂NC(=O)CH₂-, H₂NC(=O)CH₂CH₂-, benzyl, para-hydroxy-benzyl,



C₃-C₆-cycloalkyl (preferably cyclohexyl), aryl (preferably phenyl), or heteroaryl, and p is 1, 2, 3, 4, 5, and wherein the configuration at CR⁷R⁸ can be R or S; if R⁷ is other than H, then R⁸ is preferably H; R⁶ is preferably H; if p is greater than one, R⁷ and R⁸ are preferably H;

A and B can also be units derived from cyclic amino carboxylic acids with the structure



wherein R⁹ is H, C₁-C₆-alkyl, OH, or NH₂, and q is 1 or 2;

and wherein D is a unit derived from an amino carboxylic acid as defined for A and B, or D is an oligomer comprising two or more such units, which may be the same or different, preferably 2 to 10 such units;

and R^2 , R^3 are the same or different, and are hydrogen, hydroxyl, amino, C_1 - C_6 -alkyl, C_1 - C_6 -alkyl-amino, di- C_1 - C_6 -alkyl-amino, C_1 - C_6 -alkyl-oxy, thiol, C_1 - C_6 -alkyl-thio, $=O$, $=NH$, $-CHO$, $-COOH$, $-COOCH_3$, $-COOCH_2CH_3$, $-CONH_2$, halogen, C_3 - C_8 -cycloalkyl, aryl, or heteroaryl;

and R^4 is H, C_1 - C_6 -alkyl, C_3 - C_8 -cycloalkyl, aryl, or heteroaryl;

and Cyt' is the residue of a cytotoxic or cytostatic compound.

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Unless indicated otherwise, the simple stereoisomers as well as mixtures or racemates of the stereoisomers are included in the invention.

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" C_1 - C_6 -alkyl" generally represents a straight-chained or branched hydrocarbon radical having 1 to 6 carbon atoms which may optionally be substituted by one or several hydroxyl, amino, C_1 - C_6 -alkyl-amino, di- C_1 - C_6 -alkyl-amino, C_1 - C_6 -alkyl-oxy, thiol, C_1 - C_6 -alkyl-thio, $=O$, $=NH$, $-CHO$, $-COOH$, $-CONH_2$, $-NHC(=NH)NH_2$, halogen, C_3 - C_8 -cycloalkyl, aryl, or heteroaryl substituents, which may be identical to one another or different.

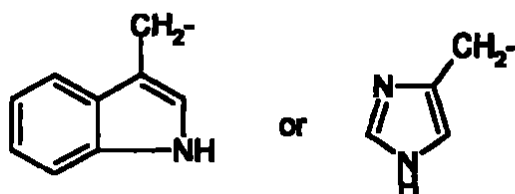
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The following radicals may be mentioned by way of example:

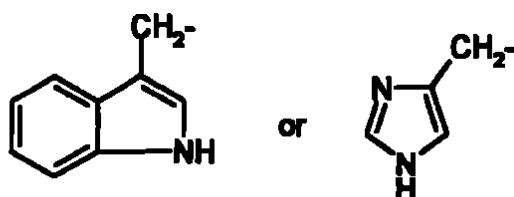
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Methyl, ethyl, propyl, 1-methylethyl (isopropyl), butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl and 1-ethyl-2-methylpropyl, $HOCH_2-$, $CH_3CH(OH)-$, $CH_3CH(OH)CH_2CH_2-$, $HOCH_2CH_2CH_2CH_2-$, $H_2NCH_2CH_2CH_2-$, $H_2NCH_2CH_2CH_2CH_2-$, $H_2NCH_2CH(OH)CH_2CH_2-$, $H_2NC(=NH)NHCH_2CH_2CH_2-$, $HSCH_2-$, $CH_3SCH_2CH_2-$, $HOOCCH_2-$, $HOOCCH_2CH_2-$, $H_2NC(=O)CH_2-$, $H_2NC(=O)CH_2CH_2-$, benzyl, para-hydroxy-benzyl,

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If C₁-C₆-alkyl is substituted, the substituents are preferably hydroxyl, amino, dimethylamino, diethylamino, thiol, methyl-thiol, methoxy, ethoxy, =O, =NH, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, -CONH₂, -NHC(=NH)NH₂, cyclohexyl, phenyl, benzyl, para-hydroxy-benzyl,



If C₁-C₆-alkyl is substituted with aryl or heteroaryl, C₁-C₆-alkyl is preferably C₁, more preferably a methylene group.

"C₃-C₈-Cycloalkyl" generally represents cyclic hydrocarbon radical having 3 to 8 carbon atoms which may optionally be substituted by one or several hydroxyl, amino, C₁-C₆-alkyl-amino, di- C₁-C₆-alkyl-amino, C₁-C₆-alkyl, C₁-C₆-alkyloxy, thiol, C₁-C₆-alkyl-thio, =O, =NH, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, -CONH₂, -NHC(=NH)NH₂, or halogen substituents, which may be identical to one another or different.

"Aryl" generally represents an aromatic ring system with 6 to 10, preferably 6 carbon atoms which may optionally be substituted by one or several hydroxyl, amino, C₁-C₆-alkyl-amino, di- C₁-C₆-alkyl-amino, C₁-C₆-alkyl, C₁-C₆-alkyloxy, thiol, C₁-C₆-alkyl-thio, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, -CONH₂, or halogen substituents, which may be identical to one another or different, and which optionally may be benzocondensed. Aryl substituents may be preferably derived from benzene, preferred examples being phenyl, 2-hydroxy-phenyl, 3-hydroxy-phenyl, 4-hydroxy-phenyl, 4-amino-phenyl, 2-amino-phenyl, 3-amino-phenyl.

If aryl is substituted, the substituents are preferably methyl, ethyl, propyl, 1-methylethyl (isopropyl), butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, hydroxyl, amino, dimethyl-amino, diethyl-amino, thiol, methyl-thiol, methoxy, ethoxy, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, or -CONH₂.

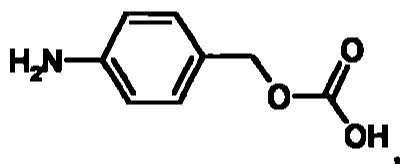
"Heteroaryl" generally represents a 5 to 10-membered aromatic heterocyclic ring system, containing 1 to 5 heteroatoms selected from the group of nitrogen, oxygen, or sulfur, which may optionally be substituted by one or several hydroxyl, amino, C₁-C₆-alkyl-amino, di- C₁-C₆-alkyl-amino, C₁-C₆-alkyl, C₁-C₆-alkyloxy, thiol, C₁-C₆-alkyl-thio, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, -CONH₂, or halogen substituents, which may be identical to one another or different, and which optionally may be benzocondensed. Heteroaryl substituents

may preferably be derived from furane, pyrrole, thiophene, pyridine, thiazole, isoxazole, pyrazole, imidazole, benzofuran, thianaphthene, indole, benzimidazole, indazole, chinoline, pyridazine, pyrimidine, pyrazin, chinazoline, pyrane, purine, adenine, guanine, thymine, cytosine, uracil.

If heteroaryl is substituted, the substituents are preferably methyl, ethyl, propyl, 1-methyl-ethyl (isopropyl), butyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl, hydroxyl, amino, dimethyl-amino, diethyl-amino, thiol, methyl-thiol, methoxy, ethoxy, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, or -CONH₂.

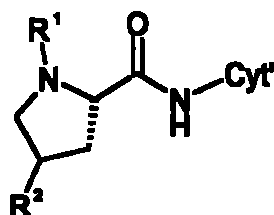
"Residue of a cytotoxic or cytostatic compound" means that the compound H₂N-Cyt', which is released upon cleavage of the amide bond shown in formula (I), is either cytotoxic or cytostatic itself, or may be converted into a cytotoxic or cytostatic compound in a subsequent step.

In the latter case, -Cyt' may be a residue of formula -L-Cyt'', wherein L is a linker residue derived from a bifunctional molecule, for instance a diamine H₂N-L'-NH₂, an amino alcohol H₂N-L'-OH, for example p-amino-benzyl alcohol (PABOH), an amino carbonate, for example



or an amino carboxylic acid H₂N-L'-COOH, like glycine, alanine, or any other naturally occurring or synthetic amino carboxylic acid. If -Cyt' is of formula -L-Cyt'', the compound H₂N-L'-Cyt'' is generated by the enzymatic cleavage of the amide bond shown in formula (I). The compound H₂N-L'-Cyt'' may be cytotoxic or cytostatic itself or the linker residue cleaved off from Cyt'' in a subsequent step releasing the cytotoxic or cytostatic agent. For example, the compound H₂N-L'-Cyt'' may be hydrolysed under physiological conditions into a compound H₂N-L'-OH and the cytotoxic or cytostatic compound H-Cyt'', which is the active therapeutic agent (In the following, only the term Cyt' is used for both Cyt' and Cyt'', and only the term L is used for both L and L', for simplicity).

Preferred compounds are of the general formula (II)



(II).

wherein the substituents have the meaning as described before,

i.e. the α carbon atom of the proline or hydroxy-proline residue is of (S) configuration.

In a preferred embodiment, the α carbon atom is of (S) configuration and R² is H.

If R² is OH, it is preferably in trans position.

R², R³ are preferably H, methyl, ethyl, propyl, isopropyl, phenyl, methoxy, ethoxy, or OH, more preferably H.

R⁴ is preferably H, methyl, ethyl, propyl, isopropyl, or phenyl, more preferably H.

H₂N-Cyt' is preferably doxorubicin (Dox). Other cytotoxic or cytostatic residues Cyt' may be derived for example from daunomycin, epirubicin, idarubicin, methotrexate, trimetrexate, pyritrexim, 5,10-dideazatetrahydrofolatepyrimetamine, trimethoprim, 10-propargyl-5,8-dideazaolate, 2,4-diamino-5(3',4'-dichlorophenyl)-6-methylpyrimidine, aminoglutethimide, goreserelin, melphalan, chlorambucil, analogs of other chemotherapeutic agents such as 9-aminocamtotecin (for examples see e.g. Burris HA, r. d. and S. M. Fields (1994). "Topoisomerase I inhibitors. An overview of the camptothecin analogs. [Review]." *Hematol. Oncol. Clin. North Am.* 8(2): 333-355; Iyer, L. and M. J. Ratain (1998). "Clinical pharmacology of camptothecins. [Review] [137 refs]." *Cancer Chemother. Pharmacol.* 42 Suppl: S31-S43.)

In formula (I), Cyt' may also be a biological effector molecule which either directly or indirectly effects destruction of tumour cells, like for example TNFα.

Preferred examples of amino carboxylic acids from which the A, B, and D units (or the two or more units making up D) may be derived are glycine (Gly), or the D- or, more preferably, the L-forms of alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), cysteine (Cys), methionine (Met), serine (Ser), threonine (Thr), lysine (Lys), arginine (Arg), histidine (His), aspartic acid (Asp), glutamic

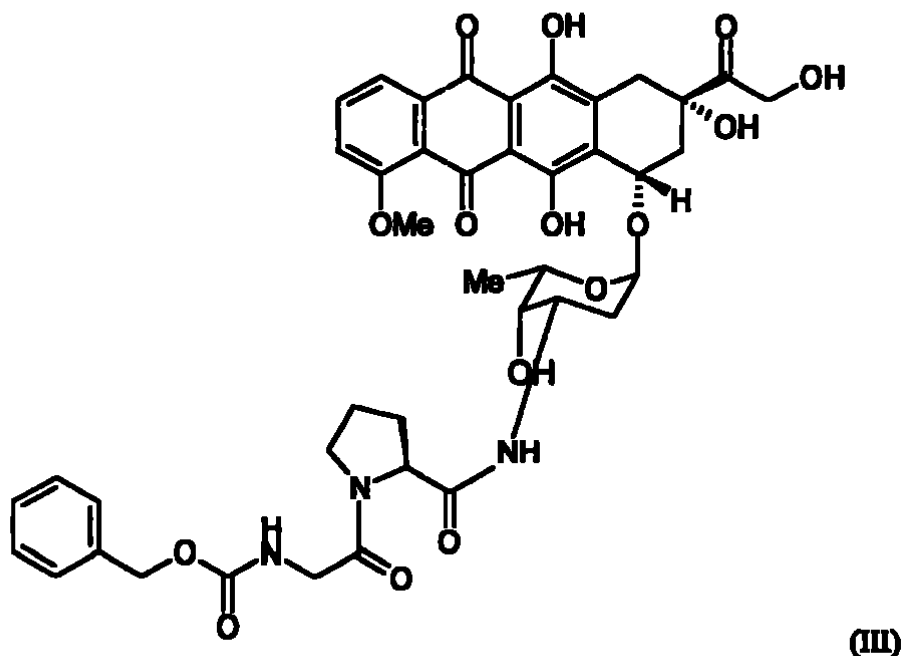
acid (Glu), asparagine (Asn), glutamine (Gln), proline (Pro), 4-hydroxy-proline, 5-hydroxy-lysine, norleucine, 5-hydroxynorleucine, 6-hydroxynorleucine, ornithine, or cyclohexylglycine.

- Preferred compounds have the general formula (I) wherein the A unit is derived from L-proline, glycine, L-norleucine, L-5-hydroxynorleucine, L-6-hydroxynorleucine, L-cyclohexylglycine, 5-hydroxylysine, L-lysine, or L-arginine.

