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(72) Feltaláló(k):
ASAMI, Taiji, Tsukuba-shi Ibaraki 3004293 (JP)
NISHIZAWA, Naoki, Tsukuba-shi Ibaraki 3004293 (JP)

(73) Jogosult(ak):
Takeda Pharmaceutical Company Limited, Osaka- shi, Osaka 541-0045 (JP)

(74) Képviselő:
dr. Török Ferenc, DANUBIA Szabadalmi és Jogi Iroda Kft., Budapest

(54) **Metasztin-származékok és azok alkalmazása**

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(73) Proprietor: **Takeda Pharmaceutical Company Limited**
Osaka- shi, Osaka 541-0045 (JP)

(72) Inventors:
- **ASAMI, Taiji**
  Tsukuba-shi
  Ibaraki 3004293 (JP)
- **NISHIZAWA, Naoki**
  Tsukuba-shi
  Ibaraki 3004293 (JP)

(74) Representative: **Brearley, Helen Rebecca**
Elkington and Fife LLP
Prospect House
8 Pembroke Road
Sevenoaks
Kent TN13 1XR (GB)

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EP 2 081 955 B1

Description

TECHNICAL FIELD

[0001] The present invention relates to metastin derivatives and use thereof.

BACKGROUND OF THE INVENTION

[0002] Human-derived metastin (also called KiSS-1 peptide) (WO00/24890) and mouse or rat-derived metastin (WO0175104) are known. Sustained release preparations containing metastin are also known (WO02/85399).

[0003] Reportedly, metastin has an effect of suppressing cancer metastasis and is thus effective for preventing/treating cancers (for example, lung cancer, gastric cancer, liver cancer, pancreatic cancer, colorectal cancer, rectal cancer, colonic cancer, prostate cancer, ovarian cancer, cervical cancer, breast cancer, renal cancer, bladder cancer, brain tumor, etc.); metastin also has an effect of controlling the pancreatic function and is effective for preventing/treating pancreatic diseases (e.g., acute or chronic pancreatitis, pancreatic cancer, etc.); and metastin further has an effect of controlling placental function and is effective for preventing/treating choriocarcinoma, hydatidiform mole, invasive mole, miscarriage, fetal hypoplasia, abnormal glucose metabolism, abnormal lipid metabolism or abnormal delivery (WO00/24890, WO01/75104, WO02/85399).

DISCLOSURE OF THE INVENTION

[0004] The present invention aims at providing stable metastin derivatives having excellent biological activities (a cancer metastasis suppressing activity, a cancer growth suppressing activity, a gonadotropic hormone secretion stimulating activity, sex hormone secretion stimulating activity, a gonadotropic hormone secretion suppressing activity, sex hormone secretion suppressing activity, etc.)

[0005] The present inventors have made extensive studies to solve the foregoing problems and as a result, have found that by substituting the constituent amino acids of metastin with specific amino acids, unexpectedly blood stability, solubility, etc. are more improved, gelation tendency is reduced, pharmacokinetics are also improved, and an excellent cancer metastasis suppressing activity or a cancer growth suppressing activity is exhibited. The present inventors have further found that unexpectedly these metastin derivatives have an effect of suppressing the gonadotropic hormone secretion, an effect of suppressing the sex hormone secretion, etc., which are totally different from the effects known so far. Based on these findings, the present inventors have continued further investigations and come to accomplish the present invention.

[0006] That is, the present invention provides the following features, and so on.

[1] A compound selected from:

Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 (Compound No. 903),
Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 (Compound No. 926), and
Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 (Compound No. 927), or a salt thereof.

[2] The compound according to [1], which is
Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 or a salt thereof.
[3] The compound according to [1], which is
Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 or a salt thereof.
[4] The compound according to [1], which is
Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 or a salt thereof.
[5] A medicament comprising the compound according to [1] or a salt thereof.

[0007] The present invention further provides the following features, and so on.

[6] The compound according to [1], or a salt thereof, for use in therapy.
[7] The compound according to [1], or a salt thereof, for use as an agent for suppressing cancer metastasis or an agent for suppressing cancer growth.
[8] The compound according to [1], or a salt thereof, for use as an agent for preventing/treating cancer.
[9] The compound according to [1], or a salt thereof, for use as an agent for down-regulating gonadotropic hormone or sex hormone.
[10] The compound according to [1], or a salt thereof, for use as an agent for preventing/treating hormone-dependent

cancer.

[0008] The metastin derivative of the present invention, and salts thereof have excellent blood stability, in addition to excellent cancer metastasis inhibiting action or cancer growth suppressing action and are useful as agents for preventing/treating cancers (e.g., lung cancer, gastric cancer, liver cancer, pancreatic cancer, colorectal cancer, rectal cancer, colonic cancer, prostate cancer, ovarian cancer, cervical cancer, breast cancer, etc.). The metastin derivative of the present invention, and salts thereof have the effects of regulating functions of the pancreas and are useful as medicaments for preventing/treating pancreatic diseases (e.g., acute or chronic pancreatitis, pancreatic cancer, etc.). The metastin derivative of the present invention, and salts thereof have the effects of regulating functions of the placenta and are useful as medicaments for preventing/treating choriocarcinoma, hydatidiform mole, invasive mole, miscarriage, fetal hypoplasia, abnormal glucose metabolism, abnormal lipid metabolism or labor induction.

[0009] Also, the metastin derivative of the present invention and salts have the effects of increasing sugar level, promoting pancreatic glucagon secretion and promoting urine formation, and are useful as agents for preventing/treating obesity, hyperlipemia, type II diabetes mellitus, hypoglycemia, hypertension, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, edema, urinary disturbances, insulin resistance, unstable diabetes mellitus, fatty atrophy, insulin allergy, insulinoma, arteriosclerosis, thrombotic disorders or lipotoxicity.

[0010] In addition, the metastin derivative of the present invention and salts have excellent activities of stimulating gonadotropic hormone secretion, stimulating sex hormone secretion, inducing ovulation or stimulating ovulation, and are useful as low toxic and stable agents, e.g., agents for improving gonadal function, agents for preventing/treating hormone-dependent cancer (e.g., prostate cancer, breast cancer, etc.), infertility, endometriosis, early puberty, myoma of the uterus, etc., agents for inducing or stimulating ovulation, gonadotropic hormone secretagogue agents, contraceptives, sex hormone secretagogue agents, or the like.

[0011] The metastin derivative of the present invention and salts are useful as agents for suppressing gonadotropic hormone secretion or suppressing sex hormone secretion; agents for down-regulating gonadotropic hormone or sex hormone; agents for down-regulating human OT7T175 (metastin receptor) protein consisting of the amino acid sequence represented by SEQ ID NO: 9; agents for preventing/treating hormone-dependent cancers (e.g., prostate cancer, breast cancer, etc.; particularly, hormone-sensitive prostate cancer, hormone-sensitive breast cancer, etc.); agents for preventing/treating endometriosis; agents for inhibiting ovarian follicular maturation; menstrual cycle-suspending agents; agents for treating myoma of the uterus; agents for treating early puberty; contraceptives, etc.

[0012] In addition, the metastin derivative of the present invention and salts are useful as agents for potentiating immunity (e.g., prophylactic agents for infection after bone-marrow transplant, agents for potentiating immunity intended for cancer, etc); immunostimulators (e.g., regeneration of the thymus, regrowth of the thymus, enhancement of T cell development, etc); agents for preventing/treating bulbospinal muscular atrophy; agents for protecting ovary; agents for preventing/treating benign prostate hypertrophy (BPH); agents for preventing/treating gender identity disorder; or agents for in vitro fertilization (IVF). In addition, they are also useful as agents for preventing/treating infertility, hypogonadism, oligospermia, azoospermia, aspermia, asthenospermia, or necrospermia. Further, they are useful for hormone-dependent diseases (e.g., sex hormone dependent cancer such as prostate cancer, uterine cancer, breast cancer, hypophyseal tumor, etc.), prostate gland enlargement, endometriosis, uterine fibroid, early puberty, dysmenorrhea, amenorrhea, menstrual syndrome, multilocular ovary syndrome, postoperative relapse of the above-mentioned cancers, metastasis of the above-mentioned cancers, hypopituitarism, dwarfism (the case where the secretion of growth hormone was compromised with hyposecretion of pituitary hormone, etc.), menopausal disorder, indefinite complaint, sex hormone dependent disorders such as calcium phosphor bone metabolic disorders. It is also applicable for contraception (or infertility when rebound effects after cessation of the drug are utilized), etc.

[0013] Moreover, metastin per se or DNA encoding metastin, etc. are also useful as agents for suppressing gonadotropic hormone secretion or sex hormone secretion; down-regulating agents for gonadotropic hormone or suppressing sex hormone; down-regulating agents for human OT7T175 (metastin receptor) protein consisting of the amino acid sequence represented by SEQ ID NO: 9; agents for preventing/treating hormone-dependent cancers (e.g., prostate cancer, breast cancer, etc.; particularly, hormone-sensitive prostate cancer, hormone-sensitive breast cancer, etc.); agents for preventing/treating endometriosis; agents for inhibiting ovarian follicular maturation; menstrual cycle-suspending agents; agents for treating myoma of the uterus; agents for treating early puberty; contraceptives, etc.

[0014] In the metastin derivatives of the present invention, the following compounds are preferred.

MS 10: Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH_2

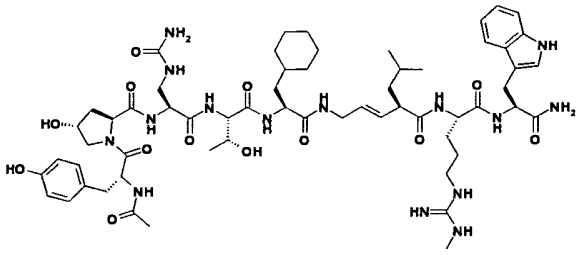
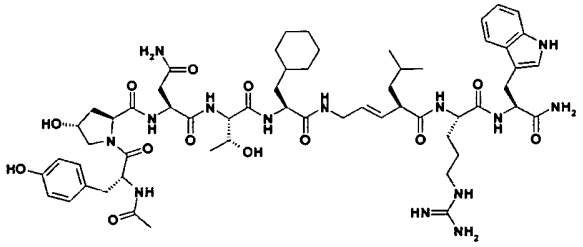
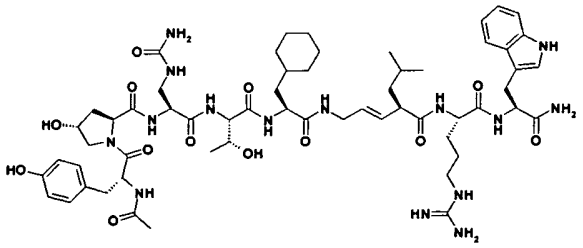
1 2 3 4 5 6 7 8 9 10

(Compound No. 903)

des(1)-Ac-[D-Tyr2,Hyp3,Alb4,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Arg(Me)9,Trp10]M S10
Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2
(Compound No. 926)
des(1)-Ac-[D-Tyr2,Hyp3,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Trp10]MS10 Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2
(Compound No. 927)
des(1)-Ac-[D-Tyr2,Hyp3,Alb4,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Trp10]MS10 Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2

[0015] The structural formulae of these compounds are shown in TABLE 1.

[TABLE 1]

Compound number	Structure
903	
926	
927	

[0016] The metastin derivatives of the present invention can be prepared by publicly known methods for peptide synthesis. As the methods for peptide synthesis, for example, either solid phase synthesis or liquid phase synthesis may be used. That is, the partial peptide or amino acids that can constitute the peptide of the present invention are repeatedly condensed with the remaining part to give the product having a desired sequence. Where the product has protecting groups, these protecting groups are removed to give the desired peptide. Publicly known methods for condensation and removal of the protecting groups includes those, e.g., described in (1) to (5) below.

(1) M. Bodanszky & M.A. Ondetti: Peptide Synthesis, Interscience Publishers, New York (1966)
(2) Schroeder & Luebke: The Peptide, Academic Press, New York (1965)
(3) Nobuo Izumiya, et al.: Peptide Gosei-no-Kiso to Jikken (Basics and experiments of peptide synthesis), published by Maruzen Co. (1975)
(4) Haruaki Yajima & Shunpei Sakakibara: Seikagaku Jikken Koza (Biochemical Experiment) 1, Tanpakushitsu no Kagaku (Chemistry of Proteins) IV, 205 (1977)
(5) Haruaki Yajima, ed.: Zoku Iyakuhin no Kaihatsu (A sequel to Development of Pharmaceuticals), Vol. 14, Peptide Synthesis, published by Hirokawa Shoten

[0017] After completion of the reaction, the product may be purified and isolated by a combination of conventional purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography and recrystallization to give the peptide of the present invention. When the peptide obtained by the above methods is in a free form, the peptide can be converted into an appropriate salt by a publicly known method; conversely when the peptide is obtained in a salt form, it can be converted into its free form by publicly known methods.

[0018] For condensation of the protected amino acids or peptides, a variety of activation reagents for peptide synthesis may be used, but trisphosphonium salts, tetramethyluronium salts, carbodiimides, etc. are particularly preferred. Examples of trisphosphonium salts include benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), bromotris(pyrrolidino) phosphonium hexafluorophosphate (PyBroP) and 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP), examples of tetramethyluronium salts include 2-(1H-benzotriazol-1-yl)-1,1,3,3-hexafluorophosphate (HBTU), 2-(7-azabenzotriazol-1-yl)-1,1,3,3-hexafluorophosphate (HATU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetraflluoroborate (TBTU), 2-(5-norbornene-2,3-dicarboxyimido)-1,1,3,3-tetramethyluronium tetrafluoroborate (TNTU) and O-(N-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU); examples of carbodiimides include DCC, N,N'-diisopropylcarbodiimide (DIPCDI) and N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI.HCl); etc. For condensation using these reagents, the addition of racemization inhibitors (e.g., HONB, HOBt, HOAt, HOOBt, etc.) is preferred. Solvents used in condensation may be appropriately chosen from solvents that are known to be usable for condensation. For example, acid amides such as anhydrous or hydrous N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, etc., halogenated hydrocarbons such as methylene chloride, chloroform, etc., alcohols such as trifluoroethanol, phenol, etc., sulfoxides such as dimethyl sulfoxide, etc., tertiary amines such as pyridine, etc., ethers such as dioxane, tetrahydrofuran, etc., nitriles such as acetonitrile, propionitrile, etc., esters such as methyl acetate, ethyl acetate, etc., or suitable mixtures thereof, etc. are used. The reaction temperature is appropriately chosen from the range known to be applicable to peptide binding reactions and is normally suitably chosen from the range of about -20°C to 50°C. The activated amino acid derivatives are used generally in 1.5 to 6 times excess. In the case of solid phase synthesis, the condensation is examined using the ninhydrin reaction; when the condensation is insufficient, the condensation can be completed by repeating the condensation reaction without removal of the protecting groups. When the condensation is yet insufficient even after repeating the reaction, the unreacted amino acids are acylated with acetic anhydride or acetylimidazole to cancel any adverse effect on the subsequent reaction.

[0019] Examples of the protecting groups used to protect amino groups in the starting amino acids include Z, Boc, tert-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, diphenylphosphinothioyl, Fmoc, trityl, etc. Examples of protecting groups for a carboxyl group include, in addition to the C_{1-6} alkyl group, C_{3-8} cycloalkyl group and C_{7-14} aralkyl group for R described above, allyl, 2-adamantyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, phenacyl group, benzyloxycarbonylhydrazide, tert-butoxycarbonylhydrazide, tritylhydrazide, etc.

[0020] The hydroxyl group of serine and threonine can be protected, for example, by esterification or etherification. Examples of groups suitable for this esterification include a group derived from organic acid such as a lower (C_{2-4}) alkanoyl group such as acetyl group, an aroyl group such as benzoyl group, etc. Examples of a group suitable for the etherification include benzyl group, tetrahydropyranyl group, tert-butyl group, trytyl group (Trt), etc.

[0021] Examples of groups for protecting the phenolic hydroxyl group of tyrosine include Bzl, 2,6-dichlorobenzyl (Cl_2-Bzl), 2-nitrobenzyl, Br-Z, tert-butyl, etc.

[0022] Examples of groups used to protect the imidazole moiety of histidine include Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr), DNP, Bom, Bum, Boc, Trt, Fmoc, etc. Examples of protecting groups for the guanidino group in arginine include Tos, Z, 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr), p-methoxybenzenesulfonyl (MBS), 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc), mesitylene-2-sulfonyl (Mts), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf), Boc, Z, NO_2, etc. Examples of protecting groups for side chain amino group of lysine include Z, Cl-Z, trifluoroacetyl, Boc, Fmoc, Trt, Mtr, 4,4-dimethyl-2,6-dioxocyclohexylideneyl (Dde), etc.

[0023] Examples of protecting groups for indolyl of tryptophan include formyl (For), Z, Boc, Mts, Mtr, etc.

[0024] Examples of protecting groups for asparagine and glutamine include Trt, xanthyl (Xan), 4,4'-dimethoxybenzhydryl (Mbh), 2,4,6-trimethoxybenzyl (Tmob), etc.

[0025] Examples of the activated carboxyl groups in the starting material include the corresponding acid anhydrides, azides, activated esters [esters with alcohols (e.g., pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccimide, 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt)], etc. Examples of activated forms of the amino groups in the starting material include the corresponding phosphorous amides.

[0026] To eliminate (split off) the protecting groups, there are used catalytic reduction under hydrogen gas flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, trimethylsilane bromide (TMSBr), trimethylsilyl trifluoromethanesulfonate, tetrafluoroboric acid, tris(trifluoro)boron, boron tribromide or a mixed solution thereof, a base treatment with diisopropylethylamine, triethylamine, piperidine, piperazine, etc., and reduction with sodium in liquid ammonia. The

elimination of protecting groups by the acid treatment described above is carried out generally at a temperature of approximately -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol, etc., dimethylsulfide, 1,4-butanedithiol, 1,2-ethanedithiol, etc. Furthermore, 2,4-dinitrophenyl group used as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of tryptophan is removed by the aforesaid acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol, etc. as well as by a treatment with an alkali such as a dilute sodium hydroxide solution, dilute ammonia, etc.

[0027] Protection of functional groups that should not be involved in the reaction of the starting materials, protecting groups, removal of the protecting groups and activation of functional groups involved in the reaction may be appropriately chosen from publicly known groups and publicly known means.

[0028] Methods for obtaining the amide of the peptide include, for example, solid phase synthesis using resins for the formation of peptide amide. In another method for obtaining the amides of the peptide, for example, the α-carboxyl group of the carboxy terminal amino acid is first protected by amidation; the peptide chain is then extended from the amino group side to a desired length. Thereafter, a peptide in which only the protecting group of the N-terminal α-amino group in the peptide chain has been removed from the peptide and a peptide (or an amino acid) in which only the protecting group of the C-terminal carboxyl group has been eliminated are prepared. The two peptides are condensed in a mixture of the solvents described above. The details of the condensation reaction are the same as described above. After the protected peptide obtained by the condensation is purified, all the protecting groups are removed by the method described above to give the desired crude peptide. This crude peptide is purified by various known purification means. Lyophilization of the major fraction gives the amide of the desired peptide.

[0029] When the metastin derivative of the present invention is present in the form of a configurational isomer, a diastereomer, a conformer, or the like, each can be isolated by the separating and purifying means described above, if desired. In addition, when the compound of the present invention is racemic, it can be separated into an S isomer and an R isomer by the conventional optical resolving means.

[0030] When steric isomers exist in the metastin derivative of the present invention, the present invention includes both of these isomers alone and the isomers present as a mixture thereof.

[0031] In addition, the metastin derivative of the present invention may also be hydrated or non-hydrated. The metastin derivative of the present invention may also be labeled with an isotope (e.g., ^{3}H, ^{14}C, ^{35}S), etc.

