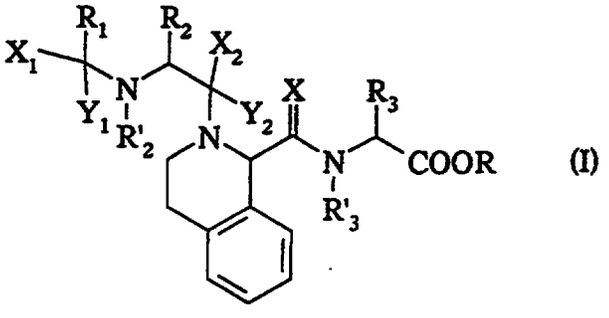
FARNESYL TRANSFERASE INHIBITORS, THEIR PREPARATION AND THE PHARMACEUTICAL COMPOSITIONS WHICH CONTAIN THEM

The present invention relates to new farnesyl transferase inhibitors of general formula:



(I)

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to their preparation and to the pharmaceutical compositions which contain them.

The inhibition of farnesyl transferase, and consequently of the farnesylation of the ras protein, blocks the capacity of the mutated ras protein to transform normal cells into cancerous cells.

The C-terminal sequence of the ras gene contains the unit "CAAX" or "Cys-Aaa$_1$-Aaa$_2$-Xaa", in which Aaa represents an aliphatic amino acid and Xaa represents any amino acid.

It is known that tetrapeptides with a CAAX sequence can inhibit the farnesylation of the ras protein. For example, peptide inhibitors of farnesyl transferase, Cys-Aaa$_1$-Aaa$_2$-Xaa, which are more particularly represented by the peptides Cys-Val-Leu-Ser, Cys-Val-Ile-Met and Cys-Val-Val-Met which manifest

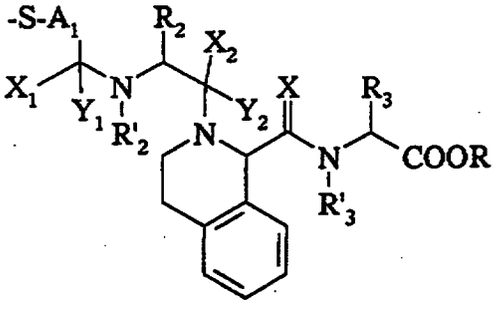
their inhibitory activity at concentrations in the region of 10^{-6}M or 10^{-7}M, have been described in PCT Application WO 91/16340 and in Application EP 0,461,869.

It has now been found, and this forms the subject of the present invention, that the peptides of general formula (I) manifest their inhibitory activity (IC_{50}) at concentrations of the order of 10^{-8}M.

In the general formula (I),

R_1 represents a radical of general formula Y-S-A_1- in which Y represents a hydrogen atom or an amino acid residue or a fatty acid residue or an alkyl or alkoxycarbonyl radical or an R_4-S- radical in which R_4 represents an alkyl radical containing 1 to 6 carbon atoms, optionally substituted by a phenyl radical, or a radical of general formula

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(II)

in which A_1, X_1, Y_1, R_2, R'_2, X_2, Y_2, X, R_3, R'_3 and R are defined as below, and A_1 represents a straight or branched alkylene radical containing 1 to 4 carbon atoms, optionally substituted at the position α to the $>C(X_1)(Y_1)$ group by an amino radical, an alkylamino radical containing 1 to 4 carbon atoms, an alkanoyl-

amino radical containing 1 to 4 carbon atoms or an alkoxycarbonylamino radical in which the alkyl part contains 1 to 4 carbon atoms,

X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_2 represents a straight or branched alkyl radical containing 1 to 4 carbon atoms, optionally substituted by a cyclohexyl radical,

R'_2 represents a hydrogen atom or a straight or branched alkyl radical containing 1 to 6 carbon atoms,

X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a straight or branched containing alkyl radical containing 1 to 4 carbon atoms, optionally substituted by a hydroxyl radical, an alkoxy radical containing 1 to 4 carbon atoms, a mercapto radical, an alkylthio radical containing 1 to 4 carbon atoms, an alkylsulphinyl radical containing 1 to 4 carbon atoms or an alkylsulphonyl radical containing 1 to 4 carbon atoms, it being understood that, when R_3 represents an alkyl radical substituted by a hydroxyl radical, R_3 can form a lactone with the carboxyl radical at the α position,

R'_3 represents a hydrogen atom or a straight or branched alkyl radical containing 1 to 6 carbon atoms,