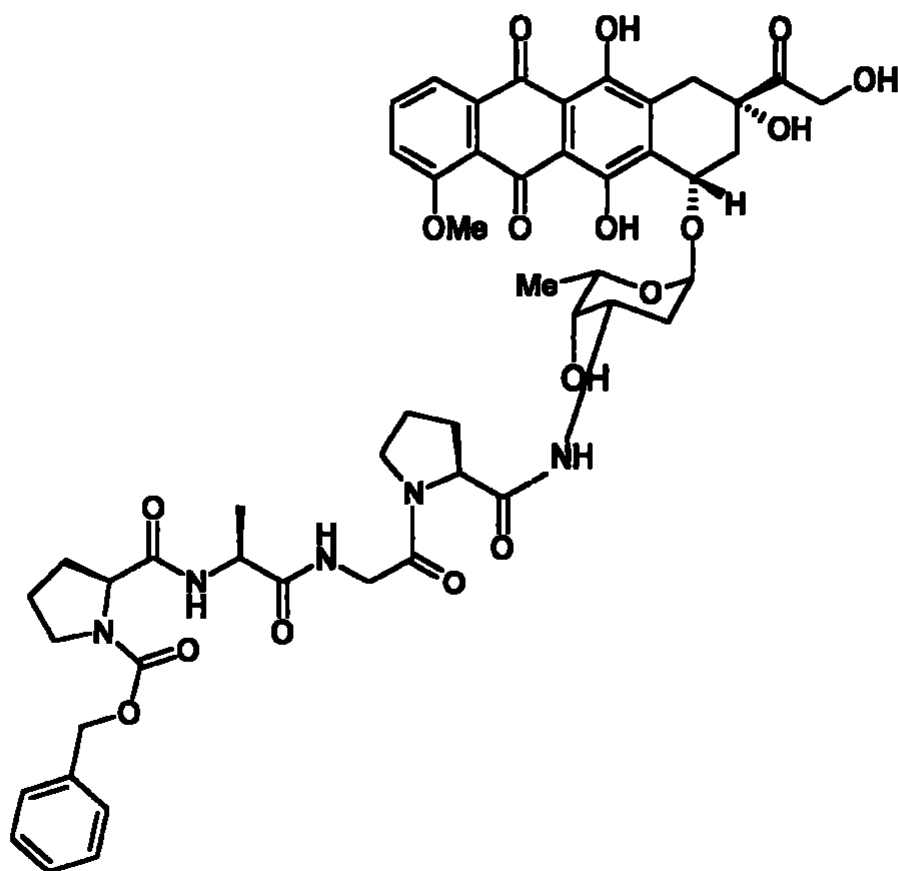
- In preferred embodiments, D is either not present, or D consists of one unit derived from an amino carboxylic acid as defined.

Preferred capping groups Cg are acetyl (Ac), succinimidyl (Suc), D-alanyl, benzyloxycarbonyl (Cbz or Z), or macromolecules such as polyethylene glycol.

- Particularly preferred compounds of the invention are Z-Gly-(L)-Pro-Doxorubicin of formula (III)



and Z-(L)-Pro-(L)-Ala-Gly-(L)-Pro-Doxorubicin of formula (IV)



(IV)

If the part Cg-D-B-A of formula (I) contains two or more sulfur atoms, the compound of the invention may contain one or more disulfide bonds.

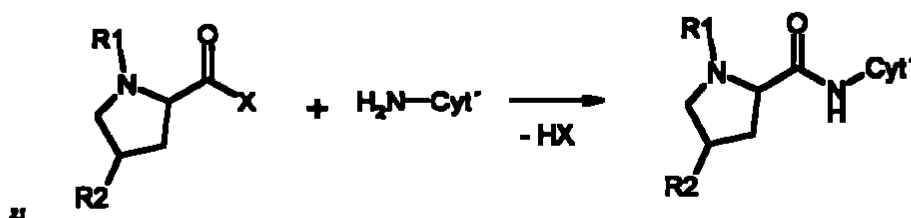
One class of cytotoxic or cytostatic compounds which may be used for the present invention has a primary amino function which is available for formation of an amidic bond as shown in formula (I), like doxorubicin. In this case, a linker molecule L is not necessary. If a cytostatic or cytotoxic compound does not have such an amino function, such a function may be created in such a compound by way of chemical modification, e.g. by introducing or converting a functional group or attaching a linker molecule to the compound. A linker molecule may also be inserted between the oligomeric part (i.e. the part comprising the amino carboxylic residues) and the cytostatic or cytotoxic part of the compound of the invention to ensure or optimise cleavage of the amide bond between the oligomeric part and the cytotoxic or cytostatic part. If a linker molecule is present, i.e. in compounds containing the structure L-Cyt', the bond between L and Cyt' is preferably an amidic or ester bond. In a preferred embodiment, such a linker molecule is hydrolysed off the cytostatic or cytotoxic compound under physiological conditions after the enzymatic cleavage and thus the free cytostatic or cytotoxic compound is generated. In any case, the compound of the invention must have the property of being cleavable upon the catalytic action of FAP α and, as a direct

or indirect consequence of this cleavage, releasing under physiological conditions a cytostatic or cytotoxic compound.

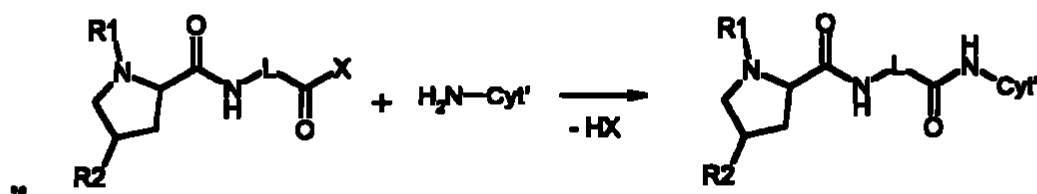
In a further aspect, the present invention relates to a prodrug that is capable of being converted into a drug by the catalytic action of FAP α , said prodrug having a cleavage site which is recognised by FAP α , and said drug being cytotoxic or cytostatic under physiological conditions. Such a prodrug preferably comprises an oligomeric part comprising two or more amino carboxylic residues and a cytotoxic or cytostatic part, wherein the C-terminal amino carboxylic residue of the oligomeric part is D- or L-proline, or D- or L-hydroxyproline, and the C-terminal carboxy function is linked to the cytotoxic or cytostatic part by an amide bond which may be cleaved by the catalytic action of FAP α . The oligomeric part is preferably a peptide. Preferably, the oligomeric part comprises two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve amino carboxylic acid residues, more preferably two, three, or four amino carboxylic residues. The N-terminal amino function is preferably protected by a capping group.

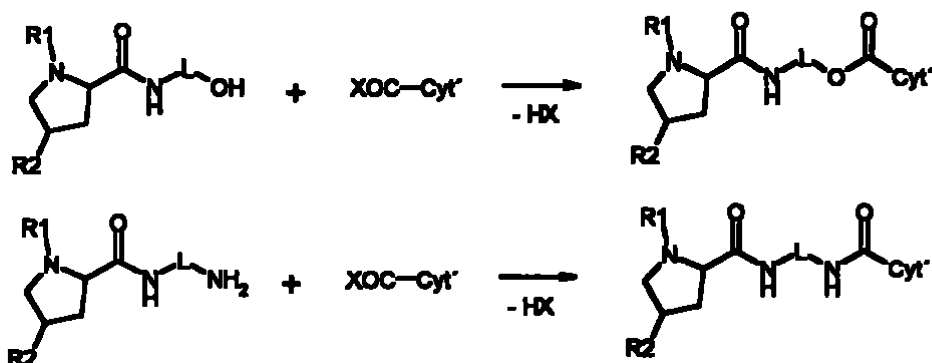
The compounds of the invention may be synthesized by processes known in the art (E. Wünsch, *Synthese von Peptiden*, in "Methoden der organischen Chemie", Houben-Weyl (Eds. E. Müller, O. Bayer), Vol. XV, Part 1 and 2, Georg Thieme Verlag, Stuttgart, 1974).

For example, the compounds could be synthesized in a block synthetic fashion by condensation of the terminal carboxy function of the oligomeric part, wherein X may be OH or an activation leaving group, with the amino group of the cytotoxic or cytostatic molecule H₂N-Cyt' resulting in an amide formation.

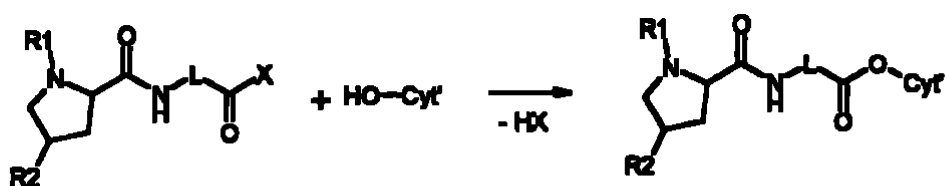


If a linker residue (L) is required between the oligomeric part and the cytotoxic or cytostatic agent the block synthesis can be done in the same manner.





If the cytotoxic or cytostatic bears a carboxy function for the attachment to the oligomeric part, the linker molecule can be an imine or an amino alcohol and the block synthesis of such compounds can be carried out in a similar way by reaction of the activated XOC-Cyt' with either the hydroxy or the amino component.



- 19 If the cytotoxic or cytostatic reagent has a hydroxy function which is suitable for the coupling to the oligomeric part the linker residue may be an amino carboxylic acid and a block synthesis can be done similarly.

20 If necessary, other functional groups in the units Cyt', L, hydroxyproline, A, B and D which shall not react during the assembly of the target molecules may be protected by suitable protecting groups. Suitable protecting groups are well known from the state of the art (P.G.M. Wuts, "Protective groups in organic synthesis", John Wiley and Sons Inc., New York 1991). These protecting groups are removed at the end of the synthesis.

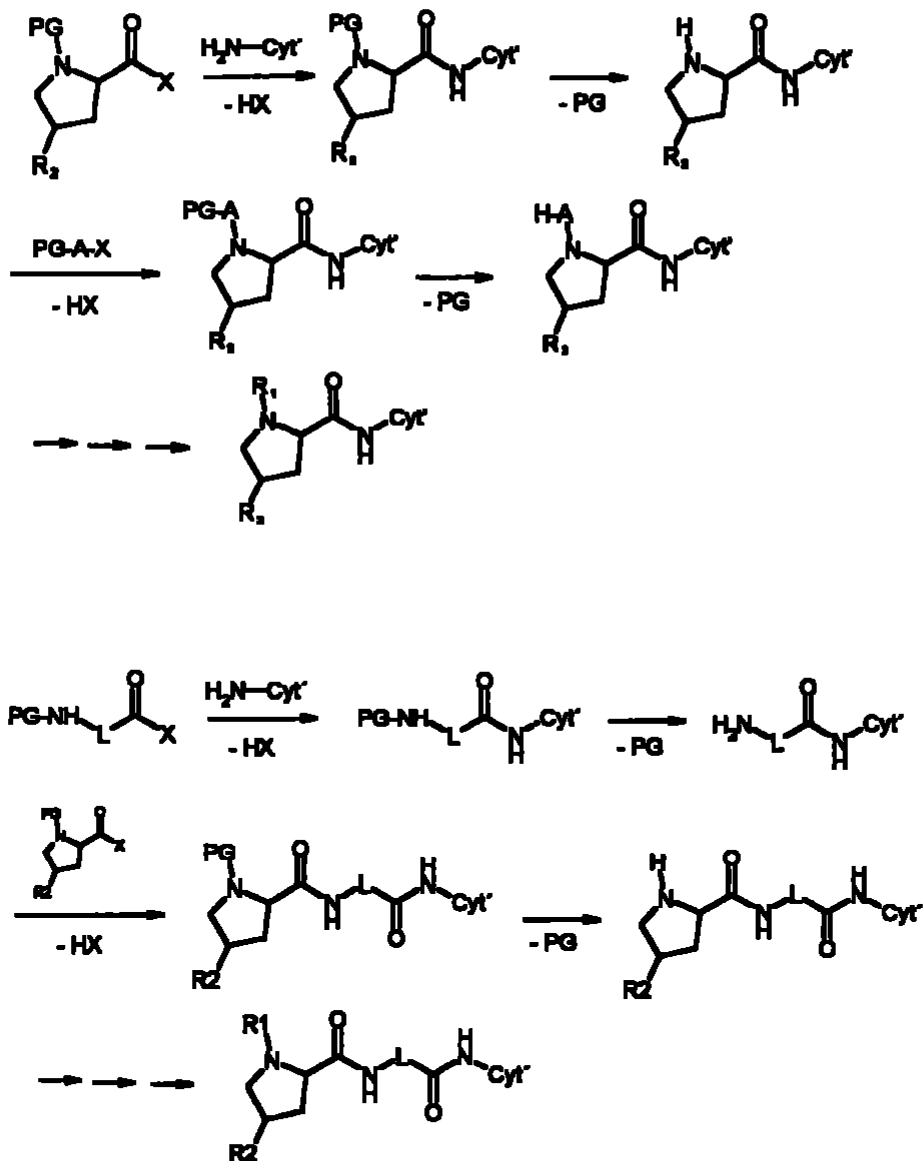
- 21 To achieve such ester- or amide formations, it may be necessary to activate the carbonyl group of the carboxylic acid for a nucleophilic attack of an amine or alcohol, i.e. X to be an activation group or leaving group which is suitable to be substituted by an amino group. This activation can be done by conversion of the carboxylic acid into an acid chloride or acid fluoride or by conversion of the carboxylic acid into an activated ester, for instance a N-hydroxysuccinimidyl ester or a pentafluorophenyl ester. Another method of activation is the transformation into an symmetrical or unsymmetrical anhydride. Alternatively, the formation of the amide- or ester bonds can be achieved by the use of in situ coupling reagents like benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)

(E. Frerot et al., *Tetrahedron*, 1991, 47, 259-70), 1,1'-carbonyldimidazole (CDI) (K. Akaji et al., *THL*, 35, 1994, 3315-18), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (R. Knorr et al., *THL*, 30, 1989, 1927-30), 1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) (B. Blankenmeyer-Menge et al., *THL*, 31, 1990, 1701-04).

