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(54) **COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN**

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(52) **U.S. Cl.** **514/16**; 530/311; 514/2; 540/456

(58) **Field of Search** 514/15

(56) **References Cited**

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(57) **ABSTRACT**

A combination of a compound of the somatostatin class and a rapamycin macrolide is useful for the prevention or treatment of cell hyperproliferation.

13 Claims, No Drawings

COMBINATION OF A SOMATOSTATIN ANALOGUE AND A RAPAMYCIN

The present invention relates to a pharmaceutical combination and its use in the treatment of disorders associated with excess benign and malignant cell proliferation, e.g. tumors or intimal cell proliferation.

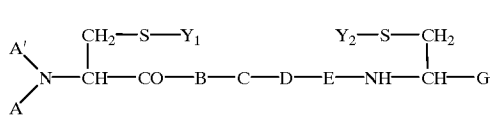
There is a continuing need for the development of drugs having increased effectiveness in inhibiting or slowing down undesired cell proliferation, particularly in the cancer field and in vasculopathies.

Accordingly, there is provided a pharmaceutical combination comprising a compound of the somatostatin class, and a rapamycin macrolide.

The somatostatin class is a known class of small peptides comprising the naturally occurring somatostatin-14 and analogues having somatostatin related activity, e.g. as disclosed by A. S. Dutta in Small Peptides, Vol. 19, Elsevier (1993). By "somatostatin analogue" as used herein is meant any straight-chain or cyclic polypeptide having a structure based on that of the naturally occurring somatostatin-14 wherein one or more amino acid units have been omitted and/or replaced by one or more other amino radical(s) and/or wherein one or more functional groups have been replaced by one or more other functional groups and/or one or more groups have been replaced by one or several other isosteric groups. In general, the term covers all modified derivatives of the native somatostatin-14 which exhibit a somatostatin related activity, e.g. they bind to at least one somatostatin receptor (hSST-1, hSST-2, hSST-3, hSST4 or hSST-5), preferably in the nMolar range, more preferably to at least the hSST-2 receptor in the nMolar range.

Cyclic, bridge cyclic and straight-chain somatostatin analogues or derivatives are known and have been described together with processes for their production e.g. in U.S. Pat. Nos. 4,310,518 and 4,235,886, in European Patent Specifications EP-A-1295; 23,192; 29,310; 29,579; 30,920; 31,303; 63,308; 70,021; 83,305; 215,171; 203,031; 214,872; 143, 307; 298,732; 277,419; 389,180; 395,417; 450,480A2; in Belgian Patent Specification BE-A-900,089; and in WO 91/09056; WO 97/01579; WO 97/14715, the contents thereof, in particular with respect to the compounds, being incorporated herein by reference.

Preferred somatostatin analogues are e. g. compounds of formula I



wherein

A is C_{1-12}alkyl, C_{7-10}phenylalkyl or a group of formula RCO—, whereby

i) R is hydrogen, C_{1-11}alkyl, phenyl or C_{7-10}phenylalkyl, or

ii) RCO— is

a) a D-phenylalanine residue optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy; or

b) the residue of a natural or a synthetic α-amino-acid other than defined under a) above, or of a corresponding D-amino acid, or

c) a dipeptide residue in which the individual amino acid residues are the same or different and are selected from those defined under a) and/or b) above, the α-amino group of amino acid residues a) and b) and the N-terminal amino group of dipeptide residues c) being optionally mono- or di-C_{1-12}alkylated or substituted by C_{1-8}alkanoyl;

A' is hydrogen or C_{1-3}alkyl,

Y_1 and Y_2 represent together a direct bond or each of Y_1 and Y_2 is hydrogen

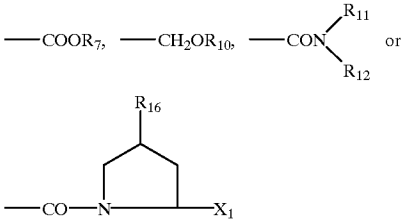
B is -Phe- optionally ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and /or C_{1-3}alkoxy (including pentafluoroalanine), naphthylalanine or pyridylalanine,

C is (L)-Trp- or (D)-Trp- optionally α-N-methylated and optionally benzene-ring-substituted by halogen, NO_2, NH_2, OH, C_{1-3}alkyl and/or C_{1-3}alkoxy,