[0032] Throughout the specification, the peptides are represented in accordance with the conventional way of describing peptides, that is, the N-terminus (amino terminus) at the left hand and the C-terminus (carboxyl terminus) at the right hand. In the peptides, the C-terminus is usually in the form of an amide (-$CONH_2$), a carboxyl group (-COOH), a carboxylate (-COO^-), an alkylamide (-CONHR) or an ester (-COOR) and the amide (-$CONH_2$) is particularly preferred. Examples of R in the ester or alkylamide include a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C_{6-12} aryl group such as phenyl, α-naphthyl, etc.; a C_{7-14} aralkyl group such as a phenyl-C_{1-2}-alkyl group, e.g., benzyl, phenethyl, etc., or an α-naphthyl-C_{1-2}-alkyl group such as α-naphthylmethyl, etc.; pivaloyloxymethyl group, which are widely used as an ester for oral use, and the like.

[0033] Examples of salts of the metastin derivative of the present invention include a metal salt, an ammonium salt, a salt with an organic base, a salt with inorganic acid, a salt with organic acid, a salt with basic or acidic amino acid, and the like. Preferred examples of the metal salts include alkali metal salts such as sodium salts, potassium salts, etc.; alkaline earth metal salts such as calcium salts, magnesium salts, barium salts, etc.; aluminum salts; and the like. Preferred examples of the salts with organic bases include salts with trimethylamine, triethylamine, pyridine, picoline, 2,6-lutidine, ethanolamine, diethanolamine, triethanolamine, cyclohexylamine, dicyclohexylamine, N,N'-dibenzylethylenediamine, etc. Preferred examples of the salts with inorganic acids include salts with hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, etc. Preferred examples of salts with organic acids include salts with formic acid, acetic acid, trifluoroacetic acid, phthalic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc. Preferred examples of salts with basic amino acids include salts with arginine, lysine, ornithine, etc., and preferred examples of salts with acidic amino acids include salts with aspartic acid, glutamic acid, etc.

[0034] Of these salts, pharmaceutically acceptable salts are preferable. For example, when the compound has an acidic functional group, inorganic salts such as alkali metal salts (e.g., sodium salts, potassium salts, etc.), alkaline earth metal salts (e.g., calcium salts, magnesium salts, barium salts, etc.), ammonium salts, and the like are preferable. When the compound has a basic functional group, salts with inorganic acids with hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, etc., and salts with organic acids such as acetic acid, phthalic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, methanesulfonic acid, p-toluenesulfonic acid, etc. are preferable.

[0035] The compound of the present invention has the effects of regulating functions of the pancreas and is useful as therapeutic/preventive agents for various pancreatic diseases (e.g., acute or chronic pancreatitis, pancreatic cancer, etc.).

[0036] Also, the compound of the present invention has the effects of increasing sugar level, promoting pancreatic

glucagon secretion and promoting urine formation, and, is useful as a hyperglycemic agent, a pancreatic glucagon secretagogue agent or an agent for promoting urine formation, e.g., as medicaments including agents for preventing or treating, obesity, hyperlipemia, type II diabetes mellitus, hypoglycemia, hypertension, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, edema, urinary disturbances, insulin resistance, unstable diabetes mellitus, fatty atrophy, insulin allergy, insulinoma, arteriosclerosis, thrombotic disorders or lipotoxicity.

[0037] In addition, the compound of the present invention has the effects of stimulating gonadotropic hormone (e.g., FSH, LH, etc.) secretion, stimulating sex hormone [e.g., androgens (e.g., testosterone, androstenedione, etc.), estrogens (e.g., estradiol, estrone, etc.), progesterones, etc.] secretion, improving gonadal function and inducing or stimulating ovulation, as well as a sexual maturation effect, etc., and thus can be used as an agent for improving gonadal function, an agent for inducing or stimulating ovulation, a gonadotropic hormone secretagogue agent or a sex hormone secretagogue agent, or an agent for preventing/treating hormone-dependent cancers [e.g., prostate cancer, breast cancer, etc.], infertility [e.g., irregular menstruation, dysmenorrhea, amenorrhea, weight loss-induced amenorrhea, secondary amenorrhea, anovulation, hypoovarianism, hypogonadism, spermatogenetic failure, hypogonadism (e.g., impotence, etc.), genital atrophy, testicular atrophy, testicular function disorder, azoospermia, hypoandrogenemia, etc.], endometriosis, early puberty, myoma of the uterus, etc.

[0038] The compound of the present invention is also useful as:

an agent for preventing/treating hyperlipemia, type II diabetes mellitus, hypertension, diabetic neuropathy, diabetic nephropathy or diabetic retinopathy; an antianxiety agent; an antistress agent; an anti-insomnia agent; an antimanic-depressive agent; an agent for preventing/treating hypertension (e.g., essential hypertension, renal hypertension, salt sensitive hypertension, etc.), angina pectoris (e.g., stable angina, unstable angina, etc.), myocardial infarction, cerebrovascular disorders (e.g., asymptomatic cerebrovascular disorder, transient ischemic attack, apoplexy, cerebrovascular dementia, hypertensive encephalopathy, cerebral infarction, etc.), venous insufficiency, obliterative peripheral circulatory disturbances, Raynaud's disease, arteriosclerosis including atherosclerosis (e.g., aneurysm, coronary arteriosclerosis, cerebral arteriosclerosis, peripheral arteriosclerosis, etc.), vascular thickening or occlusion and organ impairments after intervention (e.g., percutaneous coronary intervention, stent placement, coronary thrombolytic therapy, etc.), portal hypertension, respiratory disorders (e.g., asthma, pulmonary hypertension, etc.); an agent for preventing/treating impaired glucose tolerance (IGT); an insulin secretagogue, an inhibitor for transition from IGT to diabetes;

an agent for preventing/treating diabetic complications [e.g., neuropathy, nephropathy, retinopathy, cataract, macroangiopathy, osteopenia, hyperosmolar diabetic coma, infectious diseases (e.g., respiratory infection, urinary tract infection, digestive tract infection, skin soft-tissue infection, lower leg infection), diabetic gangrene, xerostomia, hypacusis, cerebrovascular disorder, peripheral circulatory disturbance], osteoporosis, cachexia (e.g., cancerous cachexia, tuberculous cachexia, diabetic cachexia, blood disease cachexia, endocrine disease cachexia, infectious disease cachexia or cachexia due to acquired immunodeficiency syndrome), fatty liver, polycystic ovary syndrome, renal diseases (e.g., diabetic nephropathy, glomerulonephritis, glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis, end stage kidney disease), muscular dystrophy, cardiac infarction, angina pectoris, cerebrovascular disorders (e.g., cerebral infarction, apoplexy), Alzheimer's disease, Parkinson's disease, dementia, insulin resistance syndrome, X Syndrome X, metabolic syndrome, hyperinsulinemia, hyperinsulinemia-induced sensory disorders, tumors (e.g., leukemia, skin cancer), irritable bowel syndrome, acute or chronic diarrhea, inflammatory diseases (e.g., deforming spondylitis, arthritis deformans, lumbago, gout, postoperative or posttraumatic inflammation, swelling, neuralgia, pharyngolaryngitis, cystitis, hepatitis (including nonalcoholic steatohepatitis), pneumonia, enteritis, inflammatory bowel disorder (including inflammatory bowel diseases), ulcerative colitis, gastric mucosa damage (including gastric mucosa damage induced by aspirin)), damages of small intestinal mucosa, malabsorption, testicular function disorder, visceral obesity syndrome, etc.;

[0039] The compounds described in WO2004/063221, the compounds described in WO2006/001499 and the compounds described in WO2007/072997, or salts thereof, are also useful as agents for preventing/treating rheumatic diseases (e.g., rheumatoid arthritis, osteoarthritis, gout, etc.) or the like.

[0040] The compounds of the present invention can be used in combination with drugs, e.g., chemotherapeutic agents for treating cancer, hormonal therapeutic agents, immunotherapeutic agents, drugs for inhibiting the actions of cell growth factors and their receptors, etc. (hereinafter simply referred to as concomitant agents). Examples of the "chemotherapeutic agents" include alkylating agents, antimetabolites, anticancer antibiotics, anticancer agents derived from plants, etc. Specifically, the drugs described later can be used.

[0041] Furthermore, the compound of the present invention has excellent blood stability, solubility and solution stability, as compared to native metastin such as metastin 54 (1-54) or metastin 10 (45-54).

[0042] The metastin derivative of the present invention or its salt or prodrug, metastin per se, or DNA encoding metastin, etc. is useful as an agent for suppressing gonadotropic hormone (e.g., FSH, LH) secretion or sex hormone [e.g., androgen

(e.g., testosterone, androstenedione), estrogen (e.g., estradiol, estrone), progesterone] secretion; etc.; in particular, it is useful for suppressing gonadotropic hormone secretion or sex hormone secretion via down-regulation of gonadotropic hormone or sex hormone (wherein, the down-regulation of gonadotropic hormone or sex hormone may be pulse loss of LHRH or depletion of LHRH) or down-regulation of human OT7T175 (metastin receptor) protein consisting of the amino acid sequence represented by SEQ ID NO: 9; particularly useful as an agent for preventing or treating hormone-dependent cancers (e.g., prostate cancer, breast cancer, etc.; especially a prostate cancer, hormone-sensitive prostate cancer, etc.); an agent for preventing or treating endometriosis; an agent for inhibiting ovarian follicular maturation; a menstrual cycle-suspending agent; an agent for treating myoma of the uterus; an agent for treating early puberty; or as a contraceptive, etc. Where the metastin derivative of the present invention or its salt or prodrug, metastin per se, or DNA encoding metastin, etc. has normal agonist activity, an effective dose of the metastin derivative sufficient to suppress the secretion of gonadotropic hormone or sex hormone is administered at the site or tissue where the therapeutic effects are to be exerted, so that the metastin derivative is present in a dose more than required (i.e., the metastin derivative is administered in an excess over the normal effective dose, at which the metastin derivative exerts the effects of suppressing cancer metastasis, suppressing cancer growth, etc.; or the effect of promoting gonadotropic hormone secretion, the effect of promoting sex hormone secretion, etc.) to exhibit the effects of suppressing gonadotropic hormone secretion or sex hormone secretion. Specific examples include sustained or continuous administration of the normal effective dose (including an administration technique to gradually release the pharmaceutical ingredients by bolus administration); and the like. Further when the metastin derivative of the present invention or its salt or the prodrug thereof, etc. has a sufficient agonist activity more than required (a super-agonist activity), it becomes possible to sustain the activities more than exhibited by the necessary dose at the site or tissue where the therapeutic effect are to be exhibited. It is therefore sufficient even by normal effective dose administration to suppress the secretion of gonadotropic hormone or sex hormone, whereby the effect of suppressing gonadotropic hormone secretion or sex hormone secretion is exhibited.

[0043] In other words, an effective dose of the metastin derivative of the present invention or its salt or prodrug, metastin per se, or DNA encoding metastin, etc. sufficient to suppress the secretion of gonadotropic hormone or sex hormone is administered so that the metastin derivative is present in a dose more than required at the site or tissue where the therapeutic effects are to be exerted, or its activities can be sustained more than required, which enables to exhibit the effects of suppressing gonadotropic hormone secretion or suppressing sex hormone secretion.

[0044] The medicament comprising the compound of the present invention is low toxic. Therefore, the compound of the present invention can be safely administered either directly as it is or as a mixture with pharmacologically acceptable carriers, orally or parenterally (e.g., topically, rectally, intravenously, etc.), in the form of pharmaceutical preparations such as tablets (including dragees and film-coated tablets), powdery dosage forms, granules, capsules (including soft capsules), liquid dosage forms, injections, suppositories, sustained release dosage forms, etc., in accordance with publicly known means generally used in process for producing pharmaceutical preparations.

[0045] The compound of the present invention is contained in the pharmaceutical preparation of the present invention in about 0.01 to about 100 wt%, based on the total weight of the preparation.

[0046] A dose of the compound of the present invention may vary depending upon subject to be administered, target organ, conditions, route of administration, etc., and in oral administration, the compound is generally administered to the patient (as 60 kg body weight) with cancer in a daily dose of about 0.01 to about 100 mg, preferably about 0.1 to about 50 mg and more preferably about 0.1 to about 20 mg. In parenteral administration, a single dose of the compound may vary depending upon subject to be administered, target organ, conditions, route of administration, etc., and in the form of an injectable dosage form, it is advantageous to administer the compound to the patient (as 60 kg body weight) with cancer generally in a daily dose of about 0.001 to about 30 mg, preferably about 0.01 to about 20 mg, and more preferably about 0.01 to about 10 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered.

[0047] Pharmacologically acceptable carriers, which may be used in manufacturing the pharmaceutical preparation of the present invention, include various organic or inorganic carrier substances conventionally used as materials for pharmaceutical preparations. These substances include, e.g., an excipient, a lubricant, a binder and a disintegrating agent in a solid dosage form, and a solvent, a dissolution aid, a suspending agent, an isotonizing agent, a buffer, a soothing agent, etc. in a liquid dosage form. In addition, conventional additives such as a preservative, an antioxidant, a colorant, a sweetener, an adsorbent, a wetting agent, etc. can be appropriately used in suitable amounts, if necessary. Examples of excipients include lactose, saccharose, D-mannitol, starch, cornstarch, crystalline cellulose, light anhydrous silicic acid, etc. Examples of lubricants include magnesium stearate, calcium stearate, talc, colloidal silica, etc.

[0048] Examples of binders include crystalline cellulose, saccharose, D-mannitol, dextrin, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpyrrolidone, starch, sucrose, gelatin, methylcellulose, sodium carboxymethylcellulose, etc.

[0049] Examples of disintegrating agents include starch, carboxymethylcellulose, carboxymethylcellulose calcium, sodium carboxymethyl starch, L-hydroxypropylcellulose, etc.

[0050] Examples of solvents include water for injection, alcohol, propylene glycol, Macrogol, sesame oil, corn oil, olive

oil, etc.

[0051] Examples of dissolution aids include polyethylene glycol, propylene glycol, D-mannitol, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate, etc.

[0052] Examples of suspending agents include surfactants such as stearyl triethanolamine, sodium lauryl sulfate, lauryl aminopropionate, lecithin, benzalkonium chloride, benzethonium chloride, glycerin monostearate, etc.; hydrophilic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, sodium carboxymethylcellulose, methylcellulose, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, etc.

[0053] Examples of isotonizing agents include glucose, D-sorbitol, sodium chloride, glycerin, D-mannitol, etc.

[0054] Examples of buffers include buffering solutions of a phosphate, acetate, carbonate, citrate, etc.

[0055] Examples of soothing agents include benzyl alcohol, etc.

[0056] Examples of preservatives include p-hydroxybenzoates, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid, sorbic acid, etc.

[0057] Examples of antioxidants include a sulfite, ascorbic acid, α-tocopherol, etc.

[0058] Furthermore, the compound of the present invention can be used in combination with drugs other than the compound of the present invention.

[0059] Examples of the drugs, which can be used in combination with the compound of the present invention (hereinafter sometimes simply referred to as concomitant drugs), include chemotherapeutic agents for treating cancer, hormonal therapeutic agents, immunotherapeutic agents, drugs for inhibiting the actions of cell growth factors and their receptors, etc. (hereinafter simply referred to as concomitant agents).

[0060] Examples of "chemotherapeutic agents" include alkylating agents, antimetabolites, anticancer antibiotics, anticancer agents derived from plants, etc.

[0061] Examples of "alkylating agents" include nitrogen mustard, nitrogen mustard-N-oxide hydrochloride, chlorambutyl, cyclophosphamide, ifosfamide, thiotepa, carboquone, improsulfan tosylate, busulfan, nimustine hydrochloride, mitobronitol, rnelphalan, dacarbazine, ranimustine, estramustine sodium phosphate, triethylenemelamine, carmustine, lomustine, streptozocin, pipobroman, etoglucid, carboplatin, cisplatin, miboplatin, nedaplatin, oxaliplatin, altretamine, ambamustine, dibrospidium hydrochloride, fotemustine, prednimustine, pumitepa, ribomustin, temozolomide, treosulphan, trophosphamide, zinostatin stimalamer, carboquone, adozelesin, cystemustine, bizelesin, etc.

[0062] Examples of "antimetabolites" include mercaptopurine, 6-mercaptopurine riboside, thioinosine, methotrexate, enocitabine, cytarabine, cytarabine ocfosfate, ancitabine hydrochloride, 5-FU drugs (e.g., fluorouracil, tegafur, UFT, doxifluridine, carmofur, gallocitabine, emmitefur, etc.), aminopterin, leucovorin calcium, tabloid, butocine, folinate calcium, levofolinate calcium, cladribine, emitefur, fludarabine, gemcitabine, hydroxycarbamide, pentostatin, piritrexim, idoxuridine, mitoguazone, thiazophrine, ambamustine, etc.

[0063] Examples of "anticancer antibiotics" include actinomycin D, actinomycin C, mitomycin C, chromomycin A3, bleomycin hydrochloride, bleomycin sulfate, peplomycin sulfate, daunorubicin hydrochloride, doxorubicin hydrochloride, aclarubicin hydrochloride, pirarubicin hydrochloride, epirubicin hydrochloride, neocarzinostatin, mithramycin, sarcomycin, carzinophilin, mitotane, zorubicin hydrochloride, mitoxantrone hydrochloride, idarubicin hydrochloride, etc.

[0064] Examples of "anticancer agents derived from plants" include etoposide, etoposide phosphate, vinblastine sulfate, vincristine sulfate, vindesine sulfate, teniposide, paclitaxel, docetaxel, vinorelbine, etc.

[0065] Examples of "hormonal therapeutic agents" include fosfestrol, diethylstylbestrol, chlorotrianisene, medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, cyproterone acetate, danazol, allylestrenol, gestrinone, mepartricin, raloxifene, ormeloxifene, levormeloxifene, anti-estrogens (e.g., tamoxifen citrate, toremifene citrate, etc.), pill dosage forms, mepitiostane, testrolactone, aminoglutethimide, LH-RH agonists (e.g., goserelin acetate, buserelin, leuprorelin, etc.), droloxifene, epitiostanol, ethinylestradiol sulfonate, aromatase inhibitors (e.g., fadrozole hydrochloride, anastrozole, retrozole, exemestane, vorozole, formestane, etc.), anti-androgens (e.g., flutamide, bicartamide, nilutamide, etc.), 5α-reductase inhibitors (e.g., finasteride, epristeride, etc.), adrenocorticohormone drugs (e.g., dexamethasone, prednisolone, betamethasone, triamcinolone, etc.), androgen synthesis inhibitors (e.g., abiraterone, etc.), retinoid and drugs that retard retinoid metabolism (e.g., liarozole, etc.), and among others, LH-RH agonists (e.g., goserelin acetate, buserelin, leuprorelin, etc.) are preferable.

[0066] Examples of "immunotherapeutic agents (BRM)" include picibanil, krestin, sizofiran, lentinan, ubenimex, interferons, interleukins, macrophage colony-stimulating factor, granulocyte colony-stimulating factor, erythropoietin, lymphotoxin, BCG vaccine, Corynebacterium parvum, levamisole, polysaccharide K, procodazole, etc.

[0067] The "cell growth factors" in the "drugs for inhibiting the actions of cell growth factors and their receptors" can be any substance so long as it is a material for stimulating the cell growth and, normally, peptides which have a molecular weight of 20,000 or less and bind to their receptors to exhibit the actions in a lower level can be used as the factors. Specific examples are (1) EGF (epidermal growth factor) or substances having substantially the same activity as EGF [e.g., EGF, hereglin, etc.], (2) insulin or substances having substantially the same activity as insulin [e.g., insulin, IGF (insulin-like growth factor)-1, IGF-2, etc.], (3) FGF (fibroblast growth factor) or substances having substantially the same activity as FGF [e.g., acidic FGF, basic FGF, KGF (keratinocyte growth factor), FGF-10, etc.], (4) other cell growth factors

[e.g., CSF(colony stimulating factor), EPO (erythropoietin), IL-2 (interleukin-2), NGF (nerve growth factor), PDGF (platelet-derived growth factor), TGFβ (transforming growth factor β), HGF (hepatocyte growth factor),VEGF (vascular endothelial growth factor), etc.] and the like.