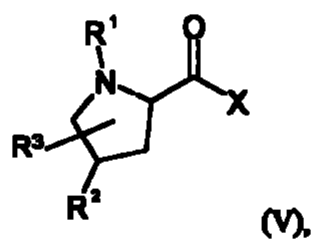
As an alternative to the block synthesis the molecules in the general formula (I) can be assembled in a step by step manner starting at the right hand side by stepwise condensation reactions of the respective monomers Cyt', L, proline or hydroxyproline, A, B and D. If D comprises more than one amino carboxylic unit, these may also be added stepwise. For the condensation reaction the same above mentioned coupling methods can be applied. Since the units L, proline/hydroxyproline, A, B and D are at least bifunctional molecules containing an amino- and (at least the units A, B, D, and proline/hydroxyproline) a carboxy group, the amino group needs to be blocked by a protecting group (PG) prior to the activation of the carboxylic function. For the protection of the amino groups the Fmoc or the Boc group can be applied. After the coupling reaction the amino protecting group has to be removed and the coupling with the next Fmoc- or Boc-protected unit can be carried out. If necessary, other functional groups in the units Cyt', L, hydroxyproline, A, B and D which shall not react during the assembly of the target molecules may be protected by suitable protecting groups. These protecting groups are removed at the end of the synthesis.

Capping groups as defined in the context of formula (I) may also serve as protection groups, in particular when the last (N-terminal) amino carboxylic acid unit is added. In this latter case the protecting group is not removed as it is a part of the target molecule. Alternatively, the capping group may be added after the last amino carboxylic acid unit has been coupled and deprotected.

The step by step synthesis is outlined in the following schemes. The second scheme is exemplary as the linker residue as well as the Cyt' residue may contain other functional groups as indicated in this scheme (see above):



Accordingly, a further aspect of the invention is a process for the production of a compound of formula (I), characterised in that a compound of the general formula (V)



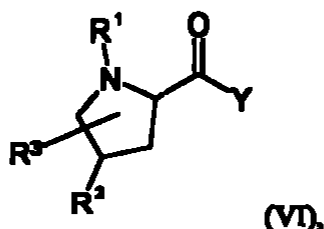
wherein X may be OH, or a leaving group which is suitable to be substituted by an amino group,

and wherein the other substituents have the meaning as described before,

- is reacted with a compound $\text{HN}(\text{R}^4)\text{-Cyt}'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

Preferably, X within formula (V) is a leaving group, for example -Cl, -F, N-hydroxysuccinimidyl, pentafluorophenyl, or a carboxylate. Alternatively, X may be OH, and condensation is achieved by the use of an in situ coupling reagent, for example benzotriazole-1-yloxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), 1,1'-carbonyldimidazole (CDI), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), or 1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT).

- A further aspect of the invention is a process for the production of a compound of formula (I), characterised in that a compound of the general formula (VI)



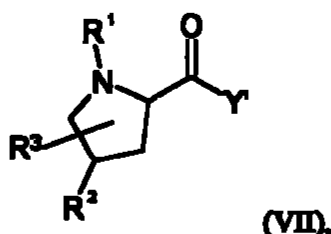
wherein Y is of formula L-COX , wherein L is a linker residue, and X may be OH, or a leaving group which is suitable to be substituted by a amino group or a hydroxy group, and wherein the other substituents have the meaning as described before,

- is reacted with a compound $\text{H}_2\text{N-Cyt}'$ or with a compound $\text{HO-Cyt}'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

Preferably, X within formula (VI) is a leaving group, for example -Cl, -F, N-hydroxysuccinimidyl, pentafluorophenyl, or a carboxylate. Alternatively, X may be OH and condensation is achieved by the use of an in situ coupling reagent, for example benzotriazole-1-yloxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), 1,1'-carbonyldimidazole (CDI), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), or 1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT).

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A further aspect of the invention is a process for the production of a compound of formula (I), characterised in that a compound of the general formula (VII)



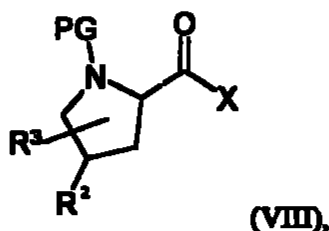
wherein Y' is of formula L-OH or L-NH₂, wherein L is a linker residue, and wherein the other substituents have the meaning as described before,

is reacted with a compound XOC-Cyt', wherein X may be OH, or a leaving group which is suitable to be substituted by a amino group or a hydroxy group, and wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

Preferably, X of the compound XOC-Cyt' is a leaving group, for example -Cl, -F, N-hydroxysuccinimidyl, pentafluorophenyl, or a carboxylate. Alternatively, X may be OH and condensation is achieved by the use of an in situ coupling reagent, for example benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), 1,1'-carbonyldimidazole (CDI), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), or 1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT).

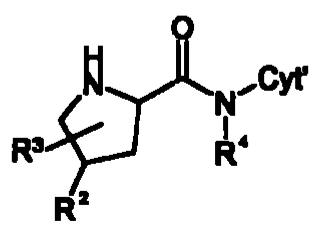
A further aspect of the invention is a process for the production of a compound of formula (I), characterised in that a compound H₂N-Cyt' is condensed stepwise with the units making up the compound of formula (I). Before each coupling step, it may be necessary to remove a protecting group PG, if present.

Accordingly, a further aspect of the invention is a process for the production of a compound of formula (I), characterised in that a compound of the general formula (VIII)



wherein PG is a protection group, and the other the substituents have the meaning as described before, is reacted with a compound $\text{HN}(\text{R}^4)\text{-Cyt}'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound;

the protecting group PG is then removed and the resulting compound



is then reacted with a compound PG-A-X , wherein PG is a protection group, and X may be OH, or a leaving group suitable to be substituted by a secondary amino group. Further coupling steps may follow, if necessary, to build up the complete molecule.

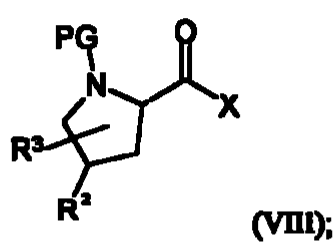
PG may be, for example, Fmoc or Boc.

Accordingly, a further aspect of the invention is a process for the production of a compound of formula (I), characterised in that

a compound of formula $\text{PG-NR}^4\text{-L-COX}$, wherein the substituents have the meaning as described before, is reacted with a compound of formula $\text{H}_2\text{N-Cyt}'$, or $\text{HO-Cyt}'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound;

the protecting group PG is then removed;

and the resulting compound $\text{HN}(\text{R}^4)\text{-L-NH-Cyt}'$ or $\text{HN}(\text{R}^4)\text{-L-COO-Cyt}'$ is reacted with a compound of the general formula (VIII)



or

10 A further aspect of the invention is a process for the production of a compound of formula (I), characterised in that

the protecting group PG is then removed;


(VIII):




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is then reacted with a compound PG-A-X, wherein PG is a protection group, and X may be OH, or a leaving group suitable to be substituted by a secondary amino group. Further coupling steps may follow, if necessary, to build up the complete molecule.

The compounds of the invention are intended for medical use. In particular, these compounds are useful for the treatment of tumours which are associated with stromal fibroblasts that express FAP α and which are generally not optimally treated with available cytotoxic and/or cytostatic agents. Tumours with this property are, for example, epithelial cancers, such as lung, breast, and colon carcinomas. Tumours, such as bone and soft tissue sarcomas which express FAP α , may also be treated with these compounds.

Consequently, another aspect of the present invention are pharmaceutical compositions comprising a compound of the present invention and optionally one or more suitable and pharmaceutically acceptable excipients, as exemplified in: *Remington: the science and practice of pharmacy. 19th ed. Easton : Mack Publ., 1995*. The pharmaceutical compositions may be formulated as solids or solutions. Solid formulations may be for preparation of a solution before injection. Preferably, the pharmaceutical compositions of the invention are solutions for injection. They may be administered systemically, e.g. by intravenous injection, or topically, e.g. by direct injection into the tumour site. The dosage will be adjusted according to factors like body weight and health status of the patient, nature of the underlying disease, therapeutic window of the compound to be applied, solubility, and the like. It is within the knowledge of the expert to adjust dosage appropriately. For doxorubicin conjugates, for example, the dose will preferably be in the range from 10 mg/m² to 1350 mg/m², but also higher or lower doses may be appropriate.

Accordingly, a further aspect of the present invention is the use of a compound of the invention in the preparation of a pharmaceutical composition for the treatment of cancer. Furthermore, an aspect of the invention is a method of treatment of cancer, comprising administering an effective amount of a pharmaceutical composition of the invention to a patient.

A further aspect of the invention is a method of treatment of cancer, wherein a prodrug is administered to a patient wherein said prodrug is capable of being converted into a cytotoxic or cytostatic drug by an enzymatic activity, said enzymatic activity being the expression product of cells associated with tumour tissue. Preferably, said enzymatic activity is the proteolytic activity of FAP α .

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Figures

Figure 1: Cleavage of doxorubicin-peptide conjugates by FAPα. Chromatograms for ZGP-Dox (Z-Gly-(L)-Pro-Doxorubicin) after incubation with purified FAPmCD8 fusion protein (A), or with buffer (B). See example 5.

Figure 2: Reduction of doxorubicin cytotoxicity by conjugation of doxorubicin to FAPα-cleavable peptides. See example 6.

Figure 3: Demonstration of cytotoxicity of doxorubicin released from FAP α -cleavable doxorubicin-peptide conjugates by FAP α -expressing HT1080 clone 33 cells versus parental HT1080 cells. See example 8.

Figure 4: Plasma stability of *N*-Cbz-Gly-(L)-Pro-Doxorubicin and *N*-Cbz-(L)-Pro-(L)-Ala-Gly-(L)-Pro-Doxorubicin in mouse and human plasma. See example 9.

Figure 5: Demonstration of FAP α enzyme activity and confirmation of its apparent molecular weight in human tumour tissue samples. See Example 12.

Examples

Example 1: Synthetic procedures of doxorubicin conjugates

***N*-Cbz-Gly-Pro-Doxorubicin:** *N*-Cbz-Gly-Pro (116.1 mg, 0.37mmol) and *N*-hydroxy succinimide (44 mg, 0.37 mmol) were weighed out and placed in a 2 neck-round bottom flask under dinitrogen. Anhydrous *N,N*-dimethylformamide (20 ml) was added and the flask was cooled to 0 °C in an ice bath. Dicyclohexylcarbodiimide (78 mg, 0.37 mmol) was added as a 1 ml solution in *N,N*-dimethylformamide. The solution was stirred at 0 °C for 40 minutes.

Doxorubicin-HCl (100 mg, mmol) was weighed into a vial with a small stir bar and placed under dinitrogen. *N,N*-Dimethylformamide (3ml) and *N,N*-diisopropylethylamine (33.1 μ l, 0.19 mmol) were added to the vial with stirring. The doxorubicin solution was added *via* syringe to the peptide solution, and the vial was rinsed with an additional 2 ml of *N,N*-di-

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methylformamide. The ice bath was removed and reaction mixture was stirred for approximately 48 hours at room temperature.

The reaction solution was extracted with ethyl acetate (500 ml). The ethyl acetate was washed with of 10% aqueous citric acid solution (250 ml), saturated aqueous sodium bicarbonate (250 ml) and brine (250 ml) sequentially. The organic extract was dried with anhydrous MgSO_4 and the solvent was removed with a roto-evaporator. The product, which was rich in DMF contaminant, was chromatographed on a C-18 reversed phase flash column with 8:2 methanol : water as the eluent. One orange spot, $\text{rf} \approx 0.3$, which fluoresced under long wave UV light was isolated. The methanol was removed with the roto-evaporator and the last traces of solvent were removed with the high vacuum pump overnight.

N-Cbz-Pro-Ala-Gly-Pro: *N*-Cbz-Pro-Ala (5 g, 15 mmol) and carbonyldiimidazole (2.43 g, 15 mmol) were placed in a 250 ml, 3-neck round bottom flask under an argon atmosphere. Anhydrous tetrahydrofuran (50 ml) was added and the solution was stirred at room temperature for approximately 45 minutes. Rigorous evolution of a gas (CO_2) was observed.

Into a separate flask was weighed Gly-Pro- $\text{OCH}_3 \cdot \text{HCl}$ (2.9 g, 15 mmol). Tetrahydrofuran (5 ml) and *N,N*-diisopropylethylamine (5.23 ml, 30 mmol) were added and the solution was stirred for several minutes. The material that dissolved was added *via* syringe to the activated peptide, the remaining material was dissolved in a minimum amount of CH_2Cl_2 and also added to the activated peptide *via* syringe.

