

SPECIFICATION

RET INHIBITOR

TECHNICAL FIELD

[0001]

The present invention relates to a RET inhibitor, an inhibitor of RET tyrosine kinase, a prophylactic or therapeutic agent for diseases including cancers with a mutation in RET and their metastasis, a method for identifying a target patient, and the like, each of which comprises a tetracyclic compound or a salt thereof or a solvate thereof.

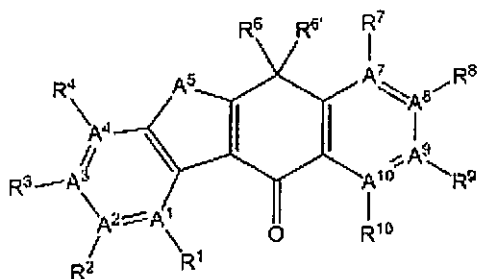
BACKGROUND ART

[0002]

Rearranged during transfection (RET) is a member of the receptor tyrosine kinases belonging to the cadherin superfamily (Surgery, 2007, vol. 141, p. 96-99). RET tyrosine kinase has a transmembrane region in the middle and has a tyrosine kinase region at the carboxyl-terminal side and an extracellular region at the amino-terminal side. It is known that there are three types of proteins due to differences in carboxyl-terminal splicing (TRENDS in Genetics, 2006, vol. 22, p. 627-636: Reference a). RET forms a dimer via a ligand/GFR complex to thereby phosphorylate and activate its own tyrosine (Reference a).

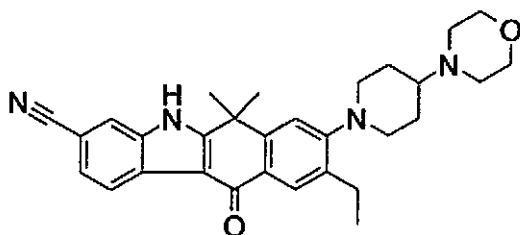
There are reports showing that RET will be involved in oncogenesis upon alterations (point mutation, chromosomal translocation, chromosomal inversion, gene amplification) in RET gene. For example, in thyroid medullary cancer, it is reported that a point mutation in RET gene results in the expression of RET tyrosine kinase with oncogenic ability (Reference a). Moreover, in thyroid papillary cancer, it is reported that RET gene is fused with another gene (e.g., coiled-coil domain containing 6 (CCDC6) gene or nuclear receptor coactivator 4 (NCOA4) gene) by chromosomal inversion or chromosomal translocation to cause the expression of fused tyrosine kinase RET/PTC with oncogenic ability (European Journal of Endocrinology, 2006, vol. 155, p. 645-653). Further, in non-small cell lung cancer,

On the other hand, a tetracyclic compound having the following general formula is reported as an inhibitor of anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase belonging to the insulin receptor family (Patent Document 1: WO2010/143664, Patent Document 2: WO2012/023597, Patent Document 3: Japanese Patent Laid-Open No. 2012-126711). This compound is useful as a therapeutic and/or prophylactic agent for tumors with a mutation in ALK gene.



(see the above patent gazette for details of substituents, etc.)

Moreover, it is reported that the following compound with a high concentration (1,000 nM) inhibits many kinases including RET in the Ambit Kinase Screening test (Non-Patent Document 3: Cancer Cell, 19 (5), p. 679-690, 2011, Supplemental Information):



However, there is no report showing that the tetracyclic compound found in Patent Document 1 and Non-patent Document 3 is useful as a therapeutic or prophylactic agent for cancers with a mutation in RET.

Moreover, it is reported that the ALK inhibitor crizotinib has no cell growth inhibitory activity against KIF5B-RET-expressing cells (Non-Patent Document 4: Nature Medicine. 2012, 18, p. 378-381).

[Document List]

[Patent Document]

[0003]

[Patent Document 1] WO2010/143664

[Patent Document 2] WO2012/023597

[Patent Document 3] JP2012-126711A

[Non-Patent Document]

[0004]

[Non-Patent Document 1] J Clin Oncol 30, 2012, suppl; Abstract no: 7510

[Non-Patent Document 2] Cancer Discov, 3 (6), Jun 2013, p. 630-5

[Non-Patent Document 3] Cancer Cell, 19 (5), p. 679-690, 2011, Supplemental Information

[Non-Patent Document 4] Nature Medicine. 2012, 18, p. 378-381

SUMMARY OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

[0005]

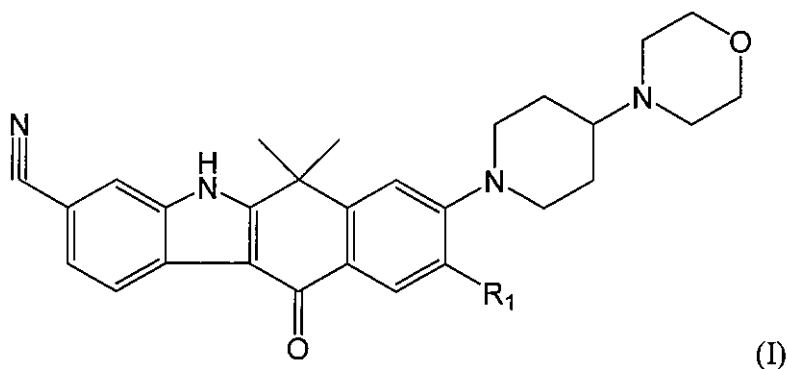
Cancers caused by a mutation in ALK gene and cancers caused by a mutation in RET gene differ in their mechanism of cancer development, the three-dimensional protein structure of their respective kinases, etc., and hence a specific therapeutic and/or prophylactic method is required for each cancer. In lung cancer, it is reported that a group of patients with a mutation in ALK gene does not overlap with a group of patients with a mutation in RET (Nat Med, 2012 Feb 12, 18 (3), 375-7). These patient groups are clearly distinguished from each other for treatment, and the patients in each group require a specific treatment and/or prevention method.

On the other hand, a compound which inhibits multiple kinases at the same time is known to show a lower therapeutic effect in some cases, because its effective therapeutic range is narrow. Thus, a drug which selectively inhibits a small number of kinases can be regarded as having desired properties in terms of therapeutic effect, and hence there is a demand for such a drug.

MEANS TO SOLVE THE PROBLEM

[0006]

As a result of extensive and intensive efforts made to solve the above problem, the inventors of the present invention have found, ahead of others, that a tetracyclic compound represented by the following formula (I) or a salt thereof or a solvate thereof has not only inhibitory activity against ALK but also potent inhibitory activity against RET, selectively inhibits RET, is useful for treatment and prevention of diseases including cancers with a mutation in RET and their metastasis, and also has high therapeutic efficacy on these diseases. This finding led to the completion of the present invention.



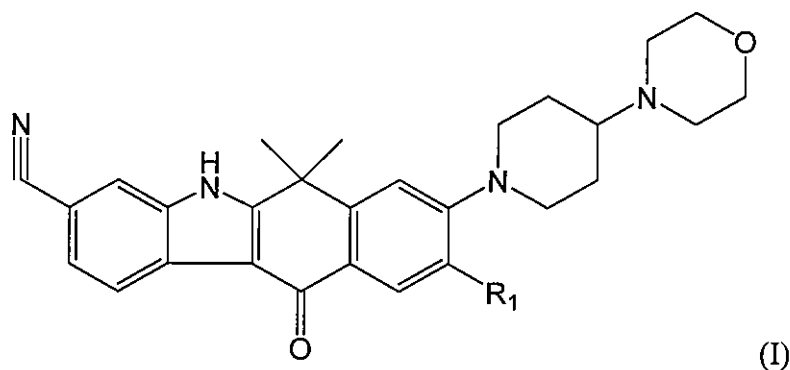
wherein R_1 is a C_{1-6} alkyl group.

Namely, according to one aspect of the present invention, the present invention is directed to a therapeutic or prophylactic agent for cancers with a mutation in RET and their metastasis, which comprises a tetracyclic compound shown blow or a salt thereof, etc. According to another aspect, the present invention provides a method for identifying a cancer or a target patient responsive to treatment with the above compound or the like.

[0007]

More specifically, the present invention is as follows.

[1] A therapeutic and/or prophylactic agent for a tumor with a mutation in RET or for metastasis of the tumor, which comprises a compound represented by formula (I), a salt thereof or a solvate thereof as an active ingredient:



wherein R_1 is a C_{1-6} alkyl group.

[2] The therapeutic and/or prophylactic agent according to [1] above, wherein the tumor is selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, brain tumor, neuroblastoma, glioma, thyroid cancer, myelodysplastic syndrome, head and neck cancer, esophageal

cancer, gastric cancer, colorectal cancer, , breast cancer, ovarian cancer, lung cancer, pancreatic cancer, liver cancer, gallbladder cancer, skin cancer, malignant melanoma, kidney cancer, renal pelvic and ureteral cancer, bladder cancer, uterine cancer, testicular cancer, prostate cancer, and tumors metastasized from these tumors.

[3] The therapeutic and/or prophylactic agent according to [1] or [2] above, wherein the tumor is thyroid cancer or lung cancer.

[4] The therapeutic and/or prophylactic agent according to any one of [1] to [3] above, wherein the tumor is thyroid medullary cancer or non-small cell lung cancer.

[4-1] The therapeutic and/or prophylactic agent according to [1] to [4] above, wherein the tumor is a tumor confirmed to show activated RET tyrosine kinase in the tumor tissue.

[4-2] The therapeutic and/or prophylactic agent according to [1] to [4] above, wherein the tumor is a tumor with a mutation which induces activation of RET tyrosine kinase.

[4-2-1] The therapeutic and/or prophylactic agent according to any one of [1] to [4-2] above, wherein the tumor is a tumor with (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) a fusion gene of RET and/or a fusion protein of RET.

[4-2-2] The therapeutic and/or prophylactic agent according to any one of [1] to [4-2] above, wherein the tumor is a tumor with a fusion gene of RET and/or a fusion protein of RET.

[4-2-3] The therapeutic and/or prophylactic agent according to any one of [1] to [4-2] and [4-2-2] above, wherein the tumor is a tumor with KIF5B-RET, CCDC6-RET, NCOA4-RET or TRIM33-RET.

[4-3] The therapeutic and/or prophylactic agent according to [1] to [4-2] above, wherein the tumor is a tumor with (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[0008]

[5] The therapeutic and/or prophylactic agent according to any one of [1] to [4-2] above, wherein the tumor is a tumor with a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[5-1] The therapeutic and/or prophylactic agent according to [5] above, wherein the another gene and protein are the gene and protein of KIF5B, CCDC6 or NCOA4 or TRIM33.

[5-2] The therapeutic and/or prophylactic agent according to [5] or [5-1] above, wherein the fusion gene and protein comprises the tyrosine kinase domain of RET gene or protein and the coiled-coil domain of another gene or protein.

[5-2-1] The therapeutic and/or prophylactic agent according to any one of [5] to [5-2] above, wherein a polypeptide constituting the RET protein and a polynucleotide constituting the RET gene are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4;
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the polypeptide shown in SEQ ID NO: 3 or 4; and
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 3 or 4.

[5-2-2] The therapeutic and/or prophylactic agent according to [5-1] or [5-2-1] above, wherein a polypeptide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 proteins and a polynucleotide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 genes are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

(1) the polypeptide constituting the KIF5B protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 30,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 30, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 30;

(2) the polypeptide constituting the CCDC6 protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 31,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 31, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 31;

(3) the polypeptide constituting the NCOA4 protein is

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42; and

(4) the polypeptide constituting the TRIM33 protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 45 or 46,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 45 or 46, or
- (c) a polypeptide consisting of amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 45 or 46.

[5-2-3]The therapeutic and/or prophylactic agent according to [4-2-2], [5] or [5-2] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (b) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24, or a polypeptide comprising an amino acid

sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(c) a polypeptide comprising an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;

(e) a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27 or 28, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28; and

(f) a polypeptide comprising an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28.

[5-2-4]The therapeutic and/or prophylactic agent according to [4-2-2], [5] or [5-2] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

(a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(b) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which carries activated tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;

(c) a polypeptide which comprises an amino acid sequence with 90% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;

(d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;

(e) a polypeptide which comprises the amino acid sequence shown in SEQ ID NO: 27 or 28 and which carries activated RET tyrosine kinase, or a polypeptide

which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity; and

(f) a polypeptide which comprises an amino acid sequence with 90% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity.

[5-3] The therapeutic and/or prophylactic agent according to [5-2] above, wherein the fusion gene is any of (a) to (d) shown below:

(a) a fusion gene which comprises a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14;

(b) a fusion gene consisting of a polynucleotide which hybridizes under stringent conditions to DNA consisting of a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14 and which encodes a polypeptide having tyrosine kinase activity;

(c) a fusion gene which comprises a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24; or

(d) a fusion gene comprising a polynucleotide encoding a polypeptide which has substitution, deletion or insertion of one or more (e.g., several tens, 1 to 10, 1 to 5, 1 to 3) amino acids in a polypeptide having an amino acids sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity.