D is Lys, 4-aminocyclohexylAla or 4-aminocyclohexylGly,

E is Thr, Ser, Val, Tyr, lie, Leu or an aminobutyric or aminoisobutyric acid residue,

G is a group of formula



wherein

R_7 is hydrogen or C_{1-3}alkyl,

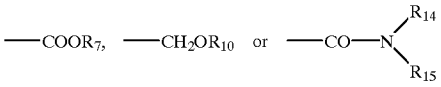
R_{10} is hydrogen or the residue of a physiologically acceptable, physiologically hydrolysable ester, e.g. formnyl, C_{2-12}alkylcarbonyl, benzoyl,

R_{11} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl

R_{12} is hydrogen, C_{1-3}alkyl or a group of formula —CH(R_{13})—X_1,

R_{13} is CH_2OH, —$(CH_2)_2$—OH, —$(CH_2)_3$—OH, —CH(CH_3)OH, isobutyl, butyl, benzyl, naphthyl-methyl or indol-3-yl-methyl, and

X_1 is a group of formula



wherein

R_7 and R_{10} have the meanings given above,

R_{14} is hydrogen or C_{1-3}alkyl,

R_{15} is hydrogen, C_{1-3}alkyl, phenyl or C_{7-10}phenylalkyl, and

R_{16} is hydrogen or hydroxy,

with the proviso that

when R_{12} is —CH(R_{13})—X_1, then R_{11} is hydrogen or methyl,

wherein the residues B, D and E have the L-configuration, and the residues in the 2- and 7-position each independently have the (L)- or (D)-configuration,

in free form or in pharmaceutically acceptable salt or complex form.

Individual compounds of formula I suitable in accordance with the present invention are the following somatostatin analogues:

a. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol

also known as octreotide

b. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

c. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-TrpNH_2

also known as vapreotide

d. (D)Trp-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

e. (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-ThrNH_2

f. 3-(2-(Naphthyl)-(D)Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-ThrNH_2

also known as lanreotide

g. (D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

h. 3-(2-naphthyl)-Ala-Cys-Tyr-(D)Trp-Lys-Val-Cys-β-Nal-NH_2

i. (D)Phe-Cys-β-Nal-(D)Trp-Lys-Val-Cys-Thr-NH_2

j. (D)Phe-Cys-Tyr-(D)Trp-Lys-Leu-Cys-Thr-NH_2

k. (D)Phe-Cys-Tyr-(D)Trp-Lys-Cys-Thr-NH_2.

A preferred compound of formula I is octreotide.

Compounds of formula I may exist e.g. in free form, salt form or in the form of complexes thereof. Acid addition salts may be formed with e.g. organic acids, polymeric acids and inorganic acids. Such acid addition salt forms include e.g. the hydrochlorides and acetates. Complexes are e.g. formed from compounds of the invention on addition of inorganic substances, e.g. inorganic salts or hydroxides such as Ca- and Zn-salts, and/or on addition of polymeric organic substances.

Further somatostatin analogues suitable for use in accordance with the present invention are:

cyclo [-Asn-Phe-Phe-DTrp-Lys-Thr-Phe-Gaba-], cyclo (Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser), and

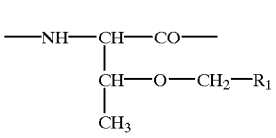
(D)Nal-Glu-Tyr-(D)Trp-Lys-Val-Lys-Thr-NH_2.

According to an alternatively preferred embodiment of the invention, the somatostatin component of the combination is a somatostatin analogue comprising the amino acid sequence of formula (II)

—(D/L)Trp-Lys-X_2—X_3— (II)

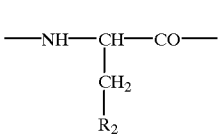
wherein

X_2 is a radical of formula (a) or (b)



(a)

or

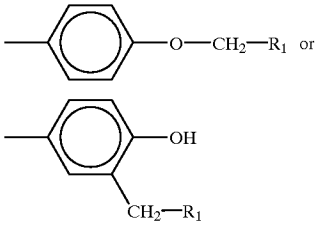


(b)

wherein

R_1 is optionally substituted phenyl,

R_2 is —Z_1—CH_2—R_1, —CH_2—CO—O—CH_2—R_1,



wherein

Z_1 is O or S, and

X_3 is an α-amino acid having an aromatic residue on the C_α side chain, or an amino acid unit selected from Dab, Dpr, Dpm, His,(Bzl)HyPro, thienyl-Ala, cyclohexyl-Ala and t.-butyl-Ala,

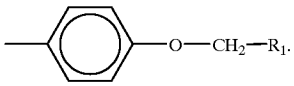
the residue Lys of said sequence corresponding to the residue Lys^9 of the native somatostatin-14.