The reaction solution was stirred overnight at room temperature (15 hours) and in the morning there was copious amounts of white precipitate. The reaction mixture was washed with 10% aqueous citric acid solution (300 ml) and extracted with ethyl acetate (500 ml). The ethyl acetate extract was washed with saturated aqueous bicarbonate solution (300 ml) and dried with brine (300 ml) and anhydrous MgSO_4 . The ethyl acetate was removed with the roto-evaporator to yield 5 g of a colourless oil, which gave satisfactory characterisation data.

The crude oil of the *N*-Cbz-Pro-Ala-Gly-Pro- OCH_3 (5g, 12 mmol) was dissolved in methanol (20 ml) in a round bottom flask. The flask was placed in an ambient temperature water bath. 1 N Sodium hydroxide solution (12 ml) was added cautiously. The solution was stirred for 3.5 hours after which time 1 N HCl solution (12 ml) was added. The solution was concentrated on the roto-evaporator and a few more drops of 1 N HCl was added until the pH is approximately 1.5 with pH paper. The water was removed with the vacuum pump to give an oil, which was recrystallized slowly from ethanol.

***N*-Cbz-Pro-Ala-Gly-Pro-Doxorubicin:** *N*-Cbz-Pro-Ala-Gly-Pro (180 mg, 0.38 mmol) was dissolved in anhydrous *N,N*-dimethylformamide (15 ml). 1-Hydroxybenzotriazole (51.3 mg, 38 mmol) and dicyclohexylcarbodiimide (78 mg, 38 mmol) were dissolved in 1 ml each of *N,N*-dimethylformamide and added to the peptide as solutions. The reaction mixture was stirred at room temperature for 45 minutes.

Doxorubicin·HCl (116 mg, 20 mmol) was weighed into a separate vial and dissolved in *N,N*-dimethylformamide (3 ml). *N,N*-Diisopropylethylamine (34.8 μ l, 20 mmol) was syringed into the vial containing the doxorubicin, and the contents were stirred for several minutes to ensure complete dissolution. The doxorubicin solution was added *via* syringe to the activated peptide. The solution was stirred for 48 hours at room temperature.

The product was extracted with ethyl acetate (2 l) and washed with 10 % aqueous citric acid solution (500 ml). The ethyl acetate layer was separated, dried with MgSO₄ and concentrated to an oil on the roto-evaporator. The oil was chromatographed on C-18 reversed phase silica gel, which gave an orange, long wave UV fluorescing spot at *rf* = 0.25. The final product gave satisfactory characterization data.

Example 2: Preparation of FAP α -expressing cell lines

Mammalian cell lines expressing recombinant FAP α were prepared. HT1080 fibrosarcoma cells, widely known and available from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) under the accession number DSMZ ACC 315, were maintained in a DMEM/F12 mix 50:50 containing 10% fetal bovine serum in an atmosphere of 95% air and 5% CO₂. HT1080 cells were transfected with FAP.38 vector (WO 97/34927, Scanlan *et al.*, *loc. cit.*) using the Lipofectin method according to the manufacturer's instructions (Gibco/BRL). Transfectants were selected for resistance to antibiotics (200 μ g/ml Geneticin) and thereafter maintained in medium containing Geneticin. Individual colonies of resistant cells were picked, grown to confluence in 10 cm tissue culture petri dishes and tested for FAP α expression in an immunofluorescence assay using the FAP α -specific monoclonal antibody F19, as described (Garin-Chesa *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87(18), 7235-7239). The parental HT1080 cell line showed no detectable FAP α expression in this immunofluorescence assay, while one clone, referred to hereafter as HT1080 clone 33, was positive for FAP α .

Similarly, human embryonic kidney 293 cells, widely known and available from American Tissue Type Collection (Rockville, MD), were maintained in a DMEM containing 10% fetal bovine serum in an atmosphere of 95% air and 5% CO₂. Cells were transfected with a FAP α expression vector, pFAP.38 using calcium phosphate transfection as described (Park, J. E., Chen, H. H., Winer, J., Houck, K. A. & Ferrara, N. (1994). Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J. Biol. Chem.* 269(41), 25646-25654). Transfectants were selected and analyzed as described above for FAP α expression. The parental 293 cell line showed no detectable FAP α expression. One clone, referred to hereafter as 293-1/2, was FAP α positive.

Example 3: Examination of FAP α expression in transfected cell lines

FAP α expression was examined in the HT1080 and HT1080 clone 33 cells. Metabolic labeling, immunoprecipitations and fluorography were performed essentially as described (Park et al. (1991) *Somatic Cell Mol. Genet.* 17(2), 137-150). HT1080 and HT1080 clone 33 cells were metabolically labelled with ³⁵S-methionine. Detergent extracts of these cells were immunoprecipitated with monoclonal antibody F19 or with mouse IgG1 antibody as a negative control. Precipitates were boiled in sample buffer and separated by sodium dodecyl sulfate gel electrophoresis (as described by Laemmli (1970) *Nature* 227(259), 680-685). Fluorographic analysis of the resulting gel confirmed that the HT1080 clone 33 cells produce FAP α protein. No FAP α protein was detectable in extracts of the parental HT1080 cells nor in immunoprecipitates with mouse IgG1.

Example 4: Soluble recombinant FAP α

A soluble recombinant form of FAP α protein was prepared as follows. A cDNA encoding the extracellular domain (ECD) of murine CD8 α (Genbank M12825), consisting of the N-terminal 189 amino acids of CD8 α , was ligated to a cDNA encoding the extracellular domain of FAP α (amino acids 27 to 760), generating a fusion protein construct, FAPmCD8, similar in structure to the CD8 α -CD40 ligand fusion protein, as previously described (Lane et al. (1993) *J. Exp. Med.* 177(4), 1209-1213). The cDNAs were verified by sequencing and inserted into the pVL1393 vector. Transfection of Sf9 cells and amplification of the resulting recombinant baculovirus were performed as described (O'Reilly (1994) *Baculovirus Expression Vectors: A Laboratory Manual*, Oxford University Press, New York). The culture supernatant of High Five cells infected with recombinant FAPmCD8 baculovirus for

four days was collected and cleared by ultracentrifugation. FAPmCD8 fusion protein was purified from such supernatants using an anti-FAP α monoclonal antibody immobilized on activated agarose beads (Pierce Chemical, Indianapolis, IN, USA). The culture supernatant was passed through the antibody affinity column and eluted by pH shift using 0.1 M citrate buffer, pH 3. The samples were immediately neutralized with a saturated Tris solution (Sigma Chemicals, St. Louis, MO) and protein-containing fractions were pooled.

Example 5: Measurement of cleavage of doxorubicin-peptide conjugates

Samples were separated by reversed-phase high performance liquid chromatographic (HPLC) assay that was established to measure cleavage of doxorubicin-peptide conjugates. The HPLC system consisted of a Waters 717 autosampler equipped with a 100 microliter (μ l) loop and two Waters model 510 pumps to deliver solvents. Separations were performed under isocratic conditions at a flow rate of 0.7 ml/min on a Nucleosil C-18 column, 100 mm long x4 mm I.D. with 5 μ m particle size (Dr. Ing. H. Knauer GmbH, Berlin). The mobile phase consisted of methanol:water (70:30, v/v) containing 0.2 M ammonium acetate, adjusted to pH 3.2. Free doxorubicin and doxorubicin-peptide conjugates were detected by fluorescence (excitation, 475 nm; emission, 585 nm) using a Waters 474 fluorescence detector. Injection, solvent delivery, data acquisition, and data analysis were all performed using the Millennium 2010 chromatography software package (Waters Corp., Milford, MA, USA). Substances to be tested were first dissolved in dimethyl sulfoxide at a concentration of 5 mM and subsequently diluted in aqueous solution before being applied to the HPLC column.

The ability of soluble recombinant FAP α enzyme to release free doxorubicin from doxorubicin-peptide conjugates was examined. Doxorubicin-peptide conjugate stock solutions (5 mM) were diluted with Hepes-buffered saline pH 7.4 to a final concentration of 50 to 100 μ M. Twenty μ l of the resulting solution was mixed with 50 μ l of purified FAPmCD8 fusion protein (approximately 20 ng) described above and 30 μ l Hepes-buffered saline, pH 7.4. The mixture was allowed to incubate at 37 $^{\circ}$ C for 1 day and release of free doxorubicin was measured in the HPLC assay described. Areas under each peak were quantified using the software package above and the initial value was set to 100%. The rate of release of free doxorubicin was measured by the appearance of a peak with the same retention time as free doxorubicin under these HPLC conditions. The areas under each peak were used to calculate the relative amounts of free doxorubicin to doxorubicin-peptide conjugate. Integration of peak areas to determine percent cleavage was carried out using the Millennium 2010 chromatography software package above. As seen in the chromatograms for ZGP-Dox (*N*-

Cbz-Gly-Pro-Doxorubicin) shown in figure 1, the doxorubicin-peptide conjugate could be converted to free doxorubicin after incubation with purified FAP α CD8 fusion protein but the retention time of the conjugate was not altered by incubation with buffer.

Example 6: Reduction of cytotoxicity of doxorubicin by conjugation to FAP α -cleavable peptides.

The ability of FAP α -cleavable peptides to block the cytotoxic action of doxorubicin on FAP α -negative, doxorubicin-sensitive cells was determined. K562 cells, available from American Type Tissue Culture Collection, Rockville, MD, USA (ATCC Number: CCL-243), were seeded in 96 well plates (Greiner Scientific) at a density of 1000 cells / well. Serum-free cell culture media containing various concentrations of free doxorubicin or equivalent molar concentrations of doxorubicin-peptide conjugates were added to the cells. Four days later, cell number was determined using an automated CASYTM cell counter (Schärfe System GmbH, Reutlingen, Germany). The results are shown in Figure 2.

Example 7: Release of free doxorubicin by cell-bound FAP α

The ability of cell-bound FAP α enzyme to release free doxorubicin from doxorubicin-peptide conjugates was examined. Each conjugate was dissolved in serum-free cell culture medium at a final concentration of 1 μ M. Ten milliliters of this solution was added to confluent monolayers of HT1080 or HT1080 clone 33 cells in 10 cm tissue culture dishes for 19 hours at 37° C. The media were removed and release of doxorubicin measured as described in Example 5. The FAP-expressing cell line, HT1080 clone 33, converted 81% and 43% of the ZGP-Dox (N-Cbz-Gly-Pro-Doxorubicin) and ZPAGP-Dox (N-Cbz-Pro-Ala-Gly-Pro-Doxorubicin) conjugates to free doxorubicin, respectively. The parental HT1080 cell line converted only 9% of ZGP-Dox to free doxorubicin under the same conditions. Little or no detectable conversion of ZPAGP-Dox to free doxorubicin by the parental HT1080 cell line was observed under these conditions.

Example 8: Killing of sensitive cells by FAP α -released doxorubicin

The ability of FAP α to generate free doxorubicin capable of killing doxorubicin-sensitive cells was determined. K562 cells, available from American Type Tissue Culture Collection, Rockville, MD, USA (ATCC Number: CCL-243), were seeded in 96 well plates (Greiner

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Scientific) at a density of 1000 cells / well. Serum-free cell culture media containing 1 μ M doxorubicin-peptide conjugate was added to HT1080 or HT1080 clone 33 cells dishes for 19 hours at 37° C. The media were removed and release of doxorubicin was confirmed as in Example 5. Sixty-six μ l of this medium was then added per well to the K562 cells. Four days later, cell number was determined using an automated CASY™ cell counter. The results are shown in figure 3.

Example 9: Plasma stability of doxorubicin-peptide conjugates

The plasma stability of doxorubicin-peptide conjugates was measured using methods described in Example 5. Samples containing doxorubicin-peptide conjugates (at a concentration of 1 μ M) were incubated in the presence of 10% (v/v) mouse or human plasma for the times indicated at 37° C. The results for ZGP-Dox and ZPAGP-Dox in mouse and human plasma are shown in Figure 4.

Example 10: FAP α -catalyzed cleavage of selected 4-methoxy- β -naphthylamide-peptide conjugates

To identify preferred FAP α peptide substrates, oligomers composed of natural and/or unnatural amino carboxylic acids were synthesized and coupled to Proline-4-methoxy- β -naphthylamine (Pro-MNA) using methods known to the art (E. Wunsch, Synthese von Peptiden, in Methoden der organischen Chemie, Houben-Weyl (Eds. E. Müller, O. Bayer), Vol. XV, Part 1 and 2, Georg Thieme Verlag, Stuttgart, 1974).

Synthesis of Pro-Pro-4-methoxy- β -naphthylamide:

Boc-Pro (32 mg, 0.15 mmol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (53 mg, 0.15 mmol) and Pro-4-methoxy- β -naphthylamide hydrochloride (46 mg, 0.15 mmol) were dissolved in anhydrous N,N-dimethylformamide/tetrahydrofuran 1:1 (4 ml). N-ethyl-diisopropylamine (0.26 ml, 0.44 mmol) dissolved in N-methylpyrrolidone (1.7 molar) was added and the mixture was stirred at room temperature overnight.