[0068] The "receptors of the cell growth factors" can be any receptor as long as it is capable of binding to the cell growth factors described above, and specific examples are EGF receptor, hereglin receptor (HER2), insulin receptor, IGF receptor, FGF receptor-1 or FGF receptor-2, etc.

[0069] As the "agent inhibiting the effect of cell growth factor" includes HER2 antibody (trastuzumab (Herceptin (trademark)), etc.), imatinib mesylate, ZD1839 or EGFR antibody (cetuximab (Erbitux (trademark)), etc.), antibody against VEGF (e.g., Bevacizumab (Avastin (trademark))), VEGFR antibody, VEGFR inhibitor, EGFR inhibitor (erlotinib (Tarceva (trademark)), gefitinib (Iressa (trademark)), etc.)

[0070] In addition to the aforesaid agents, there are also used L-asparginase, aceglatone, procarbazine hydrochloride, protoporphyrin-cobalt complex, mercury-hematoporphyrin sodium, topoisomerase I inhibitor (e.g., Irinotecan, Topotecan, etc.), topoisomerase II inhibitor (e.g., Sobzoxan, etc.), differentiation-inducing agent (e.g., retinoid, vitamin D group, etc.), angiogenesis inhibitor (e.g., thalidomide, SU11248, etc.), α-blocker (e.g., tamsulosin hydrochloride, naftopidil, urapidil, alfuzosin, terazosin, prazosin, silodosin, etc.), serine-threonine kinase inhibitor, endothelin receptor antagonist (e.g., atrasentan, etc.), proteasome inhibitor (e.g., bortezomib, etc.), Hsp90 inhibitor (e.g., 17-AAG, etc.), spironolactone, minoxidil, 11α-hydroxyprogesterone, bone resorption inhibitor/bone metastasis suppressor (e.g., zoledronic acid, alendronic acid, pamidronic acid, etidronic acid, ibandronic acid, clodronic acid), etc.

[0071] The combined use of the compound of the present invention and a concomitant drug exhibits the following excellent effects.

(1) The dose can be reduced as compared to the dose when the compound of the present invention or a concomitant drug is administered alone.
(2) A drug concomitantly administered with the compound of the present invention can be chosen depending on the condition (mild, severe, etc.) of a patient.
(3) A concomitant drug, whose functional mechanism is different from that of the compound of the present invention, can be chosen so that a treatment period can be set longer.
(4) A concomitant drug, whose functional mechanism is different from that of the compound of the present invention, can be chosen so that sustained therapeutic effects can be achieved.
(5) A synergistic effect can be obtained by the concomitant use of the compound of the present invention and a concomitant drug.

[0072] In addition, the compound of the present invention can reduce values of testosterone to emasculate level immediately after medication. Thus when the concomitant drug such as LH-RH agonist (e.g., goserelin acetate, buserelin, leuprorelin, etc.; preferably leuprorelin) is used in combination with the compound of the present invention, the values of testosterone can be reduced to emasculate level immediately after medication of the compound of the present invention. Further, since the combined use of the concomitant drug such as LH-RH agonist (e.g., goserelin acetate, buserelin, leuprorelin, etc.; preferably leuprorelin) and the compound of the present invention results in prolonged preservation of hormone-dependent period, it can advantageously be used.

[0073] Hereinafter, the combined use of the compound of the present invention and the concomitant drug is referred to as "the combined preparation of the present invention."

[0074] When the combined preparation of the present invention is used, a dosing period of the compound of the present invention and the concomitant drug is not restricted; the compound of the present invention or its pharmaceutical composition and the concomitant drug or its pharmaceutical composition may be administered to the subject to be administered either simultaneously or at certain time intervals. The dose of the concomitant drug may be modified according to the dose used clinically and may be appropriately chosen depending upon subject to be administered, route for administration, disease, combination, etc.

[0075] A mode for administration of the combined preparation of the present invention is not particularly limited, but it is sufficient that the compound of the present invention is used in combination with the concomitant drug at the time of administration. For such mode of administration, there are, for example, (1) administration of a single dosage form obtained by mixing the compound of the present invention and the concomitant drug together at the same time, (2) simultaneous administration of two dosage forms prepared separately from the compound of the present invention and the concomitant drug through the same route for administration, (3) administration of two dosage forms prepared separately from the compound of the present invention and the concomitant drug at certain time intervals through the same route for administration, (4) simultaneous administration of two dosage forms prepared separately from the compound of the present invention and the concomitant drug through different routes for administration, (5) administration of two dosage forms prepared separately from the compound of the present invention and the concomitant drug at certain time intervals (e.g., administration of the compound of the present invention and the concomitant drug in this order, or

administration in a reversed order) through different routes for administration, etc.

[0076] The combined preparation of the present invention is low toxic and thus can be safely administered orally or parenterally (e.g., topically, rectally, intravenously, etc.) in the form of pharmaceutical preparations such as tablets (including dragees and film-coated tablets), powdery dosage forms, granules, capsules (including soft capsules), liquid dosage forms, injections, suppositories, sustained release dosage forms, etc., which are obtained by mixing the compound of the present invention or (and) the concomitant drug described above with pharmacologically acceptable carriers by publicly known methods. Injectable dosage forms can be administered intravenously, intramuscularly or subcutaneously, into the organ, or directly at the focus.

[0077] Pharmacologically acceptable carriers, which may be used to manufacture the combined preparation of the present invention, include various organic or inorganic carrier substances conventionally used as materials for pharmaceutical preparations. These substances include, e.g., an excipient, a lubricant, a binder and a disintegrating agent in a solid dosage form, and a solvent, a dissolution aid, a suspending agent, an isotonizing agent, a buffer, a soothing agent, etc. in a liquid dosage form. In addition, conventional additives such as a preservative, an antioxidant, a colorant, a sweetener, an adsorbent, a wetting agent, etc. can be appropriately used in suitable amounts, if necessary.

[0078] Examples of excipients include lactose, saccharose, D-mannitol, starch, cornstarch, crystalline cellulose, light anhydrous silicic acid, etc.

[0079] Examples of lubricants include magnesium stearate, calcium stearate, talc, colloidal silica, etc.

[0080] Examples of binders include crystalline cellulose, saccharose, D-mannitol, dextrin, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpyrrolidone, starch, sucrose, gelatin, methylcellulose, sodium carboxymethylcellulose, etc.

[0081] Examples of disintegrating agents include starch, carboxymethylcellulose, carboxymethylcellulose calcium, sodium carboxymethyl starch, L-hydroxypropylcellulose, etc.

[0082] Examples of solvents include water for injection, alcohol, propylene glycol, Macrogol, sesame oil, corn oil, olive oil, etc.

[0083] Examples of dissolution aids include polyethylene glycol, propylene glycol, D-mannitol, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate, etc.

[0084] Examples of suspending agents include surfactants such as stearyltriethanolamine, sodium lauryl sulfate, lauryl aminopropionate, lecithin, benzalkonium chloride, benzethonium chloride, glycerin monostearate, etc.; hydrophilic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, sodium carboxymethylcellulose, methylcellulose, hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, etc.

[0085] Examples of isotonizing agents include glucose, D-sorbitol, sodium chloride, glycerin, D-mannitol, etc.

[0086] Examples of buffers include buffering solutions of a phosphate, acetate, carbonate, citrate, etc.

[0087] Examples of soothing agents include benzyl alcohol, etc.

[0088] Examples of preservatives include p-hydroxybenzoates, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid, sorbic acid, etc.

[0089] Examples of antioxidants include a sulfite, ascorbic acid, α-tocopherol, etc.

[0090] In the combined preparation of the present invention, a ratio of the compound of the present invention to the concomitant drug can be appropriately chosen depending upon subject to be administered, route for administration, disease, etc.

[0091] For example, the amount of the compound of the present invention contained in the combined preparation of the present invention varies depending on the dosage form of the preparation, but is usually about 0.01 to 100% by weight, preferably about 0.1 to 50% by weight, and more preferably about 0.5 to 20% by weight, based on the total weight of the preparation.

[0092] The amount of the concomitant drug contained in the combined preparation of the present invention varies depending on the dosage form of the preparation, but is usually about 0.01 to 100% by weight, preferably about 0.1 to 50% by weight, and more preferably about 0.5 to 20% by weight, based on the total weight of the preparation.

[0093] The amount of additives such as a carrier, etc. contained in the combined preparation of the present invention varies depending on the dosage form of the preparation, and is usually about 1 to 99.99% by weight, preferably about 10 to 90% by weight, based on the total weight of the preparation.

[0094] These amounts may be the same, also when the compound of the present invention and the concomitant drug are separately prepared, respectively.

[0095] These preparations can be manufactured by per se publicly known methods conventionally used in general.

[0096] For example, the compound of the present invention or the concomitant drug can be prepared into an injectable dosage form by formulating with a dispersing agent (e.g., Tween 80 (manufactured by Atlas Powder Company, USA), HCO 60 (manufactured by Nikko Chemicals Co., Ltd.), polyethylene glycol, carboxymethyl cellulose, sodium alginate, hydroxypropylmethyl cellulose, dextrin, etc.), a stabilizer (e.g., ascorbic acid, sodium pyrosulfite), a surfactant (e.g., polysorbate 80, macrogol, etc.), a solubilizing agent (e.g., glycerin, ethanol, etc.), a buffering agent (e.g., phosphoric acid or its alkali metal salt, citric acid or its alkali metal salt, etc.), an isotonizing agent (e.g., sodium chloride, potassium

chloride, mannitol, sorbitol, glucose, etc.), a pH adjusting agent (e.g., hydrochloric acid, sodium hydroxide, etc.), a preservative (e.g., ethyl p-oxybenzoate, benzoic acid, methylparabene, propylparabene, benzyl alcohol, etc.), a solubilizer (e.g., concentrated glycerin, meglumine, etc.), a dissolution aid (e.g., propylene glycol, saccharose, etc.), a soothing agent (e.g., glucose, benzyl alcohol, etc.), to prepare into aqueous injection; or by dissolving, suspending, or emulsifying with a vegetable oil such as olive oil, sesame oil, cottonseed oil, corn oil, etc., a dissolution aid such as propylene glycol or the like to prepare into an oily injection.

[0097] An oral dosage form can be produced in a conventional manner by adding to the compound of the present invention or the concomitant drug, for example, an excipient (e.g., lactose, saccharose, starch, etc.), a disintegrating agent (e.g., starch, calcium carbonate, etc.), a binder (e.g., starch, gum arabic, carboxymethyl cellulose, polyvinylpyrrolidone, hydroxypropyl cellulose, etc.), a lubricant (e.g., talc, magnesium stearate, polyethylene glycol 6000, etc.) and other additives, compressing the resulting mixture and, if necessary, coating the compressed product for the purpose of taste masking, enteric degradation or sustained release by techniques per se publicly known. Coating agents for this purpose include, for example, hydroxypropylmethyl cellulose, ethyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, polyoxyethylene glycol, Tween 80, Prulonic F68, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, hydroxymethyl cellulose acetate succinate, Eudragit (manufactured by Rohm Company, Germany, methacrylic acid/acrylic acid copolymer) and dyes (e.g., iron oxide, titanium dioxide). The oral dosage form may be either an immediate release dosage form or a sustained release dosage form.

[0098] For example, in a suppository, the compound of the present invention or the concomitant drug is prepared into an oily or aqueous solid, semi-solid or liquid composition by techniques per se publicly known. Oily bases used for the composition described above include glycerides of higher fatty acids [e.g., cacao butter, uitepsols (manufactured by Dynamite Nobel Company, Germany), etc.], moderate fatty acids [e.g., miglyols (manufactured by Dynamite Nobel Company, Germany), etc.], vegetable oils (e.g., sesame oil, soybean oil, cottonseed oil, etc.), and the like. Aqueous bases include, for example, polyethylene glycols and propylene glycol. Bases for aqueous gels include, for example, natural rubbers, cellulose derivatives, vinyl polymers, acrylic polymers, etc.

[0099] Examples of the sustained release dosage form above include sustained release microcapsules, and the like.

[0100] Sustained release microcapsules can be obtained by per se publicly known methods, and are preferably prepared in the form of, e.g., a sustained release dosage form by the method [2] shown below and administered.

[0101] Preferably, the compound of the present invention is prepared into a dosage form for oral administration such as a solid dosage form (e.g., powdery dosage form, granules, tablets, capsules) or into a dosage form for rectal administration such as a suppository, etc. A dosage form for oral administration is particularly preferred.

[0102] The concomitant drug can be prepared into the dosage form described above, depending on the kind of drug.

[0103] Hereinafter, [1] an injectable preparation of the compound of the present invention or the concomitant drug and its production, [2] a sustained release or immediate release preparation of the compound of the present invention or the concomitant drug and its production and [3] a sublingual, buccal or rapid oral disintegrating preparations of the compound of the present invention or the concomitant drug and its production will be specifically described.

[1] Injectable Preparation and its Production

[0104] An injectable preparation obtained by dissolving the compound of the present invention or the concomitant drug in water is preferred. The injectable preparation may contain a benzoate and/or a salicylate.

[0105] The injectable preparation is obtained by dissolving the compound of the present invention or the concomitant drug and optionally a benzoate and/or a salicylate in water.

[0106] Examples of the benzoate and/or salicylate described above include an alkali metal salt such as sodium and potassium salts, etc., an alkaline earth metal salt such as calcium and magnesium salts, etc., an ammonium salt, a meglumine salt, a salt of an organic acid such as trometamol, and the like.

[0107] The concentration of the compound of the present invention or the concomitant drug in the injectable preparation is about 0.05 to 50 w/v %, preferably about 0.3 to 20 w/v %. The concentration of the benzoate and/or salicylate is 0.5 to 50 w/v %, preferably 3 to 20 w/v %.

[0108] Furthermore, additives generally used in an injectable preparation such as a stabilizer (ascorbic acid, sodium pyrosulfite, etc.), a surfactant (polysorbate 80, macrogol, etc.), a solubilizing agent (glycerin, ethanol, etc.), a buffering agent (phosphoric acid and its alkali metal salt, citric acid and its alkali metal salt, etc.), an isotonizing agent (sodium chloride, potassium chloride, etc.), a dispersing agent (hydroxypropylmethyl cellulose, dextrin), a pH adjusting agent (hydrochloric acid, sodium hydroxide, etc.), a preservative (ethyl p-oxybenzoate, benzoic acid, etc.), a solubilizer (concentrated glycerin, meglumine, etc.), a dissolution aid (propylene glycol, saccharose, etc.), a soothing agent (glucose, benzyl alcohol, etc.) are appropriately added to the preparation. Any of these additives is added in an amount generally used in an injectable preparation.

[0109] The injectable preparation is adjusted to pH of 2 to 12, preferably 2.5 to 8.0 by adding a pH adjusting agent.

[0110] The injectable preparation is obtained by dissolving both the compound of the present invention or the con-

comitant drug and optionally a benzoate and/or salicylate, and, if necessary, the above additives in water. These components may be dissolved in any order according to the same manner as in a conventional injectable preparation.

[0111] An aqueous solution for injection is preferably warmed, and used as an injectable preparation after sterilization by filtration or autoclaved as in a conventional injectable preparation to provide for an injectable preparation.

[0112] An aqueous injectable preparation is preferably autoclaved, e.g., at 100 to 121°C for 5 to 30 minutes.

[0113] Moreover, the preparation may be in a solution form to which antibacterial activity is imparted to be usable as a multiple dosage form in divided dosing.

[2] Sustained Release or Immediate Release Preparation and its Production

[0114] A preferred sustained release preparation comprises a core comprising the compound of the present invention or the concomitant drug, which is optionally coated with a water-insoluble material or a swelling polymer. For example, a sustained release preparation for oral administration of a once-daily dosage form is preferred.

[0115] Examples of the water-insoluble material used for the coating agent include cellulose ethers such as ethyl cellulose, butyl cellulose, etc., cellulose esters such as cellulose acetate, cellulose propionate, etc., polyvinyl esters such as polyvinyl acetate, polyvinyl butyrate, etc., acrylic acid polymers such as an acrylic acid/methacrylic acid copolymer, a methyl methacrylate copolymer, an ethoxyethyl methacrylate/cinnamoethyl methacrylate/aminoalkyl methacrylate copolymer, a polyacrylic acid, a polymethacrylic acid, a methacrylic acid alkylamide copolymer, a poly(methyl methacrylate), a polymethacrylate, an aminoalkyl methacrylate copolymer, a poly(methacrylic anhydride), a glycidyl methacrylate copolymer, in particular, a series of Eudragits (Rohm & Pharma) such as Eudragit RS-100, RL-100, RS-30D, RL-30D, RL-PO and RS-PO (ethyl acrylate/methyl methacrylate/chlorotrimethyl methacrylate/ethyl ammonium copolymer) and Eudragit NE-30D (methyl methacrylate/ethyl acrylate copolymer), etc., hydrogenated oils such as hydrogenated castor oil (e.g., LUBRI WAX (Freund Industrial Co., Ltd.), etc.), waxes such as carnauba wax, a fatty acid glycerin ester, paraffin, etc., polyglycerin fatty acid esters, etc.

[0116] The swelling polymer is preferably a polymer having an acidic removable group and exhibiting pH-dependent swelling, and a polymer having an acidic removable group, which undergoes a less swelling at an acidic pH such as in the stomach but is swollen extensively at a neutral pH such as in the small and large intestines, is preferred.

[0117] Examples of such a polymer having an acidic removable group and exhibiting pH-dependent swelling include a crosslinked polyacrylic acid polymer such as Carbomers 934P, 940, 941, 974P, 980, 1342, etc., polycarbophil and calcium polycarbophil (all manufactured by BF Goodrich Chemicals), Hivis Wakos 103, 104, 105 and 304 (all manufactured by Wako Pure Chemical Industries, Ltd.), etc.

[0118] The coating agent used in the sustained release preparation may further contain a hydrophilic material.

[0119] Examples of the hydrophilic material include a polysaccharide which may have a sulfate group, such as pullulan, dextrin, alkali metal alginates, etc., a polysaccharide having a hydroxyalkyl group or a carboxyalkyl group such as hydroxypropyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethylcellulose, etc., methyl cellulose, polyvinyl pyrrolidone, polyvinyl alcohol, polyethylene glycol, etc.

[0120] The amount of the water-insoluble material contained in the coating agent of the sustained release preparation is about 30 to about 90% (w/w), preferably about 35 to about 80% (w/w), more preferably about 40 to about 75% (w/w), and the swelling polymer content is about 3 to about 30% (w/w), preferably about 3 to about 15% (w/w). The coating agent may further contain a hydrophilic material, and the amount of the hydrophilic material contained in the coating agent is about 50% (w/w) or less, preferably about 5 to about 40% (w/w), more preferably about 5 to about 35% (w/w). As used herein, the % (w/w) above is used to mean a % by weight based on the coating agent composition, which is the remainder of the coating agent solution after removing any solvent (e.g., water, a lower alcohol such as methanol, ethanol, etc.).

[0121] The sustained release preparation is manufactured by preparing a core containing a drug as illustrated below, followed by coating the resulting core with a coating agent solution obtained by heat-melting a water-insoluble material or a swelling polymer or by dissolving or dispersing such a material in a solvent.

I. Production of Drug-Containing Core

[0122] The shape of a core containing a drug to be coated with a coating agent (hereinafter sometimes simply referred to as a core) is not specifically limited but preferably prepared into a particulate shape such as granules, fine granules, or the like.

[0123] When the core is granules or fine granules, they have a mean particle size of preferably about 150 to about 2,000 μm, more preferably about 500 to about 1,400 μm.