Such somatostatin analogues are e.g. disclosed in WO/97/01579, the contents thereof, in particular with respect to the specifically exemplified compounds, being incorporated herein by reference.

Preferably the sequence of formula II as defined above corresponds to the residues at positions 8 through 11 of the somatostatin-14. More preferably the somatostatin analogue as disclosed above comprises a hexapeptide unit, the residues at positions 3 through 6 of said hexapeptide unit comprising the sequence of formula II. More particularly the hexapeptide unit is cyclic, e.g. having a direct peptide linkage between the α-carbonyl group of the residue at position 6 and the α-amino group of the residue at position 1.

While Lys, X_2 and X_3 in the sequence of formula II have the L-configuration, Trp may have the D- or L-configuration, preferably the D-configuration.

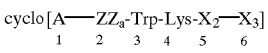
X_2 is preferably a residue of formula (a) or (b), R_2 being preferably —Z_1—CH_2—R_1 or



When X_3 comprises an aromatic residue on the C_α side chain, it may suitably be a natural or unnatural α-amino acid, e.g. Phe, Tyr, Trp, Nal, Pal, benzothienyl-Ala, Tic and thyronin, preferably Phe or Nal, more preferably Phe. X_3 is preferably an α-amino acid bearing an aromatic residue on the C_α side chain.

When R_1 is substituted phenyl, it may suitably be substituted by halogen, methyl, ethyl, methoxy or ethoxy e.g. in ortho and/or position. More preferably R_1 is unsubstituted phenyl. Z_1 is preferably O.

Representative somatostatin analogues comprising a residue of formula II are e.g compounds of formula (III)

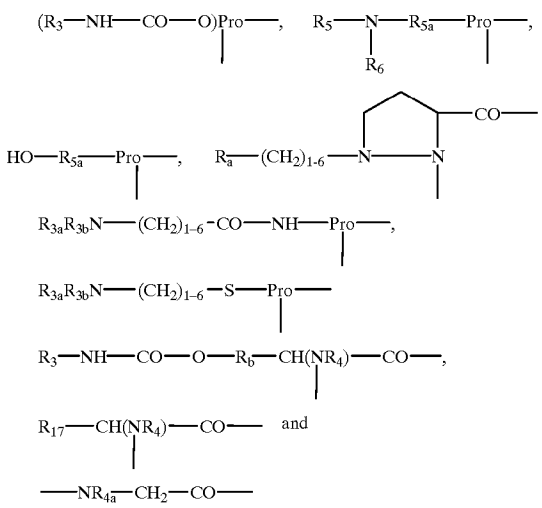


(II)

wherein

X_2 and X_3 are as defined above,

A_1 is a divalent residue selected from Pro,

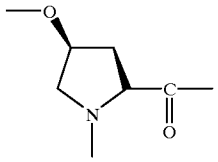


wherein R_3 is NR_8R_9—C_{2-6}alkylene, guanidino-C_{2-6} alkylene or C_{2-6}alkylene-COOH, R_{3a} is H, C_{1-4}alkyl or has independently one of the significances given for R_3 $R_{3b}$is H or C_{1-4}alkyl, R_a is OH or NR_5R_6, R_b is —$(CH_2)_{1-3}$— or —$CH(CH_3)$—, R_4 is H or CH_3, R_{4a} is optionally ring-substituted benzyl, each of R_5 and R_6 independently is H, C_{1-4}alkyl, ω-amino-C_{1-4}alkylene, ω-hydroxy-C_{1-4}alkylene or acyl, $R_{5a}$is a direct bond or C_{1-6}alkylene, each of R_8 and R_9 independently is H, C_{1-4}alkyl, ω-hydroxy-C_{2-4}alkylene, acyl or CH_2OH—$(CHOH)_c$—CH_2— wherein c is 0, 1, 2, 3 or 4, or R_8 and R_9 form together with the nitrogen atom to which they are attached a heterocyclic group which may comprise a further heteroatom, and R_{17} is optionally ring-substituted benzyl, —$(CH_2)_{1-3}$—OH, CH_3—$CH(OH)$— or —$(CH_2)_{1-5}$—NR_5R_6, and

ZZ_a is a natural or unnatural α-amino acid unit.