The solvent was removed with a roto-evaporator and the residue was dissolved in ethyl acetate (10 ml) and extracted 3 times with water. The organic layer was dried with anhydrous Na₂SO₄ and the solvent was removed with a roto-evaporator. The residue was dissolved in trifluoroacetic acid/dichloromethane (1:4, 25 ml) and allowed to react for 1 hour.

The solvent was removed with a roto-evaporator and the resulting oil was dried with a stream of nitrogen. The crude product was purified by preparative reversed phase HPLC applying a acetonitrile/water gradient. The product gave satisfactory analytical data (NMR and mass spectra).

Release of free MNA from the peptides was then measured in a Cytofluor fluorimeter (PerSeptive Biosystems, Inc.) using the 355 nm excitation /405 nm emission filter set. Enzyme kinetic parameters (Michaelis-Menten K_m and k_{cat} values) were calculated using methods known to the art (see for example, Yun, S. L. & Suelter, C. H. (1977). A simple method for calculating K_m and V from a single enzyme reaction progress curve. *Biochim. Biophys. Acta* 480(1), 1-13.) with FAP α enzyme derived from membrane extracts of the 293-I/2 transfected cells in Example 2.

Table 1: K_m and k_{cat} values for FAP α -catalyzed cleavage of selected 4-methoxy- β -naphthylamide (MNA)-peptide conjugates

Substrates	K_M	K_{cat}	K_{cat}/K_M
	[μM]	[s^{-1}]	$\times 10^4 [M^{-1} s^{-1}]$
Chg-Pro-MNA	75	53.7	71.6
Hyn-Pro-MNA	69	27.4	39.7
Pro-Pro-MNA	154	49.5	32.1
Val-Pro-MNA	95	27.7	29.2
Met-Pro-MNA	127	27.9	22.0
Arg-Pro-MNA	278	50.6	18.2
trans-Hyp-Pro-MNA	254	37.9	14.9
Gln-Pro-MNA	273	40.5	14.8
Ala-Pro-MNA	267	35.7	13.4
Lys-Pro-MNA	530	57.1	10.8
Ile-Pro-MNA	43	12.6	8.8
Met(O)-Pro-MNA	378	26.9	7.1
Ser-Pro-MNA	872	28.3	3.3

Chg = cyclohexylglycine, Hyn = 6-hydroxynorleucine, trans-Hyp = trans-4-hydroxyproline

Example 11: Specificity of MNA-coupled peptides for FAP α versus DPP-IV

Among the known prolyl-specific serine oligopeptidase family members, the most closely related enzyme to FAP α is DPP-IV. Since active DPP-IV is found in plasma and on many different cell types, optimization of the relative (optional) selectivity of prodrug peptides

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for FAP α compared to DPP-IV is necessary to reduce undesirable conversion of the pro-drug at sites other than the tumor (e.g., in the plasma). To identify peptides specific for FAP α , cleavage of MNA-coupled peptides by FAP α was compared to the ability of DPP-IV to cleave the same peptide conjugates. Release of free MNA was measured as described in Example 9. Results are shown in Table 2.

Table 2: Comparison of cleavage selectivity of MNA-peptide conjugates by FAP α and DPP-IV.

	Cleavage specificity	
	FAP	DPP IV
Ala-Pro-MNA	+	+
Z-Gly-Pro-MNA	+	-
Z-Pro-Ala-Gly-Pro-MNA	+	-

+ indicates the enzyme was able to cleave the substrate
- indicates lack of cleavage

Example 12: FAP α activity in tumour samples

Enzyme activity of FAP α measured in human tumor samples. Ninety-six-well ELISA (enzyme-linked immunosorbent assay) plates (Costar, Corning, NY) were coated overnight at 4 °C with 1 μ g/ml F19 antibody or control antibody in phosphate-buffered saline (PBS), pH 7.4. Wells were then rinsed with wash buffer (PBS, 0.1 % Tween 20, pH 7.4) and excess binding sites were blocked with blocking buffer (5 % bovine serum albumin in PBS, pH 7.4) for 1 hour at room temperature. FAP α activity was measured in tumor tissue (closed symbols) or matched normal control tissue (corresponding open symbols) from Concanavalin A-enriched membrane extracts (Figure 5a). Tumor samples included breast (∇ , $\blacksquare$), colon ($\bullet$), colon metastasized to the liver ($\diamond$), and lung cancer ($\blacktriangle$, $\oplus$). Extracts were added to F19-coated plates and incubated for 1 hour at room temperature. The unbound material was removed, wells were washed thrice with wash buffer, and FAP α enzyme activity was assayed using 100 μ l Ala-Pro-AFC as described (WO 97/34927) for one hour at 37 °C. The first two Concanavalin A fractions of each extract were measured and each value individually plotted. Background fluorescence (as measured using control antibody-coated plates) was subtracted from each value.

Independent biochemical confirmation of FAP α enzymatic activity and its apparent molecular mass in tumor extracts were obtained by labelling the aforementioned tissue extracts with ^{14} C-labelled diisopropylfluorophosphate (DFP; NEN-DuPont, Cologne, Germany). DFP is known to bind covalently and irreversibly to active site serines of many serine prote-

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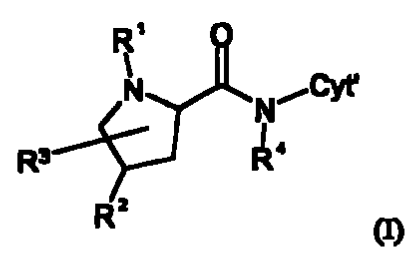
ases, thereby preventing further catalysis (Hayashi, R., Bai, Y. & Hata, T. (1975). Further confirmation of carboxypeptidase Y as a metal-free enzyme having a reactive serine residue. *J. Biochem.* 77(6), 1313-1318; Wahlby, S. & Engstrom, L. (1968). Studies on *Streptomyces griseus* protease. II. The amino acid sequence around the reactive serine residue of DFP-sensitive components with esterase activity. *Biochim. Biophys. Acta* 151(2), 402-408). ¹⁴C-DFP labelling of FAP α immunopurified from a tumor (T) corresponding to colon sample ● from Figure 5a is shown in Figure 5b. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(259), 680-685) and subsequent autoradiography (Park, J. E., Draper, R. K. & Brown, W. J. (1991). Biosynthesis of lysosomal enzymes in cells of the End3 complementation group conditionally defective in endosomal acidification. *Somatic Cell Mol. Genet.* 17(2), 137-150) of these samples revealed the presence of a labelled 95 kD protein, present in the colon cancer sample. No radiolabeled bands were observed in the normal matched control immunoprecipitate (N) nor with control antibody.

The apparent molecular mass of the immunopurified, ¹⁴C-labelled protein seen in Figure 5b agrees with previous reports for FAP α (Rettig, W. J., Su, S. L., Fortunato, S. R., Scanlan, M. J., Mohan Raj, B. K., Garin-Chesa, P., Healey, J. H. & Old, L. J. (1994). Fibroblast activation protein: Purification, epitope mapping and induction by growth factors. *Int. J. Cancer* 58(3), 385-392; WO 97/34927).

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Claims

1. Compound of the general formula (I)

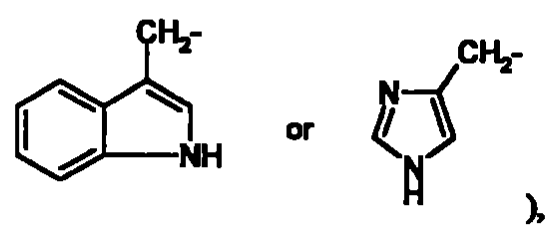


or the pharmaceutically acceptable salts thereof,

wherein R¹ is a residue of formula Cg-A, Cg-B-A, or Cg-D-B-A, wherein

Cg is H, or a capping group with the structure R⁵-CO, R⁵-O-CO-, R⁵-NH-CO-, R⁵-SO₂-, or R⁵-, wherein R⁵ is an C₁-C₆-alkyl, C₃-C₈-cycloalkyl, aryl, or heteroaryl substituent; preferably Cg is an acetyl, benzyl, D-alanyl, (R)-H₂NCH(CH₃)-, or H₂NCOCH₂CH₂-substituent or another capping group for the protection of the N-terminal amino function;

and wherein A and B are units derived from amino carboxylic acids with the formula -[NR⁶-(X)ₚ-CO]- wherein X is CR⁷R⁸ and wherein R⁶, R⁷ and R⁸ are the same or different, and are H, C₁-C₆-alkyl (preferably CH₃-, CH₃CH₂-, CH₃CH₂CH₂-, (CH₃)₂CH-, CH₃CH₂CH₂CH₂-, (CH₃)₂CHCH₂-, CH₃CH₂CH(CH₃)-, (CH₃)₃C-, HOCH₂-, CH₃CH(OH)-, CH₃CH(OH)CH₂CH₂-, HOCH₂CH₂CH₂CH₂-, H₂NCH₂CH₂CH₂-, H₂NCH₂CH₂CH₂CH₂-, H₂NCH₂CH(OH)CH₂CH₂-, H₂NC(=NH)NHCH₂CH₂CH₂-, HSCH₂-, CH₃SCH₂CH₂-, HOOCCH₂-, HOOCCH₂CH₂-, H₂NC(=O)CH₂-, H₂NC(=O)CH₂CH₂-, benzyl, para-hydroxy-benzyl,

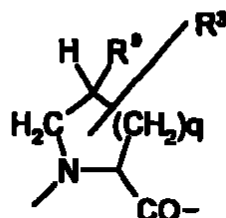


C₃-C₈-cycloalkyl (preferably cyclohexyl), aryl (preferably phenyl), or heteroaryl, and p is 1, 2, 3, 4, 5, and wherein the configuration at CR⁷R⁸ can be R or S; if R⁷ is other than H,

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then R^5 is preferably H; R^6 is preferably H; if p is greater than one, R^7 and R^8 are preferably H;

A and B can also be units derived from cyclic amino carboxylic acids with the structure



wherein R^5 is H, C_1 - C_6 -alkyl, OH, or NH_2 , R^6 has the meaning as defined below, and q is 1 or 2;

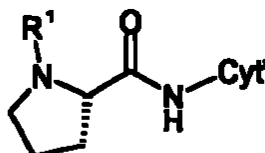
and wherein D is a unit derived from an amino carboxylic acid as defined for A and B, or D is an oligomer comprising two or more such units, which may be the same or different, preferably 2 to 10 such units;

and R^2 , R^3 are the same or different, and are hydrogen, hydroxyl, amino, C_1 - C_6 -alkyl, C_1 - C_6 -alkyl-amino, di- C_1 - C_6 -alkyl-amino, C_1 - C_6 -alkyl-oxy, thiol, C_1 - C_6 -alkyl-thio, =O, =NH, -CHO, -COOH, -COOCH₃, -COOCH₂CH₃, -CONH₂, halogen, C_3 - C_6 -cycloalkyl, aryl, or heteroaryl;

and R^4 is H, C_1 - C_6 -alkyl, C_3 - C_6 -cycloalkyl, aryl, or heteroaryl;

and Cyt' is the residue of a cytotoxic or cytostatic compound.

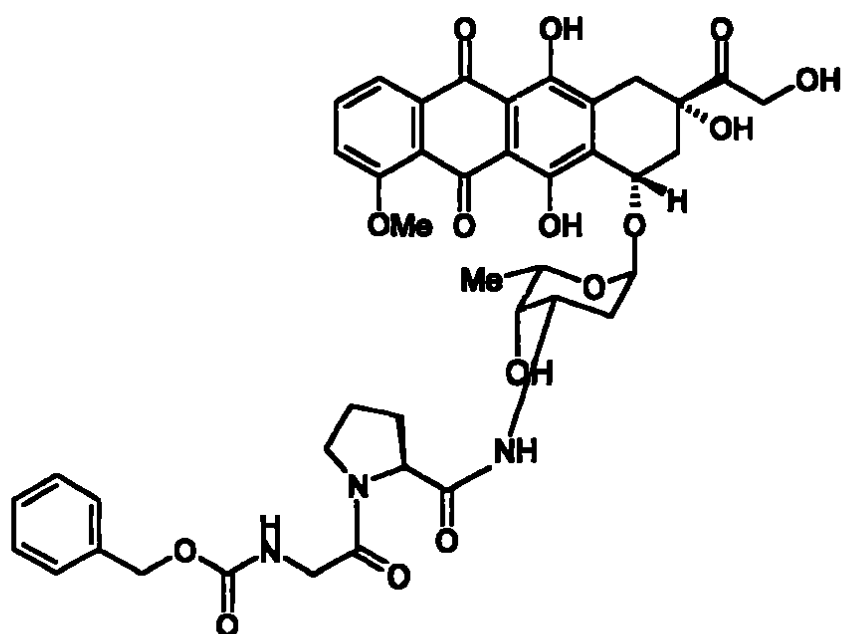
2. Compound of claim 1 with the formula



3. Compound of claim 1 or 2, wherein the units A, B, or D are derived from glycine (Gly), or the D- or L-forms of alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), cysteine (Cys), methionine (Met), serine (Ser), threonine (Thr), lysine (Lys), arginine (Arg), histidine (His), aspartic acid