[0124] The core can be prepared in a conventional manner. For example, a drug is mixed with a suitable excipient, binder, disintegrating agent, lubricant, stabilizer, etc., and then subjected to wet extrusion granulation, fluidized bed granulation, or the like.

[0125] The drug content in the core is about 0.5 to about 95% (w/w), preferably about 5.0 to about 80% (w/w), more preferably about 30 to about 70% (w/w).

[0126] Examples of the excipient contained in the core include a saccharide such as saccharose, lactose, mannitol, glucose, etc., starch, crystalline cellulose, calcium phosphate, cornstarch, etc. Among others, crystalline cellulose and cornstarch are preferred.

[0127] Examples of the binder used include polyvinyl alcohol, hydroxypropyl cellulose, polyethylene glycol, polyvinyl pyrrolidone, Pluronic F68, gum arabic, gelatin, starch, etc. Examples of the disintegrating agent include calcium carboxymethyl cellulose (ECG505), sodium croscarmellose (Ac-Di-Sol), crosslinked polyvinyl pyrrolidone (crospovidone), low substituted hydroxypropyl cellulose (L-HPC), etc. Among others, hydroxypropyl cellulose, polyvinyl pyrrolidone and low substituted hydroxypropyl cellulose are preferred. Examples of the lubricant and the anticoagulant include talc, magnesium stearate and its inorganic salts, and examples of the lubricant include polyethylene glycol, etc. Examples of the stabilizer include an acid such as tartaric acid, citric acid, succinic acid, fumaric acid, maleic acid, etc.

[0128] In addition to the technique described above, the core can be prepared by using other techniques such as an tumbling granulation technique, a pan coating technique, a fluidized bed coating technique and a melt granulation technique, wherein a drug or a mixture of the drug with an excipient, a lubricant, etc. is portionwise added to inert carrier particles as seeds for the core with spraying a binder dissolved in a suitable solvent such as water, a lower alcohol (e.g., methanol, ethanol, etc.) or the like. Examples of the inert carrier particles include those prepared from saccharose, lactose, starch, crystalline cellulose and waxes, and, preferably, these carriers have a mean particle size of about 100 μm to about 1,500 μm.

[0129] In order to separate the drug contained in the core from a coating agent, the surface of the core may be covered with a protective material. Examples of the protective material include the hydrophilic material described above and water-insoluble material. The preferred protective material is polyethylene glycol or a polysaccharide having a hydroxyalkyl group or a carboxyalkyl group, more preferably, hydroxypropylmethyl cellulose and hydroxypropyl cellulose. The protective material may contain, as a stabilizer, an acid such as tartaric acid, citric acid, succinic acid, fumaric acid, maleic acid, etc., and a lubricant such as talc. When the protective material is used, the amount thereof to be coated is about 1 to about 15% (w/w), preferably about 1 to about 10% (w/w), more preferably about 2 to about 8% (w/w) based on the core.

[0130] The protective material can be coated by a conventional coating method and specifically, the core is spray-coated with the protective material by a fluidized bed coating technique, a pan coating technique, etc.

II. Coating of Core with Coating Agent

[0131] The core obtained in I above is coated with a coating agent solution prepared by melt-heating the water-insoluble material and pH-dependent swelling polymer described above and a hydrophilic material or by dissolving or dispersing them in a solvent to obtain a sustained release preparation.

[0132] As a coating method of the core with the coating agent solution, there are, for example, spray-coating, etc.

[0133] The composition ratio of the water-insoluble material, swelling polymer and hydrophilic material in the coating agent solution can be appropriately chosen to be within the amounts of the respective components contained in the coating.

[0134] The amount of the coating agent is about 1 to about 90% (w/w), preferably about 5 to about 50% (w/w), more preferably about 5 to about 35% (w/w) based on the core (excluding the protective material coating).

[0135] As the solvent for the coating agent solution, water and an organic solvent can be used singly or as a mixture thereof. When a mixture is used, the ratio of water and the organic solvent (water/organic solvent: a weight ratio) may vary with the range of 1 to 100%, and is preferably 1 to about 30%. The organic solvent is not particularly limited so far as it can dissolve the water-insoluble material, and examples of the solvent include a lower alcohol such as methyl alcohol, ethyl alcohol, isopropyl alcohol, n-butyl alcohol, etc., a lower alkanone such as acetone, acetonitrile, chloroform, methylene chloride, etc. In particular, a lower alcohol is preferred, with ethyl alcohol and isopropyl alcohol being more preferred. Water and a mixture of water and an organic solvent are used preferably as solvents for the coating agent solution. In this case, an acid such as tartaric acid, citric acid, succinic acid, fumaric acid, maleic acid, etc. may be added to the coating agent solution, if necessary, for the purpose of stabilizing the coating agent solution.

[0136] To carry out the coating through spray coating, the coating can be made using a conventional coating method. Specifically, the core is sprayed with a coating agent solution by a fluidized bed coating technique, a pan coating technique, or the like. At this time, a lubricant such as talc, titanium oxide, magnesium stearate, calcium stearate, light silicic anhydride, etc., and a plasticizer such as glycerin fatty ester, hardened castor oil, triethyl citrate, cetyl alcohol, stearyl alcohol, etc. may also be added.

[0137] After coating with a coating agent, an antistatic agent such as talc may also be admixed, if necessary.

[0138] The immediate release preparation may be a liquid (solution, suspension, emulsion, etc.) or a solid (particles, pills, tablets, etc.). An oral preparation and a parenteral preparation such as an injectable preparation may be used, and

an oral preparation is preferred.

[0139] The immediate release preparation may usually contain a carrier, additives and an excipient (hereinafter sometimes abbreviated as excipients) which are conventionally used in the pharmaceutical field, in addition to a drug which is an active ingredient. The pharmaceutical excipients are not specifically limited so long as they are excipients conventionally used in the pharmaceutical field. Examples of the excipient for an oral solid preparation include lactose, starch, corn starch, crystalline cellulose (Avicel PH101, manufactured by Asahi Kasei Corporation, etc.), powdered sugar, granulated sugar, mannitol, light silicic anhydride, magnesium carbonate, calcium carbonate, L-cysteine, etc., with corn starch and mannitol being preferred. Any of these excipients may be employed alone or in combination with each other. The amounts of the excipients are, for example, about 4.5 to about 99.4 w/w %, preferably about 20 to about 98.5 w/w %, more preferably about 30 to about 97 w/w %, based on the total weight of the immediate release preparation. The content of drug in the immediate release preparation may appropriately be selected from the range of about 0.5% to about 95%, preferably about 1% to about 60% to the whole amount of the immediate release preparation.

[0140] When the immediate release preparation is an oral solid preparation, the preparation contains a disintegrating agent in addition to the components described above. Examples of the disintegrating agent include calcium carboxymethylcellulose (ECG505 manufactured by GOTOKU CHEMICAL Co., Ltd.), sodium croscarmellose (for example, Ac-Di-Sol manufactured by Asahi Kasei Corporation), crospovidone (for example, COLIDON CL manufactured by BASF), low-substituted hydroxypropyl cellulose (Shin-Etsu chemical Co., Ltd.), carboxymethyl starch (MATSUTANI CHEMICAL INDUSTRY Co., Ltd.), sodium carboxymethyl starch (EXORITAB manufactured by KIMURA SANGYO), partial α starch (PCS manufactured by Asahi Kasei Corporation), etc. For example, the disintegrating agent that disintegrates granules by water absorption or swelling upon contact with water, or forming a channel between the active component comprising the core and an excipient can be used. Any of these disintegrating agents can be used alone or in combination with each other. The amount of the disintegrating agent used may be appropriately chosen depending upon the type and the amount of the drug used or a particular preparation design for the intended release performance. For example, the amount is about 0.05 to about 30 w/w %, preferably about 0.5 to about 15 w/w % based on the total weight of the immediate release preparation.

[0141] When the immediate release preparation is an oral solid preparation, the oral solid preparation may optionally contain additives conventionally used in a solid preparation, in addition to the components described above. Examples of the additives include binders (for example, sucrose, gelatin, powdery gum arabic, methyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, carboxymethylcellulose, polyvinyl pyrrolidone, pullran, dextrin, etc.), lubricants (polyethylene glycol, magnesium stearate, talc, light silicic anhydride (for example, aerosil (NIPPON AEROSIL)), surfactants (for example, anionic surfactants such as sodium alkyl sulfate, nonionic surfactants such as polyoxyethylene fatty ester, polyoxyethylene sorbitan fatty ester, polyoxyethylene castor oil derivatives, etc.), colorants (for example, tar colorants, caramel, colcothar, titanium oxide, riboflavins), if necessary, corrigents (for example, sweeteners, flavors, etc.), adsorbents, preservatives, wetting agents, antistatic agents, etc. Furthermore, an organic acid such as tartaric acid, citric acid, succinic acid, fumaric acid or the like can also be added as a stabilizer.

[0142] As the binder above, hydroxypropyl cellulose, polyethylene glycol and polyvinyl pyrrolidone, etc. are preferably used.

[0143] The immediate release preparation can be prepared by mixing the components described above and kneading the mixture, if necessary, and then molding according to a conventional technique for making pharmaceutical preparations. The mixing above can be carried out in a conventional manner, e.g., by mixing, kneading, etc. Specifically, where the immediate release preparation is in the form of particles, the preparation can be prepared by mixing components with a vertical granulator, a multi-purpose kneader (manufactured by HATA IRON WORKS CO., LTD), a fluidized bed granulator FD-5S (manufactured by POWREX CORPORATION) or thee like, and then granulating the resulting by wet extrusion granulation or fluidized bed granulation by a technique similar to that for preparing the core of the sustained release preparation described above.

[0144] The immediate release preparation and the sustained release preparation thus obtained can be compounded, as they are, or, together with appropriate pharmaceutical excipients, in pharmaceutical preparations separately in a conventional manner to prepare respective preparations for administering in combination with each other simultaneously or at certain time intervals. Alternatively, both preparations may be compounded in a single dosage form for oral administration (e.g., granules, fine granules, tablets, capsules) as they are, or, together with appropriate pharmaceutical excipients. Both preparations in the form of granules or fine granules may also be filled in a single capsule for oral administration.

[3] Sublingual, Buccal or Rapid Oral Disintegrating Preparation and its Production

[0145] A sublingual, buccal or rapid oral disintegrating preparation may be in the form of a solid preparation such as a tablet, or may be in the form of an oral mucosal patch (film) or oral disintegrating film.

[0146] The sublingual, buccal or rapid oral disintegrating preparation is preferably a preparation containing the com-

pound of the present invention or the concomitant drug and an excipient. The preparation may also contain auxiliary agents such as a lubricant, an isotonizing agent, a hydrophilic carrier, a water-dispersible polymer, a stabilizer, etc. Further for the purpose of promoting the absorption and enhancing the bioavailability, the preparation may also contain β-cyclodextrin or β-cyclodextrin derivatives (e.g., hydroxypropyl-β-cyclodextrin, etc.), and the like.

[0147] Examples of the above excipient include lactose, saccharose, D-mannitol, starch, crystalline cellulose, light silicic anhydride, etc. Examples of the lubricant include magnesium stearate, calcium stearate, talc, colloidal silica, etc., with magnesium stearate and colloidal silica being preferred. Examples of the isotonizing agent include sodium chloride, glucose, fructose, mannitol, sorbitol, lactose, saccharose, glycerin and urea, with mannitol being particularly preferred. As the hydrophilic carrier, there are, for example, a swelling hydrophilic carrier such as crystalline cellulose, ethyl cellulose, crosslinked polyvinyl pyrrolidone, light silicic anhydride, silicic acid, dicalcium phosphate, calcium carbonate, etc., with crystalline cellulose (e.g., microcrystalline cellulose, etc.) being preferred. As the water-dispersible polymer, there are, for example, a gum (e.g., tragacanth gum, acacia gum, guar gum), alginate (e.g., sodium alginate), cellulose derivatives (e.g., methyl cellulose, carboxymethylcellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose), gelatin, water-soluble starch, polyacrylic acid (e.g., carbomer), polymethacrylic acid, polyvinyl alcohol, polyethylene glycol, polyvinyl pyrrolidone, polycarbophil, ascorbate palmitate salt, etc., with hydroxypropylmethyl cellulose, polyacrylic acid, alginate, gelatin, carboxymethylcellulose, polyvinyl pyrrolidone and polyethylene glycol being preferred. Hydroxypropylmethyl cellulose is particularly preferred. As the stabilizer, there are, for example, cysteine, thiosorbitol, tartatic acid, citric acid, sodium carbonate, ascrobic acid, glycine, sodium sulfite, etc., with citric acid and ascorbic acid being particularly preferred.

[0148] The sublingual, buccal or rapid oral disintegrating preparation can be prepared by mixing the compound of the present invention or the concomitant drug and an excipient by a method per se known. Furthermore, if desired, the auxiliary agents described above, such as the lubricant, isotonizing agent, hydrophilic carrier, water-dispersible polymer, stabilizer, colorant, sweetener, preservative, etc. may also be admixed. After mixing the components described above simultaneously or at certain time intervals, the mixture is compressed into tablets to obtain the sublingual, buccal or rapid oral disintegration tablet. In order to obtain a suitable hardness, a solvent such as water, an alcohol, etc. can be used to moisturize or wet the components before or after tabletting, followed by drying.

[0149] In preparing the oral mucosal patch (film), the compound of the present invention or the concomitant drug and the water-dispersible polymer (preferably, hydroxypropyl cellulose, hydroxypropylmethyl cellulose), excipient, etc. described above are dissolved in a solvent such as water, etc. and then the resulting solution is cast into a film. In addition, additives such as a plasticizer, a stabilizer, an antioxidant, a preservative, a colorant, a buffering agent, a sweeteners, etc. may be added to the preparation. A glycol such as polyethylene glycol, propylene glycol, etc. may be added to impart an appropriate elasticity to a film, and a bioadhesive polymer (e.g., polycarbophile, carbopol) may also be added to enhance the adhesion of the film to the oral mucosal lining. The casting can be carried out by pouring a solution onto a non-adhesive surface, spreading the solution using a coater such as a doctor blade in a uniform thickness (preferably, approximately 10 to 1000 microns), and then drying the solution to form a film. The film thus formed is dried at room temperature or while warming, and then cut into pieces each having a desired surface area.

[0150] A preferred rapid oral disintegrating preparation is, for example, a rapid diffusion preparation in a solid network form, which comprises the compound of the present invention or the concomitant drug and a water-soluble or water-diffusible carrier inert to the compound of the present invention or the concomitant drug. The network is formed by sublimating a solvent from the solid composition comprising a solution of the compound of the present invention or the concomitant drug in a suitable solvent.

[0151] In addition to the compound of the present invention or the concomitant drug, the composition of the rapid oral disintegrating preparation may preferably contain a matrix-forming agent and secondary components.

[0152] Examples of the matrix-forming agent include gelatins, dextrins and animal or vegetable proteins from soybean, wheat, psyllium seed, etc.; gummy materials such as gum arabic, guar gum, agar, xanthane gum, etc.; polysaccharides; alginates; carboxymethylcelluloses; carrageenans; dextrans; pectins; synthetic polymers such as polyvinyl pyrrolidones; materials derived from gelatin-gum arabic complexes, etc. The matrix-forming agent further includes saccharides such as mannitol, dextrose, lactose, galactose, trehalose, etc.; cyclic saccharides such as cyclodextrins, etc.; inorganic salts such as sodium phosphate, sodium chloride, aluminum silicate, etc.; amino acids having 2 to 12 carbon atoms such as glycine, L-alanine, L-aspartic acid, L-glutamic acid, L-hydroxyproline, L-isoleucine, L-leucine, L-phenylalanine, etc.

[0153] One or more matrix-forming agents can be incorporated into a solution or suspension before solidification. The matrix-forming agents may be present in addition to a surfactant, or may be present in the absence of a surfactant. The matrix-forming agents serve not only to form a matrix itself, but also assist to maintain diffusion of the compound of the present invention or the concomitant drug in the solution or suspension.

[0154] The composition may contain a secondary component such as a preservative, an antioxidant, a surfactant, a thickening agent, a colorant, pH adjusting agent, a flavor, a sweetener, a taste masking agent, etc. As the suitable colorant, there are, for example, iron oxide red, black and yellow, FD & C dyes available from ERIS & EVERALD such as FD & C Blue No. 2 and FD & C Red No. 40, etc. Examples of the suitable flavor include mint, raspberry, licorice,

orange, lemon, grape fruit, caramel, vanilla, cherry, grape flavor and a combination thereof. Examples of the suitable pH adjusting agent include citric acid, tartaric acid, phosphoric acid, hydrochloric acid and maleic acid. Examples of the suitable sweetener include aspartame, acesulfame K and thaumatine. Examples of the suitable taste masking agent include sodium bicarbonate, ion exchange resins, cyclodextrin inclusion compounds, adsorbents and microencapsulated apomorphine.

[0155] The preparation generally contains the compound of the present invention or the concomitant drug in an amount of about 0.1 to about 50% by weight, preferably about 0.1 to about 30% by weight and, preferably, the preparation (the sublingual tablet, buccal, etc. described above) allows 90% or more of the compound of the present invention or the concomitant drug to be dissolved (in water) within a time period of about 1 to about 60 minutes, preferably about 1 minute to about 15 minutes, more preferably about 2 minutes to about 5 minutes, or is a rapid oral disintegrating preparation which disintegrates within about 1 to about 60 seconds, preferably about 1 to about 30 seconds, more preferably about 1 to about 10 seconds, after being placed in the oral cavity.

[0156] The amount of the above excipient is about 10 to about 99% by weight, preferably about 30 to about 90% by weight based on the total weight of the preparation. The amount of β-cyclodextrin or β-cyclodextrin derivative is about 0 to about 30% by weight based on the total weight of the preparation. The amount of the lubricant is about 0.01 to about 10% by weight, preferably about 1 to about 5% by weight based on the total weight of the preparation. The amount of the isotonizing agent is about 0.1 to about 90% by weight, preferably about 10 to about 70% by weight based on the total weight of the preparation. The amount of the hydrophilic carrier is about 0.1 to about 50% by weight, preferably about 10 to about 30% by weight based on the total weight of the preparation. The amount of the water-dispersible polymer is about 0.1 to about 30% by weight, preferably about 10 to about 25% by weight based on the total weight of the preparation. The amount of the stabilizer is about 0.1 to about 10% by weight, preferably about 1 to about 5% by weight based on the total weight of the preparation. If necessary, the preparation described above may further contain additives such as a colorant, a sweetener, a preservative, etc.

[0157] A dose of the combined preparations of the present invention varies depending upon kind of the compound of the present invention, age, body weight, conditions, dosage form, route for administration, dosing period, etc.

[0158] A dose of the compound of the present invention may vary depending upon subject to be administered, target organ, conditions, route of administration, etc., and in oral administration, the compound is generally administered to the patient (as 60 kg body weight) with cancer in a daily dose of about 0.01 to about 100 mg, preferably about 0.1 to about 50 mg and more preferably about 0.1 to about 20 mg. In parenteral administration, a single dose of the compound may vary depending upon subject to be administered, target organ, conditions, route of administration, etc., and in the form of an injectable dosage form, it is advantageous to intravenously administer the compound to the patient (as 60 kg body weight) with cancer generally in a daily dose of about 0.001 to about 30 mg, preferably about 0.01 to about 20 mg, and more preferably about 0.01 to about 10 mg. For other animal species, the corresponding dose as converted per 60 kg weight can be administered. Of course, the dose may vary depending on individual conditions as described above; in such a case, a dose less than the dose given above may be sufficient, or a dose higher than the range above may be used.