X represents an oxygen or sulphur atom, and

R represents a hydrogen atom or an alkyl radical, optionally substituted by an alkoxy radical containing 1 to 4 carbon atoms, an alkylthio radical containing 1 to 4 carbon atoms, an alkylsulphinyl radical containing 1 to 4 carbon atoms, an alkylsulphonyl radical containing 1 to 4 carbon atoms, a phenyl radical, a phenoxy radical, a phenylthio radical, a phenylsulphinyl radical, a phenylsulphonyl radical, an alkylamino radical containing 1 to 4 carbon atoms or a dialkylamino radical in which each alkyl part contains 1 to 4 carbon atoms, or a phenyl radical, optionally substituted by one or a number of atoms or radicals, which are identical or different, chosen from halogen atoms and alkyl, alkoxy, alkylthio or alkanoyl radicals containing 1 to 4 carbon atoms.

More particularly,

R_1 represents a radical of formula Y-S-A_1- in which Y represents a hydrogen atom or a lysine residue or a fatty acid residue containing up to 20 carbon atoms and A_1 represents an ethylene or propylene radical optionally substituted by an amino radical,

X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_2 represents an isopropyl, 1-methylpropyl, tert-butyl or cyclohexylmethyl radical,

R'_2 represents a hydrogen atom or a methyl radical,

X_2 and Y_2 each represent a hydrogen atom or form,

together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a methyl or ethyl radical substituted by a hydroxyl, methoxy, mercapto, methylthio, methylsulphinyl or methylsulphonyl radical,

R'_3 represents a hydrogen atom or a methyl radical,

X represents an oxygen atom, and

R represents a hydrogen atom or an alkyl radical containing 1 to 4 carbon atoms, optionally substituted by an alkoxy radical, or a phenyl radical.

More particularly still,

R_1 represents a radical of formula Y-S-A_1- in which Y represents a hydrogen atom and A_1 represents an ethylene or propylene radical optionally substituted by an amino radical,

X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >CO group,

R_2 represents an isopropyl, 1-methylpropyl, tert-butyl or cyclohexylmethyl radical,

R'_2 represents a hydrogen atom,

X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a methyl or ethyl radical substituted by a hydroxyl, methoxy, mercapto or methylthio radical,

R'_3 represents a hydrogen atom, and

R represents a hydrogen atom or an alkyl radical

containing 1 to 4 carbon atoms.

The products of general formula (I) in which R_1 represents a 2-mercaptoethyl or 1-amino-2-mercaptoethyl radical, X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >CO group, R_2 represents an isopropyl radical, X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group, R'_2 represents a hydrogen atom, R_3 represents a 2-(methylthio)ethyl or 2-(methylsulphinyl)ethyl radical, R'_3 represents a hydrogen atom and R represents a hydrogen atom are very particularly advantageous.

The present invention also relates to the stereoisomeric forms of the products of general formula (I). The amino acid residues represented by $R_1C(X_1)(Y_1)$, $R_2CH(NR'_2)[C(X_2)(Y_2)]$ and $R_3CH(NR'_3)CO\text{-}OH$ preferably have the configuration of the natural amino acids.

The present invention also relates to the inorganic or organic salts, and the esters, of the products of general formula (I).

According to the invention, the new products of general formula (I) can be obtained by synthesis on a solid phase using a "9-fluorenylmethoxycarbonyl (FMOC)" synthesis strategy. In this case, the thiol groups are protected with trityl or acetamidomethyl groups, the amine functional groups with Boc (t-butoxycarbonyl) groups and the acid functional

groups in the t-butyl ester form, the alcohol functional groups with t-butyl groups and the amide and imidazole functional groups with trityl groups. The synthesis can be carried out on a resin confined in 3 cm^3 solid-phase extraction syringes made of high density polyethylene equipped with Teflon filters. The syringes are mounted on a two-way Teflon valve and are closed by a disposable high density polyethylene finned plug. Agitation of the syringes is carried out on a rotary device for haemolysis tubes. The washing and filtering operations are carried out on a solid-phase extraction work station.

The syntheses may be carried out on 50 μmol of resin. The couplings of the amino acids are carried out by treating the resin for 1 hour with 250 μmol of the suitably protected amino acid in the presence of 250 μmol of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium, hexafluorophosphate (BTUH), 250 μmol of N-hydoxybenzotriazole and 750 μmol of diisopropylethylamine in 1.2 cm^3 of an N-methyl-2-pyrrolidone (NMP)/dimethylformamide (1/1 by volume) mixture. Deprotection of the FMOC group is carried out with 3 successive treatments of the resin for 2 times 1 minute and then 20 minutes with 2 cm^3 of piperidine as a 2 % (v/v) solution in NMP.