[0009]

[6] The therapeutic and/or prophylactic agent according to any one of [1] to [4-2] above, wherein the tumor is a tumor with a point mutation in RET gene and/or protein.

[6-1] The therapeutic and/or prophylactic agent according to [6] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A, 2600G, 2861T or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-1-1] The therapeutic and/or prophylactic agent according to [6] or [6-1] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G,

2562A or 2861T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-2] The therapeutic and/or prophylactic agent according to [6] or [6-1] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2494G, 2600G or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-3] The therapeutic and/or prophylactic agent according to [6] or [6-1] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-3-1] The therapeutic and/or prophylactic agent according to [6], [6-1] or [6-3] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T or 2861T>G in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-4] The therapeutic and/or prophylactic agent according to [6], [6-1], [6-2] or [6-3] above, wherein the point mutation is 2091G>T, 2494G>C, 2600G>A or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[6-5] The therapeutic and/or prophylactic agent according to [6] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[6-5-1] The therapeutic and/or prophylactic agent according to [6] or [6-5] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, S891 or A883 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[6-6] The therapeutic and/or prophylactic agent according to [6] or [6-5] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, E768, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[6-7] The therapeutic and/or prophylactic agent according to [6] or [6-5] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F, V804M, V804L, S891A or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[6-7-1]The therapeutic and/or prophylactic agent according to [6], [6-5] or [6-7] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F or S891A in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[6-8] The therapeutic and/or prophylactic agent according to [6] or [6-6] above, wherein the point mutation is C634W, C634Y, E768D, V804M or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[0010]

[7] A therapeutic and/or prophylactic agent for a tumor used for a patient with a mutation in RET or for metastasis of the tumor, which comprises a compound represented by formula (I), a salt thereof or a solvate thereof as an active ingredient.

[8] The therapeutic and/or prophylactic agent according to [7] above, wherein the tumor is thyroid cancer or lung cancer.

[8-1] The therapeutic and/or prophylactic agent according to [7] or [8] above, wherein the patient is a patient confirmed to show activated RET tyrosine kinase in the tumor tissue.

[8-2] The therapeutic and/or prophylactic agent according to [7] or [8] above, wherein the patient is a patient with a mutation which induces activation of RET tyrosine kinase.

[8-2-1]The therapeutic and/or prophylactic agent according to any one of [7] to [8-2] above, wherein the patient is a patient with (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) a fusion gene of RET and/or a fusion protein of RET.

[8-2-2]The therapeutic and/or prophylactic agent according to any one of [7] to [8-2-1] above, wherein the patient is a patient with a fusion gene of RET and/or a fusion protein of RET.

[8-2-3]The therapeutic and/or prophylactic agent according to any one of [7] to [8-2] and [8-2-2] above, wherein the patient is a patient with KIF5B-RET, CCDC6-RET, NCOA4-RET or TRIM33-RET.

[8-3] The therapeutic and/or prophylactic agent according to any one of [7] to [8-2] above, wherein the patient is a patient with (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine

kinase, or (c) a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[9] The therapeutic and/or prophylactic agent according to [7] or [8] above, wherein the patient is a patient with a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[9-1] The therapeutic and/or prophylactic agent according to [8-3] or [9] above, wherein the another gene and protein are KIF5B, CCDC6, NCOA4 or TRIM33.

[9-2] The therapeutic and/or prophylactic agent according to [9] or [9-1] above, wherein the fusion gene and fusion protein comprises the tyrosine kinase domain of RET gene or protein and the coiled-coil domain of another gene or protein.

[9-2-1] The therapeutic and/or prophylactic agent according to any one of [9] to [9-2] above, wherein a polypeptide constituting the RET protein and a polynucleotide constituting the RET gene are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4;
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the polypeptide shown in SEQ ID NO: 3 or 4; and
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 3 or 4.

[9-2-2] The therapeutic and/or prophylactic agent according to [9-1] above, wherein a polypeptide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 proteins and a polynucleotide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 genes are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

(1) the polypeptide constituting the KIF5B protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 30,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 30, or

- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 30;
 - (2) the polypeptide constituting the CCDC6 protein is
 - (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 31,
 - (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 31, or
 - (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 31;
 - (3) the polypeptide constituting the NCOA4 protein is
 - (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42,
 - (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42, or
 - (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42; and
 - (4) the polypeptide constituting the TRIM33 protein is
 - (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 45 or 46,
 - (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 45 or 46, or
 - (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 45 or 46.
- [9-2-3]The therapeutic and/or prophylactic agent according to [8-2-2] or [9] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:
- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

- (b) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (c) a polypeptide comprising an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;
- (e) a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27 or 28, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28; and
- (f) a polypeptide comprising an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28.

[9-2-4]The therapeutic and/or prophylactic agent according to [8-2-2] or [9] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (b) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which carries activated tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;
- (c) a polypeptide which comprises an amino acid sequence with 90% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;
- (d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;

- (e) a polypeptide which comprises the amino acid sequence shown in SEQ ID NO: 27 or 28 and which carries activated RET tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity; and
- (f) a polypeptide which comprises an amino acid sequence with 90% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity.

[9-3] The therapeutic and/or prophylactic agent according to [9] or [9-2] above, wherein the fusion gene is any of (a) to (d) shown below:

- (a) a fusion gene which comprises a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14;
- (b) a fusion gene consisting of a polynucleotide which hybridizes under stringent conditions to DNA consisting of a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14 and which encodes a polypeptide having tyrosine kinase activity;
- (c) a fusion gene which comprises a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24; or
- (d) a fusion gene comprising a polynucleotide encoding a polypeptide which has substitution, deletion or insertion of one or more (e.g., several tens, 1 to 10, 1 to 5, 1 to 3) amino acids in a polypeptide having an amino acids sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity.

[0011]

[10] The therapeutic and/or prophylactic agent according to [7] or [8] above, wherein the patient is a patient with a point mutation in RET gene and/or protein.

[10-1] The therapeutic and/or prophylactic agent according to [10] above, wherein the point mutation in RET is a point mutation in the cysteine-rich domain or in the tyrosine kinase domain of RET tyrosine kinase.

[10-2] The therapeutic and/or prophylactic agent according to [10] or [10-1] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G,

2562A, 2600G, 2861T or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-2-1] The therapeutic and/or prophylactic agent according to any one of [10] to [10-2] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A or 2861T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-3] The therapeutic and/or prophylactic agent according to any one of [10] to [10-2], wherein the point mutation in RET is a mutation in the nucleotide 2091G, 2494G, 2600G or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-4] The therapeutic and/or prophylactic agent according to any one of [10] to [10-2] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-4-1] The therapeutic and/or prophylactic agent according to any one of [10] to [10-2] and [10-4] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T or 2861T>G in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-5] The therapeutic and/or prophylactic agent according to any one of [10] to [10-2], [10-4] above, wherein the point mutation in RET is 2091G>T, 2494G>C, 2600G>A or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[10-6] The therapeutic and/or prophylactic agent according to [10] or [10-1] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-6-1] The therapeutic and/or prophylactic agent according to [10], [10-1] or [10-6] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, S891 or A883 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-7] The therapeutic and/or prophylactic agent according to [10], [10-1] or [10-6] above, wherein the point mutation in RET is a mutation in the amino acid C609,

C611, C618, C620, C630, C634, E768, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-8] The therapeutic and/or prophylactic agent according to [10], [10-1] or [10-6] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F, V804M, V804L, S891A or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-8-1] The therapeutic and/or prophylactic agent according to [10], [10-1], [10-6] or [10-8] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F or S891A in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-9] The therapeutic and/or prophylactic agent according to any one of [10], [10-1] or [10-6] to [10-8] above, wherein the point mutation in RET is C634Y, E768D, V804M or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[10-10] The therapeutic and/or prophylactic agent according to any one of [10] to [10-9] above, wherein the patient is a patient detected for the presence of a mutation in RET by Sanger sequencing or FISH method.

[0012]

[11] The therapeutic and/or prophylactic agent according to any one of [1] to [10-10] above, wherein R¹ is ethyl.

[11-1] The therapeutic and/or prophylactic agent according to any one of [1] to [11] above, wherein the compound is a hydrochloride.

[11-2] The therapeutic and/or prophylactic agent according to any one of [1] to [11-1] above, wherein the therapeutic and/or prophylactic agent selectively inhibits RET.

[12] A RET inhibitor, which comprises a compound of formula (I), a salt thereof or a solvate thereof as an active ingredient.

[12-1] Use of a compound of formula (I), a salt thereof or a solvate thereof for inhibition of RET.

[12-2] A method for preventing and/or treating a tumor with a mutation in RET and metastasis of the tumor, which comprises administering a patient with an effective therapeutic amount of a compound represented by formula (I), a salt thereof or a solvate thereof.

[12-3] Use of a compound represented by formula (I), a salt thereof or a solvate thereof for prevention and/or treatment of a tumor with a mutation in RET and metastasis of the tumor.

[12-3-1] The use according to [12-3] above, wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene of RET and/or a fusion protein of RET.

[12-3-2] The use according to [12-3] or [12-3-1] above, wherein the mutation in RET results in the formation of a fusion gene of RET and/or a fusion protein of RET.

[12-3-3] The use according to any one of [12] to [12-2-2] above, wherein the mutation in RET results in the formation of KIF5B-RET, CCDC6-RET, NCOA4-RET or TRIM33-RET.

[12-3-4] The use according to any one of [12-3] to [12-3-3] above, wherein the compound of formula (I), the salt thereof or the solvate thereof selectively inhibits RET.

[12-3-5] The use according to any one of [12-3] to [12-3-4] above, wherein R¹ is ethyl.

[12-3-6] The use according to any one of [12-3] to [12-3-5] above, wherein the compound is a hydrochloride.

[12-3-7] The use according to any one of [12-3] to [12-3-6] above, wherein the tumor is thyroid cancer or lung cancer.

[0013]

[13] A method for identifying a subject to be administered with a compound represented by formula (I), a salt thereof or a solvate thereof, which comprises the step of detecting a mutation in RET in a tissue from the subject.

[13-1] The method according to [13] above, wherein the tissue is a tissue confirmed to show activated RET tyrosine kinase.

[13-2] The method according to [13] above, wherein the tissue has a mutation which induces activation of RET tyrosine kinase.

[13-2-1] The method according to any one of [13] to [13-2] above, wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine

kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene of RET and/or a fusion protein of RET.

[13-2-2] The method according to any one of [13] to [13-2-1] above, wherein the mutation in RET results in the formation of a fusion gene of RET and/or a fusion protein of RET.

[13-2-3] The method according to any one of [13] to [13-2-2] above, wherein the mutation in RET results in the formation of KIF5B-RET, CCDC6-RET, NCOA4-RET or TRIM33-RET.

[13-3] The method according to any one of [13] to [13-2] above, wherein the tissue has (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[13-4] The method according to any one of [13] to [13-3] above, wherein the mutation in RET results in the formation of a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[13-5] The method according to [13-4] above, wherein another gene and protein are KIF5B, CCDC6, NCOA4 or TRIM33.

[13-6] The method according to [13-5] above, wherein the fusion gene and fusion protein comprise the tyrosine kinase domain of RET gene or protein and the coiled-coil domain of another gene or protein.

[13-6-1] The method according to any one of [13-4] to [13-6] above, wherein a polypeptide constituting the RET protein and a polynucleotide constituting the RET gene are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4;
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the polypeptide shown in SEQ ID NO: 3 or 4; and
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 3 or 4.

[13-6-2] The method according to [13-5] above, wherein a polypeptide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 proteins and a polynucleotide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 genes are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

(1) the polypeptide constituting the KIF5B protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 30,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 30, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 30;

(2) the polypeptide constituting the CCDC6 protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 31,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 31, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 31;

(3) the polypeptide constituting the NCOA4 protein is

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42,
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42, or
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42; and

(4) the polypeptide constituting the TRIM33 protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 45 or 46,

(b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 45 or 46, or

(c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 45 or 46.

[13-6-3] The method according to [13-2-1], [13-2-2], [13-3] or [13-4] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

(a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(b) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(c) a polypeptide comprising an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;

(e) a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27 or 28, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28; and

(f) a polypeptide comprising an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28.

[13-6-4] The method according to [13-2-1], [13-2-2], [13-3], [13-4] or [13-6-3] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

(a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

- (b) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which carries activated tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;
- (c) a polypeptide which comprises an amino acid sequence with 90% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;
- (d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;
- (e) a polypeptide which comprises the amino acid sequence shown in SEQ ID NO: 27 or 28 and which carries activated RET tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity; and
- (f) a polypeptide which comprises an amino acid sequence with 90% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity.

[13-7] The method according to [13-6] above, wherein the fusion gene is any of (a) to (d) shown below:

- (a) a fusion gene which comprises a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14;
- (b) a fusion gene consisting of a polynucleotide which hybridizes under stringent conditions to DNA consisting of a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14 and which encodes a polypeptide having tyrosine kinase activity;
- (c) a fusion gene which comprises a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24; or
- (d) a fusion gene comprising a polynucleotide encoding a polypeptide which has substitution, deletion or insertion of one or more (e.g., several tens, 1 to 10, 1 to 5, 1

to 3) amino acids in a polypeptide having an amino acids sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity.