ZZ_a may have the D- or L-configuration. When ZZ_a is a natural or unnatural α-amino acid unit, it may suitably be e.g. Thr, Ser, Ala, Val, Ile, Leu, Nle, His, Arg, Lys, Nal, Pal, Tyr, Trp, optionally ring-substituted Phe or N^{α}-benzyl-Gly. When ZZ_a is Phe, the benzene ring thereof may be substituted by e.g. NH_2, NO_2, CH_3, OCH_3 or halogen, preferably in para position. When ZZ_a is Phe, the benzene ring thereof is preferably unsubstituted.

When A_1 comprises a Pro amino acid residue, any substituent present on the proline ring, e.g. R_3—NH—CO—O— etc., is preferably in position 4. Such substituted proline residue may exist in the cis form, e.g.

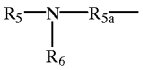


as well as in the trans form. The present invention covers each geometric isomer individually as well as mixtures thereof.

When A_1 is (NR_8R_9—C_{6-2}alkylene-NH—CO—)Pro- where NR_8R_9 forms a heterocyclic group, such group may be aromatic or saturated and may comprise one nitrogen or one nitrogen and a second heteroatom selected from nitrogen and oxygen. Preferably the heterocyclic group is e.g. pyridyl or morpholino. C_{2-6}Alkylene in this residue is preferably —CH_2—CH_2—.

Any acyl as R_5, R_6, R_8 and R_9 in A_1 may be e.g. $R_{18}CO$— wherein R_{18} is H, C_{1-4}alkyl, C_{2-4}alkenyl, C_{3-6}cycloalkyl or benzyl, preferably methyl or ethyl. When R_{4a}, or R_{17} in A_1 is ring-substituted benzyl, the benzene ring may be substituted as indicated above for ZZ_a.

A preferred group of compounds of formula III are such wherein A_1 is free of a lateral —NH—CO—O— moiety. A further group of preferred compounds of formula III are such wherein A_1 comprises a basic lateral radical, e.g. a R_3—NH—CO—O— or



moiety.

A still further group of preferred compounds of formula III are such wherein the N-terminal amino acid comprises a substituted Pro, particularly 4-substituted Pro, e.g. compounds of formula III wherein A_1 is 4-substituted Pro.

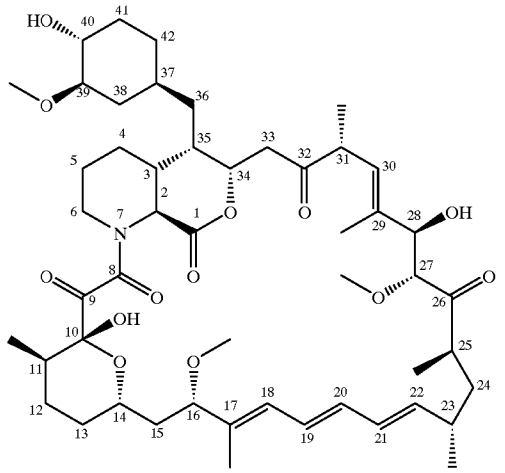
Preferably A_1 is 4-(R_3—NH—CO—O)Pro.

Examples of somatostatin analogues comprising a residue of formula II include e.g. cyclo [4—(NH_2—C_2H_4—NH—CO—O—)Pro-Phe-DTrp-Lys-Ser(Benzyl)-Phe].

The term "macrolide" as used herein, refers to a macrocyclic lactone, for example a compound having a 12-membered or larger lactone ring. Of particular interest are the "lactam macrolides", i.e. macrocyclic compounds having a lactam (amide) bond in the macrocycle in addition to a lactone (ester) bond, for example rapamycin and its numerous derivatives and analogues. Rapamycin is an immunosuppressive lactam macrolide that is produced by

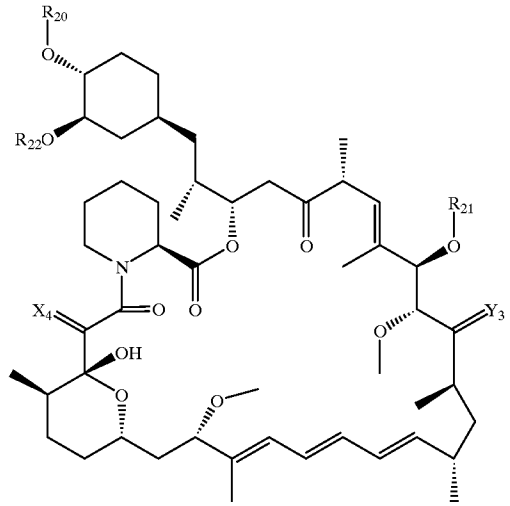
Streptomyces hygroscopicus, and having the structure depicted in Formula