For example, Cys-(NMe)Val-[(R,S)-1,2,3,4-tetrahydroisoquinolyl-1-carbonyl]-Met can be prepared in the following way:

50 μmol of Fmoc-Met resin [Wang resin; Wang et al., J. Amer. Chem. Soc., 95, 1328 (1973)] are successively subjected to the following treatments:

- deprotection of the FMOC group,
- washing with 5 times 2 cm^3 of NMP,
- coupling of FMOC-(R,S)-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid,
- washing with 5 times 2 cm^3 of NMP,
- deprotection of the FMOC group,
- washing with 5 times 2 cm^3 of NMP,
- coupling of FMOC-N-methylvaline,
- washing with 5 times 2 cm^3 of NMP,
- deprotection of the FMOC group, then
- washing with 5 times 2 cm^3 of NMP,
- coupling of FMOC-cysteine(S-trityl),
- washing with 5 times 2 cm^3 of NMP,
- deprotection of the FMOC group, then
- washing with 5 times 2 cm^3 of NMP.

On completion of the synthesis, the products are separated by treatment of the resin with 10 cm^3 of a trifluoroacetic acid/phenol/ethanedithiol/thio-anisole/-water (40/3/1/2/2 by volume) mixture for 1 hour 30 minutes. The resin is then removed by filtration. The filtrate is concentrated under reduced pressure by means of a centrifugal evaporator (RC10-10 Jouan) equipped with a vane pump and with a trap at -90°C for 1.5 hours, the temperature of the evaporation chamber being maintained at 50°C. The final

volume of the concentrate is approximately 1 cm^3. The product is then precipitated by addition of 15 cm^3 of a mixture of methyl tert-butyl ether and petroleum ether (2/1 by volume) and it is then collected by centrifuging. The pellet is then dissolved in 1 cm^3 of trifluoroacetic acid, precipitated by addition of 15 cm^3 of methyl tert-butyl ether and then washed with 15 cm^3 of methyl tert-butyl ether. The product is then dried under reduced pressure (3.5 kPa). The product is finally purified by high performance liquid chromatography (HPLC) on a C_{18} 100 Å column (250 × 10 mm, BioRad) eluted with a gradient of acetonitrile containing 0.07 % of trifluoroacetic acid (by volume) in water containing 0.07 % of trifluoroacetic acid (by volume) at a flow rate of 6 cm^3/min and then lyophilized. The product obtained is characterized by its mass spectrum (electrospray).

1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid, in the racemic form, can be prepared by hydrogenation of isoquinoline-1-carboxylic acid under the conditions described by R.T. Shuman et al., J. Med. Chem., 36, 314 (1993).

Introduction of the FMOC protective group onto an amino acid is carried out by reaction of the amino acid with 9-fluorenylmethyl chloroformate (FMOC-chloride) in the presence of a base.

The inhibitory activity with respect to farnesyl transferase and to farnesylation of the Ras

protein may be demonstrated in the following test:

Farnesyl transferase activity is determined by the quantity of [^{3}H]farnesyl transferred from [^{3}H]farnesyl pyrophosphate ([^{3}H]FPP) to the p21 H-ras protein. The standard reaction mixture is composed, for a final volume of 60 μl, of 50 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM dithiotreitol, 0.2 % octyl β-D-glucopyranoside, 200 picomol p21 H-ras, 4.5 picomol [^{3}H]FPP (activity 61000 dpm/picomol).

Reaction is initiated by adding approximately 5 ng of human farnesyl transferase purified from THP1 cell cultures. After incubation for 20 minutes at 37°C in a microtitration plate containing 96 1-cm^3 wells per plate (Titer Plate®, Beckman), the reaction is stopped by adding 0.4 cm^3 of 0.1 % SDS in methanol at 0°C. The mixture is then treated with 0.4 cm^3 of 30 % trichloroacetic acid® (TCA) in methanol. The plates are left in ice for 1 hour. The precipitated contents are then retained on Filtermat®, Pharmacia) glass fibre® membranes with the filtration unit (Combi Cell Harvester®, Skatron), and rinsed with 6 % trichloroacetic acid in distilled water. The membranes are dried in a microwave oven, then impregnated with scintillation medium by melting Meltilex® (Pharmacia) under hot air, and lastly counted in cpm in a β-Plate® counter (LKB). Each test is repeated 3 times.

The unit of activity is defined as 1 picomol of [^{3}H]FPP transferred to p21 H-ras in 20 minutes.

The percentage inhibition values are obtained by comparison of the tests with and without inhibitor after deduction of blanks, the IC_{50} values being measured on the basis of the inhibitions obtained with 9 different concentrations using Enzfitter® or Grafit® software.

The results obtained are collated in Table I.