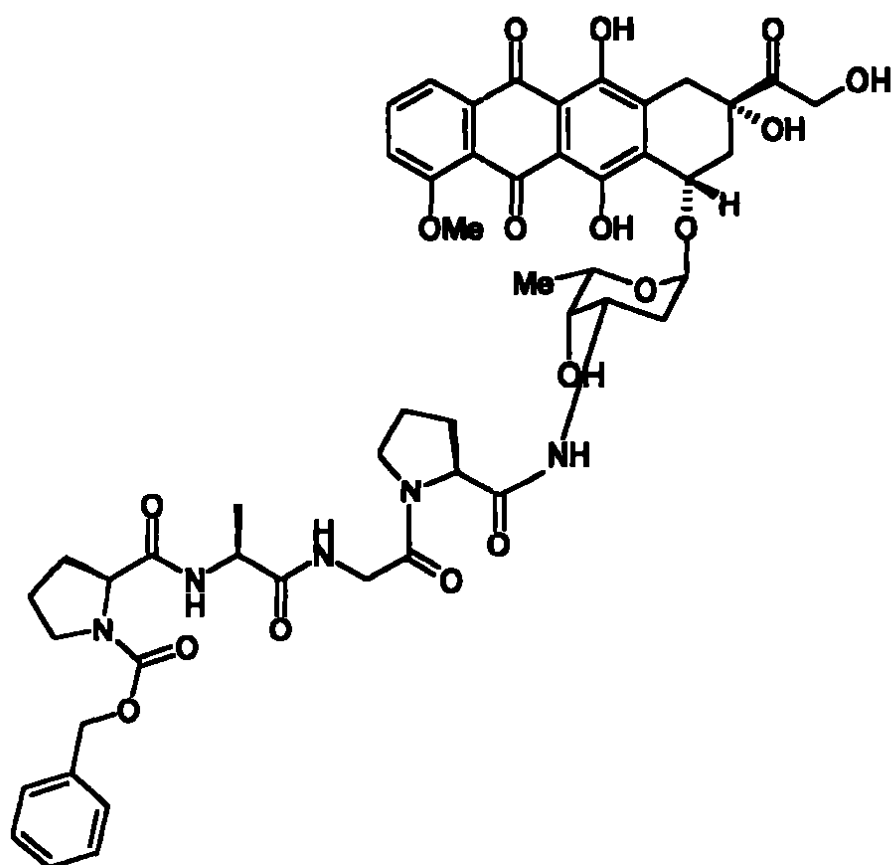
(Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), proline (Pro), 4-hydroxyproline, 5-hydroxy-lysine, norleucine, 5-hydroxynorleucine, 6-hydroxynorleucine, ornithine, or cyclohexylglycine.

4. Compound of any one of claims 1 to 3, wherein the unit A is derived from L-proline, glycine, L-norleucine, L-cyclohexylglycine, L-5-hydroxynorleucine, L-6-hydroxynorleucine, L-5-hydroxylysine, L-arginine, or L-lysine.
5. Compound of any one of claims 1 to 4, wherein the unit D is either not present or consists of one units derived from an amino carboxylic acid as defined for units A or B.
6. Compound of any one of claims 1 to 5, wherein H₂N-Cyt' is doxorubicin.
7. Compound of claim 6 which is Z-Gly-Pro-Dox of the formula (III)



(III),

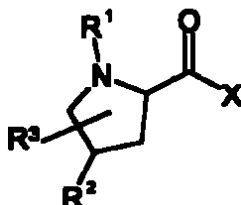
or Z-Pro-Ala-Gly-Pro-Dox of formula (IV)



(IV).

8. A prodrug that is capable of being converted into a drug by the catalytic action of FAP α , said prodrug having a cleavage site which is recognised by FAP α , and said drug being cytotoxic or cytostatic under physiological conditions.
9. The prodrug of claim 8 comprising an oligomeric part comprising two or more amino carboxylic residues, and a cytotoxic or cytostatic part, wherein the C-terminal amino carboxylic residue of the oligomeric part is D- or L-proline, or D- or L-hydroxyproline, and the C-terminal carboxy function is linked to the cytotoxic or cytostatic part by an amide bond.
10. The prodrug of claim 9, wherein the oligomeric part comprises two, three, or four amino carboxylic acid residues.
11. The prodrug of claim 9 or 10 wherein the N-terminal amino function is protected by a capping group.
12. Compound of any one of claims 1 to 11 for medical use.

13. Pharmaceutical composition comprising a compound according to any one of claims 1 to 11, and optionally one or more pharmaceutically acceptable excipients.
14. Use of a compound according to any one of claims 1 to 11 in the preparation of a pharmaceutical composition for the treatment of cancer.
15. Process for the production of a compound of any one of claims 1 to 11, characterised in that a compound of formula (V)



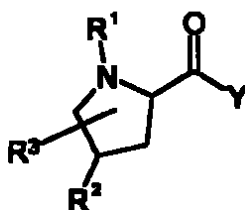
— (V),

wherein X may be OH, or a leaving group which is suitable to be substituted by a amino group,

and wherein the other substituents have the meaning as described before,

is reacted with a compound $\text{HN}(\text{R}')\text{-Cyt'}$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

16. Process for the production of a compound of any one of claims 1 to 11, characterised in that a compound of formula (VI)

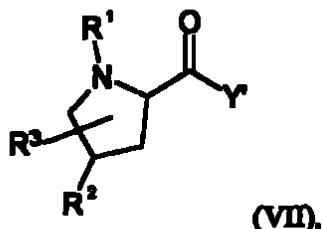


(VI),

- wherein Y is of formula L-COX , wherein L is a linker residue, and X may be OH, or a leaving group which is suitable to be substituted by a amino group or a hydroxy group, and wherein the other substituents have the meaning as described before,

is reacted with a compound H_2N-Cyt' or with a compound $HO-Cyt'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

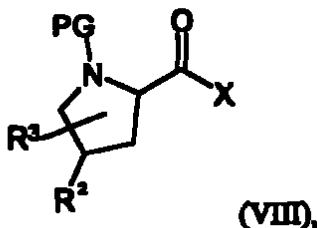
17. Process for the production of a compound of any one of claims 1 to 11, characterised in that a compound of formula (VII)



wherein Y' is of formula $L-OH$ or $L-NH_2$, wherein L is a linker residue, and wherein the other substituents have the meaning as described before,

is reacted with a compound $XOC-Cyt'$, wherein X may be OH , or a leaving group which is suitable to be substituted by an amino group or a hydroxy group, and wherein Cyt' is the residue of a cytotoxic or cytostatic compound.

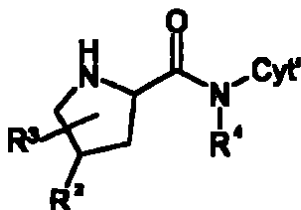
18. Process for the production of a compound of formula (I), characterised in that a compound of formula (VIII)



wherein PG is a protection group, and the other substituents have the meaning as described before, is reacted with a compound $HN(R^4)-Cyt'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound;

the protecting group PG is then removed and the resulting compound

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is then reacted with a compound PG-A-X, wherein PG is a protection group, and X may be OH, or a leaving group suitable to be substituted by an amino group;

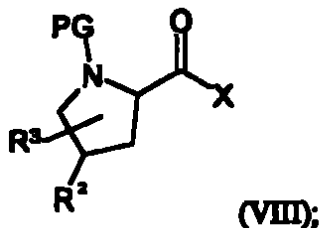
and further coupling steps are carried out, if necessary, until the complete compound is obtained.

19. Process for the production of a compound of any one of claims 1 to 11, characterised in that

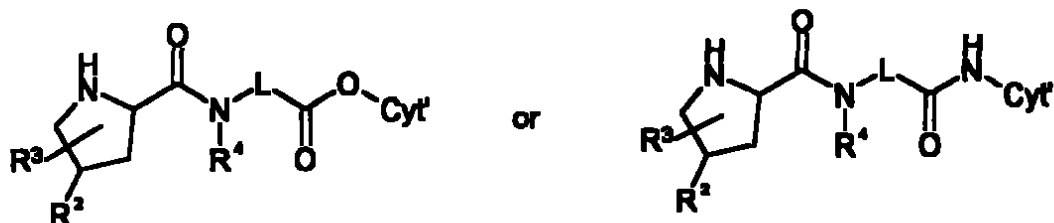
a compound of formula PG-N(R⁴)-L-COX, wherein the substituents have the meaning as described before, is reacted with a compound of formula H₂N-Cyt', or HO-Cyt', wherein Cyt' is the residue of a cytotoxic or cytostatic compound;

the protecting group PG is then removed;

and the resulting compound HN(R⁴)-L-NH-Cyt' or HN(R⁴)-L-COO-Cyt' is reacted with a compound of formula (VIII)



the protecting group is then removed and the resulting compound



is then reacted with a compound PG-A-X, wherein PG is a protection group, and X may be OH, or a leaving group suitable to be substituted by an amino group;

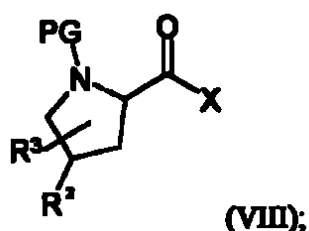
and further coupling steps are carried out, if necessary, until the complete molecule is obtained.

20. Process for the production of a compound of formula (I), characterised in that

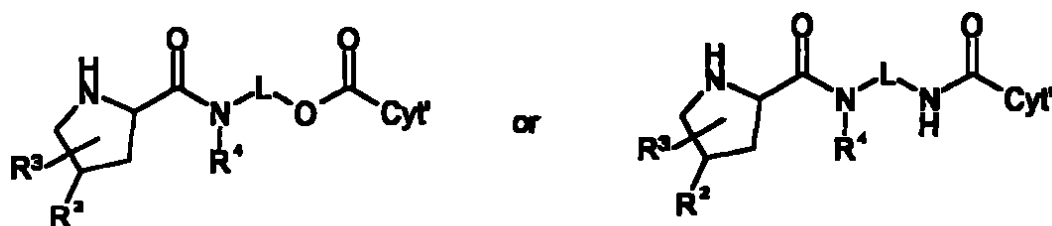
a compound of formula $\text{PG-N(R}^4\text{)-L-OH}$ or $\text{PG-N(R}^4\text{)-L-NH}_2$, wherein the substituents have the meaning as described before, is reacted with a compound of formula $\text{XOC-Cyt}'$, wherein Cyt' is the residue of a cytotoxic or cytostatic compound;

the protecting group PG is then removed;

and the resulting compound $\text{HN}(\text{R}^4)\text{-L-NH-CO-Cyt'}$ or $\text{HN}(\text{R}^4)\text{-L-OOC-Cyt'}$ is reacted with a compound of formula (VIII)



the protecting group is then removed and the resulting compound



is then reacted with a compound PG-A-X, wherein PG is a protection group, and X may be OH, or a leaving group suitable to be substituted by a amino group;

and further coupling steps are carried out, if necessary, until complete molecule is obtained.

21. Method of treatment of cancer, comprising administering a pharmaceutical composition according to claim 13 to a patient.

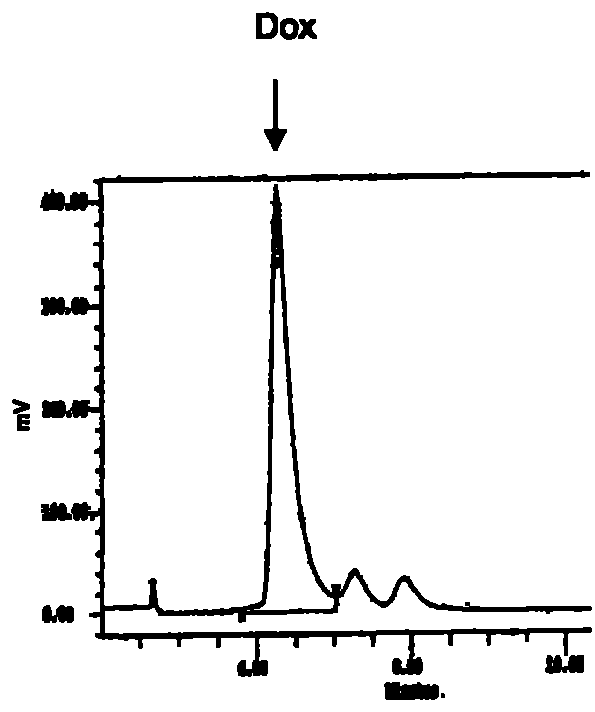
22. Method of treatment of cancer, wherein a prodrug is administered to a patient wherein said prodrug is capable of being converted into a cytotoxic or cytostatic drug by an enzymatic activity, said enzymatic activity being the activity of FAP α .