[13-8] The method according to [13] to [13-2] above, wherein the tissue has a point mutation in RET.

[13-9] The method according to [13-8] above, wherein the tissue has a point mutation in the cysteine-rich domain or in the tyrosine kinase domain of RET tyrosine kinase.

[0014]

[13-10] The method according to [13-8] or [13-9] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A, 2600G, 2861T or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-10-1] The method according to any one of [13-8] to [13-10] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A or 2861T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-11] The method according to any one of [13-8] to [13-10] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2494G, 2600G or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-12] The method according to any one of [13-8] to [13-10] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-12-1] The method according to any one of [13-8] to [13-10] and [13-12] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T or 2861T>G in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-13] The method according to any one of [13-8] to [13-12] above, wherein the point mutation is 2091G>T, 2494G>C, 2600G>A or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[13-14] The method according to [13-8] or [13-9] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634,

G691, E768, Y791, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-14-1] The method according to [13-8], [13-9] or [13-14] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, S891 or A883 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-15] The method according to [13-8], [13-9] or [13-14] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, E768, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-16] The method according to [13-8], [13-9] or [13-14] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F, V804M, V804L, S891A or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-16-1] The method according to [13-8], [13-9] or [13-14] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F or S891A in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-17] The method according to any one of [13-8] to [13-16] above, wherein the point mutation in RET is C634Y, E768D, V804M or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[13-18] The method according to any one of [13] to [13-17] above, wherein the method identifies a subject to be administered with a compound represented by formula (I), a salt thereof or a solvate thereof for treatment and/or prevention of a tumor with a mutation in RET or metastasis of the tumor.

[13-19] The method according to any one of [13] to [13-18] above, wherein the tumor is thyroid cancer or lung cancer.

[13-20] The method according to any one of [13] to [13-19] above, wherein the compound represented by formula (I), the salt thereof or the solvate thereof selectively inhibits RET.

[13-21] The method according to any one of [13] to [13-20] above, wherein R¹ is ethyl.

[13-22] The method according to any one of [13] to [13-21] above, wherein the compound of formula (I), the salt thereof or the solvate thereof is hydrochloride of the compound of formula (I).

[0015]

[14] A prophylactic and/or therapeutic method for a tumor with a mutation in RET and for metastasis of the tumor, which comprises identifying a patient with a mutation in RET and administering the patient with an effective therapeutic amount of a compound represented by formula (I), a salt thereof or a solvate thereof.

[15] A method for identifying or preliminarily identifying a patient sensitive to a compound represented by formula (I), a salt thereof or a solvate thereof, which comprises the steps of:

detecting the presence of a mutation in RET in a sample obtained from the patient;
and

determining or preliminarily determining that the patient has sensitivity to the compound, the salt thereof or the solvate thereof, on the basis of the presence of a mutation in RET in the sample.

[15-1] The method according to [15] above, further comprising the step of detecting activation of RET tyrosine kinase.

[15-2] The method according to [15] above, wherein the mutation in RET is a mutation which induces activation of RET tyrosine kinase.

[15-2-1] The method according to any one of [15] to [15-2] above, wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene of RET and/or a fusion protein of RET.

[15-2-2] The method according to any one of [15] to [15-2-1] above, wherein the mutation in RET results in the formation of a fusion gene of RET and/or a fusion protein of RET.

[15-2-3] The method according to any one of [15] to [15-2-2] above, wherein the mutation in RET results in the formation of KIF5B-RET, CCDC6-RET, NCOA4-RET or TRIM33-RET.

[15-3] The method according to any one of [15] to [15-2] above, wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine kinase,

(b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[15-4] The method according to any one of [15] to [15-3] above, wherein the mutation in RET results in the formation of a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

[15-5] The method according to [15-4] above, wherein the other gene and protein are KIF5B, CCDC6, NCOA4 or TRIM33.

[15-6] The method according to [15-4] or [15-5] above, wherein the fusion gene between RET gene and another gene and the fusion protein between RET protein and another protein comprise the tyrosine kinase domain of RET gene or protein and the coiled-coil domain of another gene or protein.

[15-6-1] The method according to any one of [15-4] to [15-6] above, wherein a polypeptide constituting the RET protein and a polynucleotide constituting the RET gene are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4;
- (b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the polypeptide shown in SEQ ID NO: 3 or 4; and
- (c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 3 or 4.

[15-6-2] The method according to [15-5] above, wherein a polypeptide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 proteins and a polynucleotide constituting each of the KIF5B, CCDC6, NCOA4 and TRIM33 genes are any of the following polypeptides and any of polynucleotides encoding the polypeptides:

(1) the polypeptide constituting the KIF5B protein is

- (a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 30,

(b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 30, or

(c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 30;

(2) the polypeptide constituting the CCDC6 protein is

(a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 31,

(b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 31, or

(c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 31;

(3) the polypeptide constituting the NCOA4 protein is

(a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42,

(b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42, or

(c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42; and

(4) the polypeptide constituting the TRIM33 protein is

(a) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 45 or 46,

(b) a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 45 or 46, or

(c) a polypeptide consisting of an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 45 or 46.

[15-6-3] The method according to [15-2-1], [15-2-2], [15-3] or [15-4] above, wherein a polypeptide constituting the fusion protein and a polynucleotide

constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (b) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (c) a polypeptide comprising an amino acid sequence with 80% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;
- (e) a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27 or 28, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28; and
- (f) a polypeptide comprising an amino acid sequence with 80% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28.

[15-6-4] The method according to [15-2-1], [15-2-2], [15-3] or [15-4] above, wherein a polypeptide constituting the fusion protein and a polynucleotide constituting the fusion gene are any of the following polypeptides (a) to (f) and any of polynucleotides encoding the polypeptides:

- (a) a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;
- (b) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which carries activated tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;

- (c) a polypeptide which comprises an amino acid sequence with 90% or higher identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity;
- (d) a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 27 or 28;
- (e) a polypeptide which comprises the amino acid sequence shown in SEQ ID NO: 27 or 28 and which carries activated RET tyrosine kinase, or a polypeptide which comprises an amino acid sequence with substitution, deletion or insertion of 1 to 10 amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity; and
- (f) a polypeptide which comprises an amino acid sequence with 90% or higher identity to the amino acid sequence shown in SEQ ID NO: 27 or 28 and which has tyrosine kinase activity.

[15-6-5] The method according to [15-3] or [15-4] above, wherein the fusion gene is any of (a) to (d) shown below:

- (a) a fusion gene which comprises a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14;
- (b) a fusion gene consisting of a polynucleotide which hybridizes under stringent conditions to DNA consisting of a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14 and which encodes a polypeptide having tyrosine kinase activity;
- (c) a fusion gene which comprises a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24; or
- (d) a fusion gene comprising a polynucleotide encoding a polypeptide which has substitution, deletion or insertion of one or more (e.g., several tens, 1 to 10, 1 to 5, 1 to 3) amino acids in a polypeptide having an amino acids sequence selected from the group consisting of SEQ ID NOs: 15 to 24 and which has tyrosine kinase activity.

[15-7] The method according to any one of [15] to [15-2] above, wherein the mutation in RET is a point mutation.

[15-8] The method according to [15-7] above, wherein the point mutation is a point mutation in the cysteine-rich domain or in the tyrosine kinase domain of RET tyrosine kinase.

[15-9] The method according to [15-7] or [15-8] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A, 2600G, 2861T or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-9-1] The method according to any one of [15-7] to [15-9] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A or 2861T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-10] The method according to any one of [15-7] or [15-9] above, wherein the point mutation is a mutation in the nucleotide 2091G, 2494G, 2600G or 2943T of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-11] The method according to any one of [15-7] to [15-9] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-11-1] The method according to any one of [15-7] to [15-9-1] and [15-11] above, wherein the point mutation is 2091G>T, 2261G>A, 2494G>C, 2562A>T or 2861T>G in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-12] The method according to any one of [15-7] to [15-11] above, wherein the point mutation is 2091G>T, 2494G>C, 2600G>A or 2943T>C in a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1.

[15-13] The method according to [15-7] or [15-8] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-13-1] The method according to [15-7], [15-8] or [15-13] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, S891 or A883 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-14] The method according to [15-7], [15-8] or [15-13] above, wherein the point mutation is a mutation in the amino acid C609, C611, C618, C620, C630, C634, E768, V804, S891, A883 or M918 of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-15] The method according to [15-7], [15-8] or [15-13] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F, V804M, V804L, S891A or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-15-1] The method according to [15-7], [15-8], [15-13] or [15-15] above, wherein the point mutation is C634W, C634Y, G691S, E768D, Y791F or S891A in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-16] The method according to any one of [15-7] to [15-15], wherein the point mutation is C634W, C634Y, E768D, V804M or M918T in a polypeptide having the amino acid sequence shown in SEQ ID NO: 3.

[15-17] The method according to any one of [15] to [15-16] above, wherein the patient is a patient with thyroid cancer or lung cancer.

[15-18] The method according to any one of [15] to [15-17] above, wherein the patient is a patient with thyroid medullary cancer or non-small cell lung cancer.

[15-19] The method according to any one of [15] to [15-18] above, wherein the compound of formula (I), the salt thereof or the solvate thereof selectively inhibits RET.

[15-20] The method according to any one of [15] to [15-18] above, wherein R¹ is ethyl.

[15-21] The method according to any one of [15] to [15-19] above, wherein the compound is a hydrochloride.

[16] A method for predicting the sensitivity of a patient to a compound of formula (I), a salt thereof or a solvate thereof, which comprises the steps of;

- (1) confirming the presence or absence of a mutation in RET in a sample obtained from the patient; and
- (2) determining or preliminarily determining that the patient has sensitivity to the compound of formula (I), the salt thereof or the solvate thereof, provided that the mutation in RET is present.

EFFECTS OF THE INVENTION

[0016]

The therapeutic and/or prophylactic agent of the present invention has a potent inhibitory effect against RET, particularly against RET tyrosine kinase, and is useful as a prophylactic or therapeutic agent (particularly therapeutic agent) for proliferative diseases. Moreover, the active ingredient in the present invention is useful as a prophylactic or therapeutic agent (particularly therapeutic agent) for diseases including various types of cancers, such as leukemia (e.g., acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia), malignant lymphoma (e.g., Hodgkin's lymphoma, non-Hodgkin's lymphoma), brain tumor, neuroblastoma, glioma, thyroid cancer, myelodysplastic syndrome, head and neck cancer, esophageal cancer, gastric cancer, colorectal cancer, breast cancer, ovarian cancer, lung cancer, pancreatic cancer, liver cancer, gallbladder cancer, skin cancer, malignant melanoma, kidney cancer, renal pelvic and ureteral cancer, bladder cancer, uterine cancer, testicular cancer and prostate cancer. The active ingredient in the present invention is further useful as a prophylactic or therapeutic agent (particularly therapeutic agent) for infiltration and metastasis of solid cancers.

The present invention achieves the identification of a cancer or a patient with a mutation in RET and achieves the effective treatment, etc. of such a patient using the compound represented by formula (I) or the like.

BRIEF DESCRIPTION OF THE DRAWING

[0017]

FIG. 1 shows the antitumor activity of compound 1 using xenograft mouse models having CCDC6-RET fusion gene (Example 6).

Mode for Carrying out the Invention

[0018]

An explanation will be given below of the therapeutic or prophylactic agent of the present invention and preparation procedures thereof.

Definitions

In the context of the present invention, the term "C₁₋₆ alkyl group" refers to a monovalent group derived from a linear or branched aliphatic hydrocarbon containing 1 to 6 carbon atoms by removing any one of the hydrogen atoms. More specifically, examples include a methyl group, an ethyl group, an isopropyl group, a butyl group, a n-butyl group, an isobutyl group, a sec-butyl group, a t-butyl group, a pentyl group, an isopentyl group, a 2,3-dimethylpropyl group, a hexyl group, a 2,3-dimethylhexyl group, a 1,1-dimethylpentyl group, a heptyl group and an octyl group. Preferred is a C₁₋₆ alkyl group, more preferred is a C₁₋₅ alkyl group, even more preferred is a C₁₋₄ alkyl group, and still even more preferred is a C₁₋₃ alkyl group.

[0019]

In the context of the present invention, the expression "with a mutation in RET", "a mutation in RET" or "a mutation of RET" is intended to mean that a mutation occurs in RET gene and/or RET protein. In the context of the present invention, the expression "with a mutation in RET", "a mutation in RET" includes a point mutation, a deletion mutation or an insertion mutation in RET gene and/or RET protein, translocation- or inversion-mediated fusion between RET gene and another gene, as well as fusion protein formation between RET protein and another protein. The expression "with a mutation in RET", "a mutation in RET" or "a mutation of RET" further includes amplification of RET gene and/or amplification of RET protein, caused by an increased number of DNA regions on the genome compared to the normal state upon cleavage and rejoining of RET gene, impairment in the repair functions for RET gene, etc.