TABLE I

Product	Inhibitory activity IC_{50}
Cys-(N-Me)Val-[(R,S)-1,2,3,4-tetrahydoisoquinolyl-1-carbonyl]-Met	2.6×10^{-8} M

The new peptides of general formula (I) can be in the form of non-toxic and pharmaceutically acceptable salts. These non-toxic salts comprise the salts with inorganic acids (hydrochloric, sulphuric, hydrobromic, phosphoric and nitric acids) or with organic acids (acetic, propionic, succinic, maleic, hydroxymaleic, benzoic, fumaric, methanesulphonic, trifluoroacetic or oxalic acid), or with inorganic bases (sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide) or organic bases (tertiary amines such as triethylamine, piperidine, benzylamine), depending on the nature of the constituent amino acids of the peptide of general formula (I).

The new peptides according to the invention, which inhibit farnesyl transferase and farnesylation of the Ras protein, are notable anticancer agents which are active as regards both solid and non-solid tumours.

The present invention also relates to pharmaceutical compositions containing at least one peptide of general formula (I), in combination with one or more pharmaceutically acceptable diluents or adjuvants, which may be either inert or physiologically active.

These compositions may be administered orally, parenterally or rectally.

The compositions for oral administration comprise tablets, pills, powders or granules. In these compositions, the active product according to the invention is mixed with one or more inert diluents such as sucrose, lactose or starch. These compositions can comprise substances other than diluents, for example lubricants such as magnesium stearate.

As liquid compositions for oral administration, solutions, suspensions, syrups, elixirs and pharmaceutically acceptable emulsions, containing inert diluents such as water or liquid paraffin, may be used. These compositions can also comprise substances other than diluents, for example wetting, sweetening or flavouring products.

The compositions according to the invention for parenteral administration can be sterile solutions,

aqueous or non-aqueous, suspensions or emulsions. As solvent or vehicle, propylene glycol, a polyethylene glycol, vegetable oils, especially olive oil, or injectable organic esters, for example ethyl oleate, may be employed. These compositions can also contain adjuvants, especially wetting, emulsifying and dispersing agents. The sterilization may be carried out in several ways, for example using a bacteriological filter, by incorporating sterilizing agents in the composition or by heating. They may also be prepared in the form of sterile solid compositions which can be dissolved at the time of use in sterile water or any other sterile injectable medium.

The compositions for rectal administration are suppositories which can contain, besides the active product, excipients such as cocoa butter.

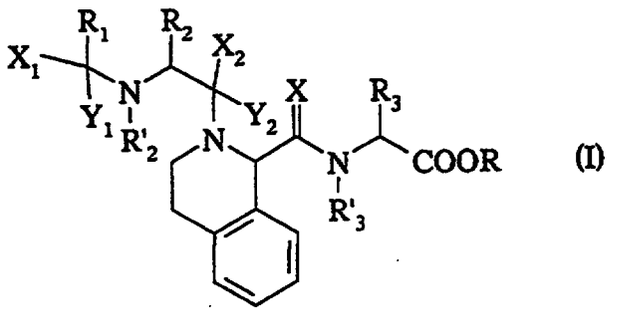
The compositions according to the invention are especially useful in human therapy in the treatment of cancers of various origins.

In human therapy, the doses depend on the effect sought, the period of treatment and factors specific to the subject to be treated.

Generally, in man, the doses are between 0.1 and 20 mg/kg per day via the intraperitoneal route.

CLAIMS

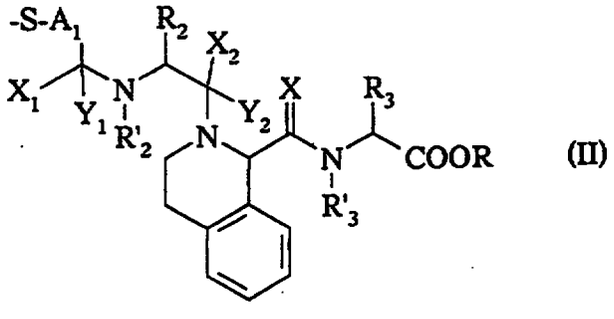
1. New peptides of general formula:



(I)

in which:

R_1 represents a radical of general formula Y-S-A_1- in which Y represents a hydrogen atom or an amino acid residue or a fatty acid residue or an alkyl or alkoxycarbonyl radical or an R_4-S- radical in which R_4 represents an alkyl radical containing 1 to 6 carbon atoms, optionally substituted by a phenyl radical, or a radical of general formula