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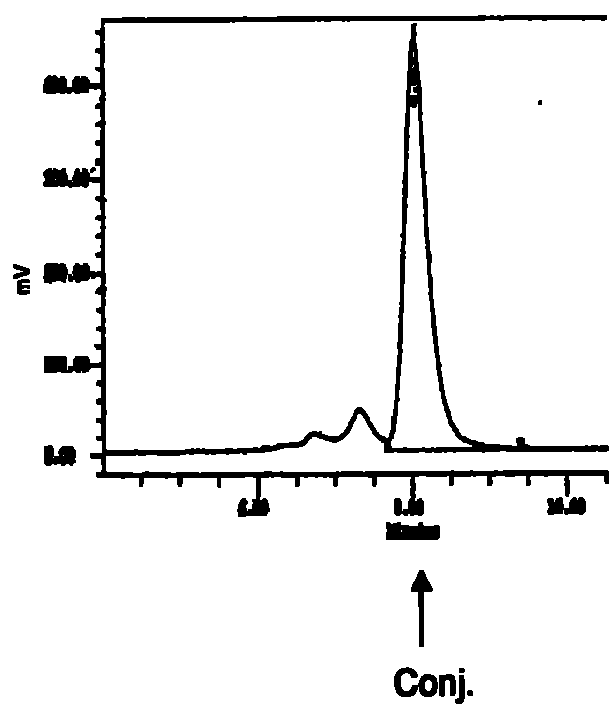
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Figure 1

Z-Gly-Pro-Dox
+ CD8FAP



Z-Gly-Pro-Dox
+ buffer

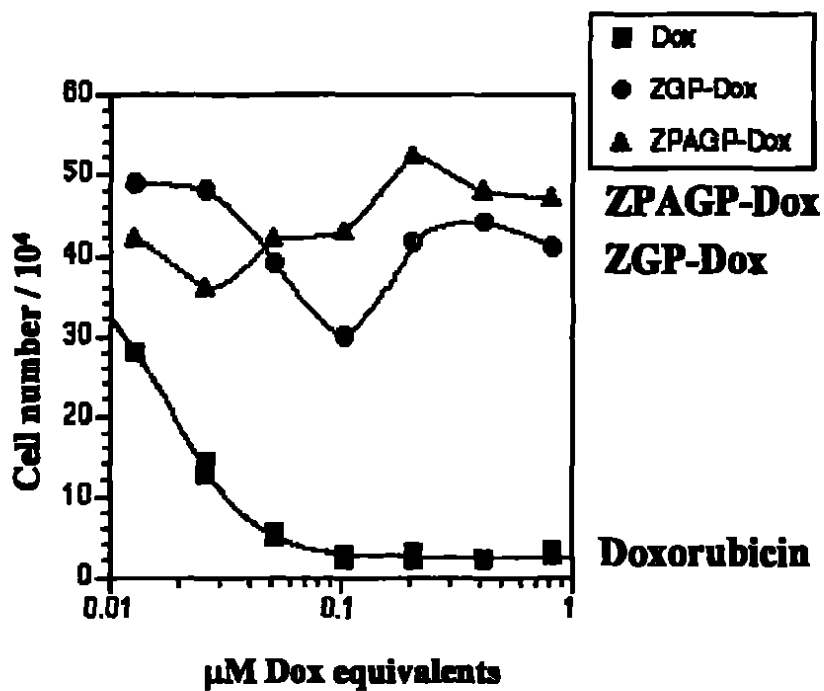


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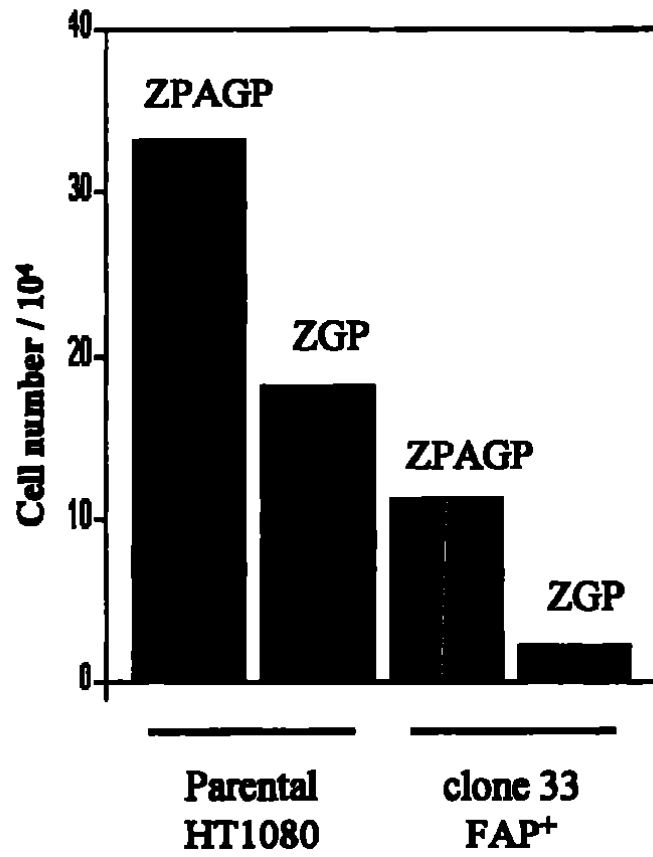
Figure 2



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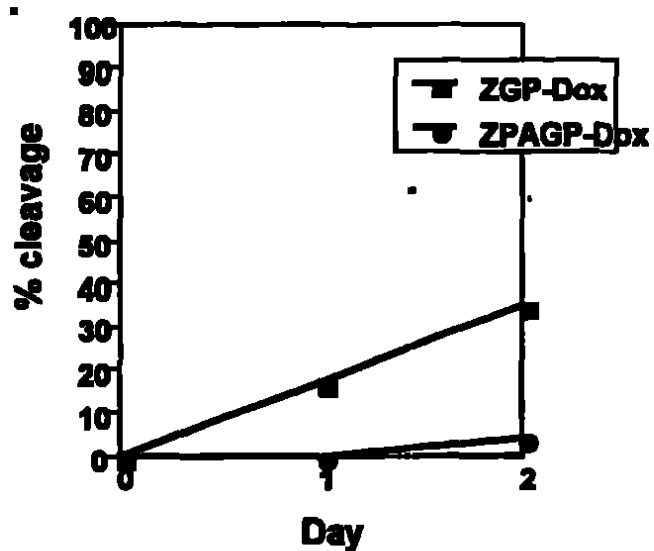
Figure 3



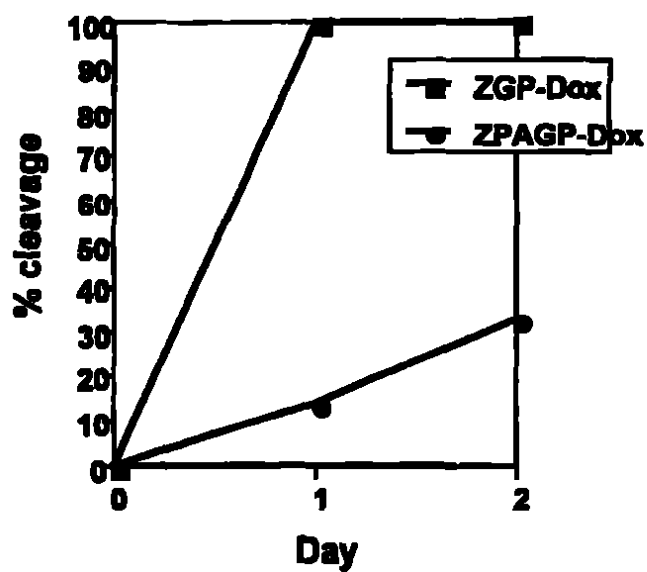
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Figure 4

Human Plasma



Mouse Plasma

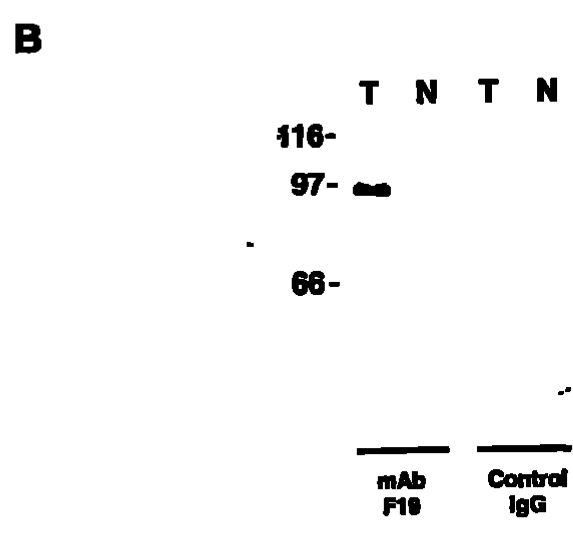
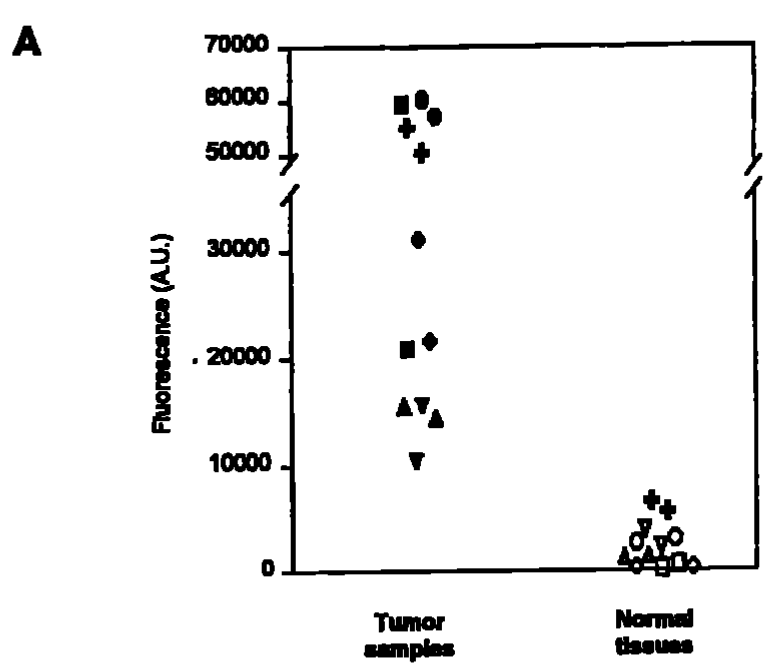


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Figure 5



TRADUCCIÓN: Se traduce la carátula y las reivindicaciones anexas a una copia de la solicitud de patente estadounidense No. 60/134.136, escrita en inglés. Para el invento denominado: **Compuestos antitumorales activados por PAF.**

LOS ESTADOS UNIDOS DE AMÉRICA
A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORQUE UNA FECHA DE PRIORIDAD BAJO 35 USC 11.

SOLICITUD NUMERO: 60/134.136
FECHA DE PRESENTACIÓN: 14 de Mayo, 1999

**Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS**

(Rubrica)

Funcionario Certificador

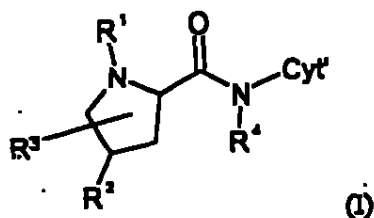
SELL EN RELIEVE

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REIVINDICACIONES

1. Compuesto de la fórmula general (I)

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o de las sales farmacéuticamente aceptables de este o,
donde R¹ es un residuo de la fórmula Cg-A., Cg-B-A. O Cg-D-B-A, en donde

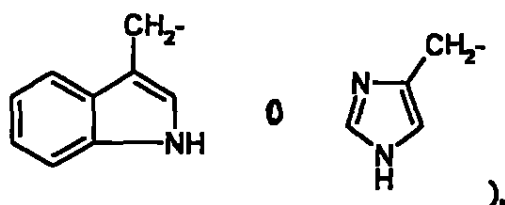
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Cg es H o un grupo tapón con la estructura R⁵-CO-, R⁵-O-CO-, R⁵-NH-CO-, R⁵-SO₂-
o R⁵-, en donde R⁵ es un alquilo C₁-C₆, cicloalquilo C₃-C₈, arilo o sustituyente de
heteroarilo; preferiblemente Cg es un sustituyente acetilo, bencilo, D-alanilo, (R)-
H₂NCH(CH₃)-, o H₂NCOCH₂CH₂- u otro grupo tapón para la protección de la función
amino N-terminal;

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y en donde A y B son unidades derivadas de ácidos amino carboxílicos con la
fórmula [NR⁶-(X)ₚ-CO]- en donde X es CR⁷ R⁸ y en donde R⁶, R⁷ y R⁸ son iguales
o diferentes, y son H, alquilo C₁-C₆ (preferiblemente CH₃-, CH₃CH₂-, CH₃CH₂CH₂-,
(CH₃)₂CH-, CH₃CH₂CH₂CH₂-, (CH₃)₂CHCH₂-, CH₃CH₂CH(CH₃)-, (CH₃)₃C-, HOCH₂-,
CH₃CH(OH)-, CH₃CH(OH)CH₂CH₂-, HOCH₂CH₂CH₂CH₂-, H₂NCH₂CH₂CH₂-,
H₂NCH₂CH₂CH₂CH₂-, H₂NCH₂CH(OH)CH₂CH₂-, H₂NC(=NHCH₂CH₂CH₂-, HSCH₂-,
CH₃SCH₂CH₂-, HOOCCH₂-, HOOCCH₂CH₂-, H₂NC(=O)CH₂-, H₂NC(=O)CH₂CH₂-,
bencilo, para-hidroxil-bencilo,

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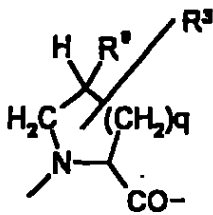


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cicloalquilo C₃-C₈ (preferiblemente ciclohexilo), arilo (preferiblemente fenilo), o
heteroarilo, y p es 1, 2, 3, 4, 5, y en donde la configuración en CR⁷ R⁸ puede ser
R o S; si R⁷ es diferente de H, entonces R⁸ es preferiblemente H; R⁶ es
preferiblemente H; si p es mayor de uno, R⁷, R⁸ son preferiblemente H;

A y B pueden ser unidades derivadas de ácidos aminocarboxílicos cíclicos con la

5 estructura



10 en donde R⁹ es H, alquilo C₁-C₆, OH, o NH₂, R₃ tiene el significado como se definió anteriormente, y q es 1 o 2;

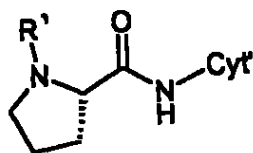
y en donde D es una unidad derivada de un ácido amino carboxílico como se definió para A y B, o D es un oligómero que comprende dos o más de tales unidades, los cuales pueden ser iguales o diferentes, preferiblemente 2 a 10 de tales unidades;

20 y R², R³ son iguales o diferentes, y son hidrógeno, hidroxilo, amino, alquilo C₁-C₆, alquil-amino C₁-C₆, di-C₁-C₆ alquil-amino, alquilo C₁-C₆-oxi, tiol, alquil C₁-C₆-tio, =O, =NH, -CHO, -COOH, -COOCH₂CH₃, -CONH₂, halogeno, cicloalquilo C₃-C₈, arilo, o heteroarilo,

y R⁴ es H, alquilo C₁-C₆, cicloalquilo C₃-C₈, arilo, o heteroarilo
y Cyt' es el residuo de un compuesto citotóxico o citoestático.