[0159] It is possible to set any range of a dose for the concomitant drug, so long as it causes no adverse side effects. A daily dose of the concomitant drug may vary depending on the severity of disease, the age, sex, body weight and susceptibility of the subject, dosing period and intervals, characteristics, formulation, type and active components of the pharmaceutical preparation, etc. and is not particularly limited. For example, in oral administration, the dose is about 0.001 to 2000 mg, preferably about 0.01 to 500 mg, and more preferably about 0.1 to 100 mg per kg body weight of mammals in terms of a drug; usually, this dose is administered by dividing 1 to 4 times per day.

[0160] When the pharmaceutical preparations of the present invention are administered, they may be administered concomitantly. Alternatively, the concomitant drug is first administered and then the compound of the present invention is administered, or the compound of the present invention is first administered and then the concomitant drug is administered. When they are administered at certain time intervals, the intervals vary depending on the active component to be administered, dosage form and route of administration; for example, when the concomitant drug is first administered, the compound of the present invention may be administered within 1 minute to 3 days, preferably 10 minutes to 1 day, more preferably 15 minutes to 1 hour after the administration of the concomitant drug. When the compound of the present invention is first administered, the concomitant drug may be administered within 1 minute to 1 day, preferably 10 minutes to 6 hours, more preferably 15 minutes to 1 hour after the administration of the compound of the present invention.

[0161] As a preferred method of administration, for example, about 0.001 to 200 mg/kg of the concomitant drug in the form of an oral dosage preparation is administered orally and, after about 15 minutes, about 0.005 to 0.5 mg/kg of the compound of the present invention in the form of a parenteral preparation is administered parenterally as a daily dose.

[0162] As the metastins, there are used, for example, human metastin described in WO 00/24890, mouse or rat metastin described in WO 01/75104, etc.

[0163] Specific examples of human metastin include a peptide comprising the N-terminal 47th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 and consisting of 8 to 54 amino acid residues, and

the like.

[0164] The "peptide comprising the N-terminal 47th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 and consisting of 8 to 54 amino acid residues" may be any peptide, as far as it is a peptide comprising the N-terminal 47th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 and consisting of 8 to 54 amino acid residues, but means that these peptides have substantially the same physiological activity (e.g., a receptor binding activity, a signal transduction action, a sugar level elevating action, a pancreatic glucagon secretion stimulating action, a urine formation promoting action, etc.). Specifically, there are used (i) a peptide having the amino acid sequence represented by SEQ ID NO: 1, (ii) a peptide comprising the N-terminal 47th-54th amino acid sequence at the C terminus in the amino acid sequence represented by SEQ ID NO: 1 and consisting of 8 to 15 amino acid residues, etc.

[0165] More specifically, human metastin used includes (i) a peptide consisting of the amino acid sequence represented by SEQ ID NO: 1 (human metastin 54 (1-54)), (ii) a peptide consisting of the N-terminal 40th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 15 (40-54); SEQ ID NO: 15), (iii) a peptide consisting of the N-terminal 45th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 10 (45-54); SEQ ID NO: 16), (iv) a peptide consisting of the N-terminal 46th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 9 (46-54); SEQ ID NO: 17), (v) a peptide consisting of the N-terminal 47th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 8 (47-54); SEQ ID NO: 18), etc.

[0166] As mouse metastin (A), there are used, for example, (i) a peptide comprising the N-terminal 134th-141st amino acid sequence in the amino acid sequence represented by SEQ ID NO: 3 and consisting of 8 to 52 amino acid residues. Specific examples of mouse metastin (A) used include (i) a peptide consisting of the N-terminal 90th-141st amino acid sequence in the amino acid sequence represented by SEQ ID NO: 3, (ii) a peptide consisting of the N-terminal 132nd-141st amino acid sequence in the amino acid sequence represented by SEQ ID NO: 3, (iii) a peptide consisting of the N-terminal 127th-141st amino acid sequence in the amino acid sequence represented by SEQ ID NO: 3, and the like.

[0167] As mouse metastin (B), there are used, for example, (i) a peptide comprising the N-terminal 138th-145th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 5 and consisting of 8 to 52 amino acid residues. Specific examples of mouse metastin (B) used include a peptide consisting of the N-terminal 94th-145th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 5, and the like. As rat metastin, there are used, for example, (i) a peptide comprising the N-terminal 112th-119th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 7 and consisting of 8 to 52 amino acid residues. Specific examples of rat metastin used include (i) a peptide consisting of the N-terminal 68th-119th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 7, (ii) a peptide consisting of the N-terminal 110th-119th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 7, (iii) a peptide consisting of the N-terminal 105th-119th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 7, and the like.

[0168] Throughout the specification, the metastins are represented in accordance with the conventional way of describing peptides, that is, the N-terminus (amino terminus) at the left hand and the C-terminus (carboxyl terminus) at the right hand. In the peptide represented by SEQ ID NO: 1, the C-terminus may be in any form of a carboxyl group (-COOH), a carboxylate ($-COO^-$), an amide ($-CONH_2$) and an ester (-COOR). Herein, examples of R of the ester group or alkyl amide include a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C_{6-12} aryl group such as phenyl, α-naphthyl, etc.; a C_{7-14} aralkyl such as a phenyl-C_{1-2} alkyl group, e.g., benzyl, phenethyl, benzhydryl, etc., or an α-naphthyl-C_{1-2} alkyl group such as α-naphthylmethyl, etc.; pivaloyloxymethyl group, which are widely used as an ester for oral use, and the like.

[0169] Furthermore, the metastins include peptides, wherein the amino group at the N-terminal methionine residue is protected with a protecting group (e.g., a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g., formyl group, acetyl group, etc.); those wherein the N-terminal region is cleaved in vivo and the glutamyl group thus formed is pyroglutaminated; those wherein a substituent (e.g., -OH, -SH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain of an amino acid in the molecule is protected with a suitable protecting group (e.g., a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g., formyl group, acetyl group, etc.), or conjugated peptides such as glycopeptides bound to sugar chains.

[0170] For salts of the metastin of the present invention, preferred are salts with physiologically acceptable bases (e.g., alkali metal salts) or acids (e.g., organic acids or inorganic acids), etc., especially preferred are physiologically acceptable acid addition salts. Examples of such salts include salts with, for example, inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid); salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

[0171] As the DNAs encoding metastins, there are used, for example, DNAs encoding human metastin described in WO 00/24890, DNAs encoding mouse or rat metastin described in WO 01/75104, etc.

[0172] The DNAs encoding the metastins may be any of genomic DNA, genomic DNA library, cDNA derived from the

cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA may also be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) using the total RNA or mRNA fraction prepared from the cells and tissues described above.

[0173] The DNA encoding human metastin, mouse metastin precursor (A), mouse metastin precursor (B) or rat metastin precursor may be any DNA, so long as each is a DNA containing a base sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, or a DNA having a base sequence hybridizable to the base sequence represented by any base sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 under highly stringent conditions and encoding the human metastin, mouse metastin (A), mouse metastin (B) or rat metastin described above.

[0174] Specific examples of the DNA hybridizable to the base sequence represented by any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 include DNAs containing a base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and the most preferably at least about 95% homology, to the base sequence represented by any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

[0175] Homology in the base sequence can be measured using the homology scoring algorithm NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) under the following conditions (an expectation value = 10; gaps are allowed; filtering = ON; match score = 1; mismatch score = -3).

[0176] The hybridization can be carried out by per se publicly known methods or by modifications of these methods, for example, according to the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). A commercially available library may also be used according to the instructions of the attached manufacturer's protocol. Preferably, the hybridization can be carried out under highly stringent conditions.

[0177] The highly stringent conditions used herein are, for example, those in a sodium concentration at about 19 to 40 mM, preferably about 19 to 20 mM at a temperature of about 50 to 70°C, preferably about 60 to 65°C. In particular, hybridization conditions in a sodium concentration of about 19 mM at a temperature of about 65°C are most preferred.

[0178] Specifically, as the DNA encoding the human metastin consisting of the amino acid sequence represented by SEQ ID NO: 1, the DNA consisting of the base sequence represented by SEQ ID NO: 2 is used. Accordingly, for the base sequence encoding the human metastin consisting of the various amino acid sequences described above, a base sequence corresponding to each of the partial amino acid sequences in the amino acid sequence represented by SEQ ID NO: 1 may be chosen from the base sequence represented by SEQ ID NO: 2.

[0179] As the DNA encoding the mouse metastin precursor (A) comprising the amino acid sequence represented by SEQ ID NO: 3, there are employed a DNA consisting of the base sequence represented by SEQ ID NO: 4, and the like. Accordingly, for the base sequence encoding the mouse metastin precursor (A) consisting of the various amino acid sequences described above, a base sequence corresponding to each of the partial amino acid sequences in the amino acid sequence represented by SEQ ID NO: 3 may be chosen from the base sequence represented by SEQ ID NO: 4.

[0180] As the DNA encoding the mouse metastin precursor (B) comprising the amino acid sequence represented by SEQ ID NO: 5, there are employed a DNA consisting of the base sequence represented by SEQ ID NO: 6, and the like. Accordingly, for the base sequence encoding the mouse metastin precursor (B) comprising of the various amino acid sequences described above, a base sequence corresponding to each of the partial amino acid sequences in the amino acid sequence represented by SEQ ID NO: 5 may be chosen from the base sequence represented by SEQ ID NO: 6.

[0181] As the DNA encoding the rat metastin comprising the amino acid sequence represented by SEQ ID NO: 7, there are employed a DNA consisting of the base sequence represented by SEQ ID NO: 8, and the like. Accordingly, for the base sequence encoding the rat metastin consisting of the various amino acid sequences described above, a base sequence corresponding to each of the partial amino acid sequences in the amino acid sequence represented by SEQ ID NO: 7 may be chosen from the base sequence represented by SEQ ID NO: 8.

[0182] More specifically, for the peptide consisting of the amino acid sequence represented by SEQ ID NO: 1 (human metastin 54 (1-54)), a DNA containing the base sequence represented by SEQ ID NO: 2, etc. is used.

[0183] For the peptide consisting of the N-terminal 40th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 15 (40-54); SEQ ID NO: 15), a DNA containing the base sequence represented by SEQ ID NO: 19, etc. is used.

[0184] For the peptide consisting of the N-terminal 45th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 10 (45-54); represented by SEQ ID NO: 16), a DNA containing the base sequence represented by SEQ ID NO: 20, etc. is used.

[0185] For the peptide consisting of the N-terminal 46th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 9 (46-54); represented by SEQ ID NO: 17), a DNA containing the base sequence represented by SEQ ID NO: 21, etc. is used.

[0186] For the peptide consisting of the N-terminal 47th-54th amino acid sequence in the amino acid sequence represented by SEQ ID NO: 1 (human metastin 8 (47-54); represented by SEQ ID NO: 18), a DNA containing the base

sequence represented by SEQ ID NO: 22, etc. is used.

[0187] As the metastin receptor, its partial peptides or salts thereof, there are used, for example, a human metastin receptor, its partial peptides or salts thereof described in WO 00/24890, a mouse or rat human metastin receptor, its partial peptides or salts thereof described in WO 01/75104, etc.

[0188] Specifically, a protein comprising the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, etc. is used as the metastin receptor.

[0189] The amino acid sequence which is substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13 includes, for example, an amino acid sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology, and most preferably at least about 95% homology, to the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13.

[0190] Homology of the amino acid sequences can be determined using the homology scoring algorithm NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) under the following conditions (an expectation value = 10; gaps are allowed; matrix = BLOSUM62; filtering = OFF).

[0191] As the protein comprising substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, preferred is a protein having substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13 and having the activity of the same nature as that of a protein having the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, etc.

[0192] As the activity of substantially the same nature, there are, for example, a ligand binding activity, a signal transduction activity, and the like. The "substantially the same nature" is used to mean that the nature of these activities is equivalent in terms of quality. Thus, the activities such as a ligand binding activity, a signal transduction activity, etc. are preferably equivalent (e.g., about 0.01 to 100 times, preferably about 0.5 to 10 times, more preferably 0.5 to 2 times), but differences in quantitative factors such as a level of these activities, or such as a molecular weight of the protein may be present and allowable.

[0193] The activities such as a ligand binding activity, a signal transduction activity, etc. can be assayed by per se publicly known method with modifications and may be determined according to methods of determining a ligand or screening methods described in, e.g., WO 00/24890 or WO 01/75104.

[0194] Examples of the metastin receptor used include proteins comprising (i) the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, of which at least 1 or 2 (preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 or 2)) amino acids are deleted; (ii) the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, to which at least 1 or 2 (preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 or 2)) amino acids are added; (iii) the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, in which at least 1 or 2 (preferably about 1 to about 30, more preferably about 1 to about 10 and most preferably several (1 or 2)) amino acids are substituted by other amino acids; or (iv) a combination of these amino acid sequences; and the like.

[0195] Throughout the specification, the metastin receptors are represented in accordance with the conventional way of describing peptides, that is, the N-terminus (amino terminus) at the left hand and the C-terminus (carboxyl terminus) at the right hand. In the metastin receptors including the metastin receptor represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13, the C-terminus may be in any form of a carboxyl group (-COOH), a carboxylate (-COO-), an amide ($-CONH_2$) and an ester (-COOR). Herein, examples of R of the ester group include a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C_{6-12} aryl group such as phenyl, α-naphthyl, etc.; a C_{7-14} aralkyl such as a phenyl-C_{1-2} alkyl group, e.g., benzyl, phenethyl, etc., or an α-naphthyl-C_{1-2} alkyl group such as α-naphthylmethyl, etc.; and pivaloyloxymethyl group, which are widely used as an ester for oral use, and the like.

[0196] Where the metastin receptors contain a carboxyl group (or a carboxylate) at a position other than the C-terminus, the carboxyl group may be amidated or esterified and such amides or esters are also included within the receptor protein of the present invention. In this case, the ester group used may be the same group as the C-terminal esters described above.

[0197] Furthermore, the metastin receptors include those wherein the amino group at the N-terminal methionine residue is protected with a protecting group (e.g., a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g., formyl group, acetyl group, etc.); those wherein the N-terminal region is cleaved in vivo and the glutamyl group thus formed is pyroglutaminated; those wherein a substituent (e.g., -OH, -SH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain of an amino acid in the molecule is protected with a suitable protecting group (e.g., a C_{1-6} acyl group such as a C_{2-6} alkanoyl group, e.g., formyl group, acetyl group, etc.), or conjugated proteins such as glycoproteins bound to sugar chains.

[0198] Specific examples of the metastin receptors include human metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 9, rat metastin receptor consisting of the amino acid sequence represented by

SEQ ID NO: 11, mouse metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 13, etc.

[0199] The partial peptides of the metastin receptor (hereinafter sometimes simply referred to as the partial peptide) may be any peptide, so long as they are partial peptides of the metastin receptor described above; there are used those such as protein molecules of the metastin receptor, which are the sites exposed outside the cell membrane, and having a ligand binding activity.

[0200] Specifically, the partial peptide of the metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13 is a peptide containing the parts analyzed to be extracellular domains (hydrophilic domains) in the hydrophobic plotting analysis. A peptide containing a hydrophobic domain in part can be used as well. In addition, the peptide may contain each domain separately or a plurality of domains together.

[0201] In the metastin receptor, preferred partial peptides are those having the number of amino acids of at least 20, preferably at least 50, and more preferably at least 100, in the amino acid sequence described above, which constitutes the metastin receptor.

[0202] The partial peptide may be a peptide having the amino acid sequence described above, of which at least 1 or 2 (preferably about 1 to about 10 and more preferably several (1 or 2)) amino acids are deleted; to which at least 1 or 2 (preferably about 1 to about 10 and more preferably several (1 or 2)) amino acids are added; or, in which at least 1 or 2 (preferably about 1 to about 10 and more preferably several (1 or 2)) amino acids are substituted by other amino acids. In the partial peptide, the C terminus may be any form of a carboxyl group (-COOH), a carboxylate (-COO), an amide ($-CONH_2$) and an ester (-COOR), as in the metastin receptor described above.

[0203] Furthermore, the partial peptides include peptides, wherein the amino group at the N-terminal methionine residue is protected with a protecting group; those wherein the N-terminal region is cleaved in vivo and the glutamyl group thus formed is pyroglutaminated; those wherein a substituent on the side chain of an amino acid in the molecule is protected with a suitable protecting group, or conjugated peptides such as glycopeptides bound to sugar chains, as in the metastin receptors described above.

[0204] For salts of the metastin receptor or the partial peptide, preferred are salts with physiologically acceptable acids, especially physiologically acceptable acid addition salts. Examples of the salts include salts with, for example, inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid); salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

[0205] As the DNA encoding the metastin receptor or its partial peptides, there are used, for example, a DNA encoding the human metastin receptor or its partial peptides described in WO 00/24890, a DNA encoding the mouse or rat metastin receptor or its partial peptides described in WO 01/75104, etc.

[0206] The DNAs encoding the metastin receptor or its partial peptides may be any of genomic DNA, genomic DNA library, cDNA derived from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA may also be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) using the total RNA or mRNA fraction prepared from the cells and tissues described above.

[0207] Specifically, the DNA encoding human metastin receptor, mouse metastin receptor or rat metastin receptor may be any DNA, so long as it is a DNA comprising each base sequence represented by SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14, or a DNA comprising a base sequence hybridizable to the base sequence represented by SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14 under highly stringent conditions and encoding a receptor having the activity of substantially the same nature (e.g., a ligand binding activity, a signal transduction activity, etc.) as that of the human metastin receptor, mouse metastin receptor or rat metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14.

[0208] Examples of the DNA hybridizable to the base sequence represented by any of SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14 include DNAs comprising a base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and the most preferably at least about 95% homology, to the base sequence represented by any of SEQ ID NO: 10, SEQ ID NO: 12 or SEQ ID NO: 14.

[0209] Homology in the base sequence can be measured using the homology scoring algorithm NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) under the following conditions (an expectation value = 10; gaps are allowed; filtering = ON; match score = 1; mismatch score = -3).

[0210] The hybridization can be carried out by per se publicly known methods or by modifications of these methods, for example, according to the method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989), etc. A commercially available library may also be used according to the instructions of the attached manufacturer's protocol. Preferably, the hybridization can be carried out under highly stringent conditions.

[0211] The highly stringent conditions used herein are, for example, those in a sodium concentration at about 19 to 40 mM, preferably about 19 to 20 mM at a temperature of about 50 to 70°C, preferably about 60 to 65°C. In particular, hybridization conditions in a sodium concentration of about 19 mM at a temperature of about 65°C are most preferred.

[0212] More specifically, as the DNA encoding the human metastin receptor consisting of the amino acid sequence

represented by SEQ ID NO: 9, the DNA consisting of the base sequence represented by SEQ ID NO: 10 is used.

[0213] As the DNA encoding the rat metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 11, the DNA consisting of the base sequence represented by SEQ ID NO: 12 is used.

[0214] As the DNA encoding the mouse metastin receptor consisting of the amino acid sequence represented by SEQ ID NO: 13, the DNA consisting of the base sequence represented by SEQ ID NO: 14 is used.

[0215] The metastin receptors, their partial peptides or salts thereof and the DNAs encoding the metastin receptors or their partial peptides can be obtained or produced by the methods described in WO 00/24890 or WO 01/75104.

[0216] The present invention will be described in detail by referring to EXAMPLES, FORMULATION EXAMPLES AND TEST EXAMPLES, but is not deemed to be limited thereto, and any modification may be made without departing from the scope of the present invention.

[0217] In the following EXAMPLES, the term "room temperature" normally means a temperature of about 10°C to about 35°C. In percentages, the yield is shown by mol/mol% and the solvent used in chromatography by vol%, and the remaining by wt%. In proton NMR spectra, data on OH, NH protons, etc. that are broad and unidentified are not shown.

[0218] The other abbreviations used in the specification mean as follows.