(A)



See, e.g., McAlpine, J. B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S. L., et al., J. Am. Chem. Soc. (1991) 113: 7433; U.S. Pat. No. 3 929 992. One group of rapamycin derivatives are 40-0-substituted derivatives of rapamycin having the structure of Formula IV:

IV



wherein

X_4 is (H,H) or O;

Y_3 is (H,OH) or O;

R_{20} and R_{21} are independently selected from H, alkyl, arylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkoxycarbonylalkyl, hydroxyalkylaryalkyl, dihydroxyalkylarylalkyl, acyloxyalkyl, aminoalkyl, alkylaminoalkyl, alkoxycarbonylaminoalkyl, acylaminoalkyl, arylsulfonamidoalkyl, allyl, dihydroxyalkylallyl, dioxolanylallyl, dialkyl-dioxolanylalkyl, di(alkoxycarbonyl)-triazolyl-alkyl and hydroxyalkoxy-alkyl; wherein "alk-" or "alkyl" refers to C_{1-6}alkyl, branched or linear, preferably C_{1-3}alkyl,; "aryl" is phenyl or tolyl; and acyl is a radical derived from a carboxylic acid; and

R_{22} is methyl or R_{22} and R_{20} together form C_{2-6}alkyl; provided that R_{20} and R_{21} are not both H; and hydroxy-alkoxyalkyl is other than hydroxyalkoxymethyl.

Such compounds are disclosed in WO 94/09010 the contents of which, in particular with respect to the specifically exemplified compounds, are incorporated herein by reference.

A preferred compound is e.g. 40-O-(2-hydroxy)ethyl-rapamycin (referred thereafter as Compound B).

Further preferred rapamycin derivatives are e.g. those disclosed in WO 96/41807, the contents thereof, in particular with respect to the specifically exemplified compounds of formula I disclosed therein, being incorporated herein by reference. Particularly preferred are 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxyethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-rapamycin and 1 6-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Further rapamycin derivatives are known, e.g. carboxylic acid esters such as disclosed in WO 92/05179, amide esters such as disclosed in U.S. Pat. No. 5,118,677, carbamates such as described in U.S. Pat. No. 5,118,678, fluorinated esters such as disclosed in U.S. Pat. No. 5,100,883, acetals, e.g. in U.S. Pat. No. 5,151,413, silyl ethers, e.g. in U.S. Pat. No. 5,120,842, arylsulfonates and sulfamates, e.g. in U.S. Pat. No. 5 177 203, derivatives wherein the methoxy group at the position 16 is replaced with alkynyloxy, e.g. in WO 95/16691 and further derivatives such as disclosed in WO 93/11130, WO 94/02136, WO 94/02385 and WO 95/14023, all incorporated herein by reference.

Rapamycin and above mentioned derivatives have been shown to have potent immunosuppressant properties. Rapamycin has also been shown to inhibit smooth muscle cell proliferation and to inhibit cancer growth.

Somatostatin analogues, e.g. octreotide, vapreotide and lanreotide, have been disclosed i.a. to inhibit growth hormone secretion and to have an inhibiting effect on malignant tumor growth, e.g. in breast cancer. Octreotide and lanreotide have also been disclosed to inhibit smooth muscle cell proliferation.

In accordance with the invention, it has now surprisingly been found that a combination of 2 active ingredients believed to act on basically different mechanisms such as a somatostatin analogue and rapamycin or a derivative thereof, can be combined and synergistically inhibit cell hyperproliferation.

In accordance with the particular findings of the present invention, there is provided in a first aspect:

1. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, for manufacturing a pharmaceutical composition for use in synergistically effective amounts in the prevention or treatment of cell hyperproliferation in combination with a rapamycin macrolide, e.g. for the manufacture of a kit as disclosed hereinafter.
2. Use of a compound of the somatostatin class, in free form or in pharmaceutically acceptable salt form, in combination in synergistically effective amounts with a rapamycin macrolide for the prevention or treatment of cell hyperproliferation.
3. A method for preventing or treating cell hyperproliferation in a subject in need of such treatment which comprises administering to such subject a synergistically

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