In the context of the present invention, the term "RET gene" is intended to mean a gene encoding RET (rearranged during transfection) tyrosine kinase. The RET gene of the present invention is intended to mean RET gene of any origin. Specifically, examples include, but are not limited to, a gene having a polynucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 1 or 2, and a polynucleotide encoding a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4.

[0020]

In the context of the present invention, the term "RET protein" is intended to mean a protein consisting of an amino acid sequence constituting RET tyrosine kinase. The RET protein of the present invention is intended to mean RET protein of any origin. Specifically, examples include, but are not limited to, a protein having a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 3 or 4. It is known that there are three types of proteins RET9, RET43 and RET51 for RET tyrosine kinase due to differences in carboxyl-terminal splicing (TRENDS in Genetics, 2006, vol. 22, p. 627-636), and polypeptides consisting of amino acids constituting these three types of proteins also fall within "RET protein."

[0021]

In the present invention, the polypeptide constituting the RET protein and the polynucleotide constituting the RET gene include the following polypeptides and genes encoding the polypeptides:

a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in the polypeptide shown in SEQ ID NO: 3 or 4; and

a polypeptide consisting of an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to the amino acid sequence shown in SEQ ID NO: 3 or 4.

In the context of the present invention, the expression "polynucleotide encoding a polypeptide" encompasses every polynucleotide capable of encoding a specific polypeptide and encompasses any of genomic DNA and cDNA. The polynucleotide includes even a degenerate polynucleotide composed of any codon encoding the same amino acid.

[0022]

In the present invention, the identity of an amino acid sequence can be calculated by: properly aligning at least two sequences to be compared with each other; determining identical amino acid residues between the sequences; determining the number of matching sites; and subsequently dividing the number of the matching sites by the total number of residues in the sequence region to be compared and multiplying the obtained numeric value by 100. For example, the identity of a specific amino acid sequence to the amino acid sequence shown in SEQ ID NO: 3

can be calculated by: determining the number of matching sites between two sequences, i.e., the amino acid sequence shown in SEQ ID NO: 3 and the specific amino acid sequence, by the above method; and subsequently dividing the number of the matching sites by the total number of residues in the amino acid sequence shown in SEQ ID NO: 3 and multiplying the obtained numeric value by 100.

[0023]

Alternatively, the identity of an amino acid sequence may be determined by the Karlin-Altschul BLAST algorithm (Proc. Natl. Acad. Sci. USA (1993) 90: 5873-7). On the basis of this algorithm, a program called BLASTN or BLASTX has been developed (Altschul et al., J. Mol. Biol. (1990) 215: 403-10). Each nucleotide sequence can be analyzed by BLASTN on the basis of BLAST using parameters set to, for example, score = 100 and wordlength = 12. Also, each amino acid sequence can be analyzed by BLASTX on the basis of BLAST using parameters set to, for example, score = 50 and wordlength = 3. In the case of using BLAST and Gapped BLAST programs, the default parameters of each program are used. Specific approaches of these analysis methods are known in the art (see information provided by the website of BLAST (Basic Local Alignment Search Tool), NCBI (National Center for Biotechnology Information)).

[0024]

In the context of the present invention, the term "hybridizing" is intended to mean hybridizing to a target DNA or polynucleotide under stringent conditions. The stringent conditions can be determined on the basis of the melting temperature (T_m) of a nucleic acid to form a complex according to a routine method. Specifically, the stringent conditions involve "5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 50% formamide, 200 µg/ml salmon sperm DNA, 42°C overnight" as conditions for hybridization and "0.5 × SSC, 0.1% SDS, 42°C" as conditions for washing. More stringent conditions involve "5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 50% formamide, 200 µg/ml salmon sperm DNA, 42°C overnight" as conditions for hybridization and "0.2 × SSC, 0.1% SDS, 65°C" as conditions for washing.

[0025]

The expression "with a mutation in RET", "a mutation in RET" or "a mutation of RET" further includes a state where a mutation which induces activation of RET tyrosine kinase or a mutation which activates RET tyrosine kinase and induces oncogenesis (e.g., thyroid cancer, lung cancer) has occurred in RET gene and/or RET protein. The activation of RET tyrosine kinase can be confirmed by detecting phosphorylated RET in a tumor tissue by immunostaining or the like using an anti-phosphorylated RET antibody.

In the context of the present invention, the expression "activation of RET tyrosine kinase" or "state where RET tyrosine kinase has been activated" is intended to mean that an amino acid residue (e.g., a tyrosine residue) contained in RET tyrosine kinase has been phosphorylated, and includes the amount of phosphorylated RET tyrosine kinase protein is increased in a subject (e.g., a sample taken from a subject) (e.g., when compared to a normal subject). In addition, the expression "activation of RET tyrosine kinase" or "state where RET tyrosine kinase has been activated" includes a state where phosphorylated RET tyrosine kinase induces phosphorylation of a protein serving as a target of RET tyrosine kinase (hereinafter referred to as a target protein). The expression "activation of RET tyrosine kinase" or "state where RET tyrosine kinase has been activated" include not only the amount of phosphorylated RET tyrosine kinase protein, but also the amount of the above target protein in a phosphorylated form is increased.

In the context of the present invention, the expression "having tyrosine kinase activity" is intended to mean having activity as an enzyme that phosphorylates an amino acid residue, for example, a tyrosine residue, contained in tyrosine kinase. The tyrosine kinase activity of a polypeptide constituting the RET protein can be confirmed by, for example, the above method. In addition, the expression "having tyrosine kinase activity" includes having activity as an enzyme that phosphorylates an amino acid residue of a targeted protein.

[0026]

Mutations reported to induce activation of RET tyrosine kinase include (1) a mutation in the cysteine-rich domain of RET, (2) a mutation in the tyrosine kinase domain of RET, and (3) formation of a fusion gene between RET gene and another gene or a fusion protein between RET protein and another protein (TRENDS in

Genetics, 2006, vol. 22, p. 627-636). The human RET gene is located on chromosome 10 (10q11.2) and composed of 21 exons. The "cysteine-rich domain of RET" refers to a region rich in cysteine found in RET tyrosine kinase, and a polynucleotide encoding this domain is located at exons 10 and 11. The "tyrosine kinase domain of RET" refers to a region having tyrosine kinase activity found in RET tyrosine kinase, and a polynucleotide encoding this domain is located at exons 12 to 18 (TRENDS in Genetics, 2006, vol. 22, p. 627-636).

[0027]

Specific examples of the above mutations (1) to (3) include those listed below. In the context of the present invention, the expression "with a mutation in RET" or "a mutation in RET" includes a state where any of these mutations (1) to (3) has occurred in RET gene and/or RET protein.

(1) Mutation in the cysteine-rich domain

A mutation in C609, C611, C618, C620, C630, C634 or elsewhere in the amino acid sequence shown in SEQ ID NO: 3 (e.g., C634W, C634Y)

(2) Mutation in the tyrosine kinase domain

A mutation in E768, V804, S891, A883, M918 or elsewhere in the amino acid sequence shown in SEQ ID NO: 3 (e.g., E768D, V804M, V804L, M918T)

(3) Formation of a fusion gene between RET gene and another gene or a fusion protein between RET protein and another protein

Formation of KIF5B-RET fusion gene and/or fusion protein comprising the coiled-coil domain of KIF5B and the tyrosine kinase domain of RET

Formation of CCDC6-RET fusion gene and/or fusion protein comprising the coiled-coil domain of CCDC6 and the tyrosine kinase domain of RET

Formation of NCOA4-RET fusion gene and/or fusion protein comprising the coiled-coil domain of NCOA4 and the tyrosine kinase domain of RET

Formation of TRIM33-RET fusion gene and/or fusion protein comprising the coiled-coil domain of TRIM33 and the tyrosine kinase domain of RET

[0028]

In the context of the present invention, the expression "gene of KIF5B" or "KIF5B gene" is intended to mean a gene encoding KIF5B (Kinesin family protein 5B), and the expression "protein of KIF5B" or "KIF5B protein" is intended to mean a

protein consisting of an amino acid sequence constituting KIF5B. These terms are intended to mean a gene or a protein of KIF5B of any origin. Examples of a polynucleotide constituting the KIF5B gene and a polypeptide constituting the KIF5B protein specifically include, but are not limited to, a polynucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 29 and a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 30. The polypeptide constituting the KIF5B protein and the polynucleotide constituting the KIF5B gene further include the following polypeptides and polynucleotides encoding the polypeptides:

a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in the amino acid sequence shown in SEQ ID NO: 30; and

a polypeptide consisting of an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to the amino acid sequence shown in SEQ ID NO: 30.

[0029]

The human KIF5B gene is located in chromosome 10 and composed of 26 exons. Candidates for the nucleotide sequence of KIF5B-RET fusion gene include, but are not limited to, those shown in SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14. Candidates for the amino acid sequence of KIF5B-RET fusion protein include, but are not limited to, those shown in SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24. Such KIF5B-RET fusion gene and fusion protein are reported to comprise the coiled-coil domain of KIF5B and the kinase domain of RET (Nature Medicine. 2012, 18, p. 378-381, Nature Medicine. 2012, 18, p. 382-384). In the present invention, the polypeptide constituting the KIF5B-RET fusion protein and the polynucleotide constituting the KIF5B-RET fusion gene additionally include the following polypeptides and polynucleotides encoding the polypeptides:

(i) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10,

particularly preferably 1 to 5) amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24;

(ii) a polypeptide comprising an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15 to 24; and

(iii) a polypeptide which is the polypeptide (i) or (ii) and which has tyrosine kinase activity.

[0030]

In the context of the present invention, the expression "gene of CCDC6" or "CCDC6 gene" is intended to mean a gene encoding CCDC6 (coiled-coil domain containing 6), and the expression "protein of CCDC6" or "CCDC6 protein" is intended to mean a protein consisting of an amino acid sequence constituting CCDC6. These terms are intended to mean a gene or a protein of CCDC6 of any origin. Examples of a polynucleotide constituting the CCDC6 gene and a polypeptide constituting the CCDC6 protein specifically include, but are not limited to, a polynucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 31 and a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 32. The polypeptide constituting the CCDC6 protein and the polynucleotide constituting the CCDC6 gene further include the following polypeptides and polynucleotides encoding the polypeptides:

a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in the amino acid sequence shown in SEQ ID NO: 31; and

a polypeptide consisting of an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to the amino acid sequence shown in SEQ ID NO: 31.

Human CCDC6 is located in chromosome 10 and composed of 9 exons.

Examples of the nucleotide sequence of CCDC6-RET fusion gene include, but are not limited to, those shown in SEQ ID NOs: 25 and 26. Examples of the amino acid sequence of the CCDC6-RET fusion protein include, but are not limited to, those shown in SEQ ID NOs: 27 and 28. Such CCDC6-RET fusion gene and

fusion protein are reported to comprise the coiled-coil domain of CCDC6 and the tyrosine kinase domain of RET (Nat Med. 2012 Feb 12; 18 (3): 378-81).

Examples of the polypeptide constituting the CCDC6-RET fusion protein and the polynucleotide constituting the CCDC6-RET fusion gene additionally include the following polypeptides and polynucleotides encoding the polypeptides:

- (i) a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27 or 28, or a polypeptide comprising an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in the amino acid sequence shown in SEQ ID NO: 27 or 28;
- (ii) a polypeptide comprising an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to the amino acid sequence shown in SEQ ID NO: 27 or 28; and
- (iii) a polypeptide which is the polypeptide (i) or (ii) and which has tyrosine kinase activity.

[0031]

In the context of the present invention, the expression "gene of NCOA4" or "NCOA4 gene" is intended to mean a gene encoding NCOA4 (nuclear receptor coactivator 4), and the expression "protein of NCOA4" or "NCOA4 protein" is intended to mean a protein consisting of an amino acid sequence constituting NCOA4. These terms are intended to mean a gene or a protein of NCOA4 of any origin. Examples of a polynucleotide constituting the NCOA4 gene and a polypeptide constituting the NCOA4 protein specifically include, but are not limited to, a polynucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 33 to 37 and a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42. The polypeptide constituting the NCOA4 protein and the polynucleotide constituting the NCOA4 gene further include the following polypeptides and polynucleotides encoding the polypeptides:

a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42; and

a polypeptide consisting of an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 to 42.

Human NCOA4 is located in chromosome 10. A gene in which NCOA4 exon 6 is fused with RET exon 12 is reported as a NCOA4-RET fusion gene. This fusion gene and its fusion protein are reported to comprise the coiled-coil domain of NCOA4 and the tyrosine kinase domain of RET (J Clin Oncol, 30 (35), Dec 10, 2012, p. 4352-9).