Abbreviation	Description
10Ψ,CSNH:	The C-terminal-$CONH_2$ at the 10-position is substituted with -$CSNH_2$.
1Ψ2,CH_2NH:	The -CONH- bond between the 1- and 2-positions is substituted with the -CH_2NH- bond.
2Ψ3,CH_2NH:	The -CONH- bond between the 2- and 3-positions is substituted with the -CH_2NH- bond.
3Ψ4,CH_2NH:	The -CONH- bond between the 3- and 4-positions is substituted with the -CH_2NH- bond.
4Ψ5,CH_2NH:	The -CONH- bond between the 4- and 5-positions is substituted with the -CH_2NH- bond.
6Ψ7,CSNH:	The -CONH- bond between the 6- and 7-positions is substituted with the -CSNH- bond.
6Ψ7,NHCO:	The -CONH- bond between the 6- and 7-positions is substituted with the -NHCO- bond.
6Ψ7,CH_2NH:	The -CONH- bond between the 6- and 7-positions is substituted with the -CH_2NH- bond.
6Ψ7,CH_2O:	The -CONH- bond between the 6- and 7-positions is substituted with the -CH_2O- bond.
7Ψ8,CH_2NH:	The -CONH- bond between the 7- and 8-positions is substituted with the -CH_2NH- bond.
8Ψ9,CH_2NH:	The -CONH- bond between the 8- and 9-positions is substituted with the -CH_2NH- bond.
9Ψ10,CH_2NH:	The -CONH- bond between the 9- and 10-positions is substituted with the -CH_2NH- bond.
Aad	: 2-aminoadipic acid
Abu	: 2-aminobutanoic acid
Abz(2)	: 2-aminobenzoic acid
Abz(3)	: 3-aminobenzoic acid
Ac	: acetyl
AcONB	: N-acetoxy-5-norbornene-2,3-dicarboximide
Acp	: 6-aminocaproic acid
AcOEt	: ethyl acetate
AcOH	: acetic acid
Aib	: α-aminoisobutanoic acid
Ala(2-Qui)	: 2-quinolylalanine
Ala(3-Bzt)	: 3-benzothienylalanine
Ala(cBu)	: cyclobutylalanine
Ala(cPr)	: cyclopropylalanine
Ala(Pip)	: (4-piperidin-1-yl)alanine
Alb	: Albizziin 2-amino-3-ureidopropionic acid

(continued)

Abbreviation	Description
Ambz(4)	: 4-aminomethylbenzoyl
Arg(Ac)	: N^{ω}-acetylarginine
Arg(Boc_2,Me)	: $N^{\omega,\omega'}$-bis-tert-butoxycarbonyl-N^{ω}-methylarginine
Arg(Et) Arg(Me)	: N^{ω}-ethylarginine : N^{ω}-methylarginine
Arg(asyMe_2) or Arg(Me_2)asym	: asymmetric-$N^{\omega,\omega'}$-dimethylarginine
Arg(symMe_2) or Arg(Me_2)sym	: symmetric-$N^{\omega,\omega'}$-dimethylarginine
Arg(NO_2)	: N^{ω}-nitroarginine
Arg(Pbf)	: N^{ω}-2,2,4,6,7-pentamethyldihydrobenzofuransulfonylarginine
Arg(n-Pr)	: N^{ω}-propylarginine
Arg(Tos)	: N^{ω}-tosylarginine
Asp(NHMe)	: N^{ω}-methylasparagine
Asp(NMe_2)	: $N^{\omega,\omega}$-dimethylasparagine
Asp(NHPen)	: N^{ω}-pentylasparagine
Asp(NHcPr)	: N^{ω}-cyclopropylasparagine
Asp(NHBzl)	: N^{ω}-benzylasparagine
AzaGly	: azaglycine
AzaPhe	: azaphenylalanine
Aze(2)	: azetidine-2-carboxylic acid
β-Ala	: β-alanine
Boc	: tert-butoxycarbonyl
Boc_2O	: di-tert-butyl dicarbonate
Br-Z	: 2-bromobenzyloxycarbonyl
Bu^t	: tert-butyl
Bzl	: benzyl
CDI	: 1,1'-carbonyldiimidazole
Cha	: cyclohexylalanine
ClP	: 2-chloro-1,3-dimethylimidazolium tetrafluoroborate
Cit	: citrulline
Clt resin	: 2-chlorotrytyl resin
Cl-Z	: 2-chlorobenzyloxycarbonyl
Dab	: 2,4-diaminobutanoic acid
Dap	: 2,3-diaminopropionic acid
Dap(Ac)	: N^{β}-acetyl-β-diaminopropionic acid
Dap(For)	: N^{β}-formyl-β-diaminopropionic acid
Dap(Gly)	: N^{β}-glycyl-β-diaminopropionic acid
Dap(GnGly)	: N^{β}-(N-guanidinoglycyl)-β-diaminopropionic acid
DCM	: dichloromethane
DEA	: diethylamine
DIEA	: N,N-diisopropylethylamine
DIPCDI	: 1,3-diisopropylcarbodiimide
DMAP	: 4-dimethylaminopyridine
DMF	: N,N-dimethylformamide
EDC	: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDT	: 1,2-ethanedithiol
Fmoc	: 9-fluorenylmethoxycarbonyl
For	: formyl
γ-Abu	: 4-aminobutanoic acid
γ-MeLeu,Leu(Me)	: γ-methylleucine
GlyΨ((E)CH=CH)Leu:	The -CONH- between Gly and Leu is substituted with (E) type alkene.
GlyΨ(CH_2CH_2)Leu:	The -CONH- between Gly and Leu is substituted with the -CH_2CH_2- bond.

(continued)

Abbreviation	Description
GlyΨ(CH_2S)Leu:	The -CONH- between Gly and Leu is substituted with the -CH_2S- bond.
Gn	: guanidino
GuAmb	: 4-guanidinomethylbenzoyl
Har	: homoarginine
Har(Me)	: N^{ω}-methylhomoarginine
His(3Me)	: 3-methylhistidine π-methylhistidine
HOAt	: 1-hydroxy-7-azabenzotriazole
HOBt	: 1-hydroxybenzotriazole
HOOBt	: 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
HONB	: N-hydroxy-5-norbornene-2,3-dicarboximide
Hph	: homophenylalanine
Hyp	: trans-4-hydroxyproline
Hyp(Bzl)	: O-benzyl-trans-4-hydroxyproline
IndPr	: 3-(indole-3-yl)propionyl
Izc	: imidazolidine-2-carboxylic acid
Lys(Me_2)	: $N^{\varepsilon,\varepsilon}$-dimethyllysine
MBHA	: p-methylbenzhydrylamine
MeOH	: methanol
Mtt	: 4-methyltrytyl
N((CH_2)$_3$Gn)Gly	: N-(3-guanidinopropyl)glycine
Nal(1)	: 1-naphthylalanine
Nal(2)	: 2-naphthylalanine
Nar	: norarginine
Nar(Me)	: N^{ω}-methylnorarginine
Nle	: norleucine
NMeAla	: N^{α}-methylalanine
NMeArg	: N^{α}-methylarginine
NMeAsn	: N^{α}-methylasparagine
NMeLeu	: N^{α}-methylleucine
NMePhe	: N^{α}-methylphenylalanine
NMeSer	: N^{α}-methylserine
NMeTrp	: N^{α}-methyltryptophan
NMeTyr	: N^{α}-methyltyrosine
Nva	: norvaline
OBu^t	: tert-butoxy
Orn	: ornithine
Orn(Mtt)	: N^{δ}-(4-methyltrytyl)ornithine
PAL	: 5-(4-(9-fluorenylmethoxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid
Pbf	: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
pGlu	: pyroglutamic acid
Phe(2Cl)	: 2-chlorophenylalanine
Phe(2F)	: 2-fluorophenylalanine
Phe(3,4Cl_2)	: 3,4-dichlorophenylalanine
Phe(3,4F_2)	: 3,4-difluorophenylalanine
Phe(3CF_3)	: 3-trifluoromethylphenylalanine
Phe(3Cl)	: 3-chlorophenylalanine
Phe(3F)	: 3-fluorophenylalanine
Phe(4Cl)	: 4-chlorophenylalanine
Phe(4CN)	: 4-cyanophenylalanine

(continued)

Abbreviation	Description
Phe(4F)	: 4-fluorophenylalanine
Phe(4Gn)	: 4-guanidinophenylalanine
Phe(4NH_2)	: 4-aminophenylalanine
Phe(4NO_2)	: 4-nitrophenylalanine
Phe(4CN)	: 4-cyanophenylalanine
Phe(F_5)	: pentafluorophenylalanine
Phe(2Me)	: 2-methylphenylalanine
Phe(3Me)	: 3-methylphenylalanine
Phe(4Me)	: 4-methylphenylalanine
PheΨ(CH_2CH_2)AzaGly:	The -CONH- between Phe and AzaGly is substituted with the -CH_2CH_2- bond.
PheΨ((E)CH=CH)Gly:	The -CONH- bond between Phe and Gly is substituted with the (E) type alkene.
PheΨ(CH_2CH_2)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH_2CH_2- bond.
PheΨ(CH_2S)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH_2S- bond.
PheΨ((R)CH(OH)-(E)CH=)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH(OH)-CH- bond, the -CH(OH)- moiety takes (R) configuration, and the moiety between the carbon atom in the -CH- moiety and α carbon atom of the Gly is (E) type alkene.
PheΨ((S)CH(OH)-(E)CH=)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH(OH)-CH- bond, the -CH(OH)- moiety takes (S) configuration, and the moiety between the carbon atom in the -CH- moiety and α carbon atom of the Gly is (E) type alkene.
PheΨ((R)CH(OH)-CH_2)Gly:	The -CONH- bond between Phe and Gly and is substituted with the -CH(OH)-CH_2- bond, and the -CH(OH)- moiety takes (R) configuration.
PheΨ((S)CH(OH)-CH_2)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH(OH)-CH_2- bond and the -CH(OH)- moiety takes (S) configuration.
PheΨ(CH_2O)Gly:	The -CONH- bond between Phe and Gly is substituted with the -CH_2O- bond.
PheΨ($COCH_2$)Gly:	The -CONH- bond between Phe and Gly is substituted with the -$COCH_2$- bond.
PheΨ(CSNH)	-NH_2: The C-terminal phenylalanylamide is substituted with phenylalanylthioamide.
Phg	: phenylglycine
PhOH	: phenol
PhSMe	: thioanisole
Pic(2)	: pipecolinic acid
Pic(3)	: 3-piperidinecarboxylic acid
Pip	: pipecolinic acid
Pro	: proline
Pro(4F)	: trans-4-fluoroproline
Pro(4NH_2)	: cis-4-aminoproline
Pya(2)	: 2-pyridylalanine
Pya(3)	: 3-pyridylalanine
Pya(4)	: 4-pyridylalanine
PyAOP	: (7-azabenzotriazole-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate
PyBOP	: (benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate
PyBrop	: bromo-tris(pyrrolidino)phosphonium hexafluorophosphate
Pzc(2)	: piperazine-2-carboxylic acid
Sar	: N-methylglycine
Ser(Ac)	: O-acetylserine
Ser(Me)	: O-methylserine

(continued)

Abbreviation	Description
Thi	: 2-thienylalanine
Thz	: thioproline
Tic	: 1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid
TIS	: triisopropylsilane
Tle	: tert-leucine
Tos	: tosyl
Trp(For)	: N^{in}-formyltryptophan
Trt	: trytyl
Tyr(Me)	: O-methyltyrosine
Tyr(PO_3H_2)	: O-phosphotyrosine
TyrΨ(CH_2NH)Asn:	The -CONH- between Tyr and Asn is substituted with the -CH_2NH- bond.
TFA	: trifluoroacetic acid
TFE	: trifluoroethanol
Z	: benzyloxycarbonyl

[0219] In the specification and drawings, where the codes of bases and amino acids are denoted by abbreviations, they are based on the abbreviations in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature or the common codes in the art. For amino acids that may have the optical isomer, L form is presented unless otherwise indicated.

[0220] The sequence identification numbers in the sequence listing of the specification indicates the following sequence, respectively.

[SEQ ID NO: 1]
This shows the amino acid sequence of human-derived metastin (Metastin).
[SEQ ID NO: 2]
This shows the base sequence of DNA encoding human metastin.
[SEQ ID NO: 3]
This shows the amino acid sequence of mouse metastin precursor (A).
[SEQ ID NO: 4]
This shows the base sequence of DNA encoding mouse metastin precursor (A), which is the base sequence in plasmid pCMV-mKiSS-1 harbored on transformant Escherichia coli DH10B/pCMV-mKiSS-1.
[SEQ ID NO: 5]
This shows the amino acid sequence of mouse metastin precursor (B).
[SEQ ID NO: 6]
This shows the base sequence of DNA encoding mouse metastin precursor (B), which is the base sequence in plasmid pCR2.1-mKiSS-1.4A harbored on transformant Escherichia coli DH5α/pCR2.1-mKiSS-1.4A.
[SEQ ID NO: 7]
This shows the amino acid sequence of rat-derived metastin precursor.
[SEQ ID NO: 8]
This shows the base sequence of DNA encoding rat metastin precursor.
[SEQ ID NO: 9]
This shows the amino acid sequence of human OT7T175 (metastin receptor).
[SEQ ID NO: 10]
This shows the base sequence of DNA encoding human OT7T175 (metastin receptor).
[SEQ ID NO: 11]
This shows the amino acid sequence of rat OT7T175 (metastin receptor).
[SEQ ID NO: 12]
This shows the base sequence of DNA encoding rat OT7T175 (metastin receptor).
[SEQ ID NO: 13]
This shows the amino acid sequence of mouse OT7T175 (metastin receptor).
[SEQ ID NO: 14]
This shows the base sequence of DNA encoding mouse OT7T175 (metastin receptor).
[SEQ ID NO: 15]

This shows the amino acid sequence of human metastin 15 (40-54).
[SEQ ID NO: 16]
This shows the amino acid sequence of human metastin 10 (45-54) (MS 10).
[SEQ ID NO: 17]
This shows the amino acid sequence of human metastin 9 (46-54).
[SEQ ID NO: 18]
This shows the amino acid sequence of human metastin 8 (47-54).
[SEQ ID NO: 19]
This shows the base sequence of DNA encoding human metastin 15 (40-54).
[SEQ ID NO: 20]
This shows the base sequence of DNA encoding human metastin 10 (45-54).
[SEQ ID NO: 21]
This shows the base sequence of DNA encoding human metastin 9 (46-54).
[SEQ ID NO: 22]
This shows the base sequence of DNA encoding human metastin 8 (47-54)

[0221] The transformant *Escherichia coli* DH10B/pCMV-mKiSS-1 has been on deposit since January 24, 2000 with International Patent Organisms Depository, National Institute of Advanced Industrial Science and Technology (the former Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH)), located at Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki (postal code 305-8566), Japan, as the Accession Number FERM BP-7003 and since December 16, 1999 with Institute for Fermentation (IFO), located at 2-17-85, Juso-Honmachi, Yodogawa-ku, Osaka-shi, Osaka, Japan, as the Accession Number IFO 16348.

[0222] The transformant *Escherichia coli* DH5α/pCR2.1-mKiSS-1.4A has been on deposit since March 6, 2000 with International Patent Organisms Depository, National Institute of Advanced Industrial Science and Technology (the former Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human Technology (NIBH)), located at Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki (postal code 305-8566), Japan, as the Accession Number FERM BP-7073 and since February 16, 2000 with Institute for Fermentation (IFO), located at 2-17-85 Juso-Honmachi, Yodogawa-ku, Osaka-shi, Osaka, Japan, as the Accession Number IFO 16360.

REFERENCE EXAMPLE 1

Production of N-methyl-N,N'-Bis-Boc-1-guanylpyrazole

[0223] In a nitrogen atmosphere, 720 mg of 60% NaH in oil was dissolved in 20 mL of dry DMF and 20 mL of dry DMF solution of 5.59 g of N,N'-Bis-Boc-1-guanylpyrazole commercially available was added to the solution at 0°C, followed by stirring for 10 minutes. After 1.68 mL of methyl iodide was added thereto, the mixture was stirred at room temperature for 24 hours. After the solvent was removed by distillation, the residue was dissolved in AcOEt and the solution was washed with 1N HCl aq. solution, satd. $NaHCO_3$ aq. solution and then satd. NaCl aq. solution. After drying over Na_2SO_4, the solvent was concentrated and the concentrate was purified by flash column chromatography (ethyl acetate/n-hexane = 1/4) using silica gel 60 (200 mL) to give 5.35 g (yield 91.6%) of N-methyl-N,N'-bis-Boc-1-guanylpyrazole.
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (br s, 1H), 7.69 (br s, 1H), 6.42 (dd, 1H, J = 2.7, 1.5 Hz), 3.25 (s, 3H), 1.53 (s, 9H), 1.30 (s, 9H)
Elemental analysis as $C_{15}H_{24}N_4O_4$

Calcd.: C, 55.54; H, 7.46; N, 17.27
Found: C, 55.36; H, 7.48; N, 17.06

Rf1: 0.64, Rf2: 0.79

Developing solvent for TLC:

[0224]

Rf1 (ethyl acetate/n-hexane = 1/2), Rf2 (methanol/chloroform = 2/98)
Elution time on HPLC: 26.7 mins.

Elution conditions:

[0225]

Column: Wakosil-II 5C18 HG (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 100/0-20/80, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (40 mins.)
Flow rate: 1.0 mL/min.

REFERENCE EXAMPLE 2

Production of N-methyl-N,N'-Bis-Z-1-guanylpyrazole

[0226] In an argon atmosphere, 40 mg of 60% NaH in oil was dissolved in 5 mL of dry DMF and 5 mL of dry DMF solution of 380 mg of N,N'-Bis-Z-1-guanylpyrazole commercially available was added to the solution at 0°C, followed by stirring for 10 minutes. After 125 μL of methyl iodide was added thereto, the mixture was stirred at room temperature for 15 hours. After the solvent was distilled off, the residue was dissolved in AcOEt and the solution was washed with 1N HCl aq. solution, satd. $NaHCO_3$ aq. solution and then satd. NaCl aq. solution. After drying over Na_2SO_4, the solvent was concentrated to give 393 mg of the crude product. From the crude product, 170 mg was purified by flash column chromatography (ethyl acetate/n-hexane = 1/4) using silica gel 60 (75 mL) to give 152 mg (yield 89.5%) of N-methyl-N,N'-bis-Z-1-guanylpyrazole.
^1H NMR (300 MHz, $CDCl_3$): δ 7.97 (br s, 1H), 7.61 (d, 1H, J = 1.0 Hz), 7.37-7.32 (m, 4H), 7.29-7.26 (m, 4H), 7.16-7.13 (m, 2H), 6.36 (dd, 1H, J = 2.8, 1.6 Hz), 5.18 (s, 2H), 5.04 (s, 2H), 3.22 (s, 3H)
Elemental analysis as $C_{21}H_{20}N_4O_4$

Calcd.: C, 64.28; H, 5.14; N, 14.28
Found: C, 64.09; H, 5.24; N, 14.43

Rf1: 0.50, Rf2: 0.86

Developing solvent for TLC:

[0227]

Rf1 (ethyl acetate/n-hexane = 1/2)
Rf2 (methanol/chloroform = 2/98)
Elution time on HPLC: 28.9 mins.

Elution conditions:

[0228]

Column: Wakosil-II 5C18 HG (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 100/0-20/80, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (40 mins.)
Flow rate: 1.0 mL/min.