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2. Compuesto de la reivindicación con la fórmula



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3. Compuesto de la reivindicación 1 o 2, en donde las unidades A, B, o D se derivan de glicina (Gly), o las formas D-L- de alanina (Ala), valina (Val), leucina (Leu), isoleucina (Ile), fenil alanina (Phe), tirosina (Tyr), triptofano (Trp), cisteína (Cys), metionina (Met), serina (Ser), treonina (Thr), lisina (Lys), arginina (Arg), histidina (His), ácido aspártico (Asp), ácido glutámico (Glu), asparagina (Asn), glutamina (Gln), prolina (Pro), 4- hidroxiprolina, 5-hidroxilisina, norleucina, 5-hidroxinorleucina, o nitina, o ciclohexilglicina.

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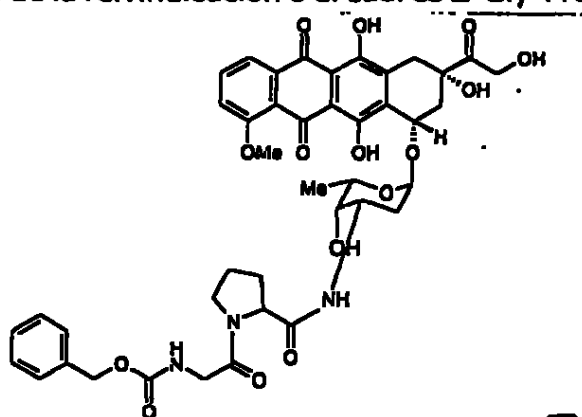
4. Un compuesto de cualquiera de las reivindicaciones 1 a la 3, en donde la

5 unidad A se deriva de L-prolina, glicina, L-norleucina, L-ciclohexilglicina, L-5-hidroxinorleucina, L-6-hidroxinorleucina, L-5-hidroxilicina, L-arginina, o L-licina.

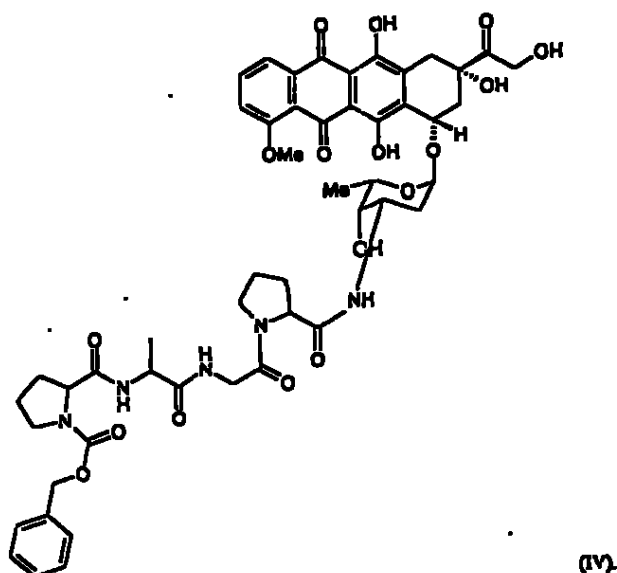
5. Compuesto de cualquiera de las reivindicaciones 1 a 4, en donde la unidad D puede estar presente o consistir de una unidad derivada de un ácido aminocarboxílico según se definió para las unidades A o B

6. Compuesto de cualquiera de las reivindicaciones 1 a 5, en donde H₂N-Cyt' es doxorubicina.

7. Compuesto de la reivindicación 6 el cual es Z-Gly-Pro-Dox de la fórmula (III)



o Z-Pro-Ala-Gly-Pro-Dox de fórmula (IV)



8. Una prodroga que es capaz de ser convertida en una droga por medio de la acción catalítica del FAP α , dicha prodroga tiene un sitio de clivaje que es reconocido por el FAP α y dicha droga es citotóxica o citoestática bajo condiciones fisiológicas.

9. La prodroga de la reivindicación 8 que comprende una parte oligomérica que

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5 comprende dos o más residuos amino carboxílicos, y una parte citotóxica o citoestática, en donde el residuo carboxílico amino C-terminal de la parte oligomérica es D- o L-prolina, o D- o L- hidroxiprolina, y la función carboxi C-terminal está unida a la parte citotóxica o citoestática por medio de un enlace amida.

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10. La prodroga de la reivindicación 9, en donde la parte oligomérica comprende 2, 3, o 4 residuos de ácido aminocarboxílico.

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11. La prodroga de la reivindicación 9 o 10 en donde la función amino N-terminal está protegida por medio de un grupo tapón.

12. Compuesto de cualquiera de la de las reivindicaciones 1 a 11 para uso médico.

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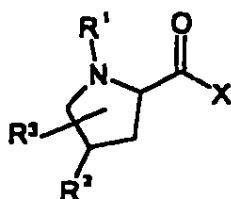
13. Composición farmacéutica que comprende un compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 11 y opcionalmente uno o más excipientes farmacéuticamente aceptables.

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14. Uso de un compuesto de acuerdo a cualquiera de las reivindicaciones 1 a 11 en la preparación de la composición farmacéutica para el tratamiento de cáncer.

15. Proceso para la producción de un compuesto de cualquiera de las reivindicaciones 1 a 11, caracterizado porque el compuesto de la fórmula (V)

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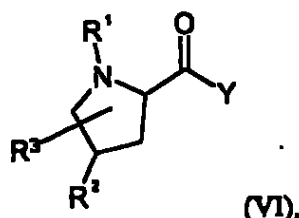
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en donde X puede ser OH, o un grupo saliente que es adecuado para ser sustituido por un grupo amino,

y en donde otros sustituyentes tienen el significado según se describió anteriormente,

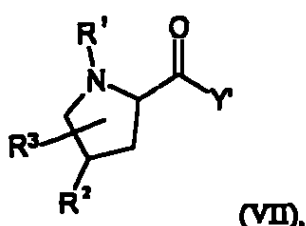
reacciona con un compuesto HN (R⁴)-Cyt', en donde Cyt' es un residuo de un compuesto citotóxico o citoestático.

- 5 16. Proceso para la producción de un compuesto de cualquiera de las reivindicaciones 1 a 11, caracterizado porque el compuesto de fórmula (VI)



en donde Y es de fórmula L-COX, en donde L es un residuo ligador, y X puede ser OH, o un grupo saliente que es adecuado para ser sustituido por medio de un grupo amino o un grupo hidroxilo, y en donde los otros sustituyentes tienen el significado según se describió anteriormente, reacciona con un compuesto H₂N-Cyt' o con un compuesto HO-Cyt', en donde Cyt' es el residuo de un compuesto citotóxico o citoestático.

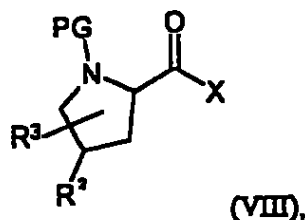
- 20 17. Proceso para la producción de un compuesto de cualquiera de las reivindicaciones 1 a 11, caracterizada porque el compuesto de fórmula (VII)



donde Y' es de fórmula L-OH o L-NH₂, donde L es un residuo ligador y en donde los otros sustituyentes tienen el significado descrito anteriormente,

reacciona con un compuesto XOC-Cyt', en donde X puede ser OH, o un grupo saliente que es adecuado para ser sustituido por medio de un grupo amino o un grupo hidroxilo, y en donde Cyt' es el residuo de un compuesto citotóxico o citoestático.

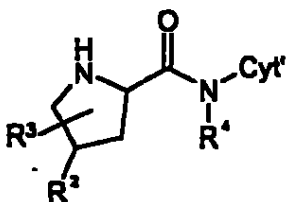
- 35 18. El proceso para la producción de un compuesto de fórmula (I), caracterizada porque el compuesto de fórmula (VIII)



en donde PG es un grupo de protección, y los otros sustituyentes tienen los significados según se describieron anteriormente, reacciona con un compuesto HN(R')-Cyt', en donde Cyt' es el residuo de un compuesto citotóxico o citoestático;

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5 El grupo protector PG es entonces removido y el compuesto resultante



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reacciona con un compuesto PG-A-X, en donde PG es un grupo de protección, y X puede ser OH, o un grupo saliente adecuado para ser sustituido por un grupo amino;

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y etapas de acople adicionales, que son llevadas a cabo, si es necesario, hasta que se completa la fórmula que se obtiene

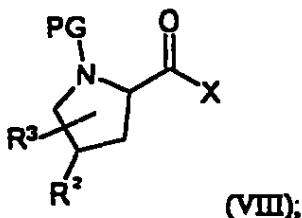
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19. Proceso para la producción de un compuesto de cualquiera de la reivindicaciones 1 a 11, caracterizado porque a parte de un compuesto de fórmula PG-N(R⁴)-L-COX, en donde los sustituyentes tienen los significados descritos anteriormente, reacciona con un compuesto de fórmula H₂N-Cyt', o HOO-Cyt', en donde Cyt' es el residuo de un compuesto citotóxico o citoestático;

El grupo protector PG es entonces removido;

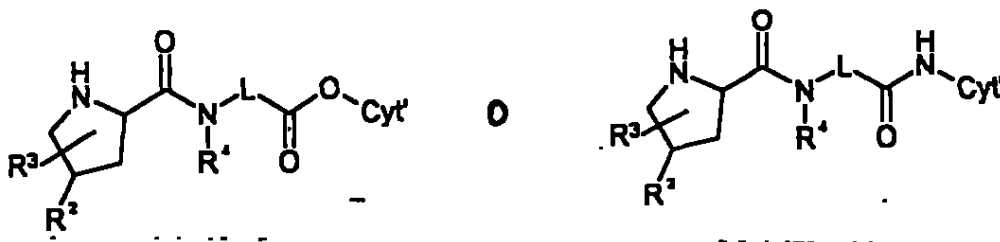
y el compuesto resultante HN(R⁴)-LNH-Cyt' o HN(R⁴)-L-COO-Cyt' reacciona con un compuesto de fórmula (VIII)

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El grupo protector es entonces removido y el compuesto resultante

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reacciona con un compuesto PG-A-X, en donde PG es un grupo protector, y X puede ser OH, o un grupo saliente adecuado para ser sustituido por un grupo amino;

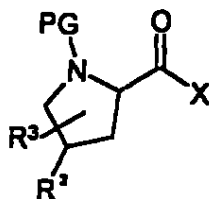
y etapas de acople adicionales llevadas a cabo, si es necesario, hasta que se completa la molécula que se obtiene.

20. Proceso para la producción de un compuesto de fórmula (I), caracterizado porque

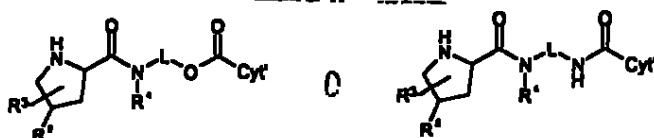
Un compuesto de fórmula PG-N(R4)-LOH o PG-N(R4)-L-NH2, en donde los sustituyentes tienen los significados como se describieron anteriormente, reacciona con un compuesto de la fórmula X-OC-Cyt', en donde Cyt' es el residuo de un compuesto citotóxico o citoestático;

El grupo protector PG es entonces removido;

y el compuesto resultante HN(R4)-L-NH-CO-Cyt' o HN(R4)-L-OOC- Cyt' reacciona con un compuesto de fórmula (VIII)



El grupo protector es entonces removido y el producto resultante



reacciona entonces con un compuesto Pg-A-X, en donde PG es un grupo de protección, y X puede ser OH, o un grupo saliente adecuado para ser sustituido por un grupo amino;

y etapas de acople adicionales, llevadas a cabo, si es necesario, hasta que se completa la molécula que se obtiene.

21. Método de tratamiento de cáncer, que comprende administrar la composición farmacéutica de acuerdo a la reivindicación 13 a un paciente.

22. El método de tratamiento de cáncer en donde se administra una prodroga a un paciente en donde dicha prodroga es capaz de ser convertida en una droga citotóxica o citoestática por medio de una actividad enzimática, dicha actividad enzimática es una actividad de FAP α .