[0032]

In the context of the present invention, the expression "gene of TRIM33" or "TRIM33 gene" is intended to mean a gene encoding TRIM33 (tripartite motif-containing 33), and the expression "protein of TRIM33" or "TRIM33 protein" is intended to mean a protein consisting of an amino acid sequence constituting TRIM33. These terms are intended to mean a gene or a protein of TRIM33 of any origin. Examples of a polynucleotide constituting the TRIM33 gene and a polypeptide constituting the TRIM33 protein specifically include, but are not limited to, a polynucleotide consisting of the nucleotide sequence shown in SEQ ID NO: 43 or 44 and a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 45 or 46. The polypeptide constituting the TRIM33 protein and the polynucleotide constituting the TRIM33 gene further include the following polypeptides and polynucleotides encoding the polypeptides:

a polypeptide consisting of an amino acid sequence with substitution, deletion or insertion of one or more (preferably 1 to 10, particularly preferably 1 to 5) amino acids in the amino acid sequence shown in SEQ ID NO: 45 or 46; and

a polypeptide consisting of an amino acid sequence with 80% or higher (preferably 85% or higher, more preferably 90% or higher, further preferably 95% or higher) identity to the amino acid sequence shown in SEQ ID NO: 45 or 46.

[0033]

Human TRIM33 is located in chromosome 1. A gene in which TRIM33 exon 14 is fused with RET exon 12 is reported as a TRIM33-RET fusion gene. This fusion gene and its fusion protein are reported to comprise the coiled-coil

domain of TRIM33 and the tyrosine kinase domain of RET (Cancer Discov, 3 (6), Jun 2013, p. 630-5).

The coiled-coil domain is a domain involved in protein dimerization. KIF5B-RET, CCDC6-RET, NCOA4-RET and TRIM33-RET are therefore considered to form dimers through their coiled-coil domains. These proteins are considered to cause the abnormal activation of RET tyrosine kinase via the dimerization between their coiled-coil domains to induce oncogenesis (Nature Medicine. 2012, 18, p. 378-381; Nature Medicine. 2012, 18, p. 382-384; J Clin Oncol, 30 (35), Dec 10, 2012, p. 4352-9; and Cancer Discov 2013 Jun, 3 (6), Jun 2013, p. 630-5).

[0034]

Examples of a mutation in RET protein in thyroid cancer (e.g., thyroid medullary cancer) include C634W, C634Y, E768D, V804M, V804L and M918T mutations in the amino acid sequence shown in SEQ ID NO: 3 (the amino acid sequence of RET). The expressions "C634W," "C634Y," "E768D," "V804M," "V804L" and "M918T" each represent an amino acid mutation, expressed with a numeral representing a specific position which is sandwiched between single-letter symbols of amino acids before and after the mutation. For example, "C634W" denotes a Cys to Trp substitution in the 634th amino acid from the N-terminus of a specific amino acid sequence. Namely, the numeral represents the amino acid position counted from the N-terminus of a specific amino acid sequence, while the single-letter symbols of amino acids appearing before and after the numeral represent amino acids before and after the substitution, respectively.

[0035]

Mutations in RET gene corresponding to the above mutations include 2091G>T, 2494G>C, 2600G>A, 2600G>C and 2943T>C mutations in the nucleotide sequence shown in SEQ ID NO: 1 (the nucleotide sequence of RET). The expressions "2091G>T," "2494G>C," "2600G>A," "2600G>C" and "2943T>C" each represent a nucleotide mutation, expressed with a numeral representing a specific position followed by bases before and after the mutation. For example, "2091G>T" denotes a G to T substitution in the 2091st nucleotide from the 5' end of a specific base sequence. Namely, the numeral represents the base position counted from the

5' end of a specific base sequence, while the base appearing after the numeral and before the symbol ">" represents a base before the substitution and the base appearing after the symbol ">" represents a base after the substitution.

Examples of a mutation in RET in lung cancer (e.g., non-small cell lung cancer) include the formation of KIF5B-RET fusion gene and/or protein, CCDC6-RET fusion gene and/or protein, NCOA4-RET fusion gene and/or protein, TRIM33-RET fusion gene and/or protein, etc. More specifically, examples include, but are not limited to, those listed below.

Formation of KIF5B-RET fusion gene and/or fusion protein comprising the coiled-coil domain of KIF5B and the tyrosine kinase domain of RET

Formation of CCDC6-RET fusion gene and/or fusion protein comprising the coiled-coil domain of CCDC6 and the tyrosine kinase domain of RET

Formation of NCOA4-RET fusion gene and/or fusion protein comprising the coiled-coil domain of NCOA4 and the tyrosine kinase domain of RET

Formation of TRIM33-RET fusion gene and/or fusion protein comprising the coiled-coil domain of TRIM33 and the tyrosine kinase domain of RET

[0036]

In the context of the present invention, the expression "fusion gene of RET," "fusion gene between RET gene and another gene" refers to a gene in which all or a part of RET gene is fused with all or a part of another gene (e.g., KIF5B gene, CCDC6 gene, NCOA4 gene).

In the context of the present invention, the term "KIF5B-RET fusion gene" refers to a gene in which all or a part of RET gene is fused with all or a part of KIF5B gene. The term "CCDC6-RET fusion gene" refers to a gene in which all or a part of RET gene is fused with all or a part of CCDC6 gene. The term "NCOA4-RET fusion gene" refers to a gene in which all or a part of RET gene is fused with all or a part of NCOA4 gene. The term "TRIM33-RET fusion gene" refers to a gene in which all or a part of RET gene is fused with all or a part of TRIM33 gene.

In the context of the present invention, the expression "fusion protein of RET," "fusion protein between RET protein and another protein" refers to a protein in which all or a part of RET protein is fused with all or a part of another protein (e.g., KIF5B protein, CCDC6 protein, NCOA4 protein, TRIM33 protein).

[0037]

In the context of the present invention, the term "KIF5B-RET fusion protein" refers to a protein in which all or a part of RET protein is fused with all or a part of KIF5B protein. The term "CCDC6-RET fusion protein" refers to a protein in which all or a part of RET protein is fused with all or a part of CCDC6 protein. The term "NCOA4-RET fusion protein" refers to a protein in which all or a part of RET protein is fused with all or a part of NCOA4 protein. The term "TRIM33-RET fusion protein" refers to a protein in which all or a part of RET protein is fused with all or a part of TRIM33 protein.

In the context of the present invention, the term "KIF5B-RET" is intended to mean KIF5B-RET fusion gene and/or KIF5B-RET fusion protein. The term "CCDC6-RET" is intended to mean CCDC6-RET fusion gene and/or CCDC6-RET fusion protein. The term "NCOA4-RET" is intended to mean NCOA4-RET fusion gene and/or NCOA4-RET fusion protein. The term "TRIM33-RET" is intended to mean TRIM33-RET fusion gene and/or TRIM33-RET fusion protein.

The expression "tumor with a mutation in RET" is intended to mean a tumor with a mutation in RET gene and/or protein in tumor cells.

[0038]

The term "therapeutic agent" is intended to mean a pharmaceutical agent for directly or indirectly ameliorating a target disease or for preventing exacerbations of the target disease. More specifically, it is intended to mean a pharmaceutical agent for use in growth inhibition or size reduction of tumor tissues, inhibition of metastasis, reduction of tumor markers, amelioration of systemic symptoms or extension of survival period in a patient, etc.

The term "prophylactic agent" is intended to mean a pharmaceutical agent for use in pre-treatment of a patient at risk of suffering from a target disease such that the target disease is not developed.

The expression "metastasis of the tumor" is intended to mean metastasis of the primary tumor to other tissues. A therapeutic agent for "metastasis of the tumor" is intended to mean a pharmaceutical agent for inhibiting or suppressing metastasis of the tumor, or a pharmaceutical agent for growth inhibition or size reduction of tumor recurring as a result of metastasis. A prophylactic agent for

"metastasis of the tumor" is intended to mean a pharmaceutical agent for use in pre-treatment such that the tumor does not metastasize or does not recur as a result of metastasis.

[0039]

The expression "tumors metastasized from tumors" is intended to mean that the tumors listed as primary tumors metastasize to other tissues and develop therein.

The expression "subject to be administered" is intended to mean a subject for which a pharmaceutical agent can be expected to provide a therapeutic effect based on its mechanism of action. More specifically, it is intended to mean a patient with a proliferative disease for which growth inhibition or size reduction of tumor tissues, inhibition of metastasis, reduction of tumor markers, and amelioration of systemic symptoms in the patient can be expected.

The expression "tissue from the subject" is intended to mean a tissue contained in blood, alveoli, a biopsy sample, a sputum sample or the like taken from a subject such as a patient.

The expression "patient with a mutation in RET" is intended to mean that the patient has a mutation in RET gene and/or protein either in tumor or non-tumor tissue taken from a patient.

[0040]

The term "RET inhibitor" is intended to mean a pharmaceutical agent which inhibits the activity of RET kinase, preferably a pharmaceutical agent which binds to RET kinase and has an inhibitory effect against the activity of RET kinase.

The expression "selectively inhibiting RET" is intended to mean that inhibitory activity against RET tyrosine kinase is high in terms of IC_{50} value when compared with inhibitory activity against many other kinases (e.g., ABL, EGFR, FGFR2, HER2, IGF1R, JAK1, KIT, MET, AKT1, MEK1) except for ALK.

The expression "preliminarily determining" or "preliminarily identifying" is intended to mean providing information about the presence of a mutation in RET in order to determine or identify a sensitive patient.

[0041]

In the present invention, the salt of the compound represented by formula (I) includes, for example: hydrochloride, hydrobromide, hydroiodide, phosphate,

phosphonate and sulfate; sulfonates such as methanesulfonate and p-toluenesulfonate; carboxylate such as acetate, citrate, malate, tartrate, succinate and salicylate; alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as magnesium salt and calcium salt; and ammonium salts such as ammonium salt, alkylammonium salt, dialkylammonium salt, trialkylammonium salt and tetraalkylammonium salt. Preferred examples include hydrochloride and methanesulfonate. Hydrochloride is more preferred.

Such a salt is produced by contacting the compound with an acid or a base available in pharmaceutical production.

In the present invention, the compound represented by formula (I) or the salt thereof may be anhydrous or may form a solvate such as a hydrate. The term "solvation" used herein refers to a phenomenon where solute molecules or ions in a solution strongly attract solvent molecules adjacent thereto to create one molecular population and refers to, for example, hydration if the solvent is water. The solvate may be a hydrate or a non-hydrate. An alcohol (e.g., methanol, ethanol, n-propanol), dimethylformamide, or the like can be used as the non-hydrate.

[0042]

Also, the compound or the salt thereof used in the present invention can exist in some tautomeric forms, for example, enol and imine forms, keto and enamine forms and mixtures thereof. The tautomers exist as a mixture of tautomeric sets in a solution. One tautomer is usually dominant in a solid form. The expression "one tautomer" in the present invention includes all tautomers of the compound used in the present invention.

The present invention includes all of stereoisomers (e.g., enantiomers, diastereomers (including cis and trans geometric isomers)) of the compound represented by formula (I), racemates of the isomers and other mixtures. The compound used in the present invention may have, for example, one or more asymmetric points in formula (I). The present invention includes racemic mixtures, diastereomeric mixtures and enantiomers of such compounds.

The compound according to the present invention may be obtained in a free form. In such a case, the free form can be converted to a salt that may be formed by the compound or to a hydrate or solvate thereof according to a routine method.

Alternatively, the compound according to the present invention may be obtained as a salt, hydrate or solvate of the compound. In such a case, these forms can be converted to a free form of the compound according to a routine method.

[0043]

Also, a substance used in the present invention includes a prodrug of the compound of formula (I). In this context, the term "prodrug" is intended to mean a derivative of the compound of formula (I) that is converted to the compound of formula (I) or a pharmaceutically acceptable salt thereof through enzymatic or nonenzymatic degradation under physiological conditions after administration. The prodrug may be inactive when administered to a patient, but exists *in vivo* as the active compound of formula (I) converted therefrom.

The prodrug, for example, converts to a desired drug form when a specific pH is reached or through the action of an enzyme.

[0044]

Typical production method

The compound represented by formula (I) that serves as the active ingredient of the therapeutic or prophylactic agent of the present invention can be produced according to a method described in International Publication No. WO2010/143664, though the method for producing the compound represented by formula (I) is not limited thereto.

[0045]

Therapeutic and/or prophylactic agent of the present invention

The term "therapeutic and/or prophylactic agent" used in the present invention refers to a pharmaceutical agent that is used for treatment or prevention or for treatment and prevention and specifically refers to a pharmaceutical agent that is used, for example, for treating or preventing the target disease or for suppressing progression (preventing exacerbations or maintaining the status quo) of the disease state.

The therapeutic and/or prophylactic agent of the present invention can be used as a pharmaceutical composition comprising a selected compound useful for the present invention and additionally a pharmaceutically acceptable carrier.

The term "pharmaceutically acceptable carrier" used herein is intended to mean one or more compatible solid or liquid excipients or encapsulating materials that are suitable for administration to mammals. The term "acceptable" used herein is intended to mean that the compound of interest and other ingredients are miscible in a composition in such a manner that reaction substantially reducing the pharmaceutical effectiveness of the composition does not occur therebetween under ordinary use conditions. As a matter of course, the pharmaceutically acceptable carrier must have sufficiently high purity and sufficiently low toxicity suitable for administration to, preferably an animal, more preferably a mammal, to be treated.