REFERENCE EXAMPLE 3

(Synthesis C): Production of des(1)-Ac-[D-Tyr2,Hyp3,Thr5,Phe6'Y(CH_2CH_2)Gly7,Arg(Me)9,Trp10]MS10 (Compound No. 850)

[0229] After 4.44g (0.45 mmol/g) of Rink Amide MBHA resin commercially available was swollen in DMF, the resin was treated with 50 mL of 20% piperidine/DMF solution for 20 minutes to remove the Fmoc group. The resulting resin was washed with DMF and treated in DMF with 4.21 g (8 mmol) of Fmoc-Trp(Boc)-OH, 1.27 mL (8 mmol) of DIPCDI and 1.31 g (8 mmol) ofHOOBt at room temperature for 90 minutes, whereby Trp(Boc) was introduced to give the Fmoc-Trp(Boc)-Rink Amide MBHA resin. In a similar manner, Orn(Mtt) was introduced to give 2 mmol of the Fmoc-

Orn(Mtt)-Trp(Boc)-Rink Amide MBHA resin. After the resin obtained was washed and swollen in toluene, 30 mL of TFA/TIS/TFE/toluene (1/5/47/47) was added, followed by shaking for 30 minutes and removing the solution by filtration. This procedure was repeated until yellow color caused by free Mtt group in a TFA/TIS/TFE/toluene (1/5/47/47) solution disappeared when the solution was added; thus the Mtt group was removed. The resulting Fmoc-Orn-Trp(Boc)-Rink Amide MBHA resin was neutralized with 5%-DIEA/toluene solution. After washing with toluene, 15 mL of toluene-TFE (4:1) and 1.95 g (6 mmol) of N-methyl-N,N'-bis-Boc-1-guanylpyrazole obtained in REFERENCE EXAMPLE 1 were added to the resin. DIEA was added to the mixture to adjust pH of the solution to 10. The solution was shaken for 15 hours to give Fmoc-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin (2 mmol). After the resin obtained was dried in MeOH, 0.03 mmol was weighed and reswollen in DMF. Leu was introduced in the same manner as described above to give 0.03 mmol of Fmoc-Leu-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin. After Fmoc deprotection in 20% piperidine/DMF solution, the resin was treated in DMF with 51.5 mg (0.12 mmol) of Fmoc-Phe-$\Psi(CH_2CH_2)$-Gly-OH obtained in REFERENCE EXAMPLE 4, 19.1 μl (0.12 mmol) of DIPCDI and 240 μl of 0.5 M HOAt/DMF solution at room temperature for 150 minutes. The resin was washed with DMF and then treated with 10.9 μl of (0.12 mmol) of Ac_2O and 20.9 μl (0.12 mmol) of DIEA for capping of residual amino groups. Subsequently, the resin was shaken overnight in 2 mL of DMF solution ofAc-D-Tyr-Hyp-Asn-Thr-OH (0.06 mmol) synthesized in a conventional manner of the liquid-phase process, 31.2 mg (0.06 mmol) of PyAOP, 120 μl (0.06 mmol) of 0.5 M HOAt/DMF solution and 10.5 μl (0.06 mmol) of DIEA to give Ac-D-Tyr-Hyp-Asn-Thr-Phe-$\Psi(CH_2CH_2)$-Gly-Leu-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin. After drying, 1.5 mL of TFA/PhSMe/m-cresol/H_2O/TIS/EDT (80/5/5/5/2.5/2.5) was added to the resin and stirred for 90 minutes.

[0230] Diethyl ether was added to each reaction solution to give the precipitate and after centrifugation, the supernatant was removed; this procedure was repeated twice for washing. The residue was extracted with an aqueous acetic acid solution and the extract was filtered to remove the resin. Thereafter, linear density gradient elution (60 minutes) to A/B: 71.5/28.5-61.5/38.5 was performed at a flow rate of 15 mL/min using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile on preparative HPLC using YMC Pack R&D-ODS-5-B S-5, 120A column (30 x 250 mm). The fractions containing the product were collected and lyophilized to give 5.5 mg of white powders.

Mass spectrum $(M+H)^+$ 1209.7 (calcd. 1209.6)
Elution time on HPLC: 12.2 mins.
Elution conditions:

Column: SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 80/20-30/70, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (25 mins.)
Flow rate: 1.0 mL/min.

REFERENCE EXAMPLE 4

Synthesis of Fmoc-Phe-$\Psi(CH_2CH_2)$-Gly-OH

[0231] After 97.4 mg (0.269 mmol) of Boc-Phe-Ψ[(E)CH=CH]-Gly-OBu^t, which is a compound known by the literature, was dissolved in 10 mL of AcOEt, 10 mg of 10% Pd/C was added and the mixture was stirred in a hydrogen gas flow at room temperature for 4 hours. The Pd catalyst was removed by filtration through celite, and the filtrate was concentrated under reduced pressure. Subsequently, the residue was dissolved in 4 mL of TFA and the solution was stirred at room temperature for 2 hours. TFA was distilled off under reduced pressure and the residue was dissolved in 2.25 mL of acetonitrile : H_2O (2:1). Under ice cooling, acetonitrile (3 mL) solution of 236 μL (1.69 mmol) of TEA and 95.1 mg (0.282 mmol) of Fmoc-OSu was dropwise added sequentially and the mixture was stirred at the same temperature for 2 hours. After 20 mL of 0.1N HCl aq. was added, the whole was extracted with AcOEt. The organic layer was washed twice with 0.1 N HCl aq. and then dried over anhydrous $MgSO_4$. After concentrating under reduced pressure, the product was purified by flash column chromatography (ethyl acetate/n-hexane = 3/2) using Wakosil C-300 to give 118 mg (quantitative) of Fmoc-Phe-$\Psi(CH_2CH_2)$-Gly-OH.

REFERENCE EXAMPLE 5

(Synthesis G): Production of des(1)-Ac-[D-Tyr2,Hyp3,Thr5,GlyΨ((E)CH=CH)Leu8,Arg(Me)9,Trp10]MS10 (Compound No. 892)

[0232] HCl.H-Ser-OMe, 3.5 g (22.6 mmol), was suspended in (40 mL) of chloroform. Under ice cooling, DIEA(8.7 mL, 49.7 mmol) and Pbf-Cl (5.86 g, 20.3 mmol) were sequentially added and stirred overnight while gradually elevating to room temperature. After the reaction was stopped by adding satd. citric acid aqueous solution, chloroform was distilled off under reduced pressure and the whole was extracted with AcOEt. The organic layer was washed sequentially with

satd. citric acid aqueous solution, satd. sodium bicarbonate aqueous solution and satd. sodium chloride aqueous solution, and then dried over anhydrous $MgSO_4$. After concentration under reduced pressure, flash chromatography was performed using AcOEt : n-hexane = 1:1 to give Pbf-Ser-OMe (6.94 g, 82.7%).

[0233] In an argon atmosphere under ice cooling, 923 μl (2.03 mmol) of a toluene solution of 2.2 M diethyl azodicarboxylate was added to a solution of 500 mg (1.35 mmol) of Pbf-Ser-OMe, 522 mg (2.03 mmol) of PPh_3 in THF (20 mL). The mixture was stirred overnight at room temperature and then THF was distilled off under reduced pressure. Flash chromatography was performed using AcOEt : n-hexane = 1:1 to give 481 mg of aziridine methyl ester (quantitative).

[0234] In an argon atmosphere, 429 μl (0.644 mmol) of a toluene solution of 1.5 M DIBAL-H was dropwise added to a toluene (10 mL) solution of aziridine methyl ester (198 mg, 0.56 mmol) at -78°C. After stirring for 20 minutes at the same temperature, the reaction was stopped with 0.1 N HCl aqueous solution. The whole was extracted with diethyl ether and the organic layer was washed sequentially with 0.1 N HCl aqueous solution and satd. sodium chloride aqueous solution. After drying over anhydrous $MgSO_4$, the reaction mixture was concentrated under reduced pressure to give the aldehyde as an oily substance. On the other hand, 146 μL (0.84 mmol) of DIEA and 197 μL (0.84 mmol) of $(EtO)_2P(O)CH_2CO_2Bu^t$ were sequentially added under ice cooling, to a suspension of 35.6 mg (0.84 mmol) of anhydrous LiCl in acetonitrile (2 mL). The mixture was stirred at the same temperature for 20 minutes and then an acetonitrile (4 mL) solution of the aldehyde obtained above was dropwise added thereto, followed by stirring at 0°C for 3 hours. After the whole was extracted with AcOEt, the organic layer was washed sequentially with satd. citric acid aqueous solution, satd. sodium bicarbonate aqueous solution and satd. sodium chloride aqueous solution, and then dried over anhydrous $MgSO_4$. The mixture was concentrated under reduced pressure and the concentrate was recrystallized from diethyl ether/n-hexane to give 86.5 mg of the objective aziridine enoate. The mother liquor was concentrated under reduced pressure and subjected to flash chromatography using AcOEt : n-hexane = 1:19 to give additional 82.4 mg of the objective aziridine enoate (total yield: 71.5%).

[0235] In an argon atmosphere, 1.59 mL (3.17 mmol) of a 2.0 M THF solution of i-BuMgCl was dropwise added at -78°C to an anhydrous THF (6 mL) solution of 284 mg (3.17 mmol) of CuCN and 269 mg (6.34 mmol) of anhydrous LiCl. The temperature was elevated to 0°C and the mixture was stirred for 10 minutes. The mixture was again cooled to -78°C, and 402 μL (3.17 mmol) of $BF_3.Et_2O$ and an anhydrous THF (6 mL) solution of 334 mg (0.792 mmol) of aziridine enoate were dropwise added sequentially. The mixture was stirred at the same temperature for 20 minutes. After quenching with satd. Ammonium chloride aqueous solution : 28% ammonia aqueous solution (1:1), the mixture was stirred at room temperature until the reaction solution turned blue. The whole was extracted with diethyl ether. The organic layer was washed with water and dried over anhydrous $MgSO_4$. After concentration under reduced pressure, the residue was dissolved in 95% TFA aqueous solution (10 mL) and stirred at room temperature for 3 hours. TFA was removed by distillation under reduced pressure and azeotropically distilled off twice with diethyl ether. The residue was recrystallized from diethyl ether/n-hexane to give 254 mg of Pbf-GlyΨ[(E)-CH=CH]Leu-OH as white powders (75.7%).

[0236] After 180 mg (0.425 mmol) of Pbf-GlyΨ[(E)-CH=CH]Leu-OH and 352 μL (3 mmol) of thioanisole were dissolved in TFA (2.65 mL), the solution was stirred at room temperature for 24 hours. TFA was distilled off under reduced pressure and the resulting residue was dissolved in acetonitrile : H_2O (2:1, 9 mL). Under ice cooling, triethylamine was added until the solution became basis and 168 mg (0.446 mmol) of Fmoc-OSu was further added thereto. While elevating to room temperature, the mixture was stirred for 4 hours and the whole was extracted with AcOEt. The organic layer was washed sequentially with 0.1 N HCl aqueous solution and satd. Sodium chloride aqueous solution, and dried over anhydrous $MgSO_4$. Following concentration under reduced pressure, flash chromatography using AcOEt: n-hexane = 3.5 : 6.5 was performed to give 145 mg of Fmoc-GlyΨ[(E)-CH=CH]Leu-OH (89%).

[0237] After 287 mg (0.1 mmol) of Fmoc-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin (0.349 mmol/g) was swollen in DMF, the mixture was treated with 20% piperidine/DMF solution to cleave the Fmoc group. Subsequently, the resin was treated in DMF with 133 mg (0.338 mmol) of Fmoc-GlyΨ[(E)-CH=CH]Leu-OH, 58.9 μl (0.338 mmol) of DIEA, 676 μl (0.338 mmol) of 0.5 M HOAt/DMF solution and 176 mg (0.338 mmol) of PyAOP at room temperature for 12 hours. After the resin was washed with DMF, the N-terminal peptide was extended by the Fmoc solid-phase synthesis to give Ac-D-Tyr(Bu^t)-Hyp(OBu^t)-Asn(Trt)-Thr(Bu^t)-Phe-GlyΨ[(E)CH=CH]Leu-Arg(Boc_2,M e)-Trp(Boc)-Rink Amide MBHA resin. After the resin was dried, 4 mL of TFA/PhSMe/m-cresol/H_2O/TIS/EDT (80/5/5/5/2.5/2.5) was added thereto and the mixture was stirred for 180 minutes. Diethyl ether was added to each reaction solution to give the precipitate and after centrifugation, the supernatant was removed; this procedure was repeated twice for washing. The residue was extracted with an aqueous acetic acid solution and the extract was filtered to remove the resin. Thereafter, linear density gradient elution (60 minutes) to A/B: 71.5/28.5-61.5/38.5 was performed at a flow rate of 15 mL/min using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile on preparative HPLC using YMC Pack R&D-ODS-5-B S-5, 120A column (30 x 250 mm). The fractions containing the product were collected and lyophilized to give 19.6 mg of white powders.

Mass spectrum $(M+H)^+$ 1207.5 (calcd. 1207.6)

Elution time on HPLC: 14.6 mins.

Elution conditions:

Column: SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 80/20-30/70, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (25 mins.)
Flow rate: 1.0 mL/min.

EXAMPLE 1

Production of des(1)-Ac-[D-Tyr2,Hyp3,Alb4,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Arg(Me)9,Trp10]MS10 (Compound No. 903)

[0238] After 4.32 g (1.5 mmol) of Fmoc-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin (0.347 mmol/g) was eqally divided into three and swollen in DMF, the mixture was treated with 20% piperidine/DMF solution to cleave the Fmoc group. Subsequently, each resin was treated in DMF with 590 mg (1.5 mmol) of Fmoc-GlyΨ[(E)-CH=CH]Leu-OH, 261 μl (1.5 mmol) ofDIEA, 3.0 ml (1.5 mmol) of 0.5 M HOAt/DMF solution and 782 mg (1.5 mmol) of PyAOP at room temperature for 12 hours. After the resin was washed with DMF and combined, Cha, Thr(Bu^t), Alb, Hyp(Bu^t) and D-Tyr(Bu^t) were introduced in this order into the obtained resin, by the Fmoc solid-phase synthesis using Fmoc-amino acid/DIPCDI/HOAt to give Fmoc-D-Tyr(Bu^t)-Hyp(OBu^t)-Asn(Trt)-Thr(Bu^t)-Cha-GlyΨ[(E)CH=CH]Leu-Arg(Boc_2,Me)-Trp(Boc)-Rink Amide MBHA resin. The resin thus obtained was deprotected by 20% piperidine/DMF treatment, and washed. The resin was then suspended in DMF, and 548 μl (6.0 mmol) of Ac_2O and 1.04 ml (6.0 mmol) of DIEA were added, respectively, followed by stirring for 20 minutes. The resin was washed with DMF. After it was confirmed that the reaction proceeded, the resin was washed in MeOH and dried to give Ac-D-Tyr(Bu^t)-Hyp(Bu^t)-Alb-Thr(Bu^t)-Cha-GlyΨ[(E)CH=CH]Leu-Arg(Boc_2,Me)-Trp (Boc)-Rink Amide MBHA resin. The resulting resin was divided into 1 g, 2 g and 2.2 g, to which 25 mL, 50 mL and 50 mL of TFA: thioanisole: m-cresol: H_2O: EDT: TIS (80:5:5:5:2.5:2.5) were added, respectively. After stirring for 180 minutes at room temperature, the reaction solution was dropwise added onto chilled ether while removing the resin by passing through a glass filter, thereby to make the crude peptide white powders. The crude peptide obtained was purified separately 10 times on preparative HPLC using SHISEIDO CAPCELL PAK MGII column (50 x 250 mm). Linear density gradient elution (60 minutes) to A/B: 68/32-58/42 was performed at a flow rate of 45 mL/min using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile on preparative HPLC. The product eluted out was fractionated in test tubes by about 14 mL each. By monitoring each fraction on HPLC, the fractions containing the product only were identified. The fractions were pooled and lyophilized to give 352.2 mg of white powders.

[0239] In AcCN-water, 352.2 mg (286.7 μmol) of the purified sample obtained was dissolved and 1.195 mL (1.433 mmol equivalent) ofAG 1x8 AcO-resin was added to the solution. While occasionally stirring manually, the solution was settled for an hour, and filtered through a PTFE membrane filter having a 3 μm pore diameter. The filtrate was transferred to a recovery flask and the solvent was distilled off. Then, 3 mL of acetic acid was added to the residue. After the mixture was sonicated for 5 minutes with an ultrasonicator, 12 mL of water was added to the solution. While cooling on a dry ice bath, the resulting 20% acetic acid solution was lyophilized to give 321.8 mg of white powders.
Mass spectrum $(M+H)^+$ 1228.4 (calcd. 1228.7)
Elution time on HPLC: 20.2 mins.
Elution conditions:

Column: SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 95/5-45/55, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (25 mins.)
Flow rate: 1.0 mL/min.
Amino acid analysis (20% hydrochloric acid containing 4% thioglycolic acid, 110°C, hydrolysis for 24 hours; figures in parentheses show theoretical values.): Thr 0.95 (1); Tyr 0.95 (1); Cha 1.00 (1)

EXAMPLE 2

Production of des(1)-Ac-[D-Tyr2,Hyp3,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Trp10]MS10 (Compound No. 926)

[0240] Using as a starting material 3.33 g (1.5 mmol) of Fmoc-Rink Amide MBHA resin (0.45 mmol/g), the Fmoc solid-phase synthesis was carried out to give Fmoc-Arg(Pbf)-Trp(Boc)-Rink Amide MBHA resin. After Fmoc-Arg(Pbf)-Trp(Boc)-Rink Amide MBHA resin was swollen in DMF, the mixture was treated with 20% piperidine/DMF solution to cleave the Fmoc group. Subsequently, the resin was treated in DMF with 1.57 g (4.0 mmol) of Fmoc-GlyΨ[(E)-CH=CH]Leu-OH, 1.39 ml (8.0 mmol) of DIEA, 8.0 ml (4.0 mmol) of 0.5 M HOAt/DMF solution and 2.09 g (4.0 mmol) of PyAOP at room temperature for 24 hours. After the resin was washed with DMF, the N-terminal peptide was

extended by the Fmoc solid-phase synthesis to give Fmoc-Cha-GlyΨ[(E)CH=CH]Leu-Arg(Pbf)-Trp(Boc)-Rink Amide MBHA resin. After deprotection of the N-terminal Fmoc group by 20% piperidine/DMF treatment, the resin was treated in DMF with Ac-D-Tyr-Hyp-Asn-Thr-OH 2.48 g (4.5 mmol), 734 mg (4.5 mmol) of HOOBt and 716 mL (4.5 mmol) of DIPCDI for 60 hours. The resulting Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ[(E)CH=CH]Leu-Arg(Pbf)-Trp(Boc)-Rink Amide MBHA resin was washed in MeOH and dried. To the whole of the resulting resin, 60 mL ofTFA: thioanisole: m-cresol: H_2O: EDT: TIS (80:5:5:5:2.5:2.5) was added. After stirring for 90 minutes at room temperature, the reaction solution was dropwise added onto chilled ether while removing the resin by passing through a glass filter, thereby to make the crude peptide white powders.

[0241] The crude peptide obtained was purified separately five times on preparative HPLC using SHISEIDO CAPCELL PAK MGII column (50 x 250 mm). Linear density gradient elution (60 minutes) to A/B: 69/31-59/41 or 68/32-58/42 was performed at a flow rate of 45 mL/min using eluant A: 0.1 % TFA-water and eluant B: 0.1% TFA-containing acetonitrile on preparative HPLC. The product eluted out was fractionated in test tubes by about 14 mL each. By monitoring each fraction on HPLC, the fractions containing the product only were identified. The fractions were pooled and lyophilized to give white powders.

[0242] The whole was dissolved in acetonitrile-water and 0.824 mL (1.005 mmol equivalent) of AG 1x8 AcO^-resin was added to the solution. While occasionally stirring manually, the solution was settled for an hour, and filtered through a PTFE membrane filter having a 3 μm pore diameter. The filtrate was transferred to a recovery flask and the solvent was distilled off. Then, 2 mL of acetic acid was added to the residue. After the mixture was sonicated for 5 minutes with an ultrasonicator, 8 mL of water was added to the solution. While cooling on a dry ice bath, the resulting 20% acetic acid solution was lyophilized to give 304.7 mg of white powders.

Mass spectrum $(M+H)^+$ 1199.6 (calcd. 1199.7)

Elution time on HPLC: 20.2 mins.