(II)

in which A_1, X_1, Y_1, R_2, R'_2, X_2, Y_2, X, R_3, R'_3 and R are defined as below, and A_1 represents a straight or branched alkylene radical containing 1 to 4 carbon atoms, optionally substituted at the position α to the $>C(X_1)(Y_1)$ group by an amino radical, an alkylamino radical containing 1 to 4 carbon atoms, an alkanoyl-

* C L M S *

amino radical containing 1 to 4 carbon atoms or an alkoxycarbonylamino radical in which the alkyl part contains 1 to 4 carbon atoms,

X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_2 represents a straight or branched alkyl radical containing 1 to 4 carbon atoms, optionally substituted by a cyclohexyl radical,

R'_2 represents a hydrogen atom or a straight or branched alkyl radical containing 1 to 6 carbon atoms,

X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a straight or branched containing alkyl radical containing 1 to 4 carbon atoms, optionally substituted by a hydroxyl radical, an alkoxy radical containing 1 to 4 carbon atoms, a mercapto radical, an alkylthio radical containing 1 to 4 carbon atoms, an alkylsulphinyl radical containing 1 to 4 carbon atoms or an alkylsulphonyl radical containing 1 to 4 carbon atoms, it being understood that, when R_3 represents an alkyl radical substituted by a hydroxyl radical, R_3 can form a lactone with the carboxyl radical at the α position,

R'_3 represents a hydrogen atom or a straight or branched alkyl radical containing 1 to 6 carbon atoms,

X represents an oxygen or sulphur atom, and

* C L M S *

R represents a hydrogen atom or an alkyl radical, optionally substituted by an alkoxy radical containing 1 to 4 carbon atoms, an alkylthio radical containing 1 to 4 carbon atoms, an alkylsulphinyl radical containing 1 to 4 carbon atoms, an alkylsulphonyl radical containing 1 to 4 carbon atoms, a phenyl radical, a phenoxy radical, a phenylthio radical, a phenylsulphinyl radical, a phenylsulphonyl radical, an alkylamino radical containing 1 to 4 carbon atoms or a dialkylamino radical in which each alkyl part contains 1 to 4 carbon atoms, or a phenyl radical, optionally substituted by one or a number of atoms or radicals, which are identical or different, chosen from halogen atoms and alkyl, alkoxy, alkylthio or alkanoyl radicals containing 1 to 4 carbon atoms.

2. New peptides according to claim 1, in which R_1 represents a radical of formula Y-S-A_1- in which Y represents a hydrogen atom or a lysine residue or a fatty acid residue containing up to 20 carbon atoms and A_1 represents an ethylene or propylene radical optionally substituted by an amino radical,
X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,
R_2 represents an isopropyl, 1-methylpropyl, tert-butyl or cyclohexylmethyl radical,
R'_2 represents a hydrogen atom or a methyl radical,
X_2 and Y_2 each represent a hydrogen atom or form,

* C L M S *

together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a methyl or ethyl radical substituted by a hydroxyl, methoxy, mercapto, methylthio, methylsulphinyl or methylsulphonyl radical,

R'_3 represents a hydrogen atom or a methyl radical,

X represents an oxygen atom, and

R represents a hydrogen atom or an alkyl radical containing 1 to 4 carbon atoms, optionally substituted by an alkoxy radical, or a phenyl radical.

3. New peptides according to claim 1, in which R_1 represents a radical of formula Y-S-A_1- in which Y represents a hydrogen atom and A_1 represents an ethylene or propylene radical optionally substituted by an amino radical,

X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >CO group,

R_2 represents an isopropyl, 1-methylpropyl, tert-butyl or cyclohexylmethyl radical,

R'_2 represents a hydrogen atom,

X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group,

R_3 represents a methyl or ethyl radical substituted by a hydroxyl, methoxy, mercapto or methylthio radical,

R'_3 represents a hydrogen atom, and

R represents a hydrogen atom or an alkyl radical

* C L M S *

containing 1 to 4 carbon atoms.

4. New peptides according to claim 1, in which R_1 represents a 2-mercaptoethyl or 1-amino-2-mercaptoethyl radical, X_1 and Y_1 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >CO group, R_2 represents an isopropyl radical, X_2 and Y_2 each represent a hydrogen atom or form, together with the carbon atom to which they are bonded, a >C=O group, R'_2 represents a hydrogen atom, R_3 represents a 2-(methylthio)ethyl or 2-(methylsulphinyl)ethyl radical, R'_3 represents a hydrogen atom and R represents a hydrogen atom.

5. Pharmaceutical composition, characterized in that it contains a sufficient amount of a peptide according to one of claims 1 to 4 in combination with one or a number of inert or physiologically active pharmaceutically acceptable diluents or adjuvants.

* C L M S *