[0046]

Examples of a material that may be used as the pharmaceutically acceptable carrier include: sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as carboxymethylcellulose sodium, ethylcellulose and methylcellulose; tragacanth gum powder; malt; gelatin; talc; solid lubricants such as stearic acid and magnesium stearate; calcium sulfate; plant oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and cacao oil; polyhydric alcohols such as propylene glycol, glycerin, sorbitol, mannitol and polyethylene glycol; alginic acid; emulsifiers such as TWEEN; wetting agents such as lecithin; colorants; flavors; tableting agents; stabilizers; antioxidants; antiseptics; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

Examples of a method for administering the therapeutic and/or prophylactic agent of the present invention include oral, rectal, parenteral (intravenous, intramuscular, subcutaneous), intracisternal, intravaginal, intraperitoneal, intravesical and local (drip, powder, ointment, gel or cream) routes and inhalation (into oral cavity or using nasal sprays). Examples of the dosage form thereof include: solid preparations such as tablets, capsules, granules, powders and pills; liquid preparations such as aqueous and nonaqueous oral solutions and suspensions, and parenteral solutions charged in containers adapted to division into individual doses; and freeze-dried preparations that can be dissolved in use. Alternatively, the dosage form may be adapted to various administration methods encompassing controlled-release formulations as in subcutaneous implantation.

[0047]

These preparations are produced by a well known method using additives such as excipients, lubricants (coating agents), binders, disintegrants, stabilizers, flavoring agents and diluents.

Examples of the excipients can include starches such as starch, potato starch and corn starch, lactose, crystalline cellulose and calcium hydrogen phosphate.

Examples of the coating agents can include ethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, shellac, talc, carnauba wax and paraffin.

Examples of the binders can include polyvinylpyrrolidone, Macrogol and the compounds similar to the excipients.

Examples of the disintegrants can include compounds similar to the excipients and chemically modified starches and celluloses such as croscarmellose sodium, carboxymethyl starch sodium and cross-linked polyvinylpyrrolidone.

Examples of the stabilizers can include: p-hydroxybenzoate esters such as methylparaben and propylparaben; alcohols such as chlorobutanol, benzyl alcohol and phenylethyl alcohol; benzalkonium chloride; phenols such as phenol and cresol; thimerosal; dehydroacetic acid; and sorbic acid.

Examples of the flavoring agents can include sweeteners, acidulants and flavors usually used.

The liquid preparations can be produced using a solvent such as ethanol, phenol, chlorocresol, purified water or distilled water.

Examples of the surfactants or emulsifiers can include polysorbate 80, polyoxyl 40 stearate, sodium lauryl sulfate and Lauromacrogol.

[0048]

The amount of the prophylactic and/or therapeutic agent of the present invention used differs depending on symptom, age, body weight, relative health conditions, the presence of other medications, administration methods, etc. In the case of oral agents, a general effective amount, for example, for a patient (warm-blooded animal, particularly a human) is preferably 0.001 to 3000 mg/kg body weight, more preferably 0.01 to 300 mg/kg body weight, per day in terms of the amount of the active ingredient (compound represented by formula (I)), and the daily dose in an adult patient having a normal body weight is in the range of preferably 1

to 800 mg. In the case of parenteral agents, a general effective amount is preferably 0.001 to 1000 mg/kg body weight, more preferably 0.01 to 300 mg/kg body weight, per day. Desirably, this amount is administered at a single dose or several divided doses per day according to symptom.

The prophylactic and/or therapeutic agent of the present invention may be formulated by a method described in International Publication No. WO2012/023597. [0049]

The therapeutic and/or prophylactic agent of the present invention may be used in combination with one or more pharmaceutical agents selected from, for example, other chemotherapeutic agents, hormone therapeutic agents, immunotherapeutic agents and molecular target drugs (hereinafter, collectively referred to as concomitant agents). Such an active ingredient may be a low-molecular-weight compound. Alternatively, such an active ingredient may be a low-molecular-weight compound, may be a high-molecular-weight protein, polypeptide or antibody, or may be a vaccine or the like. Moreover, two or more of these active ingredients may be mixed for use at an appropriate ratio.

Examples of the "chemotherapeutic agents" include alkylating agents, platinum preparations, metabolic antagonists, topoisomerase inhibitors, anticancer antibiotics and plant-derived anticancer agents. Examples of the "alkylating agents" include nitrogen mustard, nitrogen mustard-N-oxide hydrochloride, chlorambucil, cyclophosphamide, ifosfamide, thiotepa, carboquone, improsulfan tosilate, busulfan, nimustine hydrochloride, mitobronitol, melphalan, dacarbazine, ranimustine, estramustine sodium phosphate, triethylenemelamine, carmustine, lomustine, streptozocin, pipobroman, ethoglucid, altretamine, ambamustine, dibrospidium hydrochloride, fotemustine, prednimustine, pumitepa, ribomustin, temozolomide, treosulfan, trofosfamide, zinostatin stimalamer, adozelesin, cystemustine and bizelesin. Examples of the "platinum preparations" include carboplatin, cisplatin, miboplatin, nedaplatin and oxaliplatin. Examples of the "metabolic antagonists" include mercaptopurine, 6-mercaptopurine riboside, thioinosine, methotrexate, enocitabine, cytarabine, cytarabine ocfosfate, ancitabine hydrochloride, 5-FU drugs (e.g., fluorouracil, tegafur, UFT, doxifluridine, carmofur, galocitabine, emitefur), aminopterin, leucovorin calcium, tabloid, butocin, calcium folinate, calcium

levofolinate, cladribine, fludarabine, gemcitabine, hydroxycarbamide, pentostatin, piritrexim, idoxuridine, mitoguazone, tiazofurin and ambamustine. Examples of topoisomerase I inhibitors (e.g., irinotecan, topotecan), topoisomerase II inhibitors (e.g., sobuzoxane) and the "anticancer antibiotics" include anthracycline anticancer agents (doxorubicin hydrochloride, daunorubicin hydrochloride, aclarubicin hydrochloride, pirarubicin hydrochloride, epirubicin hydrochloride, etc.), actinomycin D, actinomycin C, mitomycin C, chromomycin A3, bleomycin hydrochloride, bleomycin sulfate, peplomycin sulfate, neocarzinostatin, mithramycin, sarkomycin, carzinophilin, mitotane, zorubicin hydrochloride, mitoxantrone hydrochloride and idarubicin hydrochloride. Examples of the "plant-derived anticancer agents" include vinca alkaloid anticancer agents (vinblastine sulfate, vincristine sulfate, vindesine sulfate, etc.), taxane anticancer agents (paclitaxel, docetaxel, etc.) etoposide, etoposide phosphate, teniposide and vinorelbine.

[0050]

Examples of the "hormone therapeutic agents" include adrenocortical hormone drugs (e.g., dexamethasone, prednisolone, betamethasone, triamcinolone). Among them, prednisolone is preferred.

Examples of the "immunotherapeutic agents (biological response modifiers: BRMs)" include Picibanil, Krestin, sizofiran, lentinan, ubenimex, interferons, interleukins, macrophage colony-stimulating factors, granulocyte colony-stimulating factors, lymphotoxins, BCG vaccines, *Corynebacterium parvum*, levamisole, polysaccharide K and procodazole.

The "molecular target drugs" include, for example, "pharmaceutical agents that inhibit the action of cell growth factors and their receptors". The "cell growth factors" may be any substance that promotes cell growth, and examples typically include peptides with a molecular weight of 20,000 or lower which are factors that exert action at lower concentrations through binding to their receptors and specifically include (1) EGF (epidermal growth factor) and substances having substantially the same activity thereof [e.g., EGF, heregulin (HER2 ligand)], (2) insulin and substances having substantially the same activity thereof [e.g., insulin, IGF (insulin-like growth factor)-1, IGF-2], (3) FGF (fibroblast growth factor) and substances having substantially the same activity thereof [e.g., acidic FGF, basic

FGF, KGF (keratinocyte growth factor), FGF-10], (4) VEGF (vascular endothelial growth factor) and (5) 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), TGF β (transforming growth factor β), HGF (hepatocyte growth factor)].

The "cell growth factor receptors" may be any receptor having the ability to bind to the above cell growth factors, and specific examples include EGF receptors, heregulin receptors (HER2), insulin receptors, IGF receptors, FGF receptor-1 or FGF receptor-2, HGF receptors (c-met), VEGF receptors and SCF receptors (c-kit). Examples of the "pharmaceutical agents that inhibit the action of cell growth factors" include Herceptin (HER2 antibody), GLEEVEC (c-kit, abl inhibitor) and Tarceva (EGF receptor inhibitor).

The molecular target drugs also include pharmaceutical agents each inhibiting the actions of a plurality of cell growth factors, and pharmaceutical agents that block intracellular signals generated by cell growth factors.

[0051]

In addition to the above pharmaceutical agents, L-asparaginase, aceglatone, procarbazine hydrochloride, protoporphyrin-cobalt complex salts, mercury-hematoporphyrin sodium, differentiation inducers (e.g., retinoid, vitamin Ds), angiogenesis inhibitors, α -blockers (e.g., tamsulosin hydrochloride), and the like can also be used.

Among those described above, a platinum complex (e.g., carboplatin, cisplatin, oxaliplatin), a metabolic antagonist (e.g., gemcitabine, pemetrexed), a topoisomerase I inhibitor (e.g., irinotecan, topotecan), a plant-derived anticancer agent (taxane drugs (e.g., paclitaxel, docetaxel), vinorelbine), an anticancer antibiotic (e.g., mitomycin C), a hormone therapeutic agent (e.g., prednisolone), an immunotherapeutic agent (e.g., Picibanil, Krestin), a molecular target drug (e.g., anti-VEGF antibodies such as bevacizumab, EGFR inhibitors such as erlotinib, VEGFR inhibitors such as sunitinib), or the like is preferred as a concomitant agent. Cisplatin, gemcitabine, paclitaxel, bevacizumab, or the like is more preferred. Alternatively, the therapeutic and/or prophylactic agent of the present invention may be used in combination with a combination therapy using these pharmaceutical

agents. Examples include combined use with a combination therapy of cisplatin, vinblastine and mitomycin C, cisplatin and vinorelbine, cisplatin and paclitaxel, cisplatin and gemcitabine, carboplatin and paclitaxel, pemetrexed and cisplatin, or bevacizumab, cisplatin and pemetrexed.

[0052]

In the present invention, the timings of administration of the active ingredient of the present invention and the concomitant agent are not limited, and they may be administered simultaneously or at a time interval to a subject to be administered. Alternatively, the active ingredient of the present invention and the concomitant agent may be administered as a single preparation comprising them to a subject to be administered. For example, they may be administered by a multidrug therapy which involves drip-injecting a plurality of drugs in combination over 3 to 6 months or by a method which involves taking oral agents over approximately 2 years.

Also, a preoperative adjuvant therapy such as "chemotherapy" may be performed before execution of surgery in order to inhibit already spread tumor (cancer) cells to prevent recurrence as a result of metastasis or for the purpose of reducing the extent of surgery.

A postoperative adjuvant therapy such as "chemotherapy" may be further performed in order to inhibit the growth of tumor (cancer) cells that have not been removed by local treatment such as surgery or radiation to prevent recurrence as a result of metastasis.

The anticancer agent that may be used in combination with the active ingredient of the present invention may act on not only cancer cells but also normal cells, resulting in the occurrence of adverse reactions. Typical adverse reactions include nausea caused by gastrointestinal mucosal damage, vomiting, anorexia, stomatitis, diarrhea or constipation, taste abnormality, decrease in leukocyte, erythrocyte or platelet level or alopecia caused by bone marrow damage, immune compromise, etc. The active ingredient of the present invention and the concomitant agent may be used in combination with an adverse reaction-reducing agent in order to prevent these adverse reactions. Examples include antiemetics effectively suppressing nausea (e.g., granisetron hydrochloride) and drugs promoting recovery from bone marrow damage (e.g., erythropoietin, G-CSF, GM-CSF).

The dose of the concomitant agent can be appropriately selected with reference to a dose clinically used. The mixing ratio between the active ingredient of the present invention and the concomitant agent can be appropriately selected according to the subject to be administered, administration routes, the target disease, symptom, combination of the pharmaceutical agents, etc. For example, when the subject to be administered is a human, 0.01 to 100 parts by weight of the concomitant agent may be used with respect to 100 parts by weight of a preparation comprising the active ingredient of the present invention.

[0053]

Method for detecting mutation in RET

The therapeutic and/or prophylactic agent of the invention of the present application is expected to be therapeutically and/or prophylactically effective by administration to a subject confirmed to have a mutation in RET. This suggests that a patient confirmed to have a mutation in RET is sensitive to the therapeutic and/or prophylactic agent of the invention of the present application. In the present invention, the method for detecting a mutation in RET includes a method for detecting a mutation in RET gene and a method for detecting a mutation in RET protein.