Elution conditions:

Column: SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100mm)

Eluant: Linear density gradient elution with eluants A/B = 95/5-45/55, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (25 mins.)

Flow rate: 1.0 mL/min.

Amino acid analysis (20% hydrochloric acid containing 4% thioglycolic acid, 110°C, hydrolysis for 24 hours; figures in parentheses show theoretical values.): Asp:0.93 (1); Thr 0.89 (1); Tyr 0.96 (1); Cha 1.00 (1); Arg 0.98 (1)

EXAMPLE 3

Production of des(1)-Ac-[D-Tyr2,Hyp3,Alb4,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Trp10]MS10 (Compound No. 927)

[0243] After 3.66 g (0.55 mmol) of Fmoc-Rink Amide MBHA resin was swollen in DMF, the peptide chain was extended by the Fmoc solid-phase synthesis. The N-terminal Fmoc group of Fmoc-D-Tyr(Bu^t)-Hyp(Bu^t)-Alb-Thr(Bu^t)-Cha-GlyΨ[(E)-CH=CH]Leu-Arg(Pbf)-Trp(Boc)-Rink Amide MBHA resin thus obtained was deprotected by 20% piperidine/DMF treatment, and washed. The resin was then suspended in about 10 mL of DMF, and 208 μl (2.2 mmol) of AC_2O and 383 μl (2.2 mmol) of DIEA were added, respectively, followed by stirring for 20 minutes. The resin was washed with DMF. After it was confirmed that the reaction proceeded, the resin was washed in MeOH and dried.

[0244] To 5.9695 g of the resulting resin, 50 mL of TFA: thioanisole: m-cresol: H_2O: EDT: TIS (80:5:5:5:2.5:2.5) was added and the mixture was stirred for 90 minutes at room temperature. While removing the resin by passing through a glass filter, the reaction solution was dropwise added to chilled ether with stirring to make the crude peptide white powders. The resin was thoroughly washed with a deprotection solution and then returned to the reaction solution. The resin was treated again with the same volume of the deprotection solution at room temperature for 20 hours and ether was likewise added dropwise to make the product white powders.

[0245] The mixture of white powders and ether was separated by centrifugation, respectively. The ether was removed by decantation and this procedure was repeated twice to remove the acid and scavenger. The residue was dried and extracted with acetic acid aqueous solution. The extract was passed through a 0.45 μm disk filter to remove fine particles, followed by concentration with an evaporator. The residue was diluted in acetonitrile-aqueous solution and lyophilized to give total 1.646 g of white to brown powders.

[0246] The crude peptide obtained was purified separately six times on preparative HPLC using SHISEIDO CAPCELL PAK MGII column (50 x 250 mm). Linear density gradient elution (60 minutes) to AB: 68/32-58/42 was performed at a flow rate of 45 mL/min using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile on preparative HPLC. The product eluted out was fractionated in test tubes by about 14 mL each. By monitoring each fraction on HPLC, the fractions containing the product only were identified. The fractions were pooled and lyophilized to give 436.9 mg of white powders.

[0247] In AcCN-water, 416.9 mg (343.3 μmol) of the purified sample obtained, and 1.430 mL (1.717 mmol equivalent) of AG 1x8 AcO^- resin was added to the solution. While occasionally stirring manually, the solution was settled for an hour, and filtered through a PTFE membrane filter having a 3 μm pore diameter. The filtrate was transferred to a recovery flask and the solvent was distilled off. Then, 4 mL of acetic acid was added to the residue. After the mixture was sonicated for 5 minutes with an ultrasonicator, 16 mL of water was added to the solution. While cooling on a dry ice bath, the resulting 20% acetic acid solution was lyophilized to give 368.4 mg of white powders.
Mass spectrum $(M+H)^+$ 1214.6 (calcd. 1214.7)
Elution time on HPLC: 20.1 mins.
Elution conditions:

Column: SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100 mm)
Eluant: Linear density gradient elution with eluants A/B = 95/5-45/55, using eluant A: 0.1% TFA-water and eluant B: 0.1% TFA-containing acetonitrile (25 mins.)
Flow rate: 1.0 mL/min.
Amino acid analysis (20% hydrochloric acid containing 4% thioglycolic acid, 110°C, hydrolysis for 24 hours; figures in parentheses show theoretical values.): Thr 0.95 (1); Tyr 0.97 (1); Cha 1.00 (1); Arg 0.99 (1)

[0248] The compounds synthesized in EXAMPLES 1 to 3 are shown in TABLE 2 below, in terms of their structures, physicochemical properties, etc.

[0249] The description "Synthesis" in the table indicates that:

the compounds described in EXAMPLES 1-3 were synthesized by Synthesis G described at the column "Synthesis".

[0250] The description "HPLC condition" in the table indicates that:
the compounds described in EXAMPLES 1-3 can be eluted under the condition by d described at the column "HPLC Conditions".

TABLE 2

Compound number	Structure	M+H+ (obs)	M+H+ (cal)	HPLC (min)	HPLC condition	Synthesis method
903	des(1)-Ac-[D-Tyr2,Hyp3,Abl4,Thr5,Chal,Gly7Ψ((E)CH=CH)Leu8,Arg(Me)9,Trp10]MS10	1228.4	1228.7	20.2	d	G
926	des(1)-Ac-[D-Tyr2,Hyp3,Thr5,Cha6,GlyΨ((E)CH=CH)Leu8,Trp10]MS10	1199.6	1199.7	202	d	G
927	des(1)-Ac-[D-Tyr2,Hyp3,Alb4,Thr5,Cha6,Gly7Ψ((E)CH=CH)Leu8,Trp10]MS10	1214.6	1214.7	20.1	d	G
	d:5-55% AcCN/25min flow 1ml/min					
	SHISEIDO CAPCELL PAK C18 MGII (4.6 x 100mm)					

TEST EXAMPLE 1

Measurement of Agonist Activity by Assaying Changes in Intracellular Calcium Ion Level

1) Preparation of human metastin and rat metastin stable expression cell lines

[0251] Human metastin and rat metastin stable expression cell lines were obtained by transfecting the expression plasmid for animal cells into CHO/dhfr$^-$ cells using CellPhect Transfection Kit (manufactured by GE Healthcare). First, 240 μL of Buffer A (attached to CellPhect Transfection Kit) was added to 9.6 μg of plasmid DNA dissolved in 240 μL of distilled water followed by stirring. After the mixture was settled for 10 minutes, 480 μL of Buffer B (attached to CellPhect Transfection Kit) was added to the mixture, which was vigorously stirred to form liposomes containing the DNA. Then, 4 x 10^5 CHO/dhfr$^-$ cells (obtained from ATCC) were inoculated on a 60 mm Petri dish. After culturing the cells in Ham's F-12 medium (manufactured by Nissui Seiyaku Co., Ltd.) supplemented with 10% fetal bovine serum (manufactured by BIO WHITTAKER, Inc.) at 37°C for 2 days in 5% carbon dioxide gas, 480 mL of the liposomes were dropwise added to the cells on the Petri dish. After culturing the cells in a CO_2 incubator (5% CO_2, 37°C) for 6 hours, the cells were washed twice with serum-free Ham's F-12 medium and 3 mL of 15% glycerol was added to the cells on the Petri dish followed by treatment for 2 minutes. The cells were again washed twice with serum-free Ham's F-12 medium followed by incubation in Ham's F-12 medium supplemented with 10% fetal bovine serum in a CO_2 incubator (5% CO_2, 37°C) for 15 hours. The cells were dispersed by trypsin treatment to recover from the Petri dish. The recovered cells were inoculated on a 6-well plate in 1.25 x 10^4 cells each/well and incubation was initiated in a CO_2 incubator (5% CO_2, 37°C) in Dulbecco's modified Eagle medium (DMEM, manufactured by Nissui Seiyaku) containing 10% dialyzed fetal bovine serum (manufactured by JRH BIOSCIENCES, Inc.). The plasmid-transfected CHO transformants grew in the medium but the non-transfected cells gradually died, accordingly the medium was exchanged on Days 1 and 2 after the initiation of incubation to remove the dead cells. Approximately 20 colonies of the CHO transformants that kept growing on Days 8 to 10 after the incubation were isolated. From the cells in these colonies, cells showing high reactivity with the ligand peptide metastin (hereinafter merely referred to as h175KB19 and h175KB29 strains) were selected to provide for the following experiment.

2) Cell seeding

[0252] Human metastin expression CHO cell line (h175KB19 strain is described in a separate section) and rat metastin expression CHO cell line (h175KB29 strain is described in a separate section) were seeded in a 96-well plate (type 3904, manufactured by Corning) at 3 x 10^4 cells/well, followed by incubation in a CO_2 incubator (5% CO_2, 37°C) for 24 hours. For the medium, MEMα medium (nucleic acid-free, manufactured by Nikken Bio Medical Laboratory) supplemented with dialyzed 10% fetal bovine serum (manufactured by Thermo, MultiSer) was used.

3) Loading with Fluo-4 NW in cells

[0253] As an assay buffer, 1 x Hanks' balanced salt solution (HBSS, manufactured by GIBCO) supplemented with 0.1% BSA, 20mM HEPES (pH 7.4, manufactured by GIBCO) and 1mM Probenecid (manufactured by Molecular Probes) was prepared. The medium in the wells of the cell-seeded plate was removed and 100 μL each of the assay buffer (kept warm at 37°C) was added to each well. After 10 mL of the assay buffer (the volume for assaying two plates) was charged in one Fluo-4NW dye mix (Component A, Fluo-4 NW calcium assay kit (starter pack), manufactured by Molecular Probes) bottle, the mixture was gently stirred to prepare the Fluo-4 NW loading solution. This loading solution was charged in each well by 50 μl each and reacted in a CO_2 incubator (5% CO_2, 37°C) for 30 minutes for loading with Fluo-4 NW into the cells. Subsequently, the cells were settled at room temperature (25°C) for 15 minutes and then used for assay.

4) Measurement of agonist activity

[0254] To monitor the agonist activity of a test compound, the test compound diluted in the assay buffer described above was dispensed into a 96-well plate (type 3363, manufactured by Corning) by 80 μl each/well to prepare the compound plate. Fluo-4 NW-loaded cell plate and compound plate were set on a Fluorometric Imaging Plate Reader (FLIPR, manufactured by Molecular Devices) and 50 μl each was dispensed in each well through an automated pipettor in the FLIPR. The agonist response when stimulated with the compound was monitored via CCD camera in the FLIPR in terms of changes in intracellular calcium ion levels (changes in fluorescence of Fluo-4 NW).

[0255] Human Metastin (45-54)* specific agonist activity refers to the value obtained by subtracting the fluorescence change in the control group without any additive from the fluorescence change induced by Metastin (45-54). The specific agonist activity of a test compound refers to the value obtained by subtracting the fluorescence change observed in the

control group in the absence of any test compound from the fluorescence change observed when added with the test compound. The compound level showing the 50% agonist response activity (EC_{50} value) was calculated from the dose-response curve. When the maximum response of human Metastin (45-54) specific agonist activity was made 100%, the EC_{50} was calculated on a test compound showing 70% or more of the maximum activity as compared to the maximum response.

[0256] * Peptide used and synthesized from the 45th to 54th in the amino acid sequence for human metastin [human Metastin (45-54)] is the synthetic product from Peptide Institute, Inc.

[0257] The agonist activity of each test compound [expressed by the specific activity of the EC_{50} of a test compound based on the EC_{50} of Metastin (45-54)] is shown in TABLE 3. The data reveal that the compounds of the present invention have excellent agonist activities on the metastin receptors.

TABLE 3

Compound No.	Specific Activity
903	0.4
926	0.6
927	0.5

TEST EXAMPLE 2

Evaluation of Blood Testosterone Level Reducing Effect of Metastin Peptide Derivatives Using Mature Male Rats

[0258] In the metastin peptide derivatives listed in TABLES 1 and 2, the blood testosterone level reducing effects were evaluated on the compounds.

[0259] A metastin peptide derivative (hereinafter referred to as peptide) was dissolved in 50% DMSO aqueous solution (DMSO: Sigma-Aldrich, distilled water for injection: Otsuka Pharmaceutical) to prepare a peptide solution with the concentration of 0.1, 0.03 or 0.01 mM. This peptide solution was filled in five ALZET osmotic pumps (Model 2001, 0.2 mL in volume, release rate: 0.001 mL/hr, DURECT Corporation). The ALZET pump filled with the peptide solution was implanted subcutaneously in 5 CD(SD)IGS male rats of 9 weeks old after birth (Charles River Japan, Inc.) on the back under ethereal anesthesia by one pump/animal. For negative control, 50% DMSO aqueous solution was filled in 5 ALZET osmotic pumps, which were similarly implanted in 5 male CD(SD)IGS rats (Charles River Japan, Inc.), respectively. These rats were fed for 6 days under normal feeding conditions. After weighing, the animal was decapitated to collect blood. After 0.03 mL/mL blood of aprotinin (Trasylol, Bayer) solution containing 0.1 g/mL EDTA.2Na was added to blood, the plasma was separated and recovered by centrifugation at 1,800 x g for 30 minutes. From the plasma obtained, 0.05 mL was applied to radioimmunoassay (DPC.Total Testosterone Kit, Diagnostic Products Corporation) to measure the plasma testosterone level in each rat. The peptides are listed in TABLE 4, when the number of rats showing the testosterone level below the measurement limit (0.04 ng/mL of plasma level) in radioimmunoassay was 3 or more in the 5 rats receiving the peptides.

TABLE 4

Compound No.
903
926
927

INDUSTRIAL APPLICABILITY

[0260] According to the present invention, there are provided stable metastin derivatives having excellent biological activities (a cancer metastasis suppressing activity, a cancer growth suppressing activity, a gonadotropic hormone secretion stimulating activity, sex hormone secretion stimulating activity, a gonadotropic hormone secretion suppressing activity, sex hormone secretion suppressing activity, etc.

SEQUENCE LISTING

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Claims

1. A compound selected from:

   Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2,
   Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2, and
   Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2,
   or a salt thereof,
   wherein Alb means Albizziin 2-amino-3-ureidopropionic acid and
   GlyΨ((E)CH=CH)Leu means that the -CONH- between Gly and Leu is substituted with (E) type alkene.

2. The compound according to claim 1, which is
   Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 or a salt thereof.

3. The compound according to claim 1, which is
   Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2
   or a salt thereof.

4. The compound according to claim 1, which is
   Ac-D-Tyr-Hyp-Alb-Thr-Cha-Gly¥((E)CH=CH)Leu-Arg-Trp-NH_2
   or a salt thereof.

5. A medicament comprising the compound according to claim 1, or a salt thereof.

6. The compound according to claim 1, or a salt thereof, for use in therapy.

7. The compound according to claim 1, or a salt thereof, for use as an agent for suppressing cancer metastasis or as an agent for suppressing cancer growth.

8. The compound according to claim 1, or a salt thereof, for use as an agent for preventing/treating cancer.

9. The compound according to claim 1, or a salt thereof, for use as an agent for down-regulating gonadotropic hormone or sex hormone.

10. The compound according to claim 1, or a salt thereof, for use as an agent for preventing/treating hormone-dependent cancer.

Patentansprüche

1. Verbindung, ausgewählt aus:

   Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH2,
   Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH2 und
   Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH2,
   oder ein Salz davon,
   wobei Alb für Albizziin = 2-Amino-3-ureidopropionsäure steht und GlyΨ((E)CH=CH)Leu bedeutet, dass das

-CONH- zwischen Gly und Leu mit einem Alken des (E)-Typs substituiert ist.

2. Verbindung gemäß Anspruch 1, bei der es sich um Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 oder ein Salz davon handelt.

3. Verbindung gemäß Anspruch 1, bei der es sich um Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 oder ein Salz davon handelt.

4. Verbindung gemäß Anspruch 1, bei der es sich um Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 oder ein Salz davon handelt.

5. Medikament, das die Verbindung gemäß Anspruch 1 oder ein Salz davon umfasst.

6. Verbindung gemäß Anspruch 1 oder ein Salz davon zur Verwendung in der Therapie.

7. Verbindung gemäß Anspruch 1 oder ein Salz davon zur Verwendung als Mittel zur Unterdrückung von Krebsmetastasen oder als Mittel zur Unterdrückung von Krebswachstum.

8. Verbindung gemäß Anspruch 1 oder ein Salz davon zur Verwendung als Mittel zur Prävention/Behandlung von Krebs.

9. Verbindung gemäß Anspruch 1 oder ein Salz davon zur Verwendung als Mittel zur Herunterregulierung eines gonadotropen Hormons oder Sexualhormons.

10. Verbindung gemäß Anspruch 1 oder ein Salz davon zur Verwendung als Mittel zur Prävention/Behandlung von hormonabhängigem Krebs.

Revendications

1. Composé choisi parmi les suivants :

   - Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2
   - Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2
   - et Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 ou sel d'un tel composé,

   étant entendu que
   - Alb désigne l'albizziine, autrement dit l'acide 2-amino-3-uréido-propionique,
   - et que GlyΨ((E)CH=CH)Leu signifie que le raccord -CONH- entre Gly et Leu est remplacé par un raccord alcénique de type E.

2. Composé conforme à la revendication 1, qui est Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 ou un sel de ce composé.

3. Composé conforme à la revendication 1, qui est Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 ou un sel de ce composé.

4. Composé conforme à la revendication 1, qui est Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 ou un sel de ce composé.

5. Médicament comprenant un composé conforme à la revendication 1 ou un sel d'un tel composé.

6. Composé conforme à la revendication 1, ou sel d'un tel composé, pour utilisation thérapeutique.

7. Composé conforme à la revendication 1, ou sel d'un tel composé, pour utilisation en tant qu'agent de suppression de métastases cancéreuses ou en tant qu'agent d'inhibition de la prolifération cancéreuse.

8. Composé conforme à la revendication 1, ou sel d'un tel composé, pour utilisation en tant qu'agent de prévention/traitement d'un cancer.

9. Composé conforme à la revendication 1, ou sel d'un tel composé, pour utilisation en tant qu'agent de régulation à la baisse d'une hormone gonadotrope ou d'une hormone sexuelle.

10. Composé conforme à la revendication 1, ou sel d'un tel composé, pour utilisation en tant qu'agent de prévention/traitement d'un cancer hormono-dépendant.

REFERENCES CITED IN THE DESCRIPTION

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Szabadalmi igénypontok

1. Vegyület a következők közül választva:

Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2,

Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2, és

Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2,

vagy sója,

ahol Alb jelentése Albizziin-2-amino-3-ureidopropionsav és

GlyΨ((E)CH=CH)Leu jelentése az, hogy a Gly és Leu közötti -CONH- (E)-típusú alkénnel szubszituált.

2. Az 1. igénypont szerinti vegyület, amely a Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg(Me)-Trp-NH_2 vagy sója.

3. Az 1. igénypont szerinti vegyület, amely a Ac-D-Tyr-Hyp-Asn-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 vagy sója.

4. Az 1. igénypont szerinti vegyület, amely a Ac-D-Tyr-Hyp-Alb-Thr-Cha-GlyΨ((E)CH=CH)Leu-Arg-Trp-NH_2 vagy sója.

5. Gyógyszer, amely tartalmazza az 1. igénypont szerinti vegyületet vagy sóját.

6. Az 1. igénypont szerinti vegyület vagy sója terápiában történő alkalmazásra.

7. Az 1. igénypont szerinti vegyület vagy sója olyan szerként való alkalmazása, amely gátolja a rák metasztázist vagy gátolja a rák növekedését.

8. Az 1. igénypont szerinti vegyület vagy sója rák megelőzésére/kezelésére használható szerként történő alkalmazásra.

9. Az 1. igénypont szerinti vegyület vagy sója gonadotrop hormon vagy szexhormon alulszabályzó szerként történő alkalmazásra.

10. Az 1. igénypont szerinti vegyület vagy sója hormonfüggő rák megelőzésére/kezelésére szolgáló szerként történő alkalmazásra.