The method for detecting a mutation in RET gene comprises the step of detecting the presence of a RET gene-related specific polynucleotide shown below in a sample obtained from the subject and the amplification of the specific polynucleotide.

Specifically, the method comprises the following steps (1) to (3):

- (1) A sample (blood, pulmonary alveolus, a biopsied sample, an expectoration sample, etc.) is taken from the subject;
- (2) Genomic DNA or a transcript thereof (e.g., mRNA, cDNA, protein) is extracted from the sample. The genomic DNA can be extracted by a method known in the art. This extraction can be conveniently performed using a commercially available DNA extraction kit.
- (3) The presence of a specific polynucleotide in the extracted genomic DNA or transcript thereof (e.g., mRNA, cDNA, protein) and the presence or absence of amplification of the specific polynucleotide are detected.

The presence of the specific polynucleotide can be detected using gene analysis methods known in the art (e.g., methods such as PCR, reverse transcription PCR, Sanger sequencing, *in situ* hybridization and microarray method) singly or in combination.

In the event of detecting the presence of the specific polynucleotide sequence by using mRNA, the detection can be performed by gene amplification reaction such as reverse transcription PCR using primers designed to be capable of specifically amplifying the polynucleotide sequence to be detected. The primer design can be performed using primer design software (e.g., Primer Express; PE Biosystems) or the like.

In addition, the PCR products which are obtained by PCR and reverse transcription PCR are analyzed by agarose gel electrophoresis, and the successful obtainment of an amplification fragment with a size of interest can be confirmed by ethidium bromide staining or the like. The successful obtainment of an amplification fragment with a size of interest shows that the specific polynucleotide is present in the sample obtained from the subject.

A point mutation, deletion mutation or insertion mutation in RET gene can be detected by detecting the presence of the specific polynucleotide using, for example, a combined method of the above reverse transcription PCR and Sanger sequencing, or a single-nucleotide extension reaction method known in the art.

[0054]

A fusion gene between RET gene and another gene can be detected by a method for detecting the presence of the specific polynucleotide using a combined method of the above reverse transcription PCR and Sanger sequencing or using an *in situ* hybridization technique. The detection using the *in situ* hybridization technique can be performed by, for example, fluorescent *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH) or silver *in situ* hybridization (SISH) known in the art.

A probe which can be used in hybridization is a nucleic acid molecule of at least 32 consecutive nucleotides (16 upstream nucleotides and 16 downstream nucleotides flanking the fusion point) that hybridizes under stringent conditions (preferably under more stringent conditions) to the specific polynucleotide or its

complementary strand, but not limited to. A probe comprising the sequence of a specific portion in a polynucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 5 to 14, 25 and 26, or its complementary strand can also be used.

The stringent conditions can be determined on the basis of the melting temperature (T_m) of a nucleic acid to form a complex according to a routine method. Specifically, the stringent conditions involve "5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 50% formamide, 200 µg/ml salmon sperm DNA, 42°C overnight" as conditions for hybridization and "0.5 × SSC, 0.1% SDS, 42°C" as conditions for washing. More stringent conditions involve "5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 50% formamide, 200 µg/ml salmon sperm DNA, 42°C overnight" as conditions for hybridization and "0.2 × SSC, 0.1% SDS, 65°C" as conditions for washing.

[0055]

Detection of the amplification of RET gene can be conducted by detecting the amplification of the specific polynucleotide by the above *in situ* hybridization technique or by comparative genomic hybridization (CGH) using genomic DNA.

The specific polynucleotide to be detected refers to a polynucleotide having a base varied due to a point mutation, deletion mutation or insertion mutation in polynucleotide constituting RET gene, a polynucleotide constituting the fusion gene between RET gene and another gene (e.g., KIF5B gene, CCDC6 gene, NCOA4 gene, TRIM33 gene), or amplified polynucleotide constituting RET gene. Examples of such a polynucleotide include, but are not limited to, the polynucleotides shown below and polynucleotides hybridizing under stringent conditions to polynucleotides consisting of sequences complementary to these polynucleotides (particularly polynucleotides encoding polypeptides having tyrosine kinase activity).

(1) Polynucleotide constituting fusion gene between RET gene and KIF5B gene:

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 5

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 6

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 7

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 8

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 9

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 10

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 11

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 12

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 13

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 14

(2) Polynucleotide constituting fusion gene between RET gen and CCDC6 gene:

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 25

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 26

(3) Polynucleotide constituting RET gene:

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1

Polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 2

(4) Polynucleotide having a base varied due to a point mutation, deletion mutation or insertion mutation in polynucleotide constituting RET gene:

Polynucleotide comprising a nucleotide sequence with a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A, 2600G, 2861T or 2943T (e.g., 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C mutation) of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1

Polynucleotide comprising a polynucleotide encoding a polypeptide with a mutation in the amino acid C609, C611, C618, C620, C630, C634, E768, V804, S891, A883 or M918 (e.g., C634W, C634Y, E768D, V804M, V804L or M918T mutation) of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3 [0056]

The method for detecting a mutation in RET further includes a method for detecting a mutation in RET protein in addition to the above method.

The method for detecting a mutation in RET comprises the step of detecting the presence of a specific polypeptide (hereinafter, referred to as a polypeptide to be detected) in a sample obtained from the subject. The step of detecting a polypeptide to be detected involves preparing a lysate from a sample obtained from the subject (e.g., cancer tissues or cells obtained from the subject).

In the event that a polypeptide to be detected is fusion protein between RET protein and another protein, the presence of polypeptide to be detected can be detected by for example, immunoassay or enzyme activity assay using an antibody against KIF5B, CCDC6, NCOA4 or TRIM33 and an anti-RET antibody in combination. Preferably, an approach such as enzyme immunoassay, two-antibody sandwich ELISA, fluorescent immunoassay, radioimmunoassay or Western blotting using a monoclonal or polyclonal antibody specific for the polypeptide to be detected can be used. In addition to above mentioned method, detection of a mutation (including a point mutation, a deletion mutation and an insertion mutation) in RET protein can be conducted by detecting the presence of a polypeptide to be detected using Western blotting, mass spectrometry, or the like.

[0057]

Examples of the polypeptide to be detected include, but are not limited to, the polypeptides shown below and polypeptides with deletion, substitution and/or insertion of one or more amino acids (e.g., 1 to 10, 1 to 5, or 1 to 3 amino acids) in these polypeptides (particularly polypeptides having tyrosine kinase activity).

(1) Polypeptide constituting fusion protein between RET protein and KIF5B protein:

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 15

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 16

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 17

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 18

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 19

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 20

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 21

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 22

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 23

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 24

(2) Polypeptide constituting fusion protein between RET protein and CCDC6 protein:

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 27

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 28

(3) Polypeptide constituting RET protein

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 3

Polypeptide comprising the amino acid sequence shown in SEQ ID NO: 4

(4) Polypeptide having a point mutation in a polypeptide constituting RET protein

Polypeptide encoded by a polynucleotide comprising a nucleotide sequence with a mutation in the nucleotide 2091G, 2261G, 2494G, 2562A, 2600G, 2861T or 2943T (e.g., 2091G>T, 2261G>A, 2494G>C, 2562A>T, 2600G>A, 2600G>C, 2861T>G or 2943T>C mutation) of a polynucleotide having the nucleotide sequence shown in SEQ ID NO: 1

Polypeptide with a mutation in the amino acid C609, C611, C618, C620, C630, C634, G691, E768, Y791, V804, S891, A883 or M918 (e.g., C634W, C634Y, G691S, E768D, Y791F, V804M, V804L, S891A or M918T mutation) of a polypeptide having the amino acid sequence shown in SEQ ID NO: 3
[0058]

In addition, the presence of the fusion gene between RET gene and KIF5B gene or other fusion genes can be detected by methods described in International Publication No. WO2012/014795, Nature Medicine, 2012, 18, p. 378-381, J Clin Oncol, 30 (35), Dec 10, 2012, p. 4352-9, Cancer Discov 2013 Jun, 3 (6), Jun 2013, p. 630-5, etc.

In addition to the above method, the activation of RET tyrosine kinase may be detected. The activation of RET tyrosine kinase can be confirmed by detecting phosphorylated RET in a tumor tissue by immunostaining or the like using an anti-phosphorylated RET antibody.

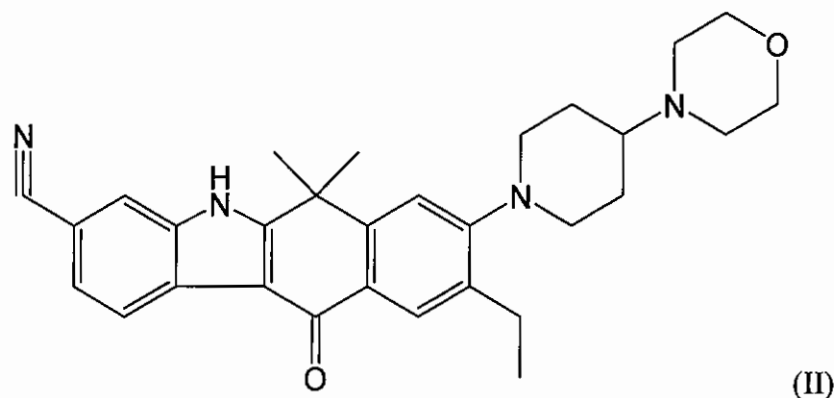
Examples

[0059]

Hereinafter, the present invention will be described more specifically with reference to Examples. However, the present invention is not intended to be limited to these Examples.

Compound 1 (9-ethyl-6,6-dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile, or 9-ethyl-6,6-dimethyl-8-[4-(morpholin-4-yl)piperidin-1-yl]-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile) represented by formula II (compound described in Example 366 of

WO2010/143664) was subjected to pharmacological tests described in Examples 1 to 6.



[0060]

Example 1

Evaluation of RET kinase inhibitory activity and binding affinity

The RET kinase inhibitory activity of compound 1 was evaluated with inhibitory activity against the phosphorylation reaction of a biotinylated peptide (EGPWLEEEEEEAYGWMDF) as an index using RET kinase containing a N-terminally GST-tagged RET kinase domain (Carna Biosciences). The phosphorylated biotinylated peptide was detected by the TR-FRET (time-resolved fluorescence resonance energy transfer) method using an europium labeled anti-phosphorylation antibody. 50% inhibition concentration (IC_{50} value) was calculated by the logistic regression method. As a result, compound 1 exhibited RET kinase inhibitory activity with an IC_{50} value of 4.8 nM.

The dissociation constant (K_d value) of compound 1 for RET kinase was also measured by KINOME_{SCAN}(TM) (DiscoverX). As a result, compound 1 exhibited binding affinity to RET with K_d value of 7.6 nM.

[0061]

Example 2

Establishment of KIF5B-RET-expressing cells and evaluation of cell growth inhibitory activity against these cells

An expression plasmid CS-GS104J-M67 (GeneCopoeia) containing KIF5B-RET variant 1 (gene in which a region from the N-terminus of KIF5B CDS to exon 15 was fused with a region from exon 12 of RET CDS to the C-terminus) (SEQ ID

NO: 5) was transfected into mouse lymphocytes Ba/F3 (RIKEN Cell Bank) by electroporation. After transfection, the cells were cultured overnight in an RPMI-1640 medium (Sigma-Aldrich) containing 10% FBS (Bovogen Biologicals) and 1 ng/mL recombinant mouse IL-3 (R&D systems). Then, the culture supernatant was replaced with an RPMI-1640 medium containing 10% FBS. The cells were seeded into a 96-well plate by the limiting dilution method. Approximately 2 weeks later, the expression of phosphorylated RET in cells grown in the absence of IL-3 was detected by Western blotting. This was used as an index to establish a cell line Ba/F3 KIF5B-RET stably expressing KIF5B-RET.

The Ba/F3 KIF5B-RET cells were seeded into a 96-well plate at a concentration of 2,500 cells/well in an RPMI-1640 medium containing 10% FBS. A hydrochloride salt of compound 1 was diluted to 1 nM to 10 μ M (final concentrations) with dimethyl sulfoxide and added to the 96-well plate. Dimethyl sulfoxide was added as a negative control. After culture at 37°C for 2 days in the presence of 5% CO₂, a cell counting reagent CellTiter-Glo(R) Luminescent Cell Viability Assay (Promega Corporation) was added thereto and stirred, followed by the measurement of luminescence intensity using a luminescence measurement apparatus Envision (PerkinElmer). The measurement value in a well supplemented with only a medium was defined as a cell viability of 0%, while the measurement value in a well supplemented with dimethyl sulfoxide was defined as a cell viability of 100%. The cell viability of the Ba/F3 KIF5B-RET cells was calculated at each concentration of compound 1. The IC₅₀ value was determined from the obtained values by the logistic regression method. As a result, compound 1 inhibited cell growth of Ba/F3 KIF5B-RET cells with IC₅₀ value of 86 nM.

These results demonstrated that compound 1 can inhibit the kinase activity of RET and can inhibit the growth of a cell line expressing KIF5B-RET.

[0062]

Example 3

Evaluation of cell growth inhibitory activity against thyroid medullary cancer cell line TT

Cell growth inhibitory activity was evaluated using a thyroid medullary cancer cell line TT (American Type Culture Collection) with a RET kinase active mutation (C634W).

The TT cells were seeded into a 96-well plate at a concentration of 5,000 cells/well in F-12K Nutrient Mixture (Life Technologies Corporation) containing 10% FBS and cultured overnight at 37°C in the presence of 5% CO₂. Then, a hydrochloride salt of compound 1 was diluted to 1 nM to 10 µM (final concentrations) with dimethyl sulfoxide and added to the 96-well plate. Dimethyl sulfoxide was added as a negative control. After culture at 37°C for 5 days in the presence of 5% CO₂, a cell counting reagent CellTiter-Glo(R) Luminescent Cell Viability Assay was added thereto and stirred, followed by the measurement of luminescence intensity using a luminescence measurement apparatus Envision. The measurement value in a well supplemented with only a medium was defined as a cell viability of 0%, while the measurement value in a well supplemented with dimethyl sulfoxide was defined as a survival rate of 100%. The cell viability of the TT cells was calculated at each concentration of compound 1. The IC₅₀ value was determined from the obtained values by the logistic regression method. As a result, compound 1 inhibited cell growth of TT cells with IC₅₀ value of 190 nM. .

[0063]

Example 4

RET kinase mutant inhibitory activity

The RET kinase mutant inhibitory activity of compound 1 was evaluated with inhibitory activity against the phosphorylation reaction of a biotinylated peptide (EGPWLEEEEEAYGWMDf) as an index using a RET kinase mutant containing a N-terminally GST-tagged RET kinase domain (Carna Biosciences, Millipore). The phosphorylated biotinylated peptide was detected by the TR-FRET method using an europium labeled anti-phosphorylation antibody. The IC₅₀ value was calculated by the logistic regression method. The test results are shown in Table 1. Compound 1 exhibited inhibitory activity against each mutant of RET kinase with a mutation (V804L, V804M) in a gatekeeper residue.

[Table 1]

RET mutant	IC ₅₀ (nM)
RET G691S	9.5
RET Y791F	14
RET V804L	32
RET V804M	53
RET S891A	8.3
RET M918T	5.7

[0064]

Example 5

Evaluation of cell growth inhibitory activity against non-small cell lung cancer cell line LC-2/ad

Cell growth inhibitory activity was evaluated using a non-small cell lung cancer cell line LC-2/ad (RIKEN, J Thorac Oncol. 2012 Dec, 7 (12), 1872-6) harboring CCDC6-RET fusion gene.

The LC-2/ad cells were seeded into a 96-well plate at a concentration of 2,000 cells/well in a medium of a 1:1 mixture of RPMI-1640 and Ham containing 15% FBS and 25 mM HEPES and cultured overnight at 37°C in the presence of 5% CO₂. Then, a hydrochloride salt of compound 1 was diluted to 1 nM to 1 μ M (final concentrations) with dimethyl sulfoxide and added to the 96-well plate. Dimethyl sulfoxide was added as a negative control. After culture at 37°C for 5 days in the presence of 5% CO₂, a cell counting reagent CellTiter-Glo(R) Luminescent Cell Viability Assay was added thereto and stirred, followed by the measurement of luminescence intensity using a luminescence measurement apparatus Envision. The measurement value in a well supplemented with only a medium was defined as a cell viability of 0%, while the measurement value in a well supplemented with dimethyl sulfoxide was defined as a cell viability of 100%. The cell viability of the LC-2/ad cells was calculated at each concentration of compound 1. The IC₅₀ value was determined from the obtained values by the logistic regression method. As a result, compound 1 inhibited cell growth of LC-2/ad cells with IC₅₀ value of 190 nM.

[0065]

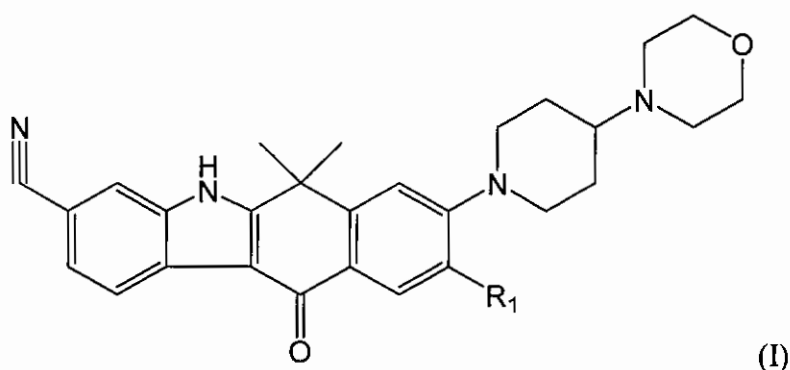
Example 6

Evaluation of antitumor activity in xenograft mouse model harboring CCDC6-RET fusion gene

The *in vivo* antitumor activity of compound 1 was evaluated in non-small cell lung cancer cell line LC-2/ad-implanted mouse models. LC-2/ad was subcutaneously implanted to SCID mice and randomized after tumor size reached 200 to 350 mm³. The administration of the compound was started on the day of randomization. The vehicles used for dissolving the compound were 0.02 N HCl, 10% dimethyl sulfoxide, 10% Cremophor EL, 15% PEG400 and 15% HPCD (2-hydroxypropyl- β -cyclodextrin) (indicated by final concentration). Compound 1 was orally administered once a day to each mouse at a dose of 20 mg/kg or 60 mg/kg for 14 days. As a result, a dose-dependent tumor growth inhibitory effect and tumor regression were confirmed for all tumors. During this test, a significant body weight loss of a mouse was not observed at any dose. The results about the antitumor activity are shown in FIG. 1.

CLAIMS

1 A therapeutic and/or prophylactic agent for a tumor with a mutation in RET and for metastasis of the tumor, which comprises a compound represented by formula (I), a salt thereof or a solvate thereof as an active ingredient:



wherein R₁ is a C₁₋₆ alkyl group.

2 The therapeutic and/or prophylactic agent according to claim 1, wherein the tumor is selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, brain tumor, neuroblastoma, glioma, thyroid cancer, myelodysplastic syndrome, head and neck cancer, esophageal cancer, gastric cancer, colorectal cancer, breast cancer, ovarian cancer, lung cancer, pancreatic cancer, liver cancer, gallbladder cancer, skin cancer, malignant melanoma, kidney cancer, renal pelvic and ureteral cancer, bladder cancer, uterine cancer, testicular cancer, prostate cancer, and tumors metastasized from these tumors.

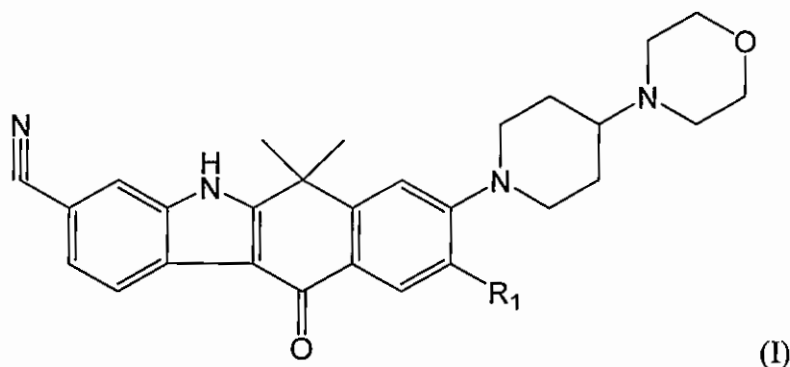
3 The therapeutic and/or prophylactic agent according to claim 1, wherein the tumor is thyroid cancer or lung cancer.

4 The therapeutic and/or prophylactic agent according to claim 1, wherein the tumor is thyroid medullary cancer or non-small cell lung cancer.

5 The therapeutic and/or prophylactic agent according to any one of claims 1 to 4, wherein the tumor is a tumor with a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

6 The therapeutic and/or prophylactic agent according to any one of claims 1 to 4, wherein the tumor is a tumor with a point mutation in RET gene and/or protein.

7 A therapeutic and/or prophylactic agent for a tumor used for a patient with a mutation in RET and for metastasis of the tumor, which comprises a compound represented by formula (I), a salt thereof or a solvate thereof as an active ingredient:



wherein R₁ is a C₁₋₆ alkyl group.

8 The therapeutic and/or prophylactic agent according to claim 7, wherein the tumor is thyroid cancer or lung cancer.

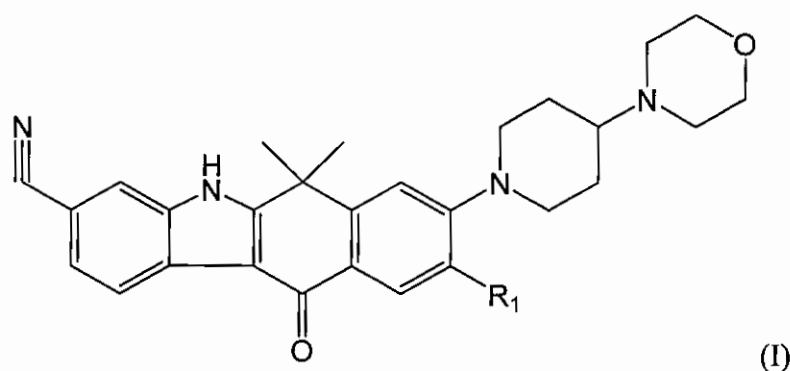
9 The therapeutic and/or prophylactic agent according to claim 7 or 8, wherein the patient is a patient with a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

10 The therapeutic and/or prophylactic agent according to claim 7 or 8, wherein the patient is a patient with a point mutation in RET gene and/or protein.

11 The therapeutic and/or prophylactic agent according to any one of claims 1 to 9, wherein R¹ is ethyl.

12 The therapeutic and/or prophylactic agent according to any one of claims 1 to 11, wherein the compound is a hydrochloride.

13 A RET inhibitor, which comprises a compound of formula (I), a salt thereof or a solvate thereof as an active ingredient:

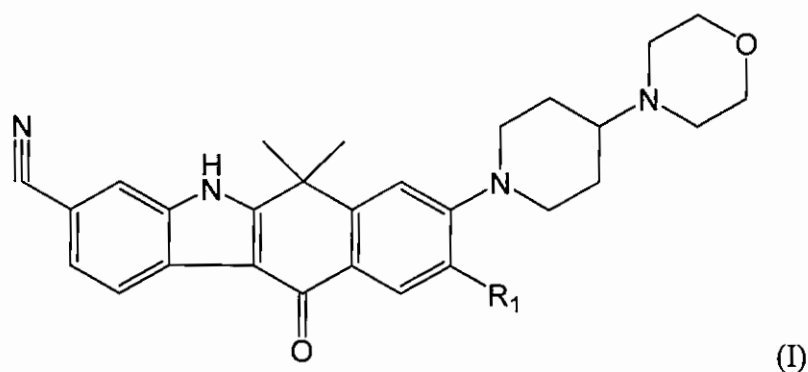


wherein R_1 is a C_{1-6} alkyl group.

14 A method for identifying a patient sensitive to a compound represented by formula (I), a salt thereof or a solvate thereof, which comprises the steps of:

detecting the presence of a mutation in RET in a sample obtained from the patient; and

determining or preliminarily determining that the patient has sensitivity to the compound, the salt thereof or the solvate thereof, on the basis of the presence of a mutation in RET in the sample:



wherein R^1 is a C_{1-6} alkyl group.

15 The method according to claim 14, wherein the mutation in RET is a mutation which induces activation of RET tyrosine kinase.

16 The method according to claim 14 or 15, wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene of RET and/or a fusion protein of RET.

17 The method according to any one of claims 14 to 16, wherein the tumor is a tumor with a fusion gene of RET and/or a fusion protein of RET.

18 The method according to claim 14 or 15 wherein the mutation in RET is (a) a mutation in the cysteine-rich domain of RET tyrosine kinase, (b) a mutation in the tyrosine kinase domain of RET tyrosine kinase, or (c) the formation of a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

19 The method according to claim 14, 15 or 18, wherein the mutation in RET results in the formation of a fusion gene between RET gene and another gene and/or a fusion protein between RET protein and another protein.

20 The method according to claim 19, wherein the another gene and protein is KIF5B, CCDC6, NCOA4 or TRIM33.

21 The method according to any one of claims 18 to 20, wherein the fusion gene between RET gene and another gene and the fusion protein between RET protein and another protein comprise the tyrosine kinase domain of RET gene or protein and the coiled-coil domain of another gene or protein.

22 The method according to claim 14 or 15, wherein the mutation in RET is a point mutation.

23 The method according to claim 22, wherein the point mutation is a point mutation in the cysteine-rich domain or in the tyrosine kinase domain of RET tyrosine kinase.

24 The method according to any one of claims 14 to 23, wherein the patient is a patient with thyroid cancer or lung cancer.

25 The method according to any one of claims 14 to 24, wherein the patient is a patient with thyroid medullary cancer or non-small cell lung